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Retrograde growth of myelinated fibres in experimental neuromas

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INTRODUCTION

A number of abnormal properties develop in damaged, sprouting, myelinated peripheral nerve axons, which may contribute to the dysaesthesiae experienced by some patients following various types of nerve injury. These properties, investigated in experimental neuromas, include ongoing activity, mechanosensitivity, adrenaline sensitivity and the later development of ephaptic impulse transmission between myelinated fibres (Wall & Gutnick, 1974*a*, *b*; Govrin-Lippmann & Devor, 1978; Seltzer & Devor, 1979; Korenman & Devor, 1981; Scadding, 1981). The anatomical changes in damaged peripheral nerve fibres have been extensively studied over many years. Re-investigation of some of these changes is now of interest in view of the recent advances in our understanding of the physiology of nerve injury. One of these, the retrograde growth of myelinated fibres following nerve section, is reported here.

Cajal (1928) showed many illustrations demonstrating that myelinated fibre sprouts frequently grow in a retrograde direction after nerve section, and this phenomenon has subsequently been commented on by other authors (e.g. Young, 1942; Weiss, Edds & Cavanaugh, 1945). In a quantitative study, Aitken (1949) showed, in the nerve to the gastrocnemius muscle of the rabbit, that there was an increase of more than 50 % in the number of myelinated fibres proximal to neuromas after transection of this nerve, and that this increase was accounted for by small fibres, particularly those of 2–4 μm diameter, with a smaller increase in fibres 4–6 μm in diameter. This striking increase in myelinated fibres was noted immediately proximal to the neuromas and it was not determined how far proximally these regenerating fibres grew in a retrograde direction. The mechanisms that lead to many myelinated fibre sprouts growing in a retrograde direction are not understood, but it is clear from Cajal's (1928) drawings and the recent electron microscopic observations of Friede & Bischhausen (1980) that retrograde growth begins as soon as the first sprouts are formed. There is physiological evidence for the existence of retrogradely growing sprouts at least 2–3 mm proximal to neuromas (Scadding, 1981).

It is conceivable that sprouts growing for long distances proximally along the nerve trunk could have important functional consequences. For example, the neuroma properties of ongoing activity, mechanosensitivity and adrenaline sensitivity may also apply to retrogradely growing fibres, increasing the anatomical extent of abnormal function, with important therapeutic implications, and it is conceivable

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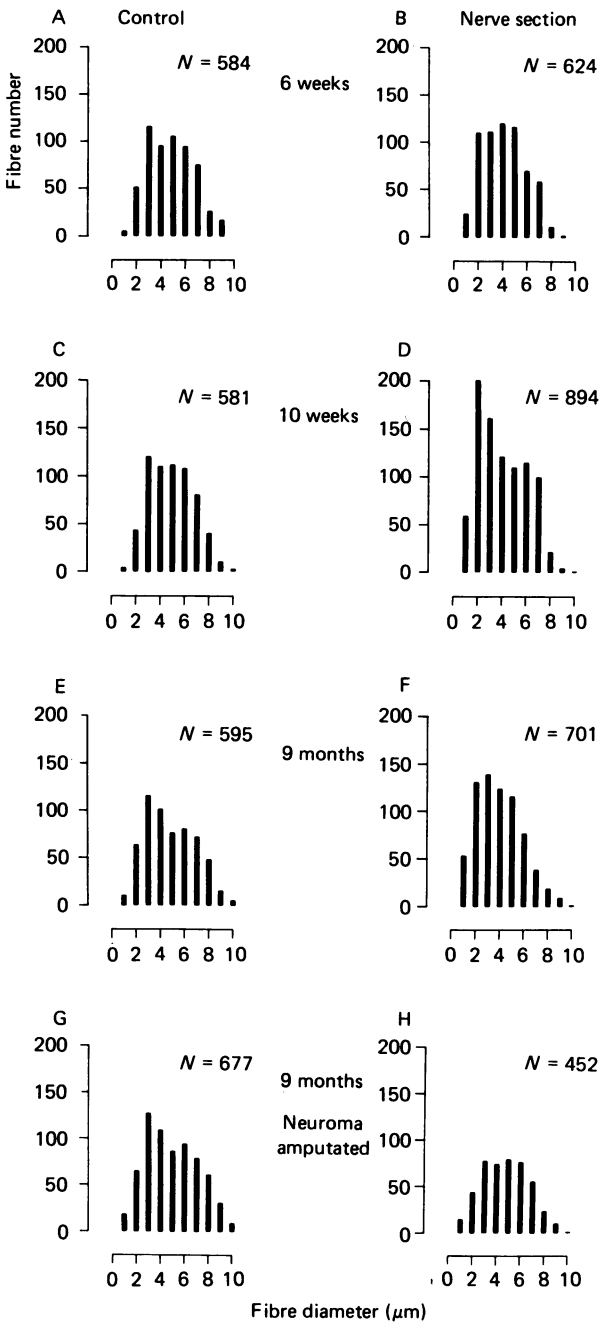


Fig. 1 (A–H). Histograms of myelinated fibre counts. (A), (C), (E) and (G) are made from counts on the unoperated right sural nerve in each mouse. (B), (D) and (F) are made from counts proximal to sural nerve neuromas at 6 weeks, 10 weeks and 9 months after nerve section respectively. (H) Counts made proximal to a neuroma 9 months after nerve section, where the neuroma had been amputated 10 days before biopsy of the nerve. See text for further explanation.

Table 1. *Myelinated fibre counts in sectioned and control sural nerves*

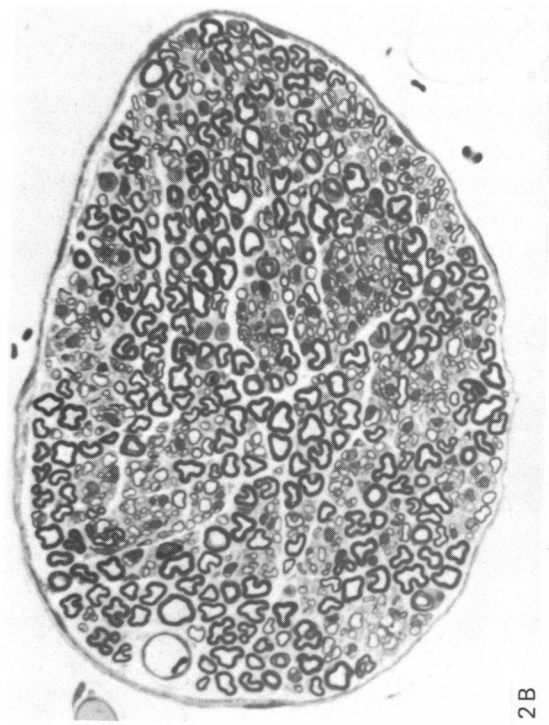
Mouse and nerve		Time after nerve section	Fibre diameter (μm) 1	2	3	4	5	6	7	8	9	10	Total fibres
M1	Right	Control	5	51	115	96	105	94	75	26	17	0	584
M1	Left	6 weeks	24	110	112	120	117	70	58	11	2	0	624
M2	Right	Control	4	43	120	110	112	107	80	41	10	2	581
M2	Left	10 weeks	59	201	162	121	110	115	100	21	4	1	894
M3	Right	Control	10	63	115	101	76	80	72	48	15	5	595
M3	Left	9 weeks	53	131	138	124	116	76	37	17	8	1	701
M4	Right	Control	17	65	127	109	86	94	78	61	31	9	677
M4	Left	9 months, and neuroma amputation 10 days before biopsy	15	43	77	74	78	76	55	23	10	1	425

that sprouts might extend as far as the dorsal root ganglia, or into dorsal and ventral roots, at which sites it is possible that afferent and efferent activity might be influenced.

The experiments described here demonstrate retrograde growth of myelinated fibre sprouts over relatively large distances in mouse sural nerve after nerve section.

MATERIALS AND METHODS

Four adult female CBA strain mice were used, aged 8–12 weeks at the time of operation. Under halothane and nitrous oxide anaesthesia, a longitudinal incision was made in the skin of the posterior aspect of the left calf. The sural nerve in the mouse originates from the main trunk either as a single fascicle or as two separate fascicles arising from tibial and peroneal nerves immediately distal to the formation of these nerves by the division of the main sciatic trunk. It then passes through the popliteal fossa and reaches the dorsal surface of the gastrocnemius muscle. It gives off no branches between its origin and mid-calf level, where small branches are given off as the nerve descends towards the foot. With a pair of fine scissors, the sural nerve was cut across at mid-calf level above the origin of the first branches of the nerve, identified under a dissecting microscope. The distal part of the nerve was then extensively avulsed. The skin was re-sutured, and the mouse was given a single 15 mg dose of chloramphenicol subcutaneously, and allowed to recover from the anaesthetic. At intervals of 6 weeks, 10 weeks and 9 months after sural nerve section respectively, three of the mice were anaesthetised. The left and right sural nerves were gently exposed between mid-calf and their origin from the sciatic nerves. The nerves were fixed *in situ* and then by immersion with 2% glutaraldehyde with formaldehyde in a cacodylate buffer (Karnovsky, 1965). A segment of the sural nerve, measuring approximately 2–3 mm near to the point of origin of the nerve from the sciatic nerve, was excised on each side, after at least 10 minutes of *in situ* fixation to minimise traumatic damage to these very small nerves during biopsy. In the left sural nerve, the distance from the point of nerve section in the calf to the point of biopsy in the thigh was between 12 and 15 mm in each case. In the fourth mouse, 10 days after biopsy at 9 months after sural nerve section, the left sural nerve was re-exposed under anaesthesia and the neuroma was identified on the proximal stump. The distal part of the neuroma was amputated using fine scissors, and removed. The skin was then sutured and the mouse was allowed to recover. Ten



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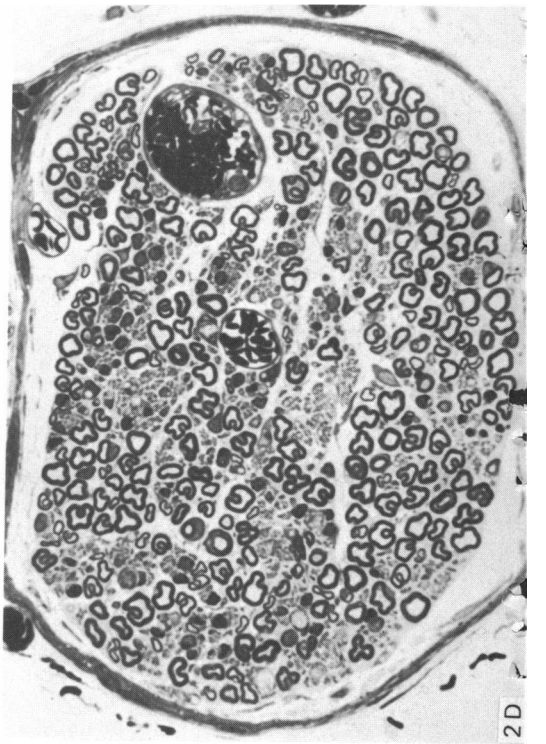

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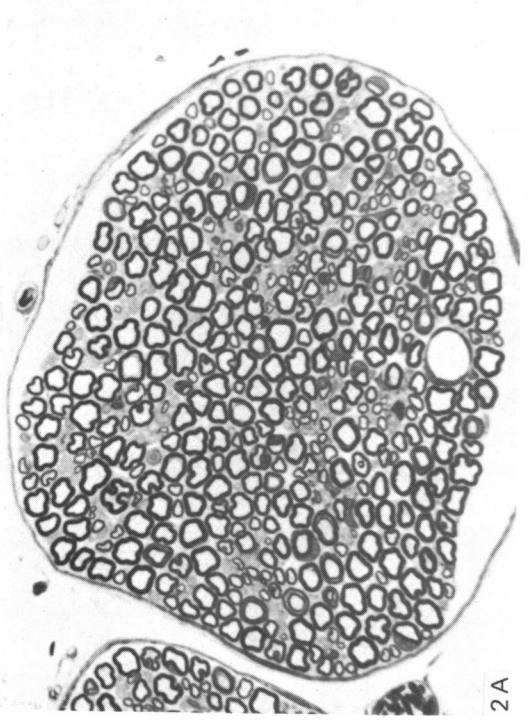

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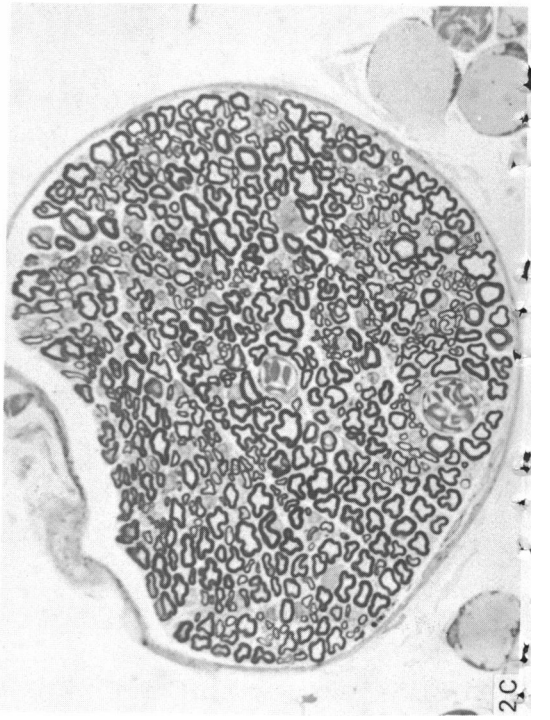

2C

days later, biopsy of the proximal segments of both sural nerves was performed, as described above. After initial fixation for 3 hours, the biopsied nerves were post-fixed in osmium tetroxide, dehydrated in alcohol and embedded in Araldite, using propylene oxide as transition medium. Transverse sections 1 μm thick were cut on a Huxley ultramicrotome using glass knives, and stained with thionin and acridine orange. Photomicrographs of the whole of each nerve were taken and prints were made from the negatives to a final magnification of $\times$ 1000. From these photographs, the total number of myelinated fibres in each nerve was counted and the external diameter of each fibre was measured. Histograms of fibre diameter distribution in each nerve were constructed. Thin sections were cut from the left sural nerve of the mouse in which the neuroma had been amputated 10 days before biopsy. These sections were stained with uranyl acetate and lead citrate, and viewed in a Siemens Elmiskop 101.

RESULTS

The results of the total myelinated fibre counts and size distributions are shown in Figure 1 and Table 1. In Figure 1, A, C, E, and G are the histograms made from measurements on the unoperated right sural nerves from each mouse. A similar distribution is present in each nerve, peaks occurring at 3 and 5–6 μm. The total numbers of fibres in each nerve ranged from 581–677, with an average 609 fibres. The fibre size distributions in the left sural nerve of each mouse are shown in Figure 1B, D, F and H. At 6 weeks after nerve section (Fig. 1B) the total number of myelinated fibres in the nerve was only slightly greater than the control nerve, but the bimodal size distribution was replaced by a unimodal one, with a peak at 4 μm. The most striking change, however, was the increase in fibres of 1–2 μm diameter, with a less marked reduction in large fibres 6–9 μm diameter. At 10 weeks after nerve section (Fig. 1D), there was a very large increase in fibres 1–3 μm in diameter, accounting for the 35 % increase in total number of fibres in the left sural nerve compared with the right sural nerve from this mouse. At 9 months after nerve section (Fig. 1F) there was an increase in all fibre sizes up to 5 μm, most marked in fibres 1–3 μm in diameter. There was also a reduction in the number of fibres of 7–10 μm diameter. Figure 1H shows the fibre size distribution in the sural nerve which had been sectioned 9 months previously and from which the resulting neuroma had been amputated 10 days before biopsy. There was a 33 % decrease in the total number of myelinated fibres, with reductions in all fibre sizes, although this was most marked in fibres 8–10 μm in diameter.

Figures 2A and B show light micrographs of the normal and experimental nerves of the mouse in which the left sural nerve was biopsied 6 weeks after nerve section (M1). The great increase in the number of small diameter fibres is obvious in the left sural nerve. Figures 2C and D compare the appearances of two experimental nerves at 9 months after nerve section (M3 left and M4 left). Figure 2C shows the

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Fig. 2 (A–D). (A) Micrograph of normal sural nerve (right) and (B) from experimental nerve (left) of mouse M1, in which section of the left sural nerve had been performed 6 weeks earlier. In (B), there is a large increase in the number of small myelinated fibres present. (C) left sural nerve proximal to a neuroma at 9 months after nerve section (M3). (D) left sural nerve proximal to a neuroma at 9 months after nerve section where the neuroma was amputated 10 days before biopsy (M4). In (D), many degenerating myelinated fibres are seen, mostly of small diameter. All micrographs $\times$ 500.

increase in the number of small myelinated fibres, while Figure 2D, from the nerve in which the neuroma was amputated 10 days before biopsy, shows many degenerating myelinated fibres, with swelling of the nerve. On electron microscopy, numerous degenerating small diameter myelinated fibres were seen.

DISCUSSION

The results indicate that after nerve section many myelinated fibres emit sprouts which grow in a retrograde direction from the injured region. This is evidenced by a great increase in the numbers of small diameter myelinated fibres well proximal to the point of nerve section. It could be argued that the sprouts seen originated from proximal parts of the severed nerve fibres and were in fact growing in an anterograde direction, but the depletion of fibre numbers to below the normal expected myelinated fibre total by amputation of a neuroma at 9 months after nerve section shows that the great majority of sprouts growing in a retrograde direction must originate from the proximal stump near to the point of nerve section.

From this limited series of myelinated fibre counts, it can be estimated that the fibre population had increased by around 37 % at 1·5 cm proximal to the point of nerve section, by 10 weeks after nerve section. By 9 months after nerve section, the excess number of myelinated fibres can be calculated at 15 %. The timing of the greatest extent of myelinated fibre retrograde sprouting, and the reasons for its later decline, require further experimental investigation. Aitken (1949) observed that around 50 % of the myelinated fibres seen just proximal to a motor nerve neuroma were growing in a retrograde direction. This is much higher than the greatest number seen further proximally in the mouse sural nerves in the present investigation. It may be that many of the proximally growing sprouts grow only short distances before arrest of growth occurs, or that there are differences in the capacities of motor and sensory fibres for retrograde growth. Aitken (1949) made his observations at 100 days after nerve section, and these may be approximately compared with the observations made here at 70 days.

The sural nerve biopsied 10 days after amputation of the neuroma which had developed following nerve section 9 months earlier contained a substantially reduced number of myelinated fibres. The original nerve section and subsequent neuroma amputation may have contributed to this, due to dorsal root ganglion cell death (see Lieberman, 1971). It is not possible to determine the importance of this factor from the present results. All the experimental nerves showed a decrease in the number of larger myelinated fibres (8–10 μm diameter), but this could be explained on the basis of fibre shrinkage rather than dorsal root ganglion cell death (Cragg & Thomas, 1961; Aitken & Thomas, 1962; Dyck *et al.* 1981). Furthermore, it is known that lesions of the nerve peripherally induce less dorsal root ganglion cell death than lesions made proximally, near to the dorsal root ganglion (Ranson, 1906; Romanes, 1941), and this may be relevant to the present investigation, in which a fairly distal lesion of the sural nerve was made. However, the reduction of the numbers of fibres of all sizes in the amputated neuroma sural nerve suggests that some dorsal root ganglion cell death had occurred.

SUMMARY

Retrograde growth of myelinated fibre sprouts following section of mouse sural nerves has been investigated. The results indicate that many sprouts grow relatively long distances proximally along the nerve trunk. The largest number of such sprouts was observed at 10 weeks after nerve section, with a decrease by 9 months. It has been shown that the retrogradely growing sprouts arise within the neuroma and not more proximally on the nerve.

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