Intact myelinated fibres in biopsies of ventral spinal roots after preganglionic traction injury to the brachial plexus. A proof that Sherrington's 'wrong way afferents' exist in man?

MICHAEL SCHENKER AND ROLFE BIRCH

Peripheral Nerve Injury Unit, Royal National Orthopaedic Hospital, Stanmore, Middlesex, UK

(*Accepted 4 April 2000*)

ABSTRACT

Bell-Magendie's law of separation of spinal function states that afferent and efferent fibres join the spinal cord separately in ventral and dorsal spinal nerve roots. For over 100 years there have been reports that challenge the exclusiveness of this law in mammals; very few studies have referred to man. We conducted a prospective morphological study in patients with preganglionic traction injuries of the brachial plexus to address this question. Avulsed ventral and dorsal roots were examined after variable intervals from the injury for histological and ultrastructural evidence for myelinated afferent fibres entering the cord via the ventral roots. Intact myelinated fibres were found in all ventral root specimens, but the majority of fibres in later biopsies are regenerative. A small number of fibres could be demonstrated that are likely to be 'wrong way ventral afferents'. Their number is falsely low due to wallerian degeneration of dorsal and ventral afferents following the mechanical and ischaemic effects of traction injury. Our findings are the first morphological evidence in human material that Bell-Magendie's law might not entirely be correct and they underline the difficulties in comparing traumatic with experimental rhizotomy.

Key words: Brachial plexus injury; ventral root afferents.

INTRODUCTION

Sherrington (1894) showed that ventral roots of cats and monkeys contained intact myelinated nerve fibres after transection of both the ventral and dorsal roots, from observations made in a large experimental series aimed to demonstrate afferent fibres in motor nerves. He concluded from this unexpected observation that some afferents reach the spinal cord the 'wrong way' via the ventral roots. This challenged the traditional view of separation of efferent and afferent fibres in the spinal roots defined by Bell and Magendie in the beginning of the 19th century. This anatomical arrangement is often referred to as the 'law of separation of spinal function' and has been recognised as one of the fundamental discoveries in the history of anatomy comparable to Harvey's treatise on the circulation (Coggeshall, 1980).

After Sherrington's description several other reports were published in agreement with his challenging view (see Coggeshall, 1980). Using transmission electron microscopy, Coggeshall et al. (1974) demonstrated that in addition to the myelinated axons (MnF), a much larger number of nonmyelinated fibres (nMnF) escaped wallerian degeneration after rhizotomy of the ventral root of the cat. These nMnF degenerated after excision of the dorsal root ganglion. Counts of nMnF in ventral roots of human necropsy material revealed a similar proportion (27%) of fibres as in the cat (29%) (Coggeshall et al. 1975). Subsequent morphological studies in animals suggested the 'wrong way' afferent fibres might not reach the dorsal horn through the cord, but either deviate through the pia mater or describe a 'U-turn' in the ventral root to travel further towards the CNS via the dorsal root (Risling et al. 1984; Vergara et al. 1986). Physiological studies in cats also gave controversial results regarding the further course of those fibres (Loeb, 1976; Chung, et al. 1983).

Brindley (1988) described a related finding in

Correspondence to Mr R. Birch, Peripheral Nerve Injury Unit, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex HA7 4LP UK. Tel: + 044 208 909 5803; e-mail margarettp@rnoh-tr.org

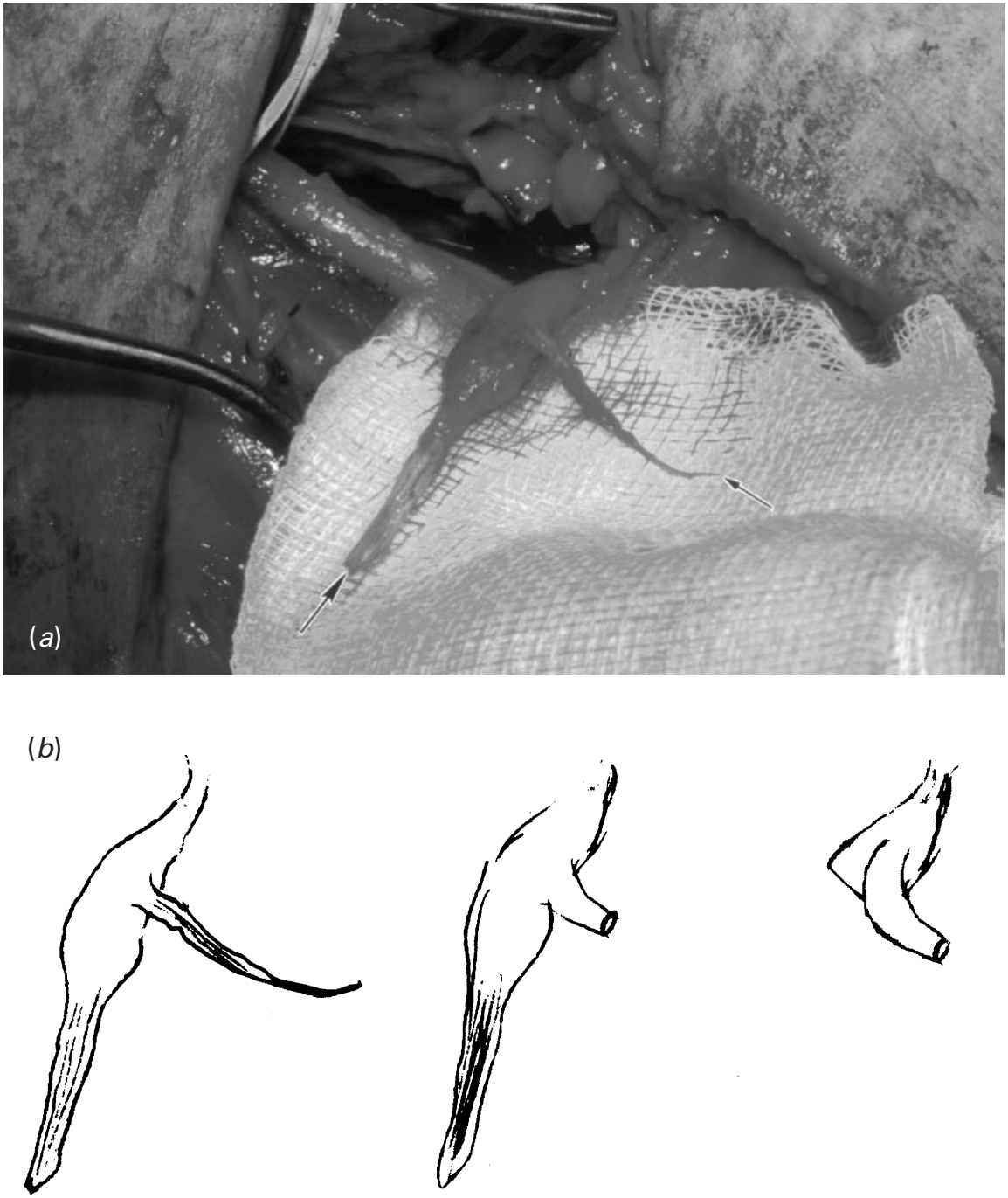


Fig. 1. (*a*) Intraoperative view of avulsed right root C7 (preganglionic injury) 4 wk after the accident. The avulsed dorsal (large arrow) and ventral roots (small arrow) are indicated. (*b*) Preparation of avulsed spinal rootlets for nerve grafts or transfer. The diagrams illustrate the situation before biopsy (left), after shortening of the ventral roots (middle) and excision of the dorsal root ganglion with attached rootlets (right).

patients with incomplete spinal lesions who underwent implantation of an electrical device to stimulate the pelvic organs. Some of his patients experienced pain during stimulation of the sacral ventral roots despite the fact that the posterior roots of the same segments had been divided at the time of implant insertion. The presence of ventral 'wrong-way' afferents was seen as a possible explanation of this phenomenon, albeit not as proof. More recently, electrophysiological recordings in children undergoing dorsal rhizotomy for spastic cerebral palsy have added further evidence for the presence of ventral afferents (Phillips et al. 2000).

Intradural injury is common in closed traction lesion of the brachial plexus. Bonney (1954) introduced the term 'preganglionic' for these injuries, and

Table 1. *Nerve biopsies after preganglionic injury*

Patient	Age	Interval to biopsy (wk)	Biopsied roots				
			C5	C6	C7	C8	T1
1	45	4/7				x	x
2	22	4/7		x			
3	47	8/7			x	x	
4	29	4			x		
5	24	5			x	x	
6	22	10			x	x	
7	29	14			x		
8	46	20		x			
9	30	50			x	x	

later (Birch et al. 1998) distinguished between preganglionic rupture peripheral to the transitional zone and preganglionic avulsion to the rootlets from the spinal cord central to the transitional zone (see Berthold et al. 1993). Between 80 and 100 patients with closed traction lesions of the supraclavicular brachial plexus are operated annually at the Royal National Orthopaedic Hospital (RNOH). The indications for surgical exploration of the plexus, the methods of repair, and the results are described elsewhere (Birch, 1996; Birch et al. 1988). Excision of the avulsed dorsal root ganglion (DRG) and the tip of avulsed ventral rootlets is an essential step in the preparation of nerve stumps to repair by graft or by nerve transfer. We examined biopsies of ventral roots damaged in this way at variable intervals from the accident by conventional histological and ultrastructural methods. The aims of our prospective study included observations to see (1) whether intact myelinated fibres persisted in human ventral roots after traumatic axonotomy; (2) to measure their number and calibre; (3) to determine their course, and (4) to speculate as to their function.

PATIENTS, MATERIALS AND METHODS

All patients that were included in this study gave informed consent to exploration and repair of the brachial plexus and they gave specific permission to study anatomical and molecular features in redundant or discarded tissue. Excision of the tips of the avulsed ventral roots and removal of the dorsal root ganglion is an essential preliminary to repair of that spinal nerve either by transfer of such uninjured adjacent nerves as the spinal accessory to supraclavicular, or by graft from the stump of an adjacent cervical spinal nerve which has sustained postganglionic rupture. This excised material is redundant, and is discarded unless specific investigation is proposed. The study was approved by the local ethical committee at the RNOH.

Biopsy material was obtained from 12 patients operated for diagnosis and repair of traction injury of the brachial plexus. All operations were performed by one or other, or both of the 2 senior surgeons (R. Birch, T. Carlstedt) of the Peripheral Nerve Injury (PNI) Unit. The damage to each root of the plexus was classified as 'intact', 'stretched', 'ruptured' or 'avulsed'. 'Rupture' means tearing of the spinal nerves within the posterior triangle of the neck, i.e. a postganglionic lesion. 'Avulsion' means that the level of lesion lay between the DRG and the spinal cord, the spinal nerves so damaged lying, displaced, in the posterior triangle, still attached to their respective DRG (Fig. 1*a*). The distinction between ventral and dorsal roots in individual avulsed spinal nerves is straightforward. The ventral root is usually shorter than the dorsal and it joins the spinal nerve at ~ 1 mm or less from the peripheral, or distal, pole of the DRG, and it is easily separable from that ganglion. The dorsal rootlets enter the proximal (central) pole of the DRG (see Fig. 1*a*). In no case were dorsal spinal nerves mistakenly biopsied as proven by the different light microscopical appearance. Biopsies were taken from the tips of the avulsed rootlets (Fig. 1*b*). The length of the biopsies varied with the level of the affected root and the distance of the intradural rupture zone from the cord. Ventral roots biopsies measured between 3 and 15 mm (mean 9 mm) and dorsal roots between 5 and 25 mm (mean 13 mm). The prepared ventral stumps were then reinnervated by grafts from nearby postganglionic ruptures or by transfer of the spinal accessory nerves. Afferent reinnervation was effected by grafts similarly placed, or by transfer of supraclavicular nerves to the dorsal component of the spinal nerve after excision of the DRG.

The biopsied material was fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer and, after 48 h fixation, small disks of tissue were cut for transverse sections. These specimen disks were cut 1–2 mm from the tips of the avulsed dorsal and ventral rootlets (most central portions) and further disks from the middle and peripheral regions of the ventral root biopsies. The distances between transverse sections from central, middle and peripheral regions of the ventral root were therefore less than half the length of the biopsy. The tissue was reimmersed in fixative and postfixed 2 h later in 1% osmium tetroxide. Stepwise dehydration in alcohol was followed by infiltration of the specimens in Spurr's resin.

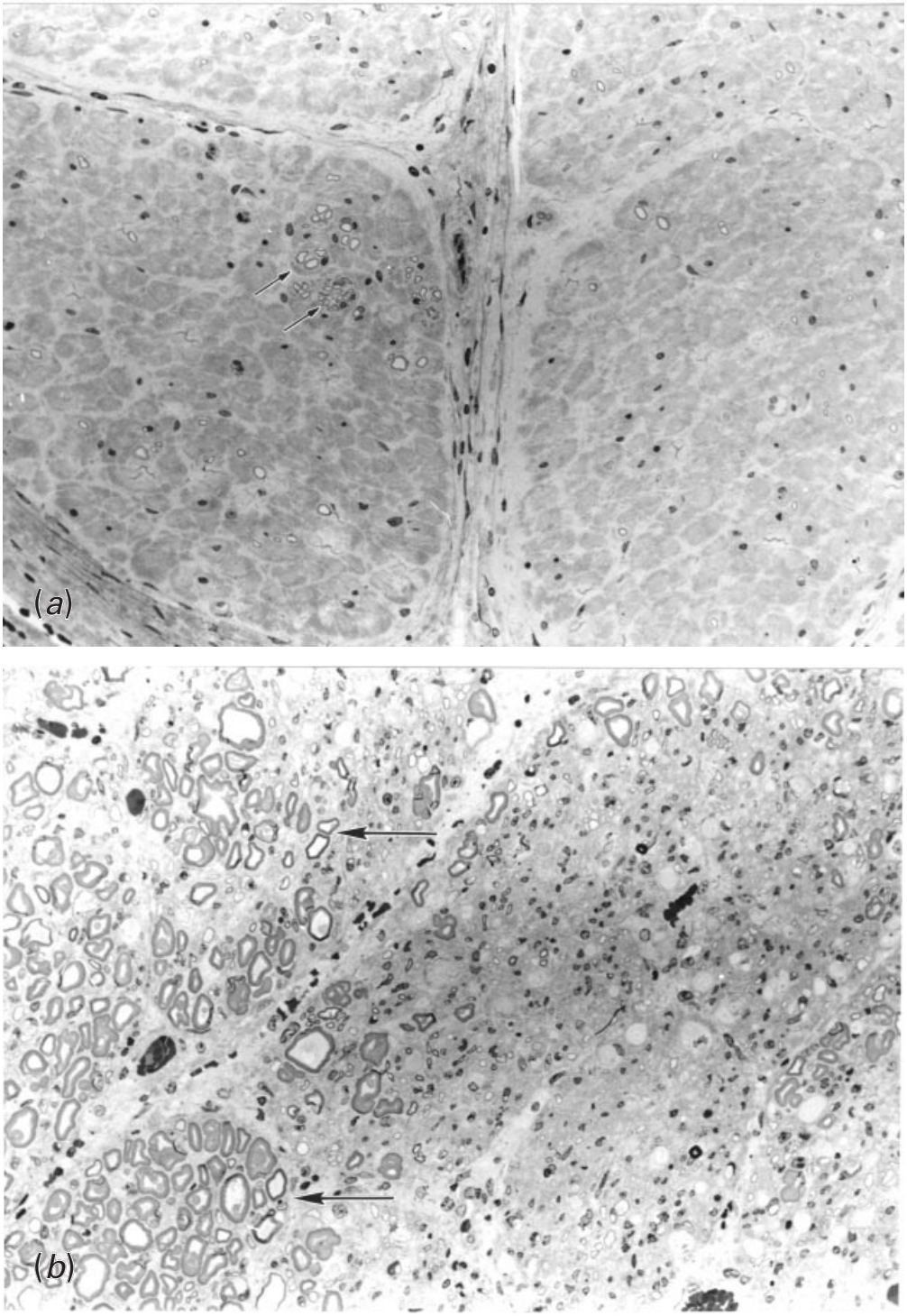


Fig. 2. (*a*) Myelinated fibres in avulsed ventral root C7 1 y after injury (J.C., transverse LM midsection, toluidine blue). Myelinated fibres (arrows) are arranged in groups and clusters of round or oval shape suggesting regenerating fibres within the basal lamina of previously degenerated fibres. ×200. (*b*) Wallerian degeneration of axons in continuity with their soma in the avulsed dorsal root C7 (J.R., transverse LM section 3 mm from the tip, 2.5 mo after injury). The preserved normal population of dorsal root axons is seen on the left of the picture (arrows). Wallerian degeneration caused by the ischaemic and mechanical effects of avulsion has affected the majority of fibres on the right. The estimated proportion of the area with preserved axons in this section is 60%, but was around 5% in most other dorsal roots. ×400.

Sections for light microscopy (LM) of 0.5 μm thickness were cut on a microtome and stained with 1% toluidine blue in borax. In suitable tissue areas ultrathin sections were cut for transmission electron microscopy (TEM). Five or more serial TEM sections were transferred onto Athenae 200 mesh copper grids

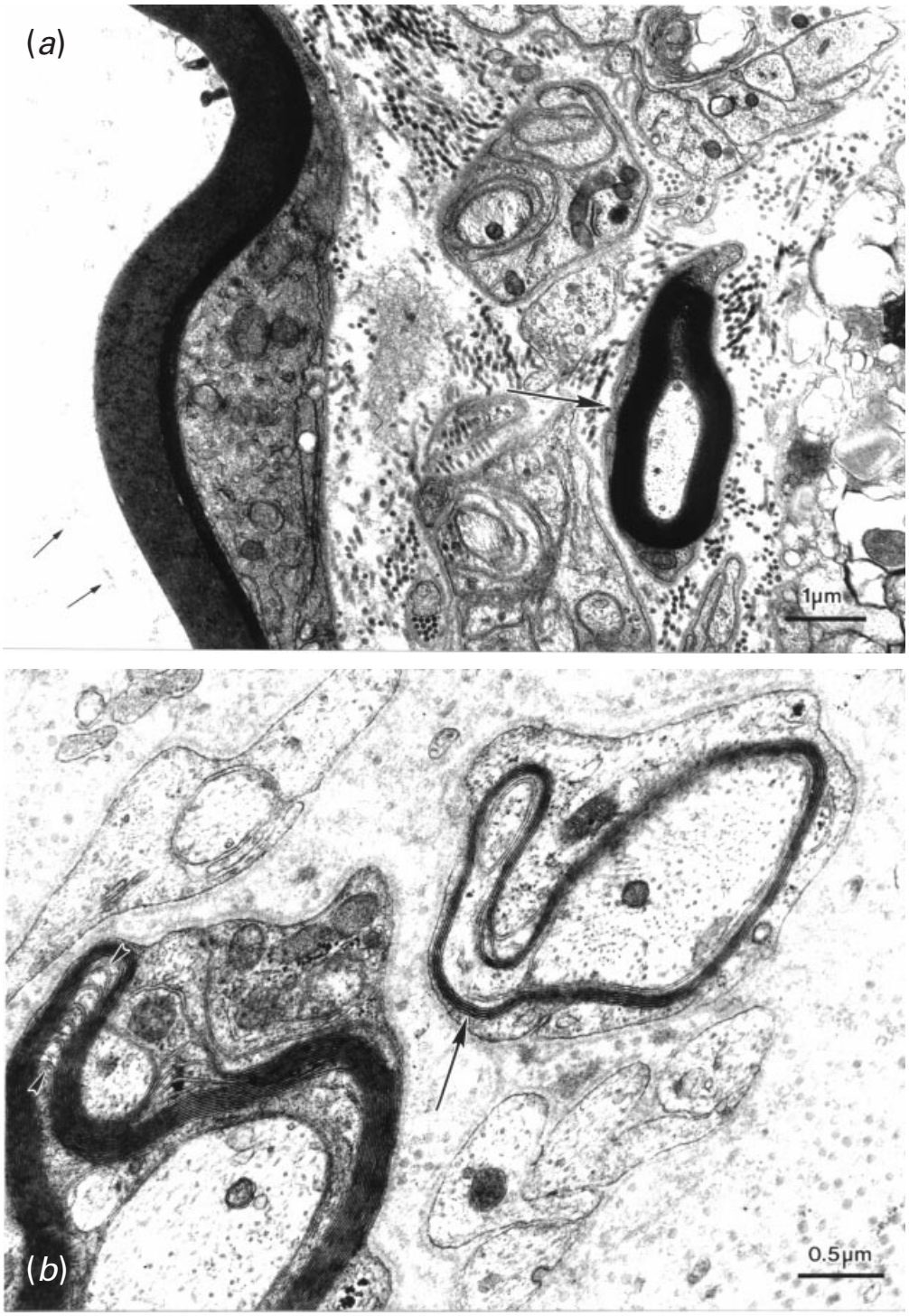
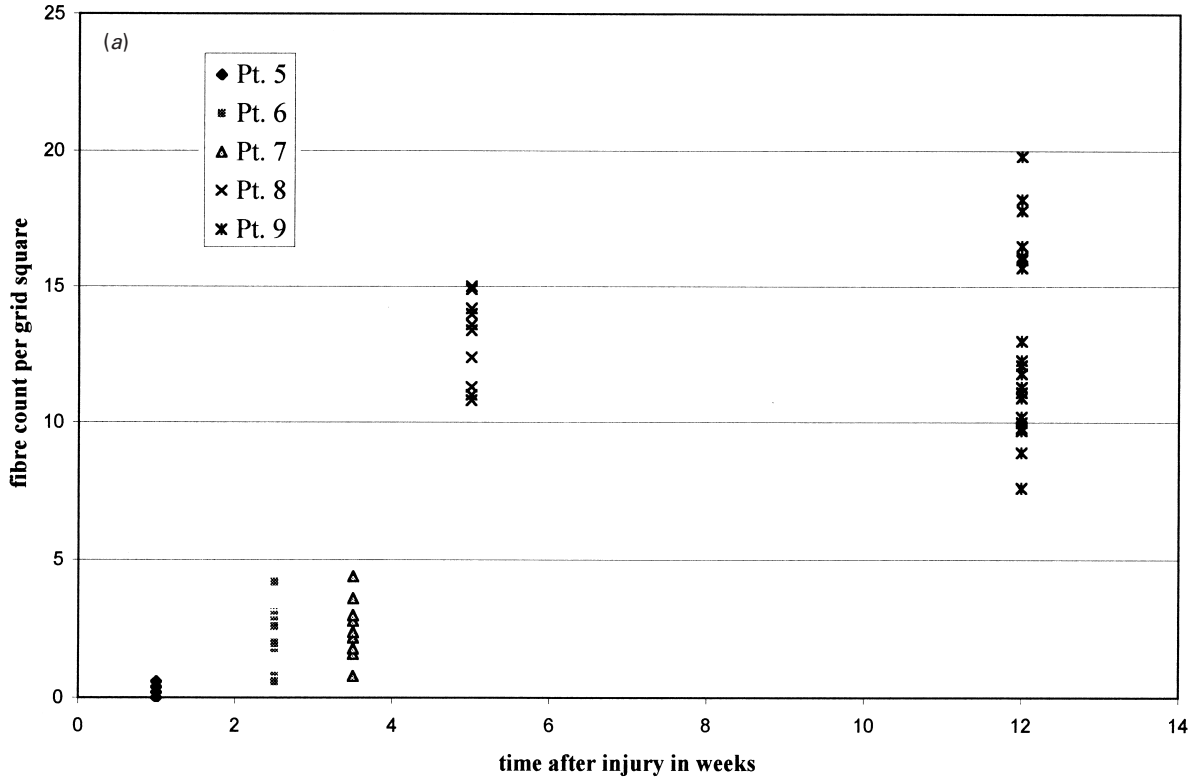


Fig. 3. (*a*) Intact myelinated fibre in ventral root C7 1 mo after avulsion (A.F., peripheral section). The small fibre (arrow) shows axoplasmic signs of its functional integrity, whereas the large fibre reveals the clumping typical of wallerian degeneration (small arrows). The thick myelin sheath of the small fibre is very unlikely to be the result of regeneration 1 mo after injury. This axon probably represents a true ventral afferent fibre. Note the morphologically intact myelin sheath of the degenerating large fibre. ×17600; bar, 1 μm. (*b*) Regenerating axons in the process of myelination in C5 ventral root 5 mo after avulsion (K.B., peripheral section). Two axons in different stages of myelination are seen. Uncompacted myelin (arrowheads) and redundant myelin loops (large arrow) are seen. ×37400; bar, 0.5 μm.

(135 μm grid size), contrasted with 2% uranyl nitrate and lead citrate and examined with a Philips CM-12 electron microscope.

The presence of regularly spaced microtubules in the TEM was used as the primary morphological indicator of the functional integrity of axons. In



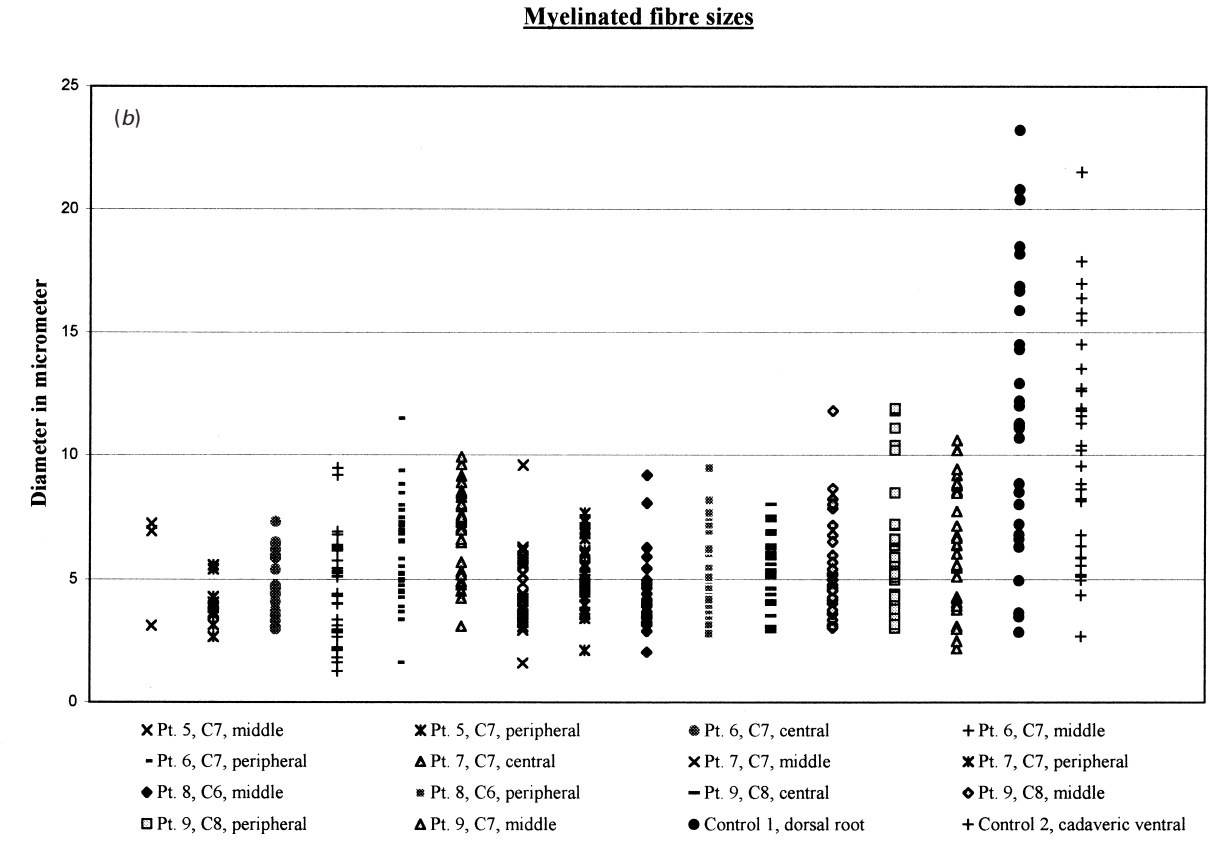


Fig. 4. (*a*) Correlation between number of intact myelinated fibres and the interval between avulsion and biopsy. Spearman's correlation for each specimen mean revealed that both variables are significantly related ($r_s = +.8564$, $n = 14$, $P < 0.01$,1-tail). (*b*) Distribution of largest external diameter of intact myelinated fibres in avulsed ventral roots compared with a dorsal root and an intact cadaveric ventral root serving as controls. The fibre diameters in injured ventral roots are significantly smaller than in the controls (on the right of the chart). For statistical analysis the observations were rank ordered and significant differences between groups found (Kruskall–Wallis test,

Table 2. *Morphologically intact nerve fibres in human ventral roots after traction injury; numbers and calibre*

Patient/root	Interval to biopsy (wk)	Fibre counts*			Fibre sizes (μm)**		
		Central	Middle	Peripheral	Central	Middle	Peripheral
1 C8/T1	4/7	Intact fibres observed (< 0.1)				2.99	3.07
2 C6	4/7	Intact fibres observed (< 0.1)					3.71
3 C7/8	8/7	Intact fibres observed (< 0.1)					
4 C7	4	Intact fibres observed (< 0.1)					
5 C7	5		0.1	0.4		5.78	4.02
6 C7	10	0.7	3	2.6	4.83	4.52	6.06
7 C7	14	2	2.7	2.2	7.01	4.59	5.21
8 C6	20		12.6	13.5		4.38	5.22
9 C7	50	9.5	11.9	15.4	5.27	5.41	6.01
9 C8	50		14.9			6.24	
Control			130.1			11.87	

*Counted as mean per grid square; **external diameter in micrometer.

contrast, nerve fibres undergoing wallerian degeneration showed axoplasmic clumping. In doubtful cases the presence of normal mitochondria was the distinctive feature to classify axons as 'intact'. The random placement of the ultrathin sections on the mesh grid was used to minimise the selection bias for fibre counts and basic morphometry. The mean number of intact fibres per grid square was calculated from 5 ultrathin sections of the same specimen. In each of these serial sections the fibres in 5 grid squares were counted manually twice at a magnification of $\times 1250$. Squares not completely filled with root tissue or containing a significant amount of connective tissue or large vessels were excluded. Vessels over 30 μm diameter were considered 'large' and a comparable area of connective tissue 'significant'. If, after such exclusion, ultrathin sections contained less than 5 suitable grid squares, squares from additional sections were selected. The maximum external diameter of the first 30 fibres counted was measured by beam deflection of the electron microscope at a magnification of $\times 2650$. The numbers and sizes MnF in a patient's dorsal root and in an uninjured ventral root from a cadaveric lower cervical cord were used as control.

For statistical analysis the average sample mean, the standard error of the mean and the ranks of each sample observation were computed with a Microsoft Excel software package. Spearman's rank correlation coefficient r_s, the Mann–Whitney U statistic and the Kruskall–Wallis T_{KW} statistic were calculated manually with the help of an electronic calculator. The critical values for the probabilities of a type I error were taken from statistical tables (Gravetter & Wallnau, 1996).

RESULTS

Nine of the 12 operated patients were found to have preganglionic ruptures in a total of 14 roots. Details regarding the biopsied nerve roots are shown in Table 1. The interval between injury and operation ranged from between 4 d to 1 y.

Intact MnF were found in all ventral root specimens. In the toluidine blue LM sections of biopsies taken after 3 mo or later they could be identified easily against the background of Büngner's bands or fibrosis (Fig. 2*a*). The ultrastructural appearance of the axoplasm allowed distinction between intact fibres and axons in the process of wallerian degeneration in biopsies from cases operated within 3 mo (Fig. 3*a*).

The results of the fibre counts and their largest diameter are given in Table 2 and Figure 4. They show that the number of intact MnF increases with time after traction injury in a statistically significant correlation of fibre count with time interval. These fibres are therefore most likely to be the result of an

$T_{KW} = 116.85$, n = 427, i = 16, $P < 0.005$). The critical value for $P < 0.005$ was taken from a χ^2 table. Significant differences exist between individual groups and the control groups. The specimen 'Pt. 9, C8, peripheral' and the cadaveric control were selected as examples, as their distribution is closest to each other. The Mann–Whitney test was used for analysis. The sum of ranks is 1205.5 for the specimen and 739 for the control (U = 219.5, n = 30). As n > 20, the location of the Mann–Whitney U-statistic was obtained with the unit normal distribution table ($z = -3.68$, $P < 0.001$).

active regenerative process after wallerian degeneration in avulsed ventral roots, which cannot come from the spinal cord. The arrangement of fibres in round clusters in the LM sections (Fig. 2*a*) and the features of remyelination in the TEM sections are further signs of axonal regeneration (Fig. 3*b*). In contrast, intact MnF in biopsies within 1 mo from injury were extremely rare, but showed a mature myelin sheath without the signs of remyelination seen in later biopsies (Fig. 3*a*). The comparison of mean fibre counts in central, middle and peripheral sections of later biopsies showed an higher number of fibres more peripherally, although this was not statistically significant. This suggests regeneration from the DRG.

The fibre diameters of the 14 specimens were compared with fibre size in the controls; this demonstrated only small fibres, less than 10 μm, in the ventral roots (Fig. 4*b*). The examination of sections of dorsal roots 3–4 mm peripheral to the rupture showed that a large number of the central processes of afferent DRG neurons underwent wallerian degeneration after traction injury, despite the fact that these axons remain in continuity with their soma in the DRG in preganglionic ruptures. Figure 2*b* shows a specimen with an exceptionally high number of intact dorsal root fibres. In all other dorsal root specimens the estimated proportion of cross-sectional area of nerve root with intact nerve fibres was only 5%.

DISCUSSION

We found intact MnF in all nerve biopsies of the ventral roots after traumatic division of the spinal roots of the brachial plexus in human. We further established that these fibres had small diameters up to ~ 10 μm, and that their number increased with the time after injury. Many axons showed morphological signs of remyelination. In contrast, a very small number of axons with mature myelin sheaths was seen in early biopsies within one month following nerve rupture. We also showed that the majority of fibres in dorsal roots undergo wallerian degeneration after traction injury in spite of the fact that the central processes of DRG neurons remain in continuity with their soma.

Our findings provide evidence that axons regenerate from the DRG into the ventral root after preganglionic avulsion injuries. The central process of DRG neurons is able to regenerate as far as to the entry to the spinal cord, despite a minimal response of the cell body to rhizotomy in contrast to axonotomy of the peripheral sensory process (Fawcett & Keynes, 1990). Regenerating fibres are attracted by degenerating peripheral nervous tissue in a nonspecific way, which explains their aberrant course into the ventral root (Abernethy et al. 1992).

The small number of intact fibres in the 4 specimens taken within 1 mo of injury cannot be attributed to regeneration. Although the experimental rate of elongation of regenerating axons varies between 0.1 and 6 mm per day (Ide, 1996), the rate of regeneration after nerve injury is delayed initially by several weeks, and remyelination does not start until 1–2 wk after the advance of the growth cone (Sunderland, 1991). It is also unlikely that the ultrastructural identification of intact fibres in our study was erronous. The granular disintegration of the axoplasm after axonotomy is triggered within hours and its completion only exceptionally exceeds 48 h (Griffin & Hoffman, 1993). Our earliest specimens were taken 4 d after injury. It is therefore likely that these few fibres in the ventral root represent afferent fibres in continuity with their somata in the DRG. However, our fibre counts show that they only represent 0.15% of the total number of fibres in the control and conclusions about their further course remains speculative.

Loeb (1976) calculated that 3.9% of all afferent fibres reach the cord via the ventral root from microelectrode recordings in cats. An explanation for the difference between this and our figures is the finding that the majority of dorsal root afferents in our biopsies degenerated after preganglionic division of the roots in a traction injury. These afferents remain in continuity with their somata after the injury, but the mechanical effects of traction as well as local ischaemia after rupture of radicular arteries are possible factors initiating wallerian degeneration. There is evidence that this does not only affect the central process of ganglionic neurons, but also the soma and the peripheral process. Berman et al. (1998) found a significant reduction in the axonal flare response in skin areas affected by preganglionic traction injury as compared with the normal side. The axonal flare depends on an intact peripheral process of sensory neurons and has been used to differentiate pre- and postganglionic ruptures in brachial plexus injuries (Lewis et al. 1927; Bonney, 1954). We observed that less than 5% of the central processes of all dorsal afferents survived the traction injury and it is safe to assume that 'wrong way' ventral afferents do just the same. Therefore the few surviving ventral afferents that we observed most likely represent only a very small proportion of their population. The ratio of our (0.15%) to Loeb's (3.9%) figures roughly represents a 5% survival rate. The insult to the nerve

tissue in traction injury makes comparison with Sherrington's experiment of ventral rhizotomy difficult.

ACKNOWLEDGEMENTS

The authors would like to thank Mr Michael Kayser at the Institute of Orthopaedics, Stanmore, for his expert technical help.

REFERENCES

ABERNETHY DA, RUD A, THOMAS PK (1992) Neurotropic influence of the distal stump of transected peripheral nerve on axonal regeneration: absence of topographic specificity in adult nerve. *Journal of Anatomy* **180**, 395–400.

BERMAN J, BIRCH R, ANAND P (1998) Pain following human brachial plexus injury with spinal cord root avulsion and the effect of surgery. *Pain* **75**, 199–207.

BERTHOLD CH, CARLSTEDT T, CORNELIUSON O (1993) Anatomy of the mature transitional zone. In *Peripheral Neuropathy*, 3rd edn (ed. Dyck PJ, Thomas PK), pp. 73–91. Philadelphia: W. B. Saunders.

BIRCH R (1996) Brachial plexus injuries. *Journal of Bone and Joint Surgery* **78B**, 986–992.

BIRCH R, BONNEY G, WYNN PARRY CB (1998) *Surgical Disorders of the Peripheral Nerves*, pp. 157–207. Edinburgh: Churchill Livingstone.

BONNEY G (1954) The value of axon responses in determining the site of lesion in traction lesions of the brachial plexus. *Brain* **77**, 588–609.

BRINDLEY GS (1988) The actions of parasympathetic and sympathetic nerves in human micturition, erection and seminal emission, and their restoration in paraplegic patients by implanted electrical stimulators (The Ferrier Lecture, 1986). *Proceedings of the Royal Society of London* **B235**, 111–120.

CHUNG JM, LEE KH, ENDO K, COGGESHALL RE (1983) Activation of central neurons by ventral root afferents. *Science* **222**, 934–935.

COGGESHALL RE (1980) Law of separation of function of the spinal roots. *Physiological Reviews* **60**, 716–755.

COGGESHALL RE, COULTER JD, WILLIS WD JR (1974) Unmyelinated axons in the ventral roots of the cat lumbosacral enlargement. *Journal of Comparative Neurology* **153**, 39–58.

COGGESHALL RE, APPLEBAUM ML, FAZEN M, STUBBS III TB, SYKES MT (1975) Unmyelinated axons in human ventral roots, a possible explanation for the failure of dorsal rhizotomy to relieve pain. *Brain* **98**, 157–166.

FAWCETT JW, KEYNES RJ (1990) Peripheral nerve regeneration. *Annual Reviews in Neurosciences* **13**, 43–60.

GRAVETTER FJ, WALLNAU LB (1996) *Statistics for the Behavioral Sciences*. Minneapolis: West Publishing.

GRIFFIN JW, HOFFMAN PN (1993) Degeneration and regeneration in the peripheral nervous system. In *Peripheral Neuropathy*, 3rd edn (ed. Dyck PJ, Thomas PK), pp. 361–376. Philadelphia: W. B. Saunders.

IDE C (1996) Peripheral nerve regeneration. *Neuroscience Research* **25**, 101–121.

LEWIS T, HARRIS KE, GRANT RT (1927) Observation relating to the influence of the cutaneous nerves on various reactions of the cutaneous vessels. *Heart* **14**, 1–18.

LOEB GE (1976) Ventral root projections of myelinated dorsal root ganglion cells in the cat. *Brain Research* **106**, 159–165.

PHILLIPS LH II, PARK TS, SHAFFREY ME, SHAFFREY CL (2000) Electrophysiological evidence for afferent nerve fibres in human ventral roots. *Muscle & Nerve* **23**, 410–415.

RISLING M, DALSGAARD CJ, CUKIERMAN A, CUELLO AC (1984) Electron microscopic and immunohistochemical evidence that unmyelinated ventral root axons make u-turns or enter the spinal pia mater. *Journal of Comparative Neurology* **225**, 53–63.

SHERRINGTON CS (1894) On the anatomical constitution of nerves of skeletal muscles; with remarks on recurrent fibres in the ventral spinal nerve-root. *Journal of Physiology* **17**, 211–258.

SUNDERLAND S (1991) *Nerve Injuries and their Repair*. Edinburgh: Churchill Livingstone.

VERGARA I, OBERPAUR B, ALVAREZ J (1986) Ventral root nonmedullated fibres: proportion, calibres and microtubular content. *Journal of Comparative Neurology* **248**, 550–554.