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Neural architecture in transected rabbit sciatic nerve after prolonged nonreinnervation

J. L. BRADLEY, D. A. ABERNETHY, R. H. M. KING, J. R. MUDDLE AND P. K. THOMAS

Clinical Neurosciences, Royal Free Hospital School of Medicine, London, UK

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ABSTRACT

Observations have been made on the rabbit sciatic nerve distal to a transection, with survival periods of up to 26 mo and prevention of reinnervation. It was confirmed that the nerve becomes compartmented by fibroblast processes and that a zone of fine collagen fibrils develops around the Schwann cell columns that constitute the Büngner bands. The Schwann cells become progressively more atrophic but after 6 mo of denervation still expressed low affinity p75 nerve growth factor receptor (NGFR), the latest stage at which this was examined. NGFR was also expressed by the processes of the fibroblasts producing the endoneurial compartmentation. By 26 mo after transection the site of previous nerve fibres was indicated by sharply demarcated domains of approximately circular outline in transverse section consisting of densely packed longitudinally oriented collagen fibrils. Some of these domains still possessed centrally situated Schwann cells or residual basal lamina but many were acellular. The central collagen fibrils in these domains were of smaller diameter than those situated peripherally but were of larger size than those that form around the Büngner bands during wallerian degeneration. The peripherally located fibrils in the domains were of the same calibre as for normal endoneurial collagen. The collagen domains were encircled by fibroblast processes or at times enclosed in a perineurial cell ensheathment. Long-standing axonal loss therefore leads to a striking reorganisation of the internal architecture of peripheral nerve trunks. The findings may be relevant for the interpretation of the appearances in chronic peripheral neuropathies in man.

Key words: Peripheral nerve; Schwann cells; fibroblasts; collagen; perineurium; nerve growth factor receptor.

INTRODUCTION

The initial studies by light microscopy on the connective tissue ensheathment of peripheral nerve fibres identified a thin inner endoneurial sheath that immediately surrounds the Schwann cells and which is inflected at the nodes of Ranvier (Plenk, 1934: Laidlaw, 1930) and a thicker outer endoneurial sheath or sheath of Key and Retzius (Young, 1942). The arrangements were analysed by transmission electron microscopy by Thomas (1963) who showed that the immediate ensheathment of myelinated nerve fibres is the Schwann cell basal lamina that is continuous across the nodes of Ranvier. External to this, around larger fibres, is a narrow zone of fine collagen fibrils with a circular and oblique orientation that extends into the nodal gap, corresponding to the inner endoneurial sheath. This is surrounded by a layer of thicker collagen fibrils with a longitudinal orientation, corresponding to the outer endoneurial sheath, that crosses the nodes. Similar findings were made using scanning electron microscopy by Friede & Bischhausen (1978). Ushiki & Ide (1986) commented that the inner collagen fibrils are closely associated with the Schwann cell basal lamina.

During the initial stages of wallerian degeneration, after nerve transection the Schwann cell basal laminal tubes persist (Thomas, 1964; Giannini & Dyck, 1990). Concomitant with removal of the axonal and myelin debris, Schwann cell proliferation occurs within these tubes, leading to longitudinally continuous columns of cells, the bands of Büngner (Nathaniel & Pease, 1963; Thomas, 1964). Schwann cells associated with myelinated axons do not express nerve growth factor

Correspondence to Prof. P. K.Thomas, Clinical Neurosciences, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK. Fax +44 171 431 1577.

(NGF). If they lose axonal contact they express low affinity p75 NGF receptors (NGFR) on their surfaces and produce NGF (Taniuchi et al. 1988). This occurs in the Büngner bands throughout the nerve distal to a transection. If regeneration takes place, once axonal contact is re-established, the Schwann cells cease to produce NGF and low affinity NGFR. This sequence may be involved in imparting directionality to axonal elongation (Taniuchi et al. 1986).

The diameter of the bands of Büngner is less than that of the original nerve fibres. In the gap outside the persisting Schwann cell basal laminae, a zone of fine collagen fibrils frequently forms internal to the original inner endoneurial collagen layer (Thomas, 1964; Salonen et al. 1987*a*). Fibroblasts proliferate (Salonen et al. 1988) and often encircle the original outer endoneurial sheaths (Thomas, 1964). This phenomenon later becomes more pronounced, producing a compartmented quasifascicular appearance (Röyttä et al. 1987). Elongated fibroblast processes then surround domains representing the site of previous nerve fibres or groups of fibres.

Weinberg & Spencer (1978) found that if reinnervation is prevented, the Schwann cells of the Büngner bands atrophy and that by 12 mo they have disappeared. In a more recent study on rats, Vuorinen et al. (1995) showed that with survival periods of up to 16 mo, although many Schwann cells had disappeared, remnants of some Büngner bands were still present. The endoneurium of the domains demarcated by the fibroblast processes was observed to contain 2 populations of collagen fibrils: thicker fibrils with a diameter of 50–60 nm surrounded areas composed of thin fibrils with a diameter of 25–30 nm. Atrophic Schwann cell columns were sometimes present within the areas of thin fibrils. It is of interest that on reinnervation of these distal stumps, the regenerating axons grow into the areas of thin fibrils (Vuorinen et al. 1995).

The present study extends these observations to an examination of the appearances at 26 mo after sciatic nerve section in the rabbit with avulsion of the proximal stump to prevent reinnervation. Some of the findings have appeared in abstract form (Thomas et al. 1996).

MATERIALS AND METHODS

All experiments were performed on adult New Zealand White rabbits. The animals were deeply anaesthetised with intramuscular pentobarbitone sodium (Sagatal, Rhône-Poulenc, France) followed by halothane by inhalation. The left sciatic nerve was exposed in the thigh under aseptic conditions and transected in the lower thigh. The proximal stump was dissected back to its intrapelvic origin from the lumbosacral plexus and transected proximal to the sciatic notch. The notch was then oversewn with a nylon suture and the operative incision closed. Observations were made on 6 animals with monthly survival times between 1 and 6 mo, and on 2 rabbits at 9 mo, 2 at 12 mo and 2 at 26 mo. At the termination of the experiment they were anaesthetised by the same method as in the initial operation. The distal stump of the transected sciatic nerve and its continuation as the tibial nerve in the lower leg were exposed. Specimens were removed, attached to pieces of card and fixed by immersion in 3% glutaraldehyde in PIPES (piperazine-*N*-*N*′-bis 2-ethane sulphonic acid) buffer (Baur & Stacey, 1977), postfixed in 1% osmium tetroxide in PIPES buffer and, after dehydration in graded concentrations of ethanol, embedded in Durcupan. Semithin sections (0.5 μm) were stained with thionin and acridine orange (Sievers, 1971). Ultrathin sections were contrasted with methanolic uranyl acetate and lead citrate and examined in a Zeiss 902A electron microscope. Portions were also taken from corresponding sites on the opposite unoperated side and processed similarly.

IMMUNOCYTOCHEMISTRY

The expression of low-affinity p75 nerve growth factor receptor (NGFR) and neurofilament (NF) protein in sectioned and unsectioned rabbit tibial nerve was examined by double labelling and for NGFR alone using the streptavidin-biotin peroxidase (StreptABC) complex technique (Scheidt & Friede, 1987). Unfixed tissues were frozen in isopentane and 7 μm sections cut on a Leica 2800 Frigocut (Leica, Milton Keynes, UK). The sections were mounted on coated glass slides and air-dried prior to fixation in acetone at room temperature for 10 min. The sections were rehydrated with trishydroxymethlyamine-buffered saline (TBS, pH 7.4). Nonspecific binding was blocked by the application of 1% bovine serum albumin (BSA) in TBS for 30 min. For double labelling the sections were then incubated with primary antibody to NF protein (Neurofilament Protein, 2F11, reacting with phosphorylated 70 and 200 kDa NF components; DAKO, Denmark) diluted 1:100 in TBS buffer for 30 min. Following this the sections were washed by agitation in TBS buffer for 5 min. The second and third layers were both applied for periods of 30 min followed by washing for 5 min in TBS buffer. For the second layer, biotinylated rabbit

antimouse immunoglobulin, diluted 1:200 in TBS, was employed to which was added 0.1% BSA. StreptABC complex horseradish peroxidase (HRP) (Dakopatts) was used as the third layer. The peroxidase reaction product was developed with AEC (3-amino-9-ethylcarbazole; Vector, USA) for 5 min. The slides were then washed in running water for 20 min followed by agitation in TBS buffer for 10 min prior to blocking with 1% BSA in TBS for 30 min. The sections were then incubated with the second primary antibody to human p75 NGF receptor (Boehringer Mannheim, Germany) diluted 1:500 in TBS buffer overnight at 4 °C. Following this the slides were washed by agitation in TBS buffer for 5 min. Application of the second and third layers was as stated for the first antibody with the exception that the peroxidase reaction product was developed with 4-chloro-1-naphthol and 0.1% hydrogen peroxide in 0.05 M TBS (pH 7.6) for 5 min. The slides were then washed in running water for 5 min and the sections counterstained with methyl green before mounting in glycerol.

For visualisation of NGFR labelling alone the sections were incubated with primary antibody to human p75 NGF receptor diluted 1:500 in TBS buffer for 30 min at room temperature. Application of the second and third layers was as stated previously but a different substrate, diaminobenzidine, was used to visualize the peroxidase reaction end-product. The slides were washed in running water for 5 min and the sections counter-stained with haematoxylin before mounting in DPX.

MEASUREMENTS OF COLLAGEN FIBRIL DIAMETER

These were made from conventional thin transverse sections of the tibial nerve which were contrasted with uranyl acetate and lead citrate. Video frames were acquired by a low light level video camera (SIT 66X, Dage-Miti Inc., Michigan City, Indiana, USA) mounted on a side port of the electron microscope. Each image was obtained by integrating 64 video frames. Images were recorded at $\times 50000$ magnification and measurements of fibril diameter made on TIFF format images using the IBAS image analysis system (Imaging Associates; Thame, Oxford). A binary image representing collagen fibrils was segmented from an enhanced edge delineated 8 bit TIFF image. Touching fibrils were automatically separated and objects not conforming to a predetermined size and shape criterion were automatically rejected. Fibril size based on the diameter of an equivalent circle was derived from measurements of fibril area. The magnification factor was determined from the image of a ruled carbon grid with 2160 lines per mm (Agar Scientific, UK).

On the nonoperated side, measurements were made of the endoneurial collagen fibril diameter. In the nonreinnervated distal stumps on the operated side, in 2 rabbits in the shorter survival time group, measurements were made of collagen fibril diameter external to the basal lamina that surrounded the larger Büngner bands, internal to the original inner endoneurial sheath. The site of this sheath was identifiable as a circumferential zone that contained a sparse population of irregularly oriented collagen fibrils (see Thomas, 1964). Because of the markedly altered intrafascicular architecture in the 2 rabbits with a prolonged survival time, measurements were obtained of collagen within domains enclosed by encircling fibroblasts (Figs 5, 6), with observations on those fibrils situated peripherally, in an intermediate position and centrally.

RESULTS

In preliminary observations on transverse sections examined both by NF immunolabelling and electron

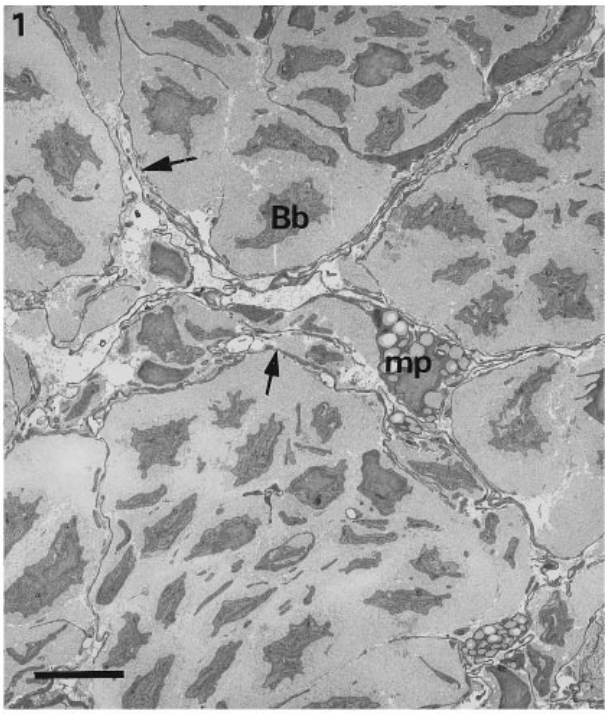

Fig. 1. Low magnification electron micrograph showing endoneurial compartmentation by fibroblast processes (arrows) in rabbit tibial nerve 6 mo after transection. Bb, Büngner band; mp, macrophage. Bar, 5 μm.

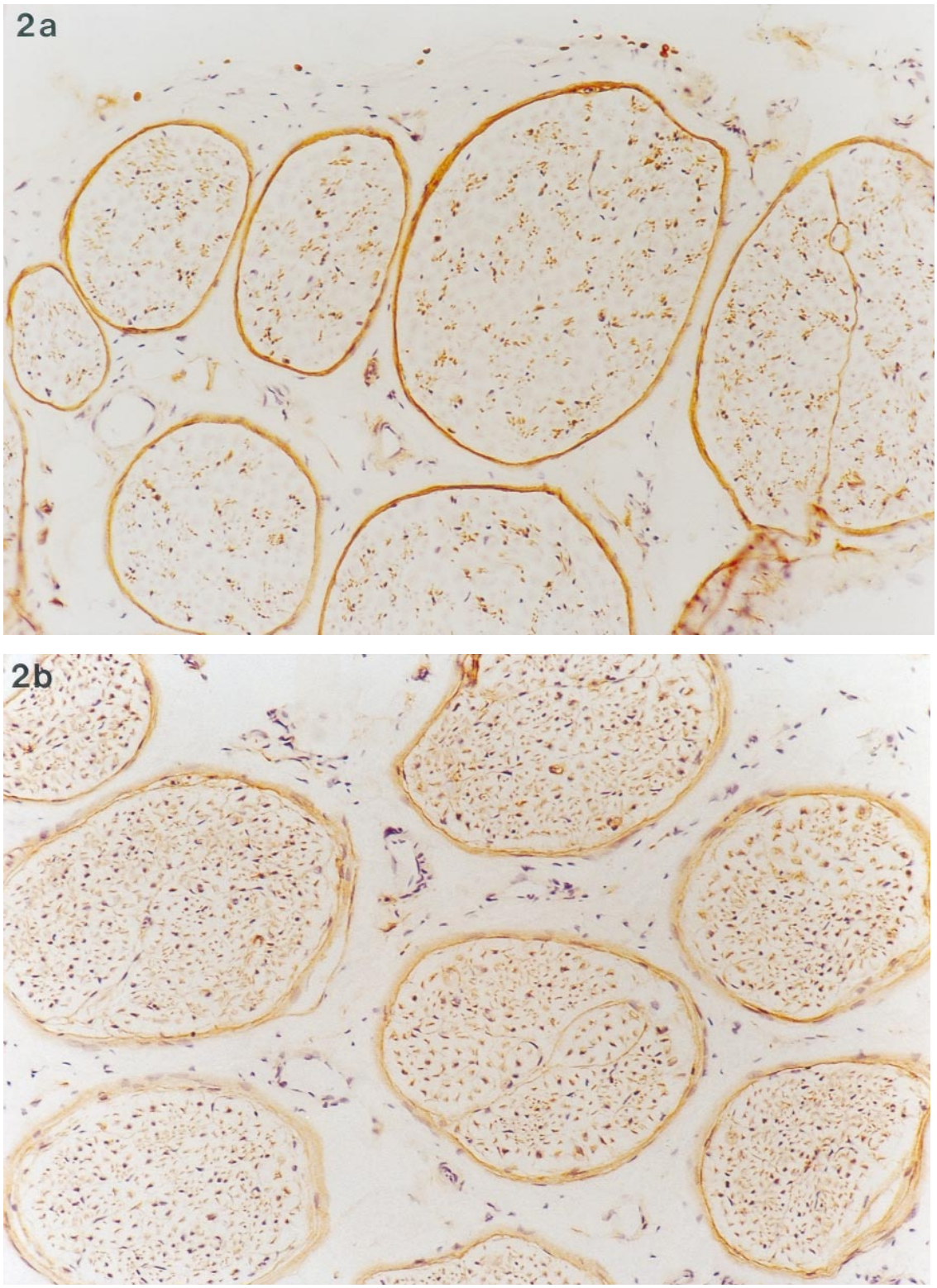

Fig. 2. Tibial nerve immunolabelled for low affinity NGFR (brown). (*a*) Unsectioned control nerve showing positive labelling for NGFR in the perineurium and on Remak fibres. (*b*) Tibial nerve 6 mo after transection showing positive labelling for NGFR on Büngner bands, endoneurial fibroblast processes and perineurium. Magnification ×400.

microscopy, any nerves that showed evidence of reinnervation were discarded. Occasional fascicles in such nerves contained a sparse population of unmyelinated axons, the origin of which was uncertain. It is possible that they had extended from the surrounding connective tissue via the vasculature.

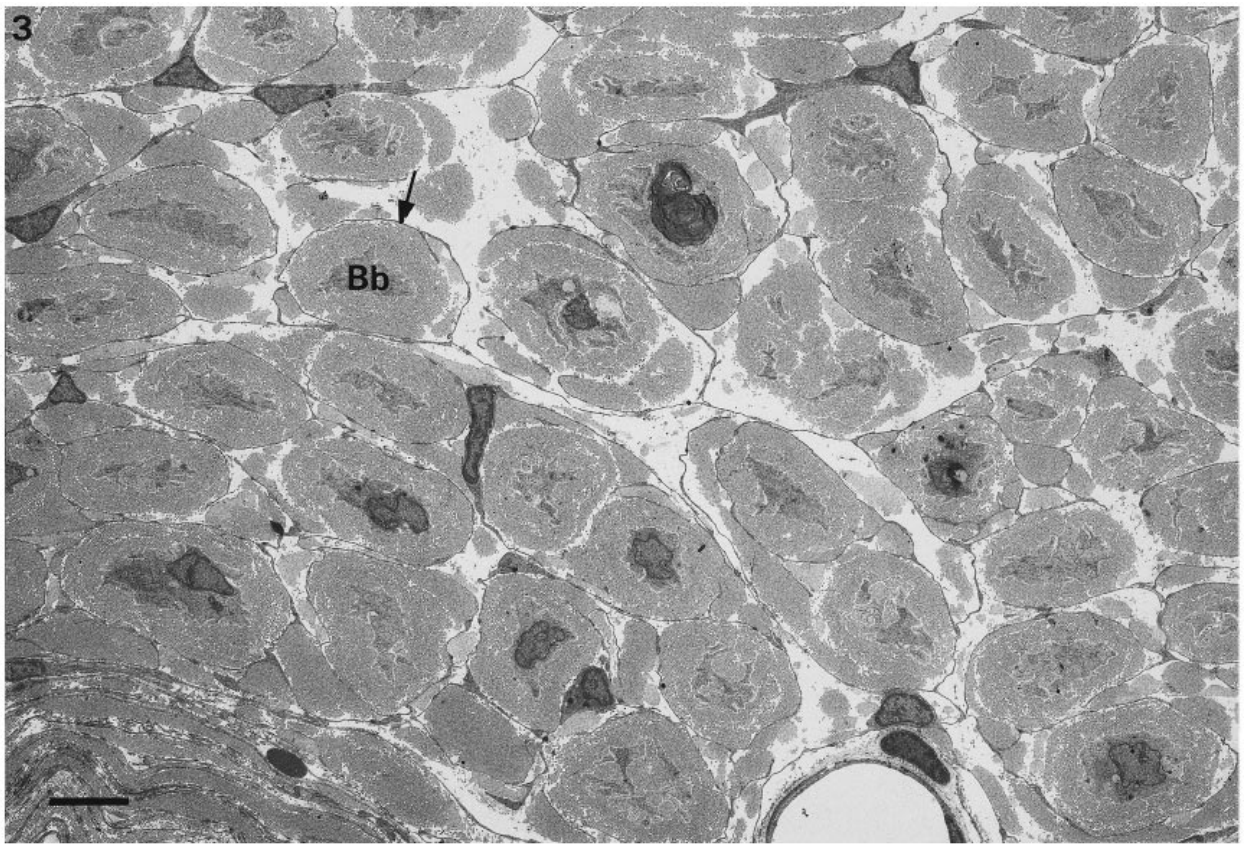

Fig. 3. Low magnification electron micrograph of tibial nerve 26 mo after transection showing multiple approximately circular domains of dense collagen containing a central Büngner band (Bb) encircled by elongated fibroblast processes (arrow). Bar, 5 μm.

Qualitative observations

Early survival periods (*1–6 mo*)

Following shorter periods of nonreinnervation, observations on the distal stump confirmed previous observations that the Schwann cells persisted within the collapsed basal laminal tubes of former nerve fibres as Büngner bands. Around the basal laminae of larger Büngner bands, a narrow zone of fine collagen fibrils was detectable within the original endoneurial sheaths, although this was not always clearly evident. External to the collagen of the original endoneurial sheaths were fibroblasts that gave rise to encircling processes that partially enclosed the Büngner bands (Fig. 1). Such fibroblasts occasionally showed short lengths of basal laminal material on their surfaces. In addition, although their processes were sometimes linked by tight junctions, they did not exhibit perineurial features such as a continuous basal laminal ensheathment or multiple pinocytotic vesicles.

Immunocytochemical staining for low affinity p75 NGFR showed that in unsectioned nerve this was expressed by the Schwann cells of Remak fibres and by the perineurium but not by Schwann cells associated with myelinated axons or by endoneurial fibroblasts (Fig. 2*a*). NGFR was expressed by the Büngner bands of the transected tibial nerves and was still evident at 6 mo after operation (Fig 2*b*), the latest stage that was examined. The perineurium continued to exhibit NGFR expression and expression was also shown by the fibroblast processes producing endoneurial compartmentation (Fig. 2*b*).

Intermediate survival period (*9–12 mo*)

In the nonreinnervated nerves from the rabbits allowed to survive for 9–12 mo, the general architecture of the nerve was similar to that at 6 mo but the Schwann cells of the Büngner bands were more atrophic. Compartmentation by fibroblasts was more complete, their elongated attenuated processes often making contact with each other. The appearances closely resembled those described by Salonen et al. (1987*b*).

Prolonged survival periods (*26 mo*)

At this stage, the fascicles were still surrounded by an intact lamellated perineurium that enclosed shrunken fascicles. Low magnification electron micrographs of transverse sections (Fig. 3) showed multiple domains

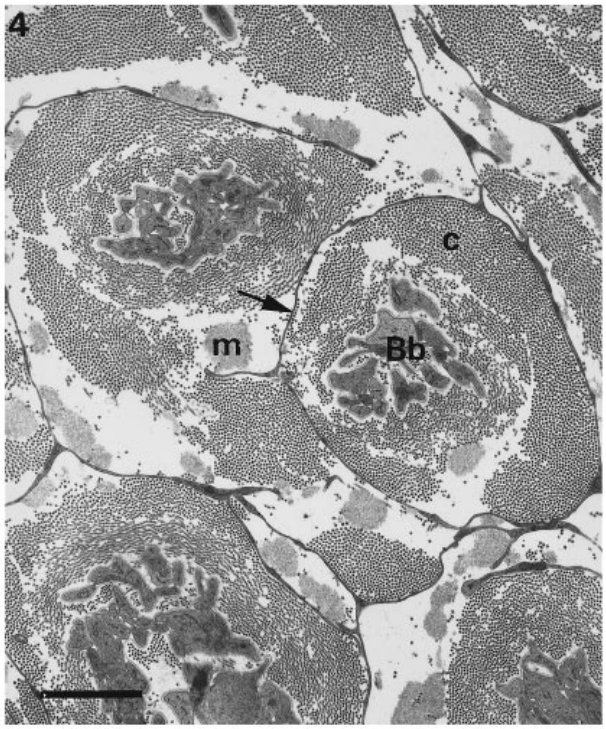

Fig. 4. Electron micrograph of collagen domains containing centrally located Büngner bands (Bb) encircled by attenuated fibroblast processes (arrow). The Schwann cells of the Büngner bands have become separated by collections of collagen fibrils and are no longer enclosed by their original basal lamina. m, microfibrils. Bar, 2 μm.

of dense collagen fibrils demarcated by cells with elongated attenuated processes. These sometimes had the appearances of fibroblasts (Figs 5, 6) and at others those of perineurial cells or mixed features (Figs 6, 7). Those with the features of perineurial cells possessed basal lamina both on their external and internal aspects or predominantly on their external face (Fig. 7). Their processes, which contained multiple pinocytotic vesicles, were linked by tight junctions. Those of fibroblastic appearance possessed occasional short lengths of basal lamina seen either in relation to the surface membrane of their cell bodies or their encircling processes. Longitudinal sections showed that a perineurial ensheathment occasionally formed a continuous tube enclosing one of these collagen domains (Fig. 8).

The collagen domains sometimes contained atrophic Büngner bands composed of isolated or small groups of Schwann cell processes surrounded by a basal lamina (Figs 4–6). In others, remnants of basal lamina were present without associated Schwann cells and, in some domains, neither Schwann cells nor persistent basal laminae were present (Fig. 7). Multiple groups of microfibrils with a longitudinal orientation were present in the endoneurium (Figs 4–6).

Quantitative observations

Measurements of collagen fibril diameter were made for the general endoneurial collagen in unsectioned tibial nerves in 3 rabbits. This was 56.1 ± 6.9 (median $\pm$ S.D.) nm. In 2 rabbits, with survival times of 5 and 6 mo, the mean diameter of the collagen fibrils immediately external to the basal lamina of the Büngner bands was 36 ± 6.0 (median $\pm$ S.D.) nm. In the 2 rabbits that had been allowed to survive for 26 mo after nerve transection, the diameter of the general endoneurial collagen was similar at 58.8 ± 7.1 nm and 51.3 ± 6.8 nm. For the fibrils within the circular domains (Figs 5, 6) the values for the outer, intermediate and inner regions were 53.7 ± 5.1, 51.3 ± 5.3 and 45.9 ± 4.5 nm, and 48.1 ± 6.1, 46.3 ± 4.8 and 42.8 ± 4.2 nm, respectively. For both animals, therefore, the collagen fibril diameter in the domains was less than for the general endoneurial collagen and smaller centrally than peripherally; the centrally located fibrils were larger than the fine collagen fibrils that had formed around the Büngner bands during wallerian degeneration and which they presumably represented.

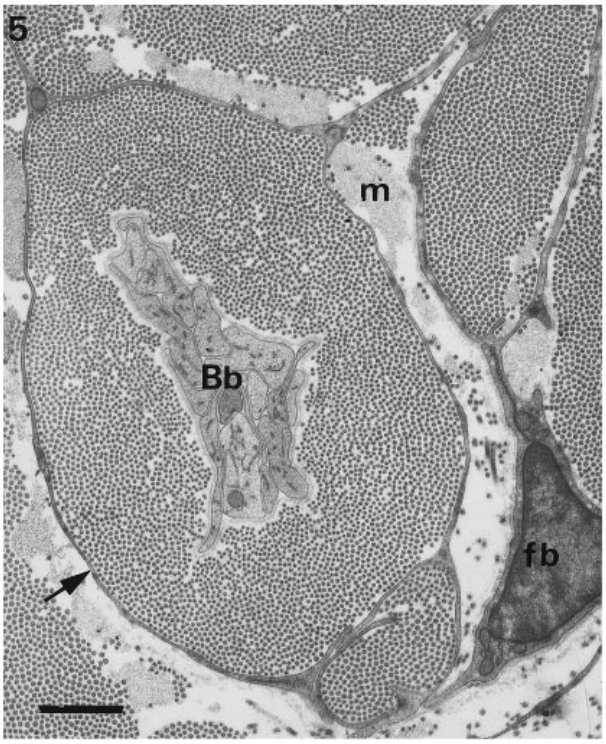

Fig. 5. Electron micrograph of a collagen domain containing a central Büngner band (Bb). The domain is encircled by thin fibroblast processes (arrow) which interdigitate in the upper right of the figure. These processes do not posses a basal laminal ensheathment whereas the fibroblast (fb) in the lower part of the figure shows definite perineurial transformation, possessing patchy basal lamina and displaying multiple pinocytotic vesicles in its processes. m, microfibrils. Bar, 1 μm.

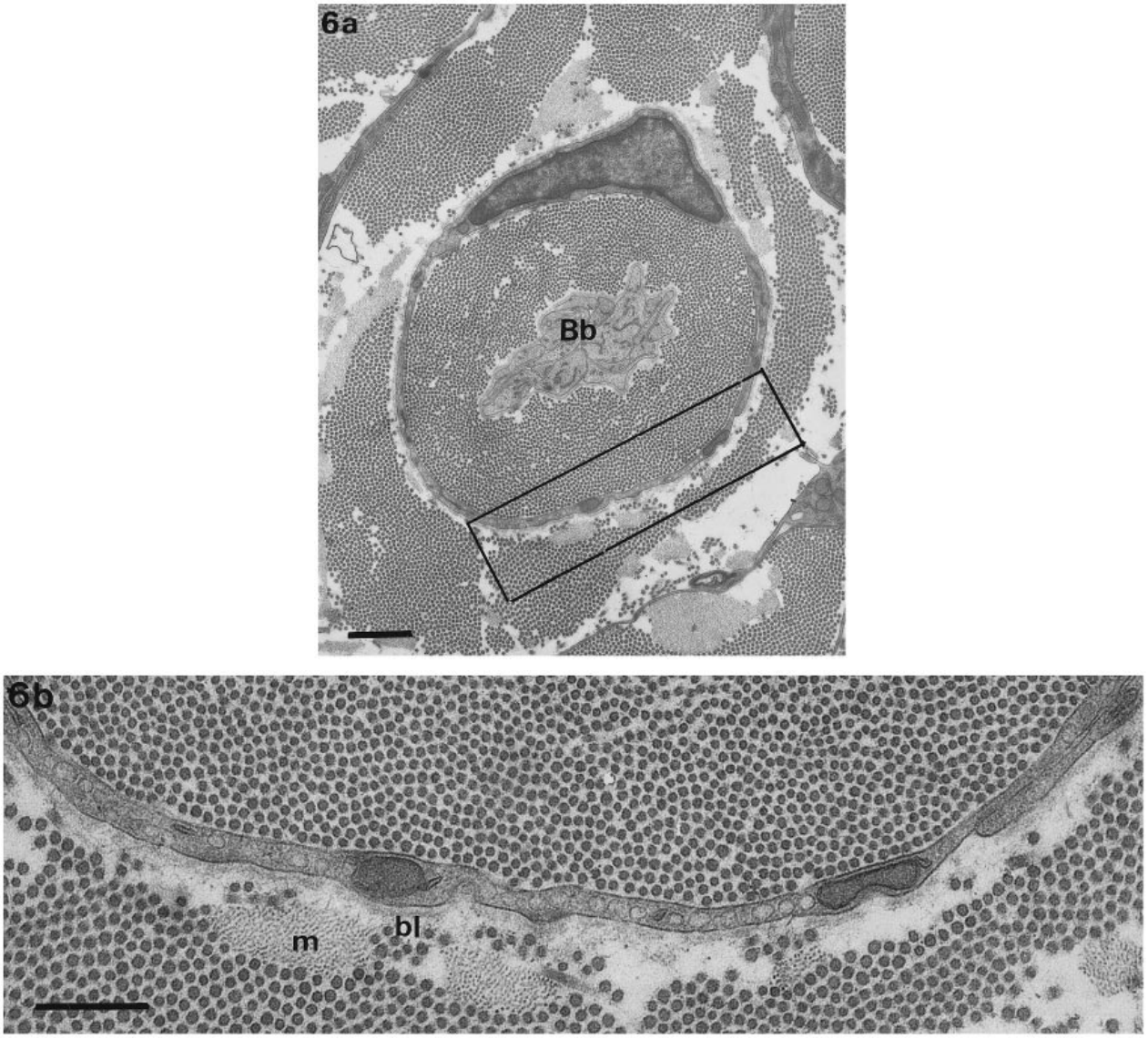

Fig. 6. (*a*) Electron micrograph of a circular collagen domain containing a central Büngner band (Bb) surrounded by a cell displaying perineurial transformation. Bar, 1 μm. (*b*) Higher magnification of part of the circular process of the cell shown in *a*. Its external aspect is partially coated by basal lamina (bl) and the process contains multiple pinocytotic vesicles. m, microfibrils. Bar, 0.5 μm.

DISCUSSION

These observations confirm those of Weinberg & Spencer (1978) that Schwann cells deprived of axonal contact atrophy and progressively disappear, although in the present experimental model some were still present even at 26 mo after nerve transection without reinnervation. The control mechanisms for Schwann cell survival have recently been analysed by Jessen & Mirsky (1997*a*). Schwann cells are derived from precursor cells that, in turn, originate from the neural crest. In medium/dense cell cultures, it was found that Schwann cell precursors die by apoptosis in the absence of neurites, whereas Schwann cells survive under similar conditions. It has been established that, in vitro, beta regulins prevent this precursor cell death. They probably act as an axon-glia survival and differentiation signal during Schwann cell differentiation in vivo. Schwann cell survival in these cell culture experiments is probably due to autocrine growth factor loops which are lacking in the precursor cells. The survival factor is unlikely to be a neuregulin. Schwann cell precursors thus rely on axon-derived neuregulins for the prevention of apoptosis, whereas Schwann cells have a dual support system involving separate autocrine and axon-derived signals.

Transforming growth factor beta (TGF_{β}) induces Schwann cell apoptosis in a dose-dependent manner. This can be prevented by β-neuregulins but not by

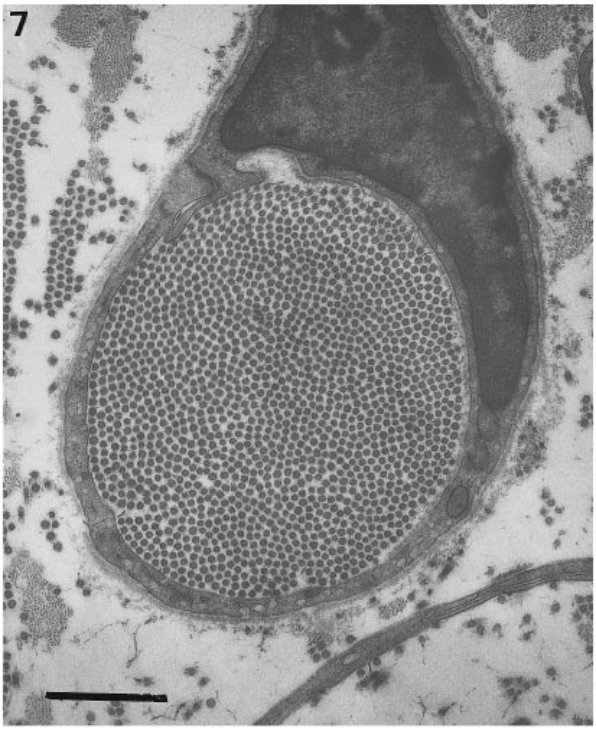

Fig. 7. Electron micrograph of a collagen domain not possessing a central Büngner band and surrounded by a cell with perineurium-like features. Bar, 1 μm.

conditioned medium containing the autocrine Schwann cell survival factor (Jessen & Mirsky, 1997*b*). Schwann cell death after axotomy might therefore result from TGF_{β} derived from macrophages or Schwann cells superimposed on a loss of the axonal support system. The present results indicate that loss of Schwann cells isolated from axonal contact is a very slow process in adult nerve. There is no information at present as to whether or not the Schwann cells that do not survive are eliminated by apoptosis.

Compartmentation of the endoneurium by fibroblasts rapidly becomes evident both in the central and peripheral stumps adjacent to the site of transection after neurotomy (Morris et al. 1972). This change subsequently takes place throughout the nerve distal to the transection (Röyttä et al. 1987) although whether this occurs in a sequential manner in a proximodistal direction or whether it takes place simultaneously throughout the nerve coincident with the development of the bands of Büngner is not known.

The functional significance of this endoneurial compartmentation is obscure, but it is likely to be related to the modifications that occur in the extracellular matrix (ECM) of the intrafascicular compartment during wallerian degeneration and the further changes that develop if reinnervation does not take place. The amount of collagen increases substantially in nerve distal to transection (Abercrombie & Johnson, 1946). The relative contribution of fibroblasts and Schwann cells to this is uncertain. Schwann cells in culture, in the presence of axons, are known to be capable of producing types I, III, IV and V collagen (Bunge et al. 1980). The collagen fibrils are of small diameter ($\sim$ 18 nm). In the earlier stages after nerve transection, the fibroblasts producing the endoneurial compartmentation display minor morphological changes of perineurial type such as short lengths of basal lamina. It is of interest that they also start to express low affinity p75 NGFR, as is shown by normal perineurial cells but not by normal endoneurial fibroblasts. The functional significance of this is again uncertain.

The most striking feature of the appearances in the distal stump seen after prolonged denervation in the present study was the presence of approximately circular domains of dense collagen when seen in transverse section, demarcated by encircling fibroblasts or cells of perineurial appearance. Similar appearances have occasionally been seen in chronic peripheral neuropathy in man (A. Hahn, personal communication and personal observations). Perineurial differentiation in the endoneurium in this animal model was unexpected. After nerve injury, for example in the gap between the 2 cut ends of a transected nerve, groups of regenerating axons and associated Schwann cells become surrounded by cells with the morphological features of fibroblasts which then acquire perineurial features (Thomas & Jones, 1967). The gap comes to consist of multiple 'minifascicles' surrounded by perineurium. The perineurium normally provides a diffusion barrier around nerve fascicles and, in association with the blood-nerve barrier, is considered to contribute to the regulation of the endoneurial microenvironment (Thomas & Olsson, 1984). In the gap between transected nerve ends the perineurial ensheathment around the minifascicles presumably isolates them from the general connective tissue space. The development of a perineurial ensheathment around the surviving Büngner bands and related collagen domains could be secondary to exposure of the intrafascicular connective tissue space at the site of transection or to the breakdown of the perineurial diffusion barrier that occurs during wallerian degeneration (Weerasuriya et al. 1980).

The bimodal distribution of collagen fibril size observed by Vuorinen et al. (1995) in the collagen domains that remained after loss of the Schwann cells of the bands of Büngner in nonreinnervated distal stumps at 12–16 mo after transection was not found in

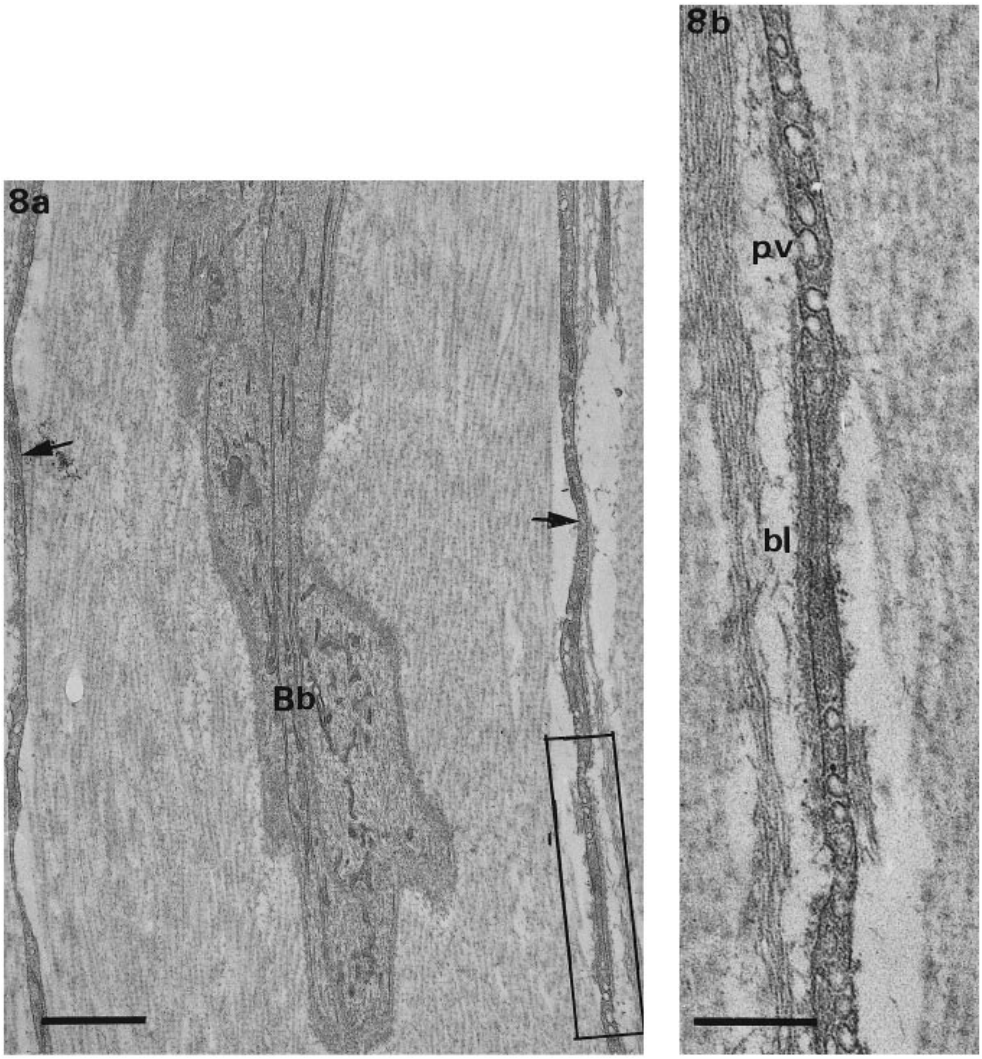

Fig. 8. (*a*) Electron micrograph of a longitudinal section through a collagen domain containing a central Büngner band (Bb) and bounded on either side by a perineurium-like ensheathment (arrows). Bar, 1 μm. (*b*) Detail of part of the perineurium-like ensheathment with patchy basal lamina (bl) mainly on its external aspect and showing multiple pinocytotic vesicles (pv). Bar, 0.5 μm.

our observations at 26 mo. In our study, the diameter of the outer collagen fibrils in the domains was equivalent to that of endoneurial collagen at $\sim$ 50 nm. The inner fibrils, although smaller at approximately 43–46 nm, were larger than the 25–30 nm found by Vuorinen et al. It therefore is likely that the fine collagen fibrils that are formed around the degenerating nerve fibres increase in size with time.

As stated in the Introduction, Vuorinen et al. (1995) found that when a viable proximal stump was linked to a transected distal stump that had remained unreinnervated for 12–16 mo, the regenerating axons grew down the zone of fine collagen fibrils in the centre of the collagen domains. Whether these central regions remain capable of accepting regenerating axons after 26 mo of nonreinnervation, and whether the surviving Schwann cells are capable of proliferation, is unknown. It is also uncertain without undertaking serial sections whether the collagen domains without a central Büngner band possess Schwann cells at a different level. It is conceivable that with Schwann cell loss the Büngner bands become discontinuous but that molecules that support axonal elongation remain attached to the ECM. Immunohistochemical studies to detect the presence of laminin or fibronectin (Bignami et al. 1984; Salonen et al. 1985, 1987*b*) in these regions of late nonreinnervated distal stumps would therefore be of interest. It is likely that expression of NGF and NGFR by the Schwann cells of the Büngner bands is important in axonal regeneration (see Introduction). In the present study, the Schwann cells continued to express low affinity NGFR at 6 mo after nerve transection. Unfortunately material was not available for us to examine this in the animals in which the nerves had remained unreinnervated for 26 mo.

ACKNOWLEDGEMENTS

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REFERENCES

Abercrombie M, Johnson ML (1946) Collagen content of rabbit nerve during Wallerian degeneration. *Journal of Neurology, Neurosurgery and Psychiatry* **9**, 113–118.

Baur PS, Stacey TR (1977) The use of PIPES buffer in the fixation of mammallian and marine tissue for electron microscopy. *Journal of Microscopy* **109**, 315–327.

Bignami A, Chi NH, Dahl D (1984) Laminin in rat sciatic nerve undergoing Wallerian degeneration. Immunofluorescence study with laminin and neurofilament antisera. *Journal of Neuropathology and Experimental Neurology* **43**, 94 -103.

Bunge MB, Williams AK, Wood PM, Jouni U, Jeffrey JJ (1980) Comparison of nerve cell and nerve cell plus Schwann cell cultures, with particular emphasis on basal lamina and collagen formation. *Journal of Cell Biology* **84**, 184–202.

Friede RL, Bischhausen R (1978) The organization of the endoneurial collagen in peripheral nerves as revealed with the scanning electron microscope. *Journal of the Neurological Sciences* **38**, 83–88.

Giannini C, Dyck PJ (1990) The fate of Schwann cell basement membrane in permanently transected nerves. *Journal of Neuropathology and Experimental Neurology* **49**, 550–563.

Jessen KR, Mirsky R (1997*a*) Embryonic Schwann cell development: the biology of the Schwann cell precursors and early Schwann cells. *Journal of Anatomy* **191**, 501–506.

Jessen KR, Mirsky R (1997*b*) Growth factor regulation of Schwann cell death and survival. Abstracts, Cambridge Meeting of the Peripheral Nerve Society, July 1997, p.202. Rochester, MN: Peripheral Nerve Society.

Laidlaw GF (1930). Silver staining of the endoneurial fibers of cerebrospinal nerves. *American Journal of Pathology* **6**, 435–444.

Morris JH, Hudson AR, Weddell G (1972) A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. IV. Changes in fascicular microtopography, perineurium and endoneurial fibroblasts. *Zeitschrift für Zellforschung* **124**, 165–203.

Nathaniel EJH, Pease DC (1963) Degenerative changes in rat dorsal roots during Wallerian degeneration. *Journal of Ultrastructure Research* **9**, 511–532.

Plenk H (1934) Die Schwannsche Scheide der markhaltigen Nervenfasern. *Zeitschrift für mikroskopische Anatomie Forschung* **36**, 191–214.

Röyttä M, Salonen V, Peltonen J (1987) Reversible endoneurial changes following nerve injury. *Acta Neuropathologica* (*Berlin*) **73**, 323–329.

Salonen V, Lehto M, Vaheri A, Aro H, Peltonen J (1985) Endoneurial fibrosis following nerve transection. An immunohistological study of collagen types and fibronectin in the rat. *Acta Neuropathologica* (*Berlin*) **67**, 315–321.

Salonen V, Röyttä M, Peltonen J (1987*a*) The effects of nerve transection on the endoneurial collagen fibril sheaths. *Acta Neuropathologica* (*Berlin*) **74**, 13–21.

Salonen V, Peltonen J, Röyttä M, Virtanen I (1987*b*) Laminin in traumatized peripheral nerve; basement membrane changes during degeneration and regeneration. *Journal of Neurocytology* **16**, 713–720.

Salonen V, Aho H, Röyttä M, Peltonen J (1988) Quantitation of Schwann cells and endoneurial fibroblast-like cells after experimental nerve trauma. *Acta Neuropathologica* (*Berlin*) **75**, 331–336.

Scheidt P, Friede RL (1987) Myelin phagocytosis in Wallerian degeneration: properties of Millipore diffusion chambers and immunocytochemical identification of cell populations. *Acta Neuropathologica* (*Berlin*) **75**, 77–84.

Sievers J (1971) Basic two-dye stains for epoxy-embedded 0.3–1 μ sections. *Stain Technology* **46**, 195–199.

Taniuchi M, Clark HB, Johnson EM (1986) Induction of nerve growth factor receptors in Schwann cells after axotomy. *Proceedings of the National Academy of Science of the USA* **83**, 4094–4098.

Taniuchi M, Clark HB, Schweitzer HB, Johnson EM (1988) Expression of nerve growth factor receptors by Schwann cells of axotomized peripheral nerves: ultrastructural location, suppression by axon contact, and binding properties. *Journal of Neuroscience* **8**, 664–681.

Thomas PK (1963) The connective tissues of peripheral nerve: an electron microscope study. *Journal of Anatomy* **97**, 35–44.

Thomas PK (1964) Changes in the endoneurial sheaths of peripheral nerve fibres during Wallerian degeneration. *Journal of Anatomy* **98**, 175–183.

Thomas PK, Jones DG (1967) The cellular response to nerve injury. 2. Regeneration of the perineurium after nerve section. *Journal of Anatomy* **101**, 45–55.

Thomas PK, Olsson T (1984) Microscopic anatomy and function of the connective tissue components of peripheral nerve. In *Peripheral Neuropathy*, 2nd edn. (ed. Dyck PJ, Thomas PK, Lambert EH, Bunge R), pp. 97–120. Philadelphia: WB Saunders.

Thomas PK, King RHM, Bradley JL, Abernethy DA (1996) Neural architechture in transected rabbit nerve after prolonged nonreinnervation. *Journal of Anatomy* **189**, 237.

Ushiki T, Ide C (1986) Three-dimensional architecture of the endoneurium with special reference to the collagen fibril arrangement in relation to nerve fibers. *Archivum Histologicum Japonicum* **491**, 553–563.

Vuorinen V, Siironen J, Röyttä M (1995) Axonal regeneration into chronically denervated distal stump. I. Electron microscope studies. *Acta Neuropathologica* (*Berlin*) **89**, 209–218.

Weerasuriya A, Rapoport SI, Taylor RE (1980) Perineurial permeability increases during Wallerian degeneration. *Brain Research* **192**, 581–585.

Weinberg HJ, Spencer PS (1978) The fate of Schwann cells isolated from axonal contact. *Journal of Neurocytology* **7**, 555–569.

Young JZ (1942) The functional repair of nervous tissue. *Physiological Reviews* **22**, 318–374.