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EXTRAJUNCTIONAL ACETYLCHOLINE SENSITIVITY OF INACTIVE MUSCLE FIBRES IN THE BABOON DURING PROLONGED NERVE PRESSURE BLOCK

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SUMMARY

1. Nerve-evoked muscular activity was abolished in the small hand muscles of the baboon for 1–2 months by a 3 hr period of nerve compression from a pneumatic tourniquet inflated round the forearm. In the large diameter nerve fibres, this produced either a prolonged conduction block due to local myelin damage at the site of compression, or (in 10–30 % of the large fibres) Wallerian degeneration.

2. At varying intervals after nerve compression the extrajunctional acetylcholine (ACh)-sensitivity of innervated but inactive muscle fibres in the fourth lumbrical muscle was measured. Observations were also made on lumbrical muscle fibres at similar intervals after surgical denervation.

3. The ACh sensitivity of nerve-blocked muscle fibres started to develop later than in denervated muscle fibres (10 *vs.* 7 days) and remained at a lower level (40–80 mV/nC, median ACh-sensitivity) than that of denervated muscle fibres (200–437 mV/nC) from 21 to 63 days after nerve block or denervation.

4. In stimulation experiments on four muscles, extrajunctional ACh-sensitivity of both denervated and innervated but inactive fourth lumbrical muscle fibres was reduced by muscular activity.

5. In four animals mild compression was used in the lower limb to produce persistent nerve block without Wallerian degeneration. With one exception (in which some Wallerian degeneration had occurred) recording with a co-axial needle from abductor hallucis showed no spontaneous fibrillation up to 28 days after compression, although the extrajunctional ACh-sensitivity of the muscle fibres appeared to reach levels similar to those observed in the forelimb. All four muscles developed a slight increase in insertion activity after 1–2 weeks.

6. It may be concluded that both muscular activity and some other neural influence, independent of muscular activity, are able to influence extrajunctional muscle properties in the baboon. The neural influence appears to be more effective in preventing spontaneous fibrillation than in preventing a rise in ACh sensitivity of the extrajunctional muscle membrane.

INTRODUCTION

It has long been assumed that the neural regulation of intrinsic properties of skeletal muscle fibres is achieved through the release of a substance, a trophic factor, from the nerve endings (Tower, 1937; Miledi, 1960*a*, *b*; Emmelin & Malm, 1965; Guth, 1968; Harris, 1974). An alternative hypothesis is that the amount and pattern of muscular activity *per se* provides the necessary information to enable the muscle to adjust its properties. Evidence in support of the latter hypothesis is that direct stimulation of denervated rat muscle fibres is able to prevent the usual development of sensitivity to acetylcholine (ACh) outside the end-plate, or reverse the process if extrajunctional ACh-sensitivity already has developed (Lømo & Rosenthal, 1972; Drachman & Witzke, 1972; Purves & Sakman, 1974*a*; Lømo & Westgaard, 1975). A similar effect of muscle activity on other intrinsic muscle properties has also been demonstrated (Jansen, Lømo, Nicolaysen & Westgaard, 1973; Westgaard, 1975). These results do not exclude the possibility that neural influences also contribute to the control of intrinsic muscle properties independently of muscle activity, and results from other types of experiments have been interpreted in favour of the trophic factor hypothesis (Albuquerque, Schuh & Kauffman, 1971; Harris & Thesleff, 1972; Bray & Harris, 1975; Deshpande, Albuquerque and Guth, 1976; Warnick, Albuquerque, and Guth, 1977). Recently, Lavoie, Collier & Tenenhause (1976, 1977) and Pestronk, Drachman & Griffin (1976) have demonstrated that denervation caused a larger increase in extrajunctional ACh-receptors than muscle paralysis induced by a nerve conduction block. The difference was attributed to a trophic influence of the intact but inactive nerve fibres in the impulse-blocked preparation. However, it is also possible that changes associated with degenerating nerve terminals in the denervated preparation were responsible for the difference (Jones & Vrbova, 1974; Lømo & Westgaard, 1976).

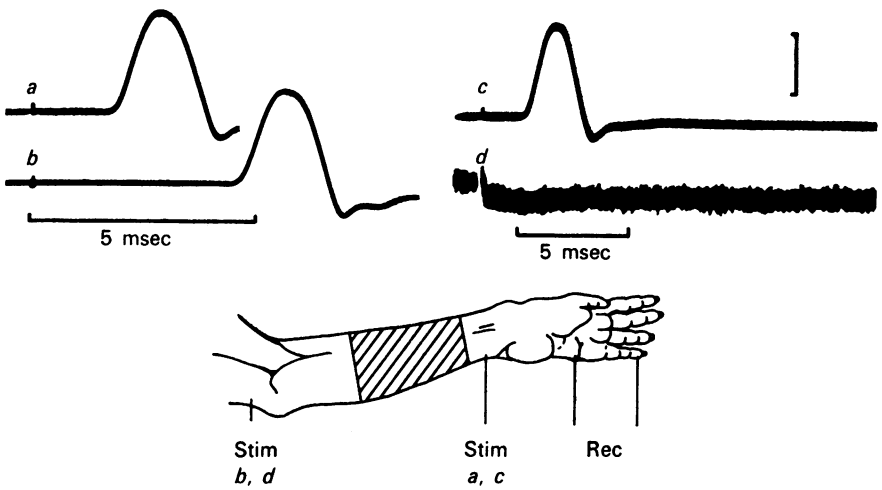
We have attempted to distinguish between the two possibilities by comparing denervated and innervated but inactive muscle fibres over long periods of time. If degenerating nerve terminals were responsible, the observed difference in the extrajunctional muscle membrane would be expected to be transitory, whereas the difference would be expected to be maintained if it is caused by the absence of a neurotrophic influence on the denervated muscle fibres. Unfortunately, the duration of the experiments with a tetrodotoxin (TTX) nerve block carried out by Lavoie *et al.* (1976, 1977) and Pestronk *et al.* (1976) was too short (5 or 7 days) to establish whether the difference in the amount of extrajunctional ACh-receptors was of a transient or more permanent nature. Consequently, the aim of this paper is to look for evidence of a lasting difference in the level of extrajunctional ACh-sensitivity of denervated versus innervated but inactive muscle fibres, using the technique of nerve compression to establish a local and long-lasting nerve conduction block (Denny-Brown & Brenner, 1944; Ochoa, Fowler & Gilliatt, 1972). A brief preliminary account of this work has been given (Gilliatt, Westgaard & Williams, 1977).

METHODS

The experiments were carried out on sexually mature female baboons (*Papio papio*) with body weights ranging from 7·5 to 17·5 kg. The animals were fed on a solid pellet diet with added fruit and milk. Monthly injections of cyanocobalamin were given. Anaesthesia during compression and in subsequent procedures was provided by an initial tranquillizing dose of ketamine (15 mg/kg) and promazine (1–2 mg/kg) followed by I.V. pentobarbitone sodium (4 mg/kg), repeated as necessary.

Preparation of nerve lesions. Initially a rubber bag (5 cm × 10 cm) in a specially reinforced sleeve (Accoson) was used, a pressure of 1000 mmHg being maintained for 3 hr round the forearm (Fowler, Danta & Gilliatt, 1972). This sometimes failed to produce a sufficiently long-lasting block. Therefore a larger cuff with a bag 10 cm × 20 cm was inflated round the forearm to a pressure of 1200–1350 mmHg for 3 hr; the resulting conduction block then persisted unchanged for periods of 1–2 months.

In order to obtain denervated muscles for comparison, the opposite ulnar nerve was divided by an aseptic procedure under anaesthesia. In three animals this was done at the elbow, approximately 25 cm from the muscle, and in the remainder the nerve was exposed just above the wrist and the motor branch divided 5–6 cm from the muscle, the cut proximal end being stitched back to prevent regeneration.



Text-fig. 1. Method of testing completeness of nerve block. Muscle action potentials in response to ulnar nerve stimulation at wrist (*a*, *c*) and at elbow (*b*, *d*) before nerve compression (left), and 25 days after compression (right) to show complete conduction block (animal P9). Calibration: 5 mV for *a*, *b*, and *c*, and 50 μV for *d*.

Completeness of nerve block. The completeness of the nerve block was usually tested at intervals of 1–2 weeks with the animal under anaesthesia, by subcutaneous stimulation of the ulnar nerve proximal and distal to the block, and recording of the response of the ulnar supplied hand muscles through subcutaneous electrodes (Text-fig. 1). Single supramaximal shocks were used for nerve stimulation, not more than five to ten being delivered to the nerve at the wrist in order to avoid unnecessary stimulation of inactive muscles.

In eleven out of thirteen animals the block remained complete throughout the period of observation. In one animal in which a 5 cm cuff was used, stimulation through the block, which had produced no response in the hand muscles when this was checked 4 and 14 days after compression, produced a small response (1% of that elicited by wrist stimulation) before the final experiment on the eighteenth day. In a second animal in which a 10 cm cuff had been used, stimulation through the block, which produced no response in the hand muscles when this was checked 17, 25, 31 and 38 days after compression, gave a small response (0·5% of that elicited by wrist stimulation) before the final experiment on the 42nd day.

In addition to routine testing with single shocks, the completeness of the conduction block was checked in a few animals using high frequency trains of stimuli (up to 300 Hz) to the ulnar nerve above the elbow. No conduction through the block occurred. This procedure was repeated under ketamine alone to exclude any possible effect of the barbiturate anaesthetic in depressing conduction through the block, and the same result was obtained. Stretching of the digits to excite muscle afferents and percussion and traction on the exposed nerve at the level of compression were also tried, and no muscle response to these procedures was observed.

Chronic stimulation. When the effects of repeated nerve and muscle stimulation on ACh sensitivity were to be studied, short trains of impulses at 5 or 20 Hz were used. On the tourniquet side the ulnar nerve was stimulated at the wrist. In one experiment denervated muscle fibres in the opposite limb were stimulated directly through subcutaneous needles placed at either end of the muscle belly. The stimulus duration was 50 μsec for nerve stimulation and 0·5 msec for direct stimulation of the denervated muscle fibres. The stimulus strength was 20–50 V when stimulating the nerve (supramaximal for the motor fibres in the nerve trunk) and 150 V when the muscle fibres were stimulated directly. This appeared to be a supramaximal stimulus, judged by palpating the contracting muscle. The frequency and duration of trains used for the individual muscles are specified in the legend to Test-fig. 3.

The acute experiment. For the measurement of extrajunctional ACh-sensitivity the fourth lumbrical muscle was used, with the exception of one denervation experiment in which there was anomalous innervation of the fourth lumbrical; in this case the third lumbrical muscle was used. In the baboon, the fourth lumbrical is a small muscle with the fibres running from end to end; the muscle nerve enters its deep surface on the ulnar side at approximately the mid-point. Unpublished studies on seven baboons by Dr J. H. J. Durston have shown that the muscle contains approximately 2,500 fibres, of which 40% are of histochemical type I and 60% of type II (classification of Engel, 1962). At the time of the acute experiment, the animal was anaesthetized and the completeness of the block was checked by nerve stimulation. The muscle was dissected out in a bloodless field together with a few mm of the muscle nerve, and put into Ringer solution. The muscle was dissected free at its origin and divided as near to the distal tendon as possible. A 20 mm length of muscle was usually preserved, with the end-plates positioned roughly in the middle of the specimen. After the muscle on the tourniquet side had been dissected out, the ulnar nerve itself was removed and placed in fixative, and the procedure repeated on the opposite side.

Measurement of extrajunctional ACh sensitivity. The two lumbrical muscles and a rat soleus muscle, denervated 5–14 days previously, were pinned out in a perspex chamber and extrajunctional ACh-sensitivity measured by iontophoretic application of ACh, the technique being similar to that described by Lømo & Westgaard (1975). Sensitivity was expressed as millivolts of depolarization per nanocoulomb charge passed through the ACh pipette (Miledi, 1960*a*). The rat soleus muscle which had fully developed ACh sensitivity was used to check the state of the ACh pipette in the course of an experiment. The ACh pipettes required a reverse current of less than 1·5 nA to prevent leakage of ACh. If extrajunctional ACh-sensitivities of less than 100 mV/nC in the denervated rat soleus fibres were found, the pipette was rejected. After each measurement of ACh sensitivity on the tourniquet side the nerve was stimulated with the recording pipette *in situ* to ensure that the individual fibre was innervated; fibres that failed to respond to nerve stimulation were rejected. The measurements of ACh sensitivity were usually made 3–5 mm away from the end-plates. The position of the end-plates was readily identified in innervated muscle fibres by the small terminal nerve branches. This identification was confirmed by intracellular recordings of miniature end-plate potentials. The likely position of the end-plate area in the denervated muscle fibres was estimated from our experience with the innervated muscle fibres, and the measurements of ACh sensitivity were made away from this area. If there was doubt as to the position of the end-plates, measurements were made at two separate sites away from the presumed position of the end-plate region, to check that the values were similar.

In some experiments the input resistance of muscle fibres was obtained by penetrating the muscle membrane by the ACh pipette and recording the change in membrane potential to a square wave of current passed through the ACh pipette. The input resistance was calculated as the ratio of the steady level of the change in membrane potential to the amplitude of the current pulse.

The Ringer solution contained (mM):
Na^+, 149; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 1; $H_2PO_4^-$, 1; HCO_3^-, 12; Cl^-, 147; D-glucose, 10. The pH was maintained at 7·2 by bubbling a gas mixture of 95% O_2, 5% CO_2 through the solution.

Histology. Nerves for histology were divided distally and proximally after stitches had been placed near the ends of the specimen to maintain its normal length. Under slight stretch the nerve was attached to a Perspex frame and immersed in 4% glutaraldehyde buffered to pH 7·4. The tissue remained in glutaraldehyde for 12 hr during which time a distal portion for transverse sections was separated from the remainder which was to be teased in glycerol.

After fixation both pieces were washed in buffer and transferred to 1% osmium tetroxide in veronal buffer. The nerves for teasing remained in the osmium tetroxide for 12–16 hr, and, after washing in distilled water, were placed in 66% glycerol in distilled water. The fibres in individual fascicles were then gently teased apart using sharp needles. Single fibres were dissected free and transferred to creosote prior to mounting on a microscope slide in DPX for photography.

The portion of nerve for transverse sections was left in the osmium tetroxide for 4–6 hr and then dehydrated and embedded in an epoxy resin (Epon). One μm sections were made and stained with toluidine blue. For measurement of fibre size and density photographs of a transverse section were enlarged to be exactly × 1000. Myelinated fibre density measurements were made in six separate areas of the section, to give a total of 500–600 fibres; histograms of fibre size distribution were constructed from measurements made in three areas (total 1500–2000 fibres).

RESULTS

Anatomical findings. Examination of single teased nerve fibres from that part of the ulnar nerve which had been under the pneumatic cuff confirmed that myelin damage occurred in two zones situated towards the edges of the cuff, with normal myelin between them. As in previous work on the lower limb, the proximal zone showed more myelin damage than the distal one. In most cases this was limited to the paranodal regions of the large myelinated fibres (Pl. 1), but demyelination of complete internodal segments was seen more often than in previous studies of the lower limb, in which it had been rare (Ochoa *et al.* 1972).

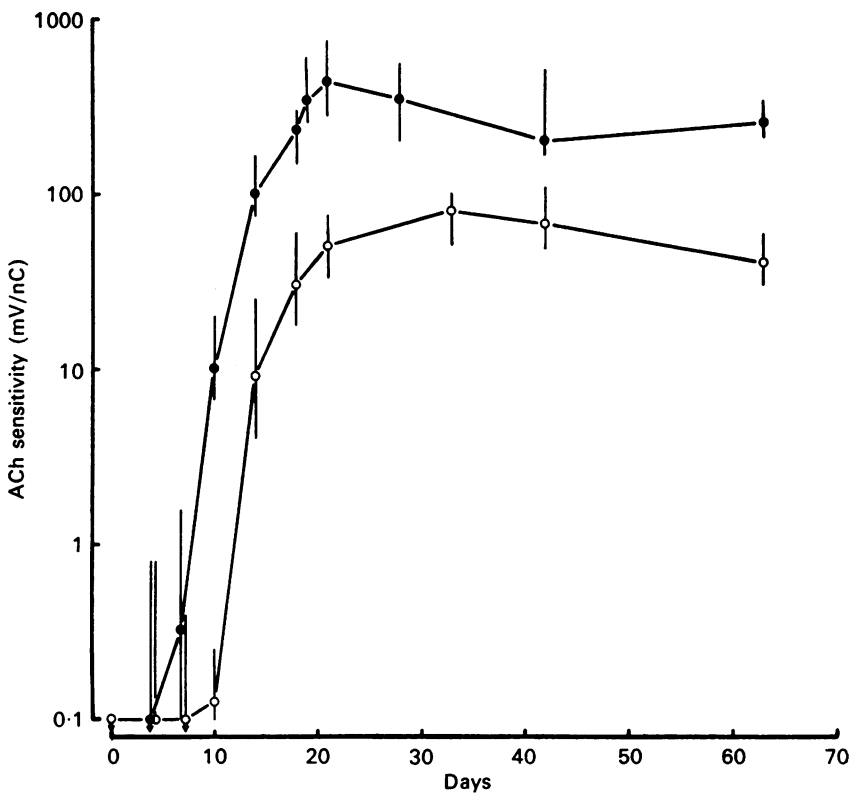
The amount of Wallerian degeneration caused by compression was assessed in transverse sections taken after 28–42 days, from the nerve approximately 2 cm distal to the cuff. Some sections appeared normal while others showed scattered myelin debris indicating Wallerian degeneration. The most severe changes occurred in the nerve illustrated in Pl. 2*B*; it can be seen that in addition to myelin debris there is loss of large diameter fibres and an increase in the number of small fibres compared with the control section shown in Pl. 2*A*.

Measurements of fibre density were carried out on transverse sections taken distal to the site of compression in three nerves; the results are shown in Table 1 in which they are compared with measurements from three normal nerves sectioned at the same level. The abnormal appearance of the nerve shown in Pl. 2*B* was confirmed by quantitative measurements of fibre density (P12 in Table 1). Although the nerve was examined only 28 days after compression, the increased number of small fibres in the section is assumed to indicate early regeneration. The other two nerves (P8, P9) showed much less change distal to the block but there appeared to be a slight decrease in the number of large diameter fibres, of the order of 10%, without a comparable change in the number of small nerve fibres, which were either similar to those of normal nerves or slightly increased. From this, it may be suggested that compression sufficient to produce long-lasting conduction block in the ulnar nerve at this level is likely to result in degeneration of 10–30% of the large diameter fibres.

Extrajunctional ACh-sensitivity in denervated and inactivated muscle fibres. The development of extrajunctional ACh-sensitivity was measured in muscle fibres from the fourth lumbrical muscles (and one third lumbrical muscle, 28 days denervated) which were either denervated by nerve section or inactivated by the pressure block of the ulnar nerve. In addition muscle fibres from four normally innervated fourth lumbrical muscles were examined. These normal fibres had undetectable

TABLE 1. Density (fibres/mm²) of large and small myelinated fibres in the ulnar nerve at the wrist

Normal				Distal to nerve block			
Animal no.	Total fibres	Small fibres ($\leq 8\ \mu m$)	Large fibres ($\geq 9\ \mu m$)	Animal no.	Total fibres	Small fibres ($\leq 8\ \mu m$)	Large fibres ($\geq 9\ \mu m$)
P10	10,900	5,348	5,552	P12	12,716	9,295	3,421
P16	9,250	4,568	4,682	P8	9,866	4,972	4,894
P17	10,600	4,940	5,660	P9	10,283	5,685	4,597
Mean	10,250	4,952	5,298		10,955	6,651	4,304



Text-fig. 2. Median extrajunctional ACh-sensitivity of denervated (filled circles) and nerve-blocked (open circles) lumbrical muscle fibres as a function of time (days) after nerve block or nerve section. Each point represents the median of ACh sensitivity from nine to twenty-two individual muscle fibres. Bars indicate 95% confidence limits of the median. The lowest level of ACh sensitivity reliably measured in these experiments was 0·1 mV/nC due to the limited capacity of the current source used.

ACh-sensitivity, the only exception being a muscle fibre with an ACh sensitivity of 0·67 mV/nC.

The median extrajunctional ACh-sensitivity is shown as a function of time for the denervated (filled circles) and inactivated (open circles) muscle fibres in Text-fig. 2. The bars indicate 95% confidence limits of the median of each group of ACh sensitivities from individual muscle fibres (Noether, 1971). The muscle fibres in a group are from the same muscle with the exception of muscle fibres 21 days nerve-blocked, 18 days denervated and 19 days denervated, in which muscle fibres from two muscles are represented.

There was no significant difference in ACh sensitivity of 18-day denervated muscle fibres from two muscles subjected to high and low nerve section respectively. This was also true for muscle fibres from two muscles denervated for 19 days after nerve section at different levels. The results from these two animals with high nerve section as well as a third animal (examined 42 days after high nerve section), have therefore been included in the calculation of the median values shown in Text-fig. 2.

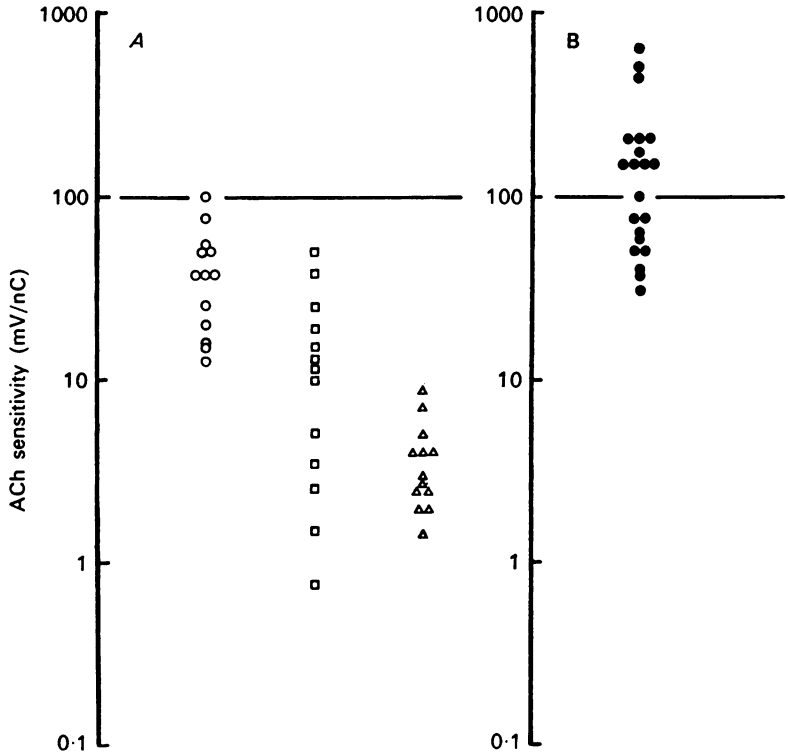
After denervation the extrajunctional ACh-sensitivity of the majority of the muscle fibres remained low for at least 4 days (with a few individual fibres developing a relatively high level of ACh sensitivity) and it then increased steadily over the next 14 days until it was fully developed 18–21 days after the nerve block. Thereafter the ACh-sensitivities of the denervated muscle fibres remained essentially unaltered up to 63 days after denervation. The absolute level of the fully developed extrajunctional ACh-sensitivity of the denervated muscle fibres, 100–1000 mV/nC, was similar to the extrajunctional ACh-sensitivity of 5–14 days-denervated rat soleus fibres which were measured with the same ACh pipettes in the course of each experiment.

The extrajunctional ACh-sensitivity of the nerve-blocked muscle fibres remained low for 3–4 days longer than the denervated muscle fibres. Between 10 and 21 days after the nerve block there was an increase in ACh sensitivity, but to a level well below that of denervated muscle fibres. From 21 days onwards the difference in the level of extrajunctional ACh-sensitivity of the two groups of muscle fibres remained essentially unchanged. This difference in level is highly significant since there was virtually no overlap in the range of ACh sensitivities from denervated and nerve-blocked muscle fibres. Accordingly, Willcoxon's two-sample test gives P-values of less than 0·001 from 10 days onwards for corresponding groups of nerve-blocked and denervated muscle fibres.

The possibility that a difference in fibre size and thereby input resistance could account for the difference in extrajunctional ACh-sensitivity between the two sides was considered. However, the input resistance of long-term nerve-blocked muscle fibres was very similar to the denervated ones, with the 63 day nerve-blocked muscle fibres having input resistances in the range of 900–3000 kΩ, median value 1300 kΩ, while the 63-day denervated muscle fibres had input resistances of 900–2170 kΩ, median 1200 kΩ. These values were well above the input resistance of normally innervated fourth lumbrical muscle fibres (from animal P10) which had input resistances in the range of 170–500 kΩ with a median value of 260 kΩ.

In order to show that our measurements of extrajunctional ACh-sensitivity 3–5 mm from the end-plate were a representative measure of the ACh sensitivity of the whole

muscle fibre away from the end-plate, ACh sensitivities were measured in 1–2 mm steps along a narrow band of muscle fibres in some muscles. For these measurements it was not possible to confirm that all the muscle fibres responded to nerve stimulation as this caused too much damage to the fibres. Instead, the end-plate region was



Text-fig. 3. Extrajunctional ACh-sensitivities of individual fibres from fourth lumbrical muscles that were either inactivated by a compression block of the ulnar nerve (three muscles, *A*) or denervated (one muscle, *B*) and then, 19–22 days later, activated either by stimulating the nerve distal to the site of the block or by direct stimulation through subcutaneous electrodes. The terminal experiment was 28 days after denervation or nerve block for all animals. The three muscles with a nerve block were stimulated with 50 (animal P12, open circles), 500 (P11, open squares) and 5000 (P13, open triangles) impulses once each day for 9, 6 and 7 days respectively. Animal P12 was stimulated with a 5 Hz train of impulses for 10 sec. Animal P11 received 20 sec trains of impulses (frequency 5 Hz) every minute for 5 min. Animal P13 was stimulated with 10 sec trains of impulses (frequency 20 Hz) every 15 sec until it had received a total of 5000 impulses. The denervated fourth lumbrical muscle (animal P11, 500 impulses a day for 6 days) was subjected to the same stimulus regimen as the contralateral nerve-blocked muscle. The horizontal line (at 100 mV/nC) indicates the lower level of extrajunctional ACh-sensitivity found in 19–42 day denervated muscle fibres which had not been stimulated.

examined first and a group of fibres chosen in which miniature end-plate potentials were present, the frequencies of release being in the range 0·5–3·0/sec. The results showed no evidence of a gradient of ACh sensitivity along the length of the muscle fibres from about 1 mm away from the end-plates onwards, either during the early

development of ACh sensitivity (in two muscles blocked and denervated for 10 days) or in long-term (42 days) nerve-blocked and denervated (63 days) muscle fibres.

The effect of stimulation on denervated and innervated muscle fibres. A total of four fourth lumbrical muscles in three animals were successfully stimulated in these experiments. The extrajunctional ACh-sensitivities of the individual muscle fibres measured in each muscle are plotted in Text-fig. 3. In one animal the denervated fourth lumbrical muscle was stimulated directly as described in Methods with 500 impulses a day, starting on the 22nd day. After 6 days of stimulation the range of extrajunctional ACh-sensitivity of the denervated, stimulated muscle fibres was significantly below that of 19–42 day denervated fibres ($P < 0{\cdot}05$, Willcoxon's 2-way test).

The effect of nerve stimulation on the tourniquet side was studied in more detail. In three animals the ulnar nerve was stimulated distal to the block with 50, 500 and 5000 impulses a day for 9, 6 and 7 days respectively.

Stimulation of the muscle with 50 impulses each day was started on the 19th day after nerve block, and the ACh-sensitivity of the muscle fibres measured 9 days later. The values obtained were similar to those of the muscle fibres from the 21–42 days nerve-blocked, unstimulated muscles ($P > 0{\cdot}1$).

Nerve stimulation with 500 impulses a day from day 22 to day 28, and with 5000 impulses a day from day 21 to day 28 resulted in a clear reduction of extrajunctional ACh-sensitivity in these muscles (median values 11·2 and 3 mV/nC respectively) compared to the 21–42 day nerve-blocked unstimulated muscle fibres (median ACh-sensitivities 50–80 mV/nC).

Since a stimulation schedule involving 50 impulses a day for 9 days had not affected the ACh sensitivity in one animal, it seemed unlikely that the much smaller number of shocks used to test the completeness of the nerve block could have affected the ACh sensitivity of the inactivated muscle fibres in our other animals. Furthermore, electrical stimulation was avoided in one animal until the day of the final experiment. The block was then found to be complete and the muscle fibres in this muscle had similar ACh sensitivities (20–75 mV/nC plus one fibre with 250 mV/nC) to the muscle fibres of another 21 day muscle as well as the 33 and 42 day nerve-blocked muscles.

Spontaneous fibrillation. Fibrillation was readily recorded with a standard coaxial needle electrode (recording area 0·07 mm^2) from any muscle which had been denervated for 7 days or longer. In the muscles on the tourniquet side fibrillation was less profuse than on the denervated side but was also present. Since, however, compression sufficient to produce prolonged conduction block in some nerve fibres would be expected to cause Wallerian degeneration in others, this result did not answer the question as to whether the fibrillation was occurring in the nerve-blocked muscle fibres themselves.

Accordingly, a separate series of experiments was carried out in which relatively mild pressure blocks were produced in the leg. The motor fibres to the abductor hallucis were compressed in the tibial nerve trunk by a 5 cm pneumatic cuff around the knee, the pressure in the cuff being approximately 1000 mm Hg and the duration of compression 90 min, a combination used previously by Fowler *et al.* (1972). Muscle action potential amplitude in response to nerve stimulation was measured

for the abductor hallucis muscle, and the proportion of the motor nerve fibres which remained blocked was calculated by comparing the muscle response to proximal nerve stimulation with that obtained by distal nerve stimulation.

From Table 2 it can be seen that after 7–11 days the block was still complete in two animals and more than 95% complete in two others. By 28 days recovery of conduction through the block had occurred in 2·5–25% of the motor fibres. On exploration with a co-axial needle electrode scanty spontaneous fibrillation was found in

TABLE 2. Spontaneous and insertion activity in m. abductor hallucis after nerve block

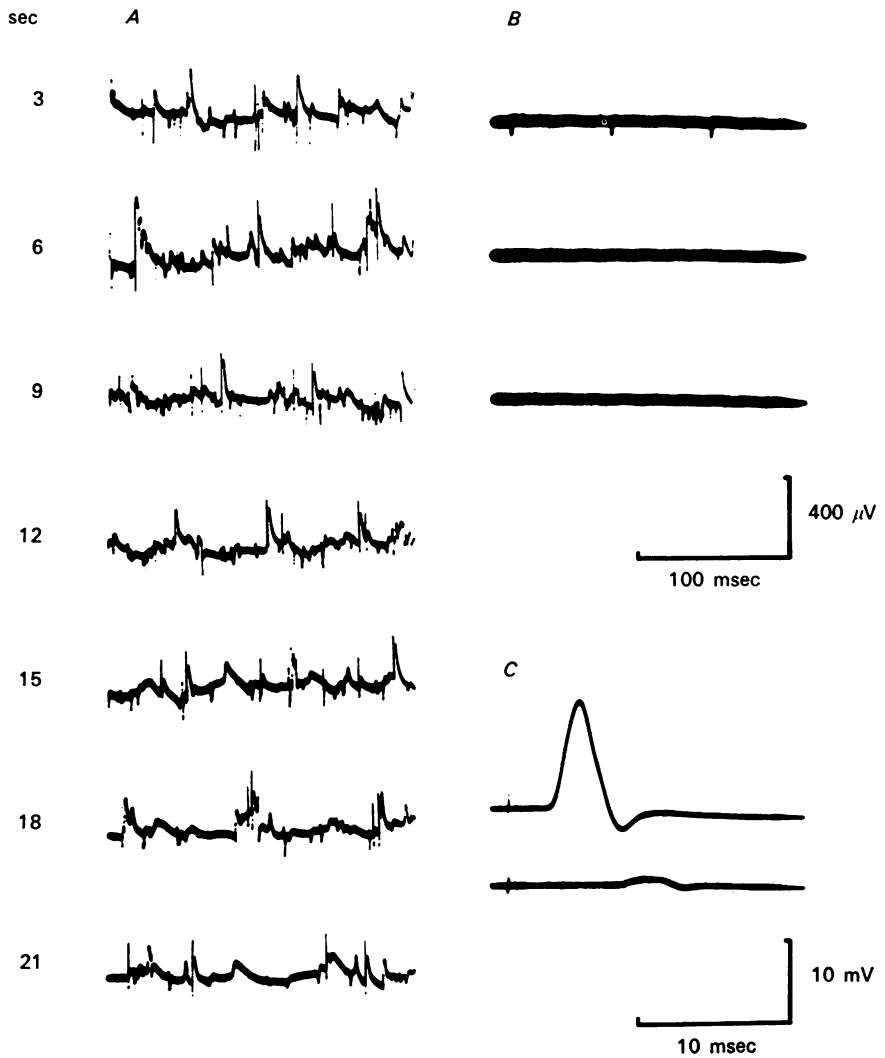
Animal no.	Days after tourniquet	Recovery of conduction (%)	Spontaneous fibrillation	Insertion activity
P11	7	4	None	None
	14	1·5	Scanty	None
	22	—	Scanty	A little increased
	28	13	Scanty	A little increased
P10	11	0	None	A little increased
	21	0·5	None	A little increased
	28	2·5	None	A little increased
P16	11	4	—	—
	21	15	None	None
	28	25	None	A little increased
P17	7	0	—	—
	17	0·5	None	A little increased
	28	5	None	A little increased

the abductor hallucis of P11, and pathological examination of the posterior tibial nerve of this animal subsequently showed occasional degenerating fibres in transverse sections. In the abductor hallucis of the remaining three animals, spontaneous fibrillation was absent, the only abnormality being a small amount of muscle fibre activity following each needle insertion (insertion activity) which tended to die away over 2–4 sec after needle movement ceased (Text-fig. 4). The posterior tibial nerve was examined histologically in one of the three animals and showed no evidence of Wallerian degeneration in teased fibres or transverse sections.

In two animals, a small superficial strip of the inactive abductor hallucis was removed on the 28th day, immediately after the muscle had been sampled with a coaxial needle electrode, and the extrajunctional ACh-sensitivity of the fibres measured in the usual way. In an animal in which the block was still virtually complete (P10 in Table 2), thirteen muscle fibres had a range of ACh sensitivities of 20–70 mV/nC, median 40 mV/nC, while input resistances were 600–900 kΩ. These values are consistent with the extrajunctional ACh-sensitivities of nerve-blocked muscles in the main series of experiments. For comparison a 6 day denervated rat soleus muscle, measured with the same ACh pipette, had extrajunctional ACh-sensitivities of 125–350 mV/nC, input resistance 160–280 kΩ.

The abductor hallucis muscle from another animal which had 25% recovery of transmission through the nerve block (P16 in Table 2), had extrajunctional ACh-sensitivities in the range of 2·7–60 mV/nC, median 8·5 mV/nC. As the lower values may be accounted for in terms of the recovery of transmission in some nerve fibres,

this result can be seen to support the result from the abductor hallucis muscle in animal P10. Taken together, the above findings indicate that the nerve-blocked muscle fibres, as well as having a reduced extrajunctional ACh-sensitivity relative to the denervated muscle fibres, also failed to develop spontaneous fibrillation.



Text-fig. 4. Fibrillation in *A*, denervated and *B*, nerve-blocked muscle, recorded at 3 sec intervals after insertion of a coaxial needle electrode. Results from two animals examined 28 days after either denervation (P15) or nerve block (P17). Muscle action potentials in response to proximal and distal nerve stimulation are shown in *C*, indicating that nerve block in P17 was 95% complete.

DISCUSSION

The results presented in this paper show that impulse-blocked motor axons are able to reduce the extrajunctional ACh-sensitivity of the muscle fibres they innervate, relative to that of denervated muscle fibres, for a period of at least 2 months. There

is no spontaneous fibrillation in the inactive but innervated muscle fibres for at least 28 days after nerve block. These findings provide evidence for a neural control of extrajunctional muscle properties, which is independent of muscle activity. One of the assumptions on which this conclusion rests, is that the nerve conduction block was complete and that the lack of fibrillation and the reduced ACh-sensitivity of the inactive muscle fibres, relative to denervated fibres, were not due to a small amount of on-going impulse activity through or distal to the block. The evidence on which this assumption is based should therefore be reviewed.

Previous studies on the lower limb, involving stimulation and recording from exposed nerve within a few days of acute compression by a pneumatic cuff, showed that conduction was blocked in two zones which had been under the edges of the cuff (Gilliatt, McDonald & Rudge, 1974) where histological evidence of myelin damage was also found (Ochoa *et al.* 1972). Percutaneous stimulation and recording have shown both in the baboon and in man that the conduction block after a pneumatic tourniquet may remain complete in the large motor fibres for as long as three months and that thereafter recovery is relatively slow, so that in some cases 50% of the fibres are still blocked at 4 or 6 months (Fowler *et al.* 1972; Rudge, 1974; Trojaborg, 1977).

These previous findings and the tests employed in the present study make it very unlikely that in the present experiments nerve impulses were transmitted through the block or that spontaneous activity arose at its distal edge.

In view of the relatively long duration of our experiments, is it possible that denervation followed by collateral reinnervation was responsible for some of the intermediate levels of ACh sensitivity which were found after nerve compression? Regeneration from the site of compression in the forearm is unlikely to have occurred within the time-scale of the experiments but the possibility of collateral reinnervation by adjacent preterminal axons cannot be excluded. However, it is unlikely that reinnervation substantially affected our results since only 10–30% of the motor fibres in the ulnar nerve underwent Wallerian degeneration as a result of compression, and most muscle fibres retained their original innervation. Furthermore, in the experiments on the abductor hallucis there was one animal (P10) with a virtually complete conduction block and no evidence of denervation, in which the level of ACh sensitivity of the individual fibres was similar to that found in the other animals. In all the nerve-blocked muscles only four fibres were found which not only responded to nerve stimulation but which also showed an ACh sensitivity within the denervated range. This combination may have been due to early reinnervation but in the remaining results, reinnervated fibres (if any were present) could not be distinguished from those which retained their original innervation.

Could the difference between denervated and nerve-blocked muscle fibres be a transient one related to the degeneration of nerve terminals on the denervated side? This may be considered as an alternative interpretation of the results of Pestronk *et al.* (1976) and Lavoie *et al.* (1976, 1977), as mentioned in the Introduction. The present results show that the nerve-blocked muscle fibres had a reduced level of extrajunctional ACh-sensitivity relative to the denervated muscle fibres for as long as 63 days after nerve block or denervation, with little change in the level during the last 40 days. The stability and the long duration of the differential levels of ACh

sensitivity make it very unlikely that a transient process such as nerve degeneration is the cause of this effect.

The results in this paper indicate that the neural influence on its own is unable to maintain normal muscle properties, since a moderately high level of ACh sensitivity develops in the nerve-blocked muscles. This effect might be due to the compression of the nerve, interfering with axonal transport. However, in independent experiments on the baboon it has been shown that the rate of regeneration and maturation of crushed nerve fibres distal to a compression block is normal, suggesting that at least slow transport is normal (Williams & Gilliatt, 1977). Furthermore, Lavoie *et al.* (1976, 1977) and Pestronk *et al.* (1976) who used local application of tetrodotoxin to the motor nerve in rats in order to block impulse activity, observed an increase in the number of extrajunctional ACh receptors in the nerve-blocked muscle fibres, albeit somewhat less than that seen in denervated muscle. Fast axonal transport was examined in both studies and appeared to be normal. Although the duration of these experiments was short (5 and 7 days respectively) these results, together with our own, make it unlikely that a disturbance of axonal transport contributed to the altered properties of the impulse-blocked muscle fibres.

One of the lines of evidence thought to be in favour of a neurotrophic factor transported down the axon has been the difference in the time of onset of muscle membrane changes with high and low nerve sections (Emmelin & Malm, 1965; Harris, 1974). No such difference was apparent in extrajunctional ACh-sensitivity of muscle fibres from two muscles denervated for 18 days by high (25 cm from the muscle) and low (5–6 cm from the muscle) nerve section respectively. A similar result was obtained from two muscles denervated for 19 days. However, the lack of any differential effect of high and low nerve section in these experiments may be explained by the relatively long survival times used.

The effect of electrical stimulation in reducing the extrajunctional ACh-sensitivity both in nerve-blocked and denervated lumbrical muscle fibres strongly indicates that muscle activity is required to prevent ACh-sensitivity developing. In the rat several extrajunctional muscle properties are restored to normal by muscle activity in the absence of any neural influence (Lømo and Rosenthal, 1972; Jansen *et al.* 1973; Westgaard, 1975; Lømo & Westgaard, 1975). In the baboon extrajunctional ACh-sensitivity of the inactivated and the denervated muscle fibres was reduced but not abolished in our limited series of stimulation experiments. However, the results indicate that baboon lumbrical muscle fibres are even more sensitive to stimulation than rat soleus muscle. In the rat stimulation for 7 days with 100 impulses every 5·5 hr (approximately 430 impulses/24 hr) had no significant effect on the extrajunctional ACh-sensitivity (Lømo & Westgaard, 1975) whereas 6 days of stimulation with 500 impulses every 24 hr gave a clear effect in the fourth lumbrical muscle of the baboon.

Fibrillation. The experiments on abductor hallucis indicate that, at least in baboons, conduction block for 28 days is not associated with more than a slight increase in muscle fibre activity evoked by needle movement (insertion activity). The only animal in our series which proved an exception to this subsequently showed some Wallerian degeneration on histological examination.

In two animals the extrajunctional ACh-sensitivity of the muscle fibres was also

measured. In one of these it was found to be similar to that recorded in the main series of experiments; in the other there were a few fibres with relatively low ACh-sensitivities (down to 2·7 mV/nC). These lower values may have been caused by activity due to the incomplete nerve block since at that stage the block was complete in only 75% of the fibres. There does therefore appear to be a differential effect of the inactive nerve on two different muscle properties, causing a moderate reduction in acetylcholine sensitivity (compared with the denervated fibres) and a much stronger effect in preventing fibrillary activity almost completely. The reason for this is unclear, but might be due to a graded effect of the nerve in reducing the underlying oscillations in membrane potential which give rise to the fibrillary activity (Purves & Sakmann, 1974b; Thesleff & Ward, 1975) so that these are no longer sufficiently large to trigger muscle action potentials.

Note added in proof. Since this paper was submitted for publication, Cangiano, Lutzemberger & Nicotra (1977) have reported the results of placing silastic cuffs round the sciatic nerve of rats to produce compression block for periods of up to 14 days. A decreased resting membrane potential, fibrillary activity and resistance to TTX developed in the impulse-blocked muscles, but these effects were less pronounced than in the denervated muscles of the opposite hind limb. The authors were unable to decide whether the observed differences were due to a factor associated with degenerating nerve terminals on the denervated side or whether they indicated that a neurotrophic factor was present on the impulse-blocked side.

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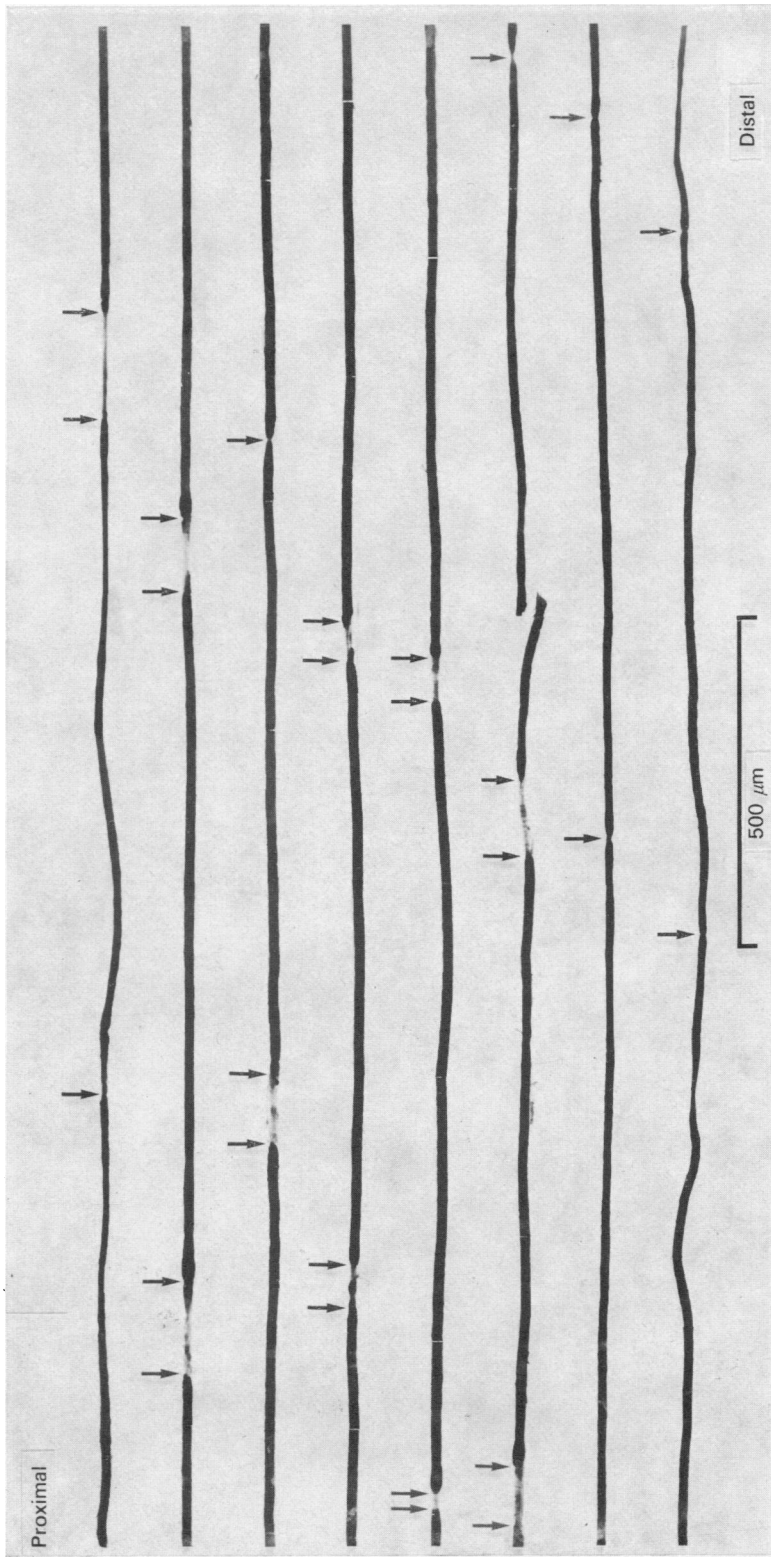
EXPLANATION OF PLATES

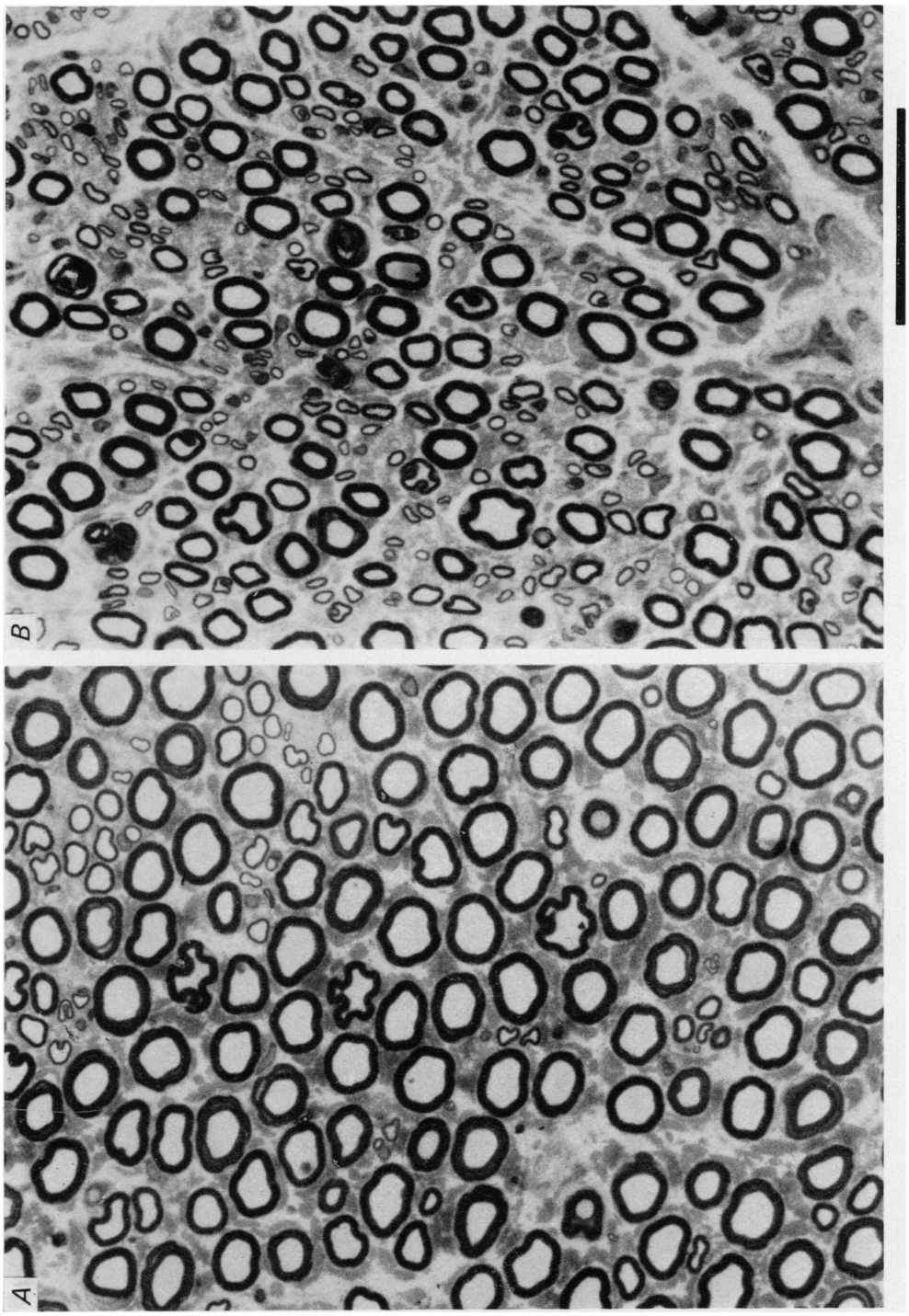
PLATE 1

Consecutive lengths of a single myelinated fibre, mounted below each other (total length shown, approx. 19 mm); from the forearm of P10, 63 days after acute compression. The portion shown is thought to have been under the distal edge of the compressing cuff and the distal four nodes of Ranvier are normal at high magnification. Proximal to this there are myelin defects up to 150 μm in length at the site of the original nodes, with early remyelination. The margins of the intact myelin have been marked with arrows.

PLATE 2

Transverse sections of the ulnar nerve at the wrist from (*A*) control nerve (P16) and (*B*) from the compressed nerve of P12 distal to site of compression, 28 days after tourniquet. Myelin debris and the reduced density of large myelinated fibres suggest Wallerian degeneration in a proportion of the compressed fibres. Bar 50 μm.


Distal
Proximal
500 μm


B
A