Arrays of chronically implanted electrodes were used to examine the time course of elongation and maturation of peripheral nerve fibers in the cat after crush of the tibial nerve in the proximal calf. Regeneration after crush alone was compared with crush 5 mm proximal to a tight constriction of the nerve. Regeneration was monitored by the progression of excitability along the electrode arrays on the tibial and plantar nerves. The sensitivity was sufficient to record the averaged activity in single nerve fibers allowing detection of the earliest regeneration. The diameters of the fastest regenerating fibers were estimated from the conduction velocity *proximal* to the site of crush. Both after crush alone, and after crush constriction, small myelinated fibers regenerated in front of large fibers. The rate of elongation after crush alone was 3.2 mm/day, whereas it was slower ($P < 0.02$) distal to crush + constriction (2.2 mm/day). In both lesions, the extrapolated delay to onset of regeneration was 8 days. In observations up to 300 days after crush, maturation was delayed or impaired by the constriction, and the compound nerve action potential had a smaller amplitude and a dispersed shape. Transverse sections of nerves after crush + constriction showed a diminished number of large and an increased number of small fibers compared with crush alone, possibly due to persistent branching of regenerated fibers. After both crush alone and crush + constriction, regenerated fibers had similar *g* ratios, suggesting that myelination developed fully in fibers of diminished diameters.

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CONDUCTION STUDIES IN PERIPHERAL CAT NERVE USING IMPLANTED ELECTRODES: II. THE EFFECTS OF PROLONGED CONSTRICTION ON REGENERATION OF CRUSHED NERVE FIBERS

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Following Wallerian degeneration, peripheral nerve fibers have the capacity to regenerate. The precise temporal sequence of this process in different fibers may have implications for the functional recovery and the reversal of atrophy occurring during the period of denervation. Recovery after traumatic nerve injury appears to be dependent on the type of lesion. Degeneration may occur as a consequence of, or concomitant with, compression or constriction of the nerve trunk. In an antomical study, Weiss and Hiscoe[35] found maturation of regenerated fibers distal to a constriction to be impaired, and Krarup and Gilliatt[19] in a physiological study found delayed and impaired reinnervation of muscle when nerve was crushed proximal to a tight ligature of the nerve. However, as regeneration was gauged from reinnervation of muscle, it was not possible to distinguish a focal delay at the constriction from a reduced rate of regeneration.

The aim of this study was to detect and follow regenerating nerve fibers at well-defined sites along the nerve trunk to measure rates of regeneration. Detection of action potentials from newly regenerated fibers requires high resolution, and

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we have therefore implanted cuff electrodes to obtain fixed anatomical and low-impedance contacts with the nerve.[23] As a result of the high temporal and spatial resolution of the method, different phases of the regeneration process could be distinguished, and we have compared these phases in crush of the nerve with crush proximal to a tight constriction. Our findings suggest that the rate of elongation throughout the segment distal to crush + constriction was slower than after crush alone and that the subsequent maturation was impaired. In both lesions, thin-caliber fibers appeared to regenerate faster than large fibers. Some of the results have been published in preliminary reports.[20–22]

MATERIALS AND METHODS

Regeneration was followed for up to 10 months in 9 nerves from 5 adult cats. The methodology and characteristics of implanted devices have been described.[23]

Nerve Lesions. At the time of implantation of electrodes in anesthetized cats, the tibial nerve was crushed 10–15 mm proximal to the cuff electrode around the tibial nerve (see Fig. 1 in Ref. 23) using 3-mm-wide smooth forceps covered with silicone rubber to avoid cutting supporting connective tissue. The forceps was held clamped for 10 seconds, after which the nerve appeared as a flattened band. In 4 of the 9 nerves, a tight silicone cuff with an internal diameter of 1 mm and a length of 3 mm was tied around the nerve 5 mm distal to the site of crush, reducing the transverse area of the nerve by 60–70%. Trophic disturbances of the limb were not observed in any of the animals.

Electrophysiological Studies. The animals were followed for at least 78 days and at most 300 days. Conduction studies were performed serially during light anesthesia,[23] starting the day after implantation, and were repeated every 5–10 days for 6–8 weeks, then at intervals of 1–3 weeks for 2–3 months, and finally every 4–5 weeks for up to 10 months.

Regenerating fibers distal to the site of crush and distal to crush + constriction were identified by stimulating the nerve at several electrode sites in the tibial cuff electrode and in the plantar patch electrodes. The presence of excitable nerve fibers was detected by recording the ascending nerve action potential at two sites along the undamaged sciatic nerve proximal to the site of crush (Fig. 1). The stimulus was first applied to the electrode 15–20 mm distal to the lesion, and if an action potential could be elicited from this site, the stimulus

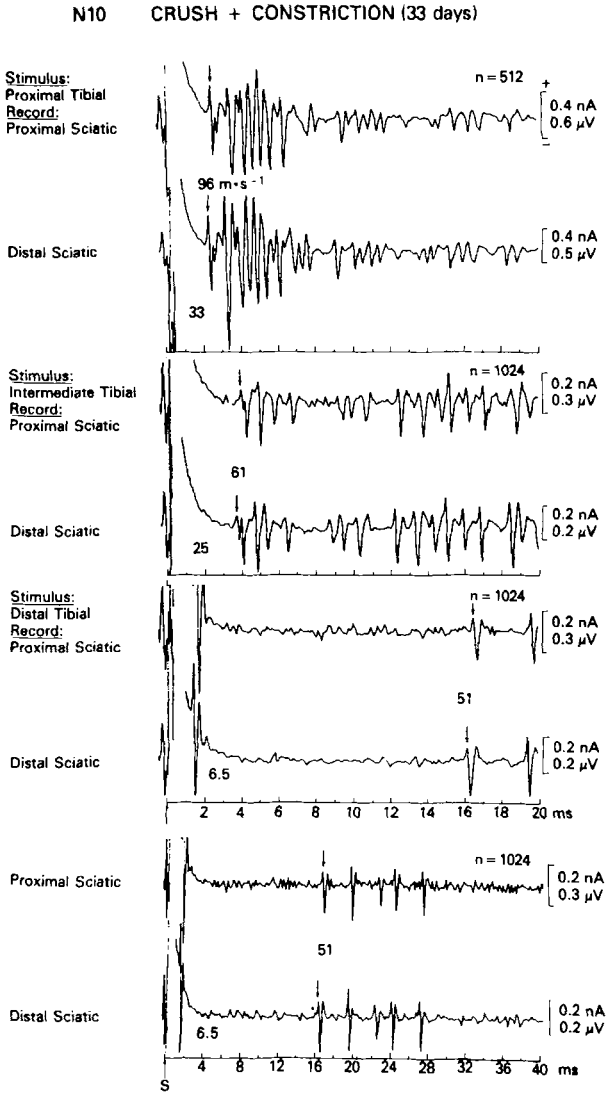


FIGURE 1. Ascending CNAPs recorded from two sites at the sciatic nerve (indicated to the left of tracings). The responses were evoked in the tibial nerve at increasing distances distal to the site of crush. *Top pair of traces:* The tibial nerve was stimulated 18 mm distal to crush. *Second pair of traces:* Stimulus 33 mm distal to crush. *Bottom two pairs of traces:* Stimulus 48 mm distal to crush (two different time bases to show entire potential in bottom traces). Stimulation further distally did not elicit an action potential. The conduction velocity from the site of stimulation to the sciatic nerve indicated below traces and included conduction along a distal regenerated portion of the nerve and along an undamaged portion of the nerve proximal to crush (see text). The conduction velocity between the two sites of sciatic nerve recording (arrows) indicated between traces. The number of averaged responses *(n)* is indicated. The responses were calibrated in voltage and current units[23] in this and other responses (cat N10, right leg after crush + constriction).

was applied to the next lead 15 mm further distally. The process was repeated until a response was absent from a stimulus site after averaging 1024–2048 responses. Reinnervation of plantar muscle was followed by recording the evoked muscle action potential.

Because of the high threshold of newly regenerated fibers, a high stimulus current of up to 10 mA (i.e., a 10–20-fold higher maximal stimulus current than in normal nerve) was necessary. Unfortunately, although the threshold was normal in the sciatic nerve proximal to the lesion, the reverse stimulus-recording paradigm had disadvantages: (1) the animal had to be curarized and ventilated to block large muscle respones from calf muscle, and (2) owing to summations between small, long-duration, and ill-defined action potentials from poorly myelinated fibers, we could not distinguish between temporal dispersion and reduction of the number of responding fibers at the different recording sites distal to the lesion.

Conduction Property Parameters. Conduction velocities were measured along the nerve distal to, across, and proximal to the site of the lesion. During early phases of regeneration, the distal conduction velocity was calculated from the difference in latency between two sites of stimulation. However, as may be seen in Fig. 1 and Table 1, the conduction velocities between the two recording sites at the sciatic nerve differed when the nerve was stimulated at different distal sites, indicating that the same fibers were not activated at the different sites of stimulation. During this phase, the conduction velocity along the distal regenerated segment was therefore also estimated by extrapolation: the sciatic nerve conduction velocity was used to calculate the conduction time proximal to the lesion between the site of crush and the site of recording at the sciatic nerve. This conduction time was subtracted from the overall stimulus-response latency and the remainder considered due to conduction along the regenerated segment distal to the lesion and converted to the corresponding average conduction velocity.

The largest peak-to-peak amplitude of the response was measured in voltage as well current units (Fig. 1).[23]

To measure the safety of transmission during regeneration, responses were recorded during double stimulation with short interstimulus intervals,[23] although the stimulus strength of the second stimulus often only could be increased slightly due to the high excitation threshold of the newly regenerated fibers.

Histological Studies. After completion of the chronic experiments, the tibial and plantar nerves were fixed in situ by perfusion or immersion.[23] The constricting cuff was gently removed from the fixed nerve, and transverse sections of the nerve including surrounding scar were carefully searched to exclude the possibility that fibers had regenerated outside the constriction.

Electronmicroscopy. Areas representing at least two-thirds of the transverse section were photographed at a final magnification of 5400× (controlled by calibration with a diffraction grid). From each nerve, 200 myelinated fibers were analyzed in a Zeiss Videophan computer for fiber and axon size distribution, for myelin thickness as a

Table 1. Sciatic nerve conduction velocities (m/sec) of ascending action potentials evoked distal to crush of the tibial nerve to show faster regeneration of small than large myelinated nerve fibers.

Mixed nerve action potential§	Conduction velocities of action potentials evoked from a given electrode site (S_n)* at different times (t)† and (t + 1)‡	
	From S_n at time t	From S_n at time t + 1
Conduction velocity	61 ± 3 (17)	83 ± 3 (17)
Paired t-test	t = 6.11, $P < 0.001$	
Mixed nerve action potential	Conduction velocities of action potentials evoked from two different electrode sites S_n and S_{n-1}‖ at the same time t	
	From S_n at time t	From S_{n-1} at time t
Conduction velocity	59 ± 3 (15)	87 ± 3 (15)
Paired t-test	t = 6.07, $P < 0.001$	

**The most distal site from which an action potential could be elicited.*
†The time at which an action potential could be elicited from the given most distal excitable site.
‡The following observation in the series.
§Mean ± standard error of the mean (number of observations in eight nerves).
‖The electrode site immediately proximal to the most distal excitable site in the array (S_n).

mean of four measurements at different sites along the circumference, and for calculation of g ratios[27] obtained from the fiber diameter (D) and the myelin thickness (m): $g = (D\text{-}2m)/D$.

RESULTS

The day after the crush injury, nerve conduction distal to the lesion was normal, whereas no response was conducted across the site of crush. When tested 5–10 days later, the distal nerve segment was inexcitable, indicating that myelinated fibers had degenerated.

Findings During Early Regeneration. During the phase of regeneration when nerve fibers elongated into the nerve distal to the crush, the action potential, evoked distal to the lesion and recorded at two sites along the sciatic nerve proximal to the lesion, showed a striking reduction of components with increasing distance from the site of crush, suggesting that the number of excitable fibers decreased distal to the lesion. In Fig. 1 the traces, obtained 33 days after crush + constriction, showed a polyspike potential when the nerve was stimulated 18 and 33 mm distal to the lesion, whereas only five individual spikes were elicited from the most distal excitable site, 48 mm from the constriction. The conduction velocity decreased the further distally the stimulus was applied (Fig. 1), being 33 m/sec from the lead situated 18 mm distal to the site of crush and only 6.5 m/sec from the site 48 mm distal to the lesion. This difference was to some extent due to the relatively longer undamaged portion of the nerve proximal to the site of crush compared with the regenerated portion of the nerve. However, even when the conduction velocity was extrapolated to the regenerated segment of the nerve distal to the lesion (see Methods), it was 11 m/sec when the tibial nerve 18 mm distal to crush was stimulated (top traces in Fig. 1), whereas it was 3 m/sec when the nerve 48 mm distal to crush was stimulated (bottom two sets of traces in Fig. 1). Hence, a higher degree of maturation had probably been attained closer to the lesion, suggesting that regenerated fibers were tapered distal to the lesion rather than abruptly reduced in diameter at the lesion.

The unitary triphasic action currents from the most distal excitable sites had amplitudes (0.06–0.8 nA) and duration (<1 msec) similar to those obtained from single fibers by spike-triggered averaging[11,26] (Fig. 4 in Ref. 23). That these potentials, evoked by maximal electrical stimulation, were recorded from single fibers was further supported by their *all-or-none* response at short stimulus intervals. During double-stimulation, the refractory period of transmission of the newly regenerated fibers was markedly prolonged to >2 msec. The characteristics of these potentials were further analyzed by relating the amplitude of the triphasic unitary responses to their conduction velocity (Fig. 2). The amplitude was obtained as an average from the two sites of recording at the sciatic nerve, and the conduction velocity was measured between these two sites. A power function fitted the data:

$$\text{Amplitude} = k \bullet \text{conduction velocity}^{x}$$

where k is a constant dependent on the size of the cuff and the position of the nerve fiber within the

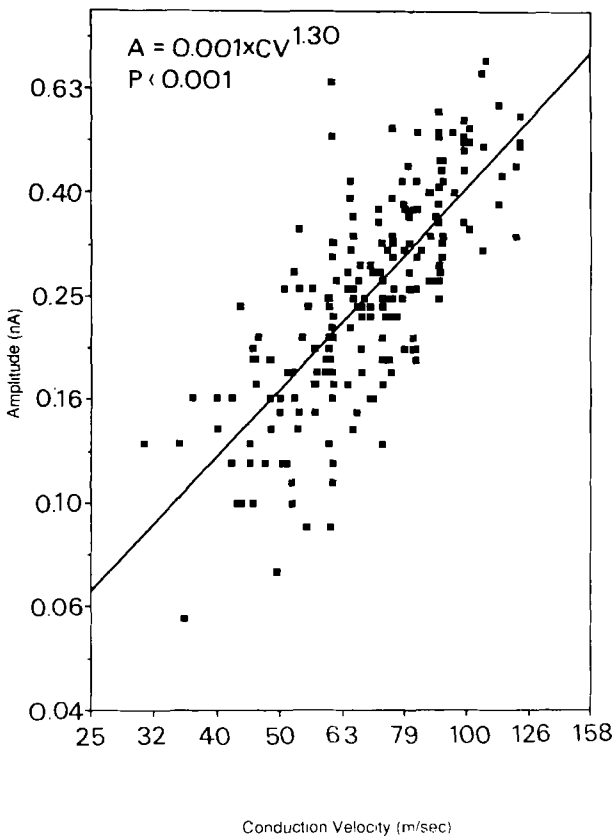


FIGURE 2. Relationship between the amplitude (nA, ordinate) and the conduction velocity (m/sec, abscissa) of unitary action potentials recorded from the sciatic nerve proximal to the site of crush. The amplitude was the average of the peak-to-peak amplitudes of the responses recorded at two sites, and the velocity was measured between the sites. The coordinates were drawn to log scale, and the regression line was calculated from least squares ($r = 0.76$, $P < 0.001$, $n = 206$). The regression line corresponds to the following function (see text): Amplitude = 0.001 • conduction velocity$^{1.30}$.

nerve. The exponent x ranged in individual cats from 1.07 to 1.47, with a mean ± SEM of 1.32 ± 0.06. With increasing spacing between leads in the electrode, the amplitude will approach the square of the conduction velocity[30] but change towards unity with short interlead spacing approaching the internodal length.[24] The exponent of 1.3 in these experiments corresponds reasonably to the predicted value considering the interlead spacing of 7.5 mm,[23] and it was similar to that found by Hoffer et al.[14] using slightly longer lead spacing for spike-triggered averaging in freely moving cats.

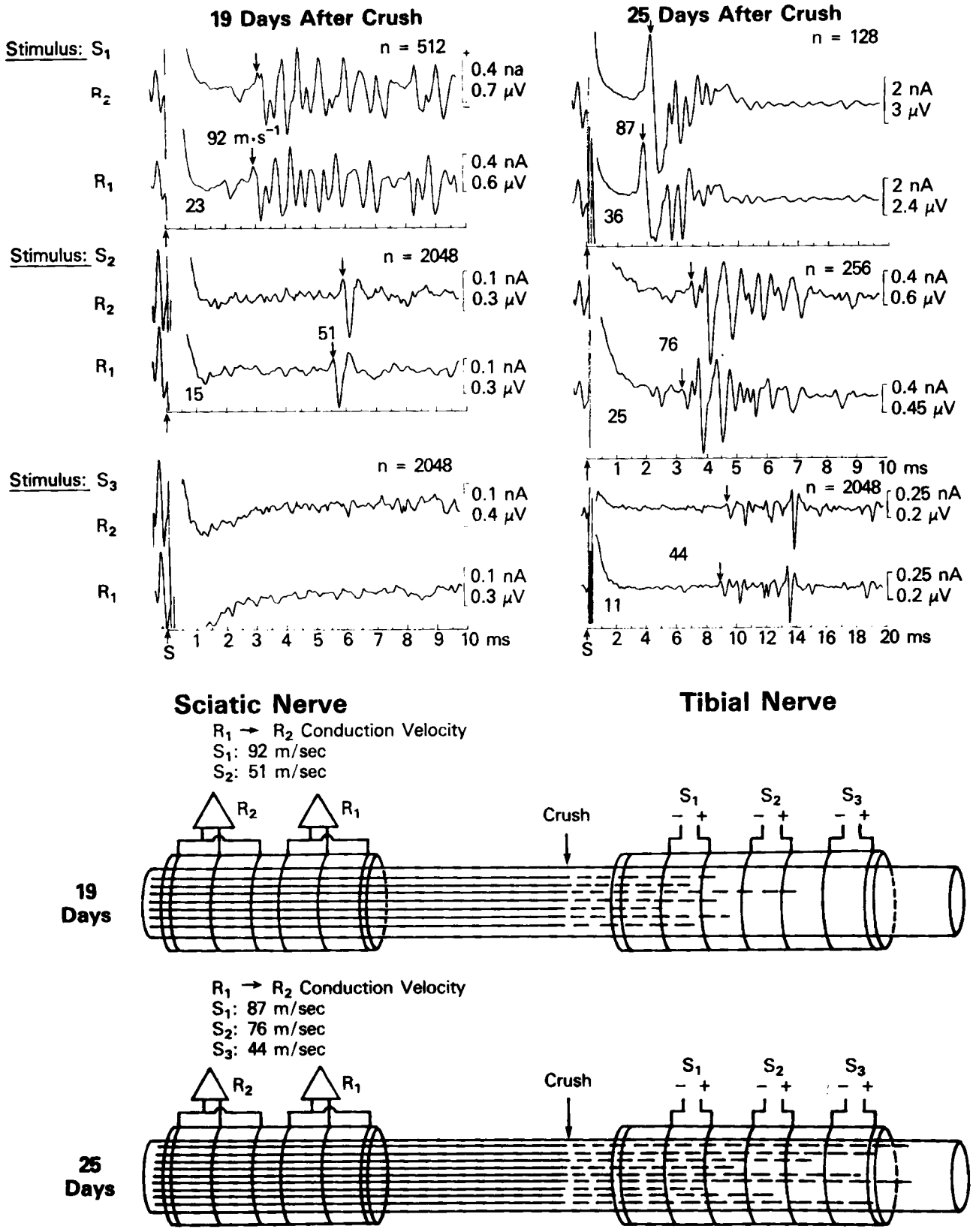
Rates of Recovery of Conduction. In serial studies, ascending action potentials were elicited from electrode sites at increasing distances from the site of the lesion. In the traces shown in Fig. 3, a response was obtained when the nerve 34 mm distal to the lesion was stimulated but not from the site 15 mm further distally. When the study was repeated 6 days later, the nerve was excitable also from this more distal site. The spatial progression of excitability was delayed after crush + constriction compared with crush alone. Similarly, reinnervation of plantar muscle occurred 41–48 days after crush alone, whereas it was more delayed at 49–69 days after crush + construction ($P < 0.05$). When the distance of recovery of excitability was related to time after crush alone in four nerves and after crush + constriction in the contralateral four nerves (Fig. 4), the data could be fitted by linear regression lines ($P < 0.001$). The scatter was somewhat larger after crush + constriction than after crush alone, which may be due to individual variations of the tightness of the constricting cuff. The time of reinnervation of plantar muscle fitted these regression lines, suggesting that there was little delay between regeneration and reestablishment of neuromuscular transmission[1] and in accordance with the finding that transmission is resumed before the structural integrity of the synaptic apparatus recovers completely.[16]

The slope of the regression line in Fig. 4 after crush alone was 3.2 ± 0.2 mm/day (mean ± SE of the estimate, $n = 16$ observations) which was significantly larger ($P < 0.02$) than the 2.2 ± 0.3 mm/day ($n = 12$) after crush + constriction. This suggested that the progression of excitability was 45% faster after crush along than after crush + constriction. When the distance versus time relationships were extrapolated to zero distance, i.e., the site of crush, the delay in regeneration after both types of lesions was about 8 days. This similarity suggests that there was no focal delay in elongation at the site of constriction placed 5 mm distal to crush.

Diameters of the Fastest Regenerating Fibers. To examine whether there was a relationship between the rate of elongation and the diameter of the parent nerve fiber, the conduction velocity of the ascending compound nerve action potential (CNAP) in the sciatic nerve was compared for nerves at different times after nerve crush. This is illustrated in Fig. 3 obtained after crush of the tibial nerve: 19 days after crush, the sciatic nerve conduction velocity of the unitary triphasic potential originating from the most distal regenerating fiber (S_2) was 51 m/sec, whereas the action potential from the next more proximal site (S_1) had a conduction velocity of 92 m/sec (Fig. 3, below). One week later, after further elongation had occurred, the conduction velocity of the potential originating from S_2 had increased from 51 to 76 m/sec, whereas the potential from S_1 had unchanged velocity. At this time, the potential originating from the most distal regenerating fibers had a conduction velocity of only 44 m/sec.

A similar slower proximal conduction velocity of action potentials from the most distal excitable site was apparent after crush + constriction (Fig. 1), and the results from the four nerves with crush alone and the four contralateral nerves with crush + constriction were pooled in Table 1. The average conduction velocities of potentials elicited from the most distal excitable site were 25% lower ($P < 0.001$) when compared with the same site after further regeneration. Similarly, the conduction velocity of the potential from the most distal site was 30% lower ($P < 0.001$) when compared with potentials from the next more proximal site.

The conduction velocity of the proximal undamaged portion of the nerve is influenced by the retrograde atrophy that generally occurs proximal to Wallerian degeneration.[3,5] In our studies, the maximum sciatic nerve conduction velocities of ascending responses evoked by stimulation of the nerve distal to the lesion gradually decreased by about 20% of normal controls to 80 ± 3 m/sec at 50–55 days after the lesion. After reinnervation of distal structures had occurred, the velocity gradually recovered over a period of about 200 days similar to findings by Davis et al.[5] In comparison, the sciatic nerve velocity of the fastest regenerating fibers was 61 ± 3 m/sec, which was about 40% lower than that seen in normal nerve and significantly ($P < 0.001$) lower than the


N10 Regeneration After a Crush Lesion
19 Days After Crush
25 Days After Crush
Stimulus: S1
R2
R1
n = 512
0.4 na
0.7 μV
92 m·s⁻¹
0.4 nA
0.6 μV
23
n = 128
2 nA
3 μV
87
2 nA
2.4 μV
36
Stimulus: S2
n = 2048
0.1 nA
0.3 μV
51
15
n = 256
0.4 nA
0.6 μV
76
0.4 nA
0.45 μV
25
Stimulus: S3
n = 2048
0.1 nA
0.4 μV
0.1 nA
0.3 μV
S 1 2 3 4 5 6 7 8 9 10 ms
1 2 3 4 5 6 7 8 9 10 ms
n = 2048
0.25 nA
0.2 μV
44
0.25 nA
0.2 μV
11
S 2 4 6 8 10 12 14 16 18 20 ms
Sciatic Nerve
Tibial Nerve
R1 → R2 Conduction Velocity
S1: 92 m/sec
S2: 51 m/sec
R2
R1
Crush
S1
S2
S3
19 Days
R1 → R2 Conduction Velocity
S1: 87 m/sec
S2: 76 m/sec
S3: 44 m/sec
25 Days

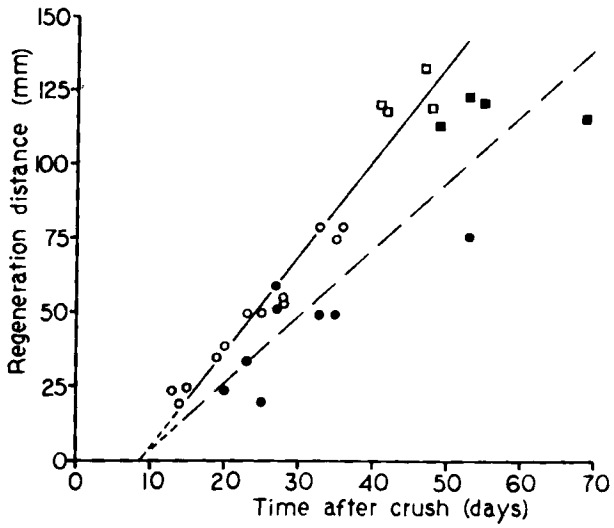


FIGURE 4. Relationship between the distance of regeneration (mm, ordinate) and the time (days, abscissa) after crush alone (open symbols) and crush + constriction (solid symbols). The distance was determined by the most distal excitable site in the nerve (○, ●) and the distance of plantar muscle from the site of crush (□, ■). Linear regression lines ($P < 0.001$) were fitted by least squares after crush alone (solid line, $r = 0.98$, $n = 16$) with a slope of 3.24 mm/day and after crush + constriction (dashed line, $r = 0.90$, $n = 12$) with a slope of 2.23 mm/day. After crush alone the slope was 45% steeper ($P < 0.02$) than after crush + constriction. The regression lines were extrapolated to zero distance to show the delay (8 days) in the start of regeneration. In crush + constriction, the constricting cuff was placed 5 mm along the distance of regeneration axis.

velocity in nerve with slowing due to retrograde atrophy.

Maturation of Regenerated Nerve Fibers. When the nerve distal to the site of crush first became excitable, the nerve action potential had a simple triphasic shape. During further regeneration, the response became polyphasic as more fibers reached the stimulus site and then subsequently synchronized as fibers became mature (Fig. 5). The changes associated with maturation occurred faster and were more complete after crush alone compared with crush + constriction (Figs. 5 and 6).

After crush alone the amplitude of the compound nerve potential evoked about 20 mm distal to the site of the lesion increased to low normal values[23] within about 100 days (Fig. 6, left), whereas that originating about 30 mm further distally recovered more lowly and did not reach low normal values until 150–200 days after the lesion (Fig. 6, middle). The lower rate of recovery of amplitude at the distal compared with the more proximal site probably reflects both a longer regeneration distance and temporal dispersion caused by the longer conduction distance along slowly conducting fibers (Fig. 6, right). After crush + constriction, the action potential recovered both more slowly and less completely compared with crush alone (Fig. 6). The lower peak-to-peak value probably reflected fewer and thinner regenerated fibers as well as more pronounced temporal dispersion of the action potential after long-term maturation.

The conduction velocity along the regenerated nerve segment gradually increased from a low value of <10 m/sec and often as low as 1–3 m/sec early after regeneration toward 80–85% of control values over a period of 250–300 days in accordance with previous studies.[1,4] After crush alone, the recovery was initially more rapid than after crush + constriction, but after prolonged observations, the conduction velocities became similar (Fig. 6).

When a muscle action potential could first be recorded from plantar muscle, the latency was 5–10 times longer than normal as found previously[19] and then recoverd to near normal values both after crush alone and after crush + constriction.

Histological Findings. Electronmicroscopic sections from plantar nerve 78 days after crush showed thin fibers with a unimodal diameter distribution (Fig. 7, left). After crush alone, the average diameter was 3.3 ± 0.08 μm (mean ± SEM, n = 195), and the largest fibers were 5–6 μm in di-

FIGURE 3 (opposite). Progression of excitability along the denervated portion of the nerve and characterization of fastest regenerating fibers. *Above:* The tibial nerve distal to crush was stimulated at three different sites (S_1, S_2, S_3), and the evoked ascending nerve action potential was recorded at two sites (R_1, R_2) along the sciatic nerve proximal to crush. The stimulus site S_1 was 19 mm (upper pair of traces), S_2 was 34 mm (middle pair of traces), and S_3 was 49 mm (lower pair of traces) distal to the lesion. The conduction velocity from the site of stimulation to R_1 is indicated below traces and between R_1 and R_2 (arrows) between the traces. The number of averaged responses *(n)* is indicated. Nineteen days after crush an action potential was evoked from S_1 and S_2 but not from S_3. When the study was repeated 6 days later, 25 days after crush, a potential was also present from S_3, but more distal stimulation did not evoke a response. *Below:* Schematic interpretation of the findings in the traces above. Nineteen days after crush a number of nerve fibers responded to the most proximal stimulus site (S_1), giving rise to the polyspike potential, but only a single fiber was excited at S_2. After further regeneration, a number of fibers responded at all three sites of stimulation. The conduction velocity proximal to the site of crush suggests that the response from the most distal excitable site was slower than that from more proximal sites of stimulation (cat N10, left leg, crush alone).

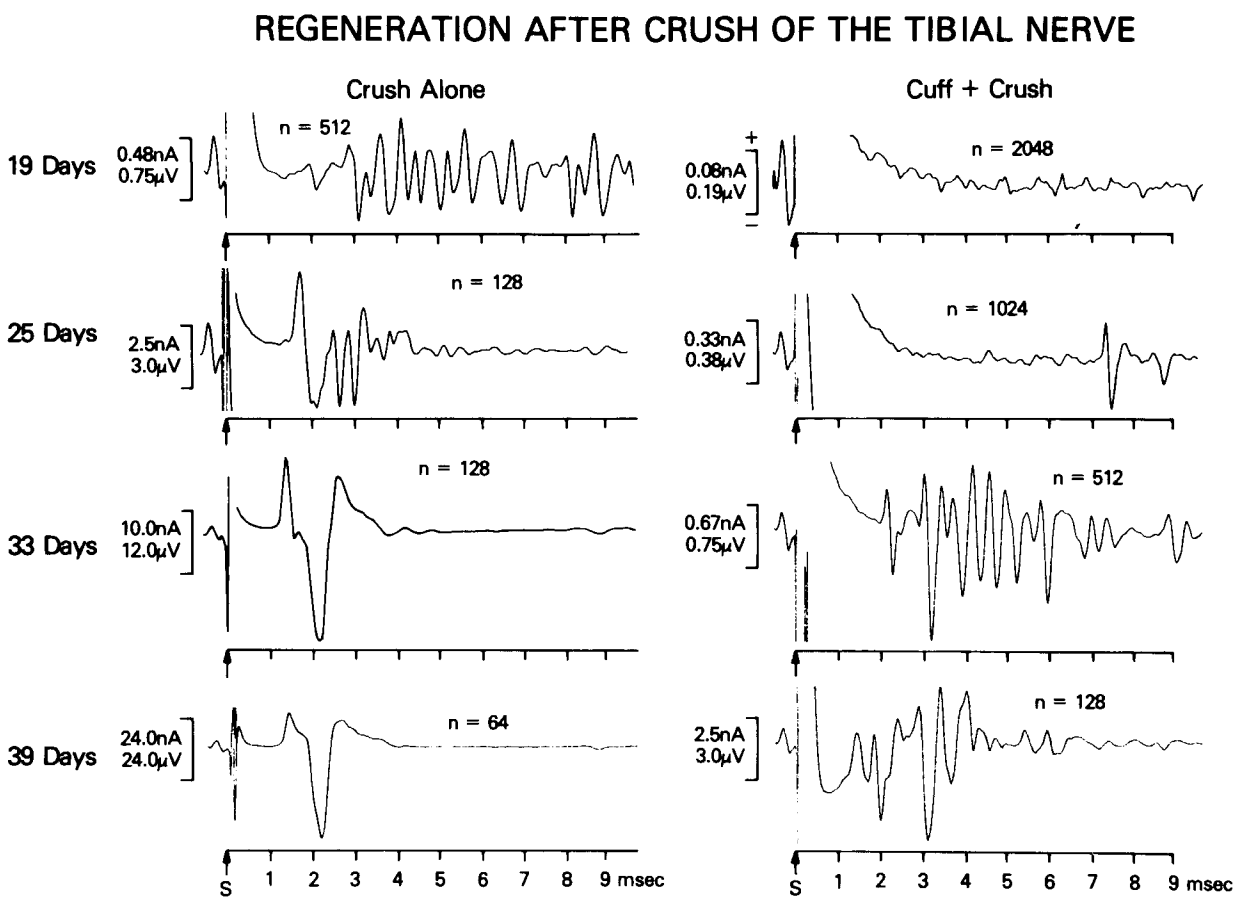


FIGURE 5. Gradual recovery of the shape and amplitude of the CNAP at different times (days after crush indicated to the left of traces) during regeneration to illustrate delays in regeneration and maturation after crush + constriction compared with crush alone. *Left:* The traces were obtained from action potentials evoked by stimulation 19 mm distal to the site of crush alone and recorded from one site at the sciatic nerve. *Right:* Stimulation 18 mm distal to crush after crush + constriction (cat N10).

ameter. The plantar nerve conduction velocity was 19 m/sec. On the contralateral side with crush + constriction, the plantar nerve was at that time inexcitable, and the average fiber diameter was only 2.7 ± 0.06 μm, which was significantly smaller ($P < 0.001$) than after crush alone.

In the nerve examined 300 days after crush, the diameter distribution was bimodal (Fig. 7, right). The largest fibers after crush alone had diameters of 11–12 μm, about 80% of control,[23] and the conduction velocity was 60 m/sec. On the contralateral side with crush + constriction, the conduction velocity was 55 m/sec, corresponding to the slightly thinner diameter of the largest fibers (9–10 μm).

The *g* ratio[27] was about 0.7 in the nerve removed 78 days after crush and remained about 10% larger than control after prolonged maturation in accordance with earlier observations.[28] The *g* ratio was the same after crush alone and after crush + constriction both early and late after nerve crush.

DISCUSSION

The purpose of this study was to develop a method to measure rates of regeneration and maturation using long-term in vivo observations. Previous in vivo studies have used reinnervation of a distal target organ as a gauge of regeneration.[2,10,19,36] However, no information can be obtained until a physiological response is present, and it is therefore not possible to analyze the complex series of events that precede reinnervation. These events include retrograde degeneration, initial delays in regeneration, rates of elongation, and the formation of functional connections to receptors and muscle. Tracing of fibers during growth, which is necessary to differentiate these factors, is technically demanding due to the small amplitude and low conduction velocities of action

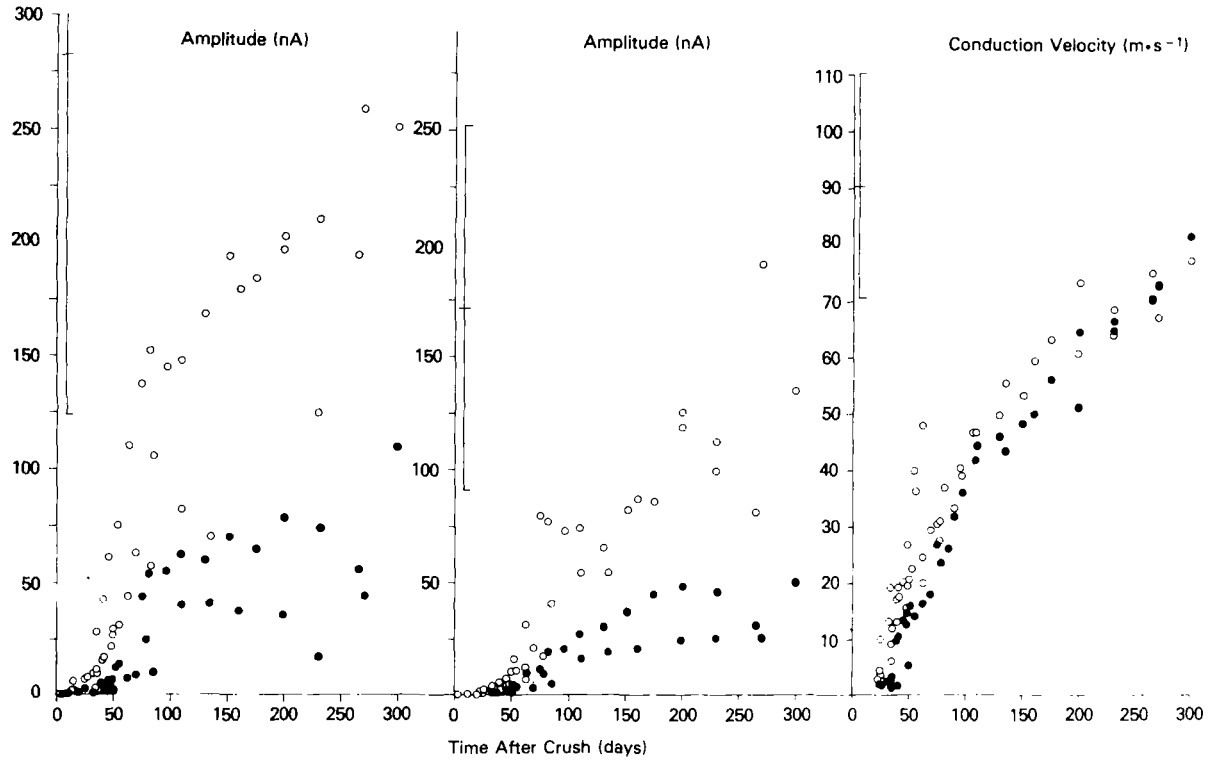


FIGURE 6. Composite of recovery of conduction properties as function of time (days, abscissa) after crush alone (○, five nerves) and crush + constriction (●, four nerves). *Left:* Amplitude (nA, ordinate) of the ascending action potential in the sciatic nerve evoked at the tibial nerve 18–25 mm distal to crush. The mean and lower 95% confidence limit in control nerves[23] are shown to the right of the ordinate. *Middle:* As in the left panel, obtained by stimulating the tibial nerve 48–55 mm distal to the lesion. Mean ± 95% confidence limits from control indicated to the right of the ordinate. *Right:* Conduction velocity (m/sec) between the two sites of stimulation. Mean ± 95% confidence limits from control indicated to the right of the ordinate.

potentials and high excitation thresholds of promyelinated regenerated fibers. Repeated percutaneous insertions of stimulation and recording probes along the length of regenerated nerve do not allow suffcient spatial resolution to measure rates of regeneration accurately. In vitro observations have been used previously to follow regeneration[1,8,15] but do not allow direct investigation of the effects of various treatment regimens in the same animal over the course of regeneration. To obtain sufficient resolution to record responses during early as well as late phases of regeneration, we have used implanted electrodes with multiple contacts having stable anatomical and functional relationships with the nerve.[23]

The first action potentials recorded after nerve crush had characteristics suggesting that they were elicited in the first few nerve fibers growing along the electrode array. Furthermore, the conduction velocities of the regenerated segment of these fibers were <10 m/sec and often as low as 1–3 m/sec, similar to findings in continuously conducting regenerated dorsal root fibers.[9] It therefore seems likely that these newly regenerated fibers were detected before myelination and that the progression of excitability along the nerve corresponded to elongation of regenerating fibers. Hence, the method appears suitable to differentiate elongation from the subsequent maturation during regeneration, at least in operational terms. The ability to propagate an action potential implies at least some degree of maturation with incorporation of sodium and potassium channels in newly formed axolemma,[34] although little lag probably occurs between formation of axolemma and incorporation of ion channels.[17,34] Similarly, the increase in amplitude of the compound nerve action potential is due to growth of additional fibers as well as to maturation of already regenerated fibers. Despite these limitations, the distinction between elongation and maturation was useful when attempting to characterize different aspects of regeneration.

The rate of elongation after crush measured here was 3.2 mm/day, which was slightly slower than the 4 mm/day measured from reflex contrac-

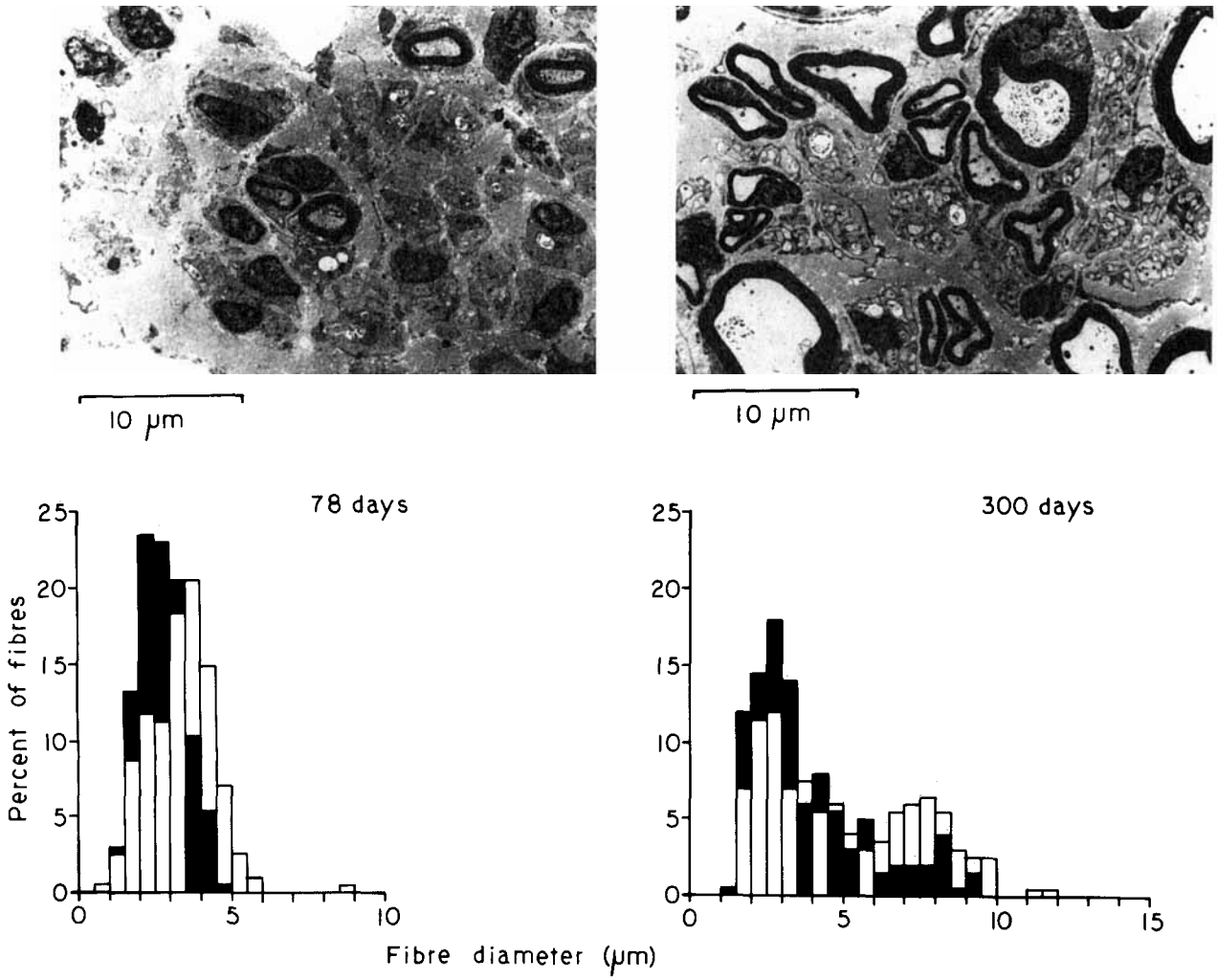


FIGURE 7. *Above:* Electronmicrographs of transverse sections from the plantar nerve taken 78 days (left, cat N13) and 300 days (right, cat N10) after crush alone of the tibial nerve. Bar = 10 μm. *Below:* Percentage distribution of fiber diameters after crush alone (open columns) and after crush + constriction (solid columns); 165–200 fibers were measured in each nerve at a final magnification of 5400×.

tions in cat elicited by pinching the distal end of growing fibers.[13,37] In general, the measured rate of regeneration is closely dependent on the parameter used to gauge recovery of function. In man, the rate of regeneration was estimated to be 1 mm/day using muscle contraction as an indicator,[29] whereas it was about twice as fast when regenerated fibers were detected using sensitive recordings of the nerve action potential.[2] The lower rate of elongation using electrical stimulation compared with pinching may be related to the low threshold of newly regenerated fibers to mechanical distortion,[18] whereas the threshold to electrical depolarization is high.[1]

The rate of elongation measured in our experiments may be somewhat underestimated since the distances between electrode sites were 15–30 mm to avoid inaccuracies due to spread of activation. Considering the high stimulus current, it is likely that some spread of the stimulus to adjacent nerve fiber segments occurred. However, the regular increase of the latency of the evoked action potential in parallel with distal shift of the stimulus site (Figs. 1 and 4) suggests that the sites of activation did not overlap.

Over the relatively limited length (110–130 mm) of regeneration in cat, the progression of excitability could be fitted by a straight regression line, indicating that the rate of elongation was constant throughout the denervated nerve segment. This finding is at variance with the suggestion that the rate of regeneration decreased[30] or

increased[15] substantially with the distance distal to the site of the lesion. However, longer distances of regeneration may reveal irregularities in the progression of excitability, and it is possible that differences in the type of lesion causing loss of axonal continuity and Wallerian degeneration may have an influence on these charateristics of fiber growth. Further work is needed to test these possibilities.

Characterization of Fastest Growing Fibers. The conduction velocities measured along the sciatic nerve for fibers activated at the distal front of regeneration suggested that myelinated fibers with diameters of 5–10 μm regenerated in front of large fibers 15–20 μm in diameter. That small fibers may regenerate faster than large fibers is in agreement with the observation that pain sensation in man recovered faster than touch sensation.[13,29,31] Fibers regenerating into a neuroma were also found to have low conduction velocity,[32] and this was shown to be due to preferential regeneration of small myelinated fibers.[6] However, the resolution of the method in our study did not permit recording of action currents of less than 0.05–0.1 nA in amplitude, which would correspond to a conduction velocity of 30–35 m/sec (Fig. 2); thus, we could not determine whether fibers with diameters smaller than 5–10 μm had even faster rates of elongation. Since the same response components could not be recognized in repeated observations, we could not determine whether large fibers with later regeneration had slower rates of elongation or whether growth was delayed compared with smaller fibers. It is possible that large fibers have longer initial delays before regeneration begins or that their retrograde degeneration extends further proximally.

However, retrograde atrophy is a general phenomenon in nerve fibers with distal degeneration, and it should be considered whether the low proximal conduction velocity might be due to such a mechanism. In our experiments the retrograde atrophy accounted for about 20% slowing of the velocity of the fastest conducting fibers, whereas the stem fiber conduction velocity of the fastest regenerating fibers was much slower. It might be speculated that the fastest regenerating fibers underwent more pronounced atrophy than slower growing fibers. Although we have no direct evidence against this possibility, it appears unlikely since other studies have shown that retrograde atrophy is most pronounced in nerves where regeneration has been prevented.[5,7]

It should be considered whether the slow conduction velocity along the stem of the fastest regenerating fibers might be due to changes in core conductor geometry at the site of the lesion causing selective conduction block of nerve action potentials in large fibers. Owing to the disparity in diameter, a large-diameter fiber might not be depolarized to threshold by an action potential ascending from a thin regenerated segment, whereas a smaller proximal fiber might be depolarized with a greater safety factor.[11] This possibility is unlikely because the gradual slowing in conduction towards the periphery suggests tapering of regenerated axon diameters rather than the abrupt transition associated with conduction block in such models.

The Effect of Constriction on Nerve Regeneration. Distal to crush + constriction, reinnervation of plantar muscle was delayed compared with crush alone, confirming previous findings in the rabbit.[19] This difference did not appear to be explained by different fiber populations having preferential regeneration as small fibers elongated fastest in both lesions. Moreover, the initial delay in regeneration was about 8 days after both lesions. If a focal effect was significant or retrograde degeneration more pronounced after crush + constriction, a longer delay would have been expected at the constriction. Measurements of the rate of progression of excitability distal to the lesion indicated that the rate of elongation was 45% faster after crush alone than after crush + constriction.

The effect of the constriction on fibers regenerating distal to it is in agreement with the findings by Reiners et al.,[25] who demonstrated a delay in reinnervation of plantar muscle in rabbit after crush of the tibial nerve distal to a tight ligature. The mechanism causing delay in elongation and maturation is unknown, but it might be speculated that fast axoplasmic transport, which provides glycoproteins and other constituents for incorporation into newly formed axolemma,[12,33] may be impaired. Another possibility may be that the loss of branching sprouts which occurs during normal maturation may be impaired as suggested in a previous study.[19] Even though many fibers in the present study had diminished diameters and the CNAP was dispersed, the *g* ratio recovered to the same extent after crush + constriction as after crush alone, in agreement with an earlier report.[25]

This would suggest that the constriction did not impair the level of maturation appropriate for the axonal diameter.

This chronic electrophysiological method of tracing regenerating axons distal to the site of lesion causing Wallerian degeneration in vivo observations while being able to determine the stem fiber caliber appears useful for quantifying various aspects of nerve regeneration during different therapeutic measures and may enable the study of determinant factors during different phases of the regenerative processes.

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