J. Anat. (1970), **106**, 3, pp. 463–470
With 5 figures
Printed in Great Britain

The cellular response to nerve injury

3. The effect of repeated crush injuries

P. K. THOMAS

Institute of Neurology, Queen Square, and Royal Free Hospital, London, W.C. 1

(*Received* 28 *May* 1969)

INTRODUCTION

During Wallerian degeneration, the cellular population of the nerve distal to the site of injury shows a substantial increase (Abercrombie & Johnson, 1946), mainly or perhaps entirely as a result of local cellular proliferation. This increase predominantly affects the Schwann cells and is greatest in nerves containing a high proportion of large myelinated fibres (Thomas, 1948) and least in those containing only unmyelinated axons (Abercrombie, Evans & Murray, 1959). The cellular population subsequently falls (Abercrombie & Johnson, 1946; Thomas, 1948) but, even if regeneration occurs, does not return to its normal level (Abercrombie, Johnson & Thomas, 1949). Although the increase in the cell population is quantitatively related to the proportion of the nerve occupied by nerve fibres, it is uncertain whether the increase is dependent upon a chemical stimulus provided by the degenerating axons (Abercrombie & Johnson, 1946) or on the size of the vacant space created by fibre degeneration (Joseph, 1950). A further sustained increase follows Wallerian degeneration if produced for a second time (Abercrombie & Santler, 1957). These authors were able to show that the number of cells added is again related to the proportion of the nerve occupied by nerve fibres and not to the number of cells present initially.

Schwann cell proliferation is a feature of many chronic neuropathies in man. Two distinct patterns are identifiable (Thomas, 1969*b*), although both may at times be seen in the same patient. In one, transverse sections through affected nerves show a concentric proliferation of Schwann cells around myelinated axons. There is now little doubt that this is caused by repeated segmental demyelination and remyelination (Thomas & Lascelles, 1967; Weller, 1967; Ballin & Thomas, 1968; Zacks, Lipshutz & Elliott, 1968), although it had been suggested that it may result from repeated axonal degeneration with partial regeneration (Webster, Schröder, Asbury & Adams, 1967; Nichols, Dyck & Miller, 1968). In the second pattern, transverse sections reveal large irregular clusters of Schwann cells, associated with which are multiple myelinated and unmyelinated axons.

This investigation was designed to assess the pattern of Schwann cell proliferation resulting from repeated Wallerian degeneration with intervening regeneration. Preliminary accounts have already been published (Thomas, 1968, 1969*a*).

MATERIAL AND METHODS

The observations were made on the peroneal nerve of adult rabbits. The nerve was exposed in the lower thigh on one side under pentobarbitone sodium (Nembutal) and ether anaesthesia and crushed for 10 s with smooth-tipped watchmakers' forceps. Regeneration was allowed to take place for 4–6 weeks. The nerve was then recrushed by the same procedure and the sequence repeated up to a maximum of nine times. Portions of nerve were removed at biopsy, employing the same anaesthesia, from the peroneal nerve in the upper part of the lower leg at a site 2 cm below the level of the crushes. This was performed either at 10 or 15 months after the final crush to allow time for adequate axonal regeneration and maturation, and to enable persistent rather than temporary changes in nuclear population to be assessed. The operations were carried out in the lower part of the thigh because of the ease of surgical access in that situation. Two animals were subjected to a single crush, two to 6 repeated crushes and two to 9 crushes. Unfortunately, the material from one of the nerves crushed once only was not suitable for quantitative assessment.

The pieces of nerve removed were divided into two parts. One was fixed in Heidenhain's Susa solution and transverse and longitudinal paraffin sections cut at 5 μm stained with haematoxylin and eosin, haematoxylin-van Gieson, cresyl violet, Masson's trichrome and by the Holmes silver impregnation method. The second portion was fixed in Flemming's solution and stained by the modified Weigert technique described by Gutmann & Sanders (1943) for the examination of myelin sheaths.

Counts of the total nuclear population were made on photographs of 5 μm transverse sections, stained with cresyl violet, at a magnification of $\times 500$. They were corrected for nuclear length (Abercrombie, 1946), estimated by measurements on approximately 100 nuclei in longitudinal sections of the same portions of each nerve. Measurements of fascicular area were also made, estimated by planimetry. The results were compared with counts obtained from two unoperated nerves.

RESULTS

The results obtained for the estimates of nuclear numbers and density, and for fascicular area, are shown in Table 1. It can be seen that a progressive increase in the total number of nuclei present and in nuclear density followed the repeated crush lesions. After 9 crushes, there was a nearly eightfold increase in nuclear numbers, but only a fourfold increase in nuclear density. This is explained by the fact that the fascicular area nearly doubled. Although the number of observations is limited, there was an approximately linear relationship between the changes in the nuclear count and the number of crushes (Fig. 1).

A transverse section of an unoperated nerve is shown in Fig. 2. The appearances following 9 repeated crushes are shown in Figs. 3 and 4. The cellular proliferation is seen to be mainly of Schwann cells, although an increase in the numbers of endoneurial fibroblasts also seems likely. Clusters of Schwann cells are evident in this nerve, the diameter of some of them being as great as 40–50 μm. Longitudinal sections reveal that these are columns of cells. Similar but less marked changes were seen after 6 repeated crushes. Myelin stains (Fig. 5) demonstrated that the Schwann

cell columns are associated with multiple axons of varying diameters, with no regular pattern of arrangement within the columns.

There was a tendency for the endoneurial spaces to be enlarged after the multiple crushes (Figs. 3, 4), and this probably contributed to the increase in fascicular area. The spaces contained finely fibrillar material that was stained yellow by van Gieson's stain. It did not exhibit metachromasia on cresyl violet staining.

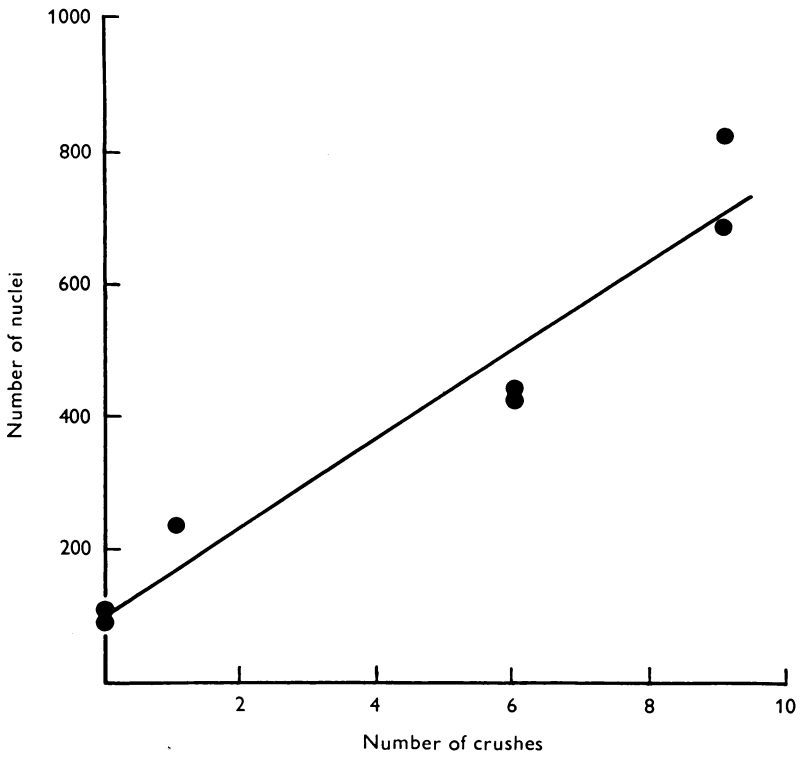

Fig. 1. Relationship between nuclear population and number of crushes.

DISCUSSION

The results obtained in this study show that the concentric type of Schwann cell proliferation around myelinated axons is not produced by repeated Wallerian degeneration and regeneration. Instead, groups of myelinated and unmyelinated axons are found aggregated together in columns made up of multiple Schwann cells. The appearances resemble those found in the second type of Schwann cell proliferation seen in chronic peripheral neuropathies in man (Thomas, 1969*b*). It is therefore possible that the latter may arise by repeated axonal degeneration and regeneration.

The precise way in which the appearances observed in the present study develop is not yet established. Thomas (1968) suggested that with each consecutive crush injury, Wallerian degeneration leads to Schwann cell proliferation, and this is con-

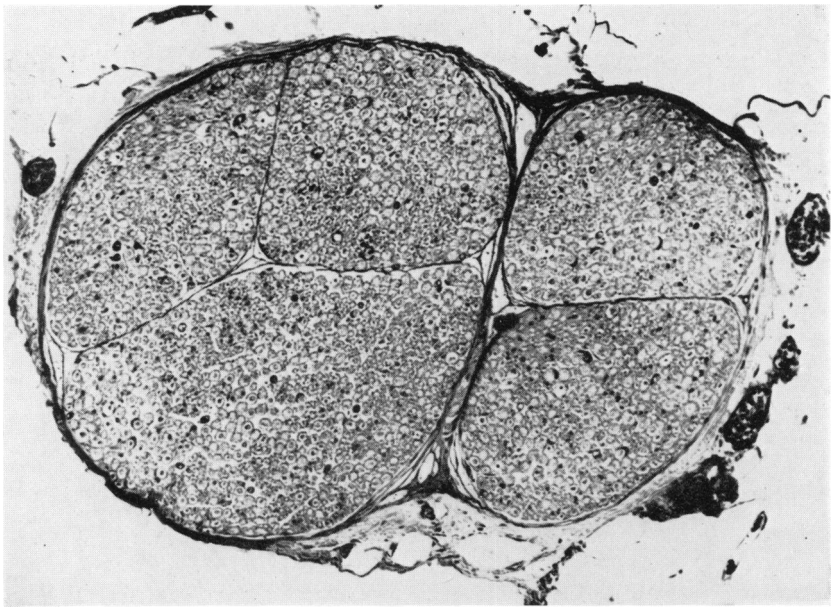

Fig. 2. Transverse section through normal peroneal nerve.
Haematoxylin and eosin. × 100.

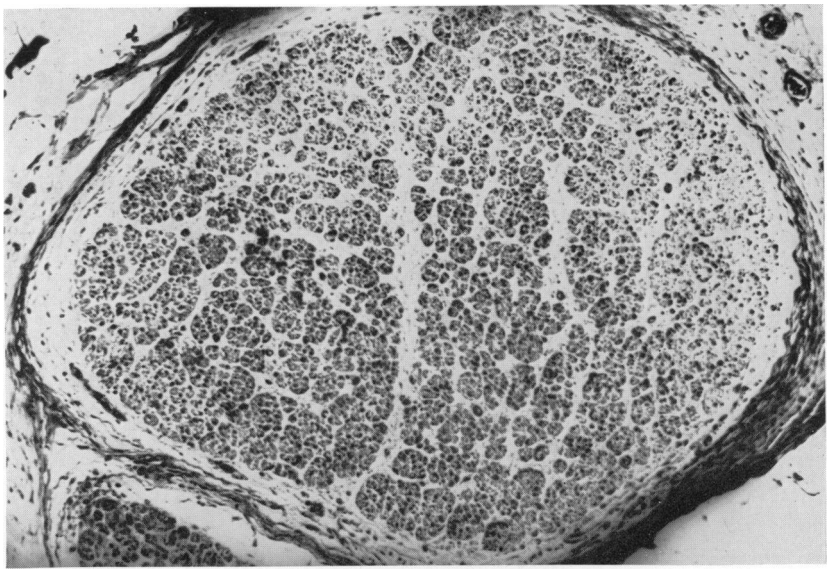

Fig. 3. Portion of transverse section through peroneal nerve following nine repeated crushes, with biopsy at 10 months after final crush. The section shows groups of Schwann cells separated by expanded endoneurial spaces. Masson trichrome. × 110.

firmed by the quantitative estimations of nuclear population reported here. It is known that following a single crush injury, multiple axon sprouts grow down the individual Büngner bands from each axon, but normally only a single axon becomes myelinated (Young, 1949). With repeated crush injuries multiple axon sprouts appear to become myelinated. The Schwann cells, once they are associated with axons, will separate from one another and collagen form between them (Thomas, 1964), but they remain closely grouped together. Subdivision of the Büngner bands presumably occurs following each repeated crush injury, giving rise to the large groups of Schwann cells and myelinated axons that are observed.

Table 1. *Nuclear population and fascicular area of rabbit peroneal nerve following repeated crush injuries*

Number of crushes	Survival time (months)	Corrected nuclear count	Average nuclear length (μm)	Fascicular area (mm^2)	Nuclear density (nuclei per mm^3)
0	—	111	13·1	0·438	50 582
0	—	87	13·8	0·357	48 871
1	15	236	13·8	0·345	136 850
6	10	445	11·8	0·392	226 707
6	15	441	11·5	0·456	193 381
9	10	703	11·1	0·662	212 043
9	15	842	12·3	0·782	215 469

'Hyperneurotization' of Büngner bands with multiple myelinated axon sprouts has recently been described by Schröder (1968) in experimental isoniazid neuropathy. This neuropathy involves a distal degeneration ('dying-back') of the axon (Cavanagh, 1967) and recovery occurs by axonal regeneration. Evidently, multiple myelinated axon sprouts are also produced in this situation. Such appearances are in addition seen after nerve section and suture, where multiple sprouts from several axons grow down each Büngner band because of interlacing at the site of the suture. The region of the crushes was examined in the present study, and the appearances did not suggest any extensive intermingling of axons between endoneurial tubes.

The thickening of the nerve trunks that occurs in cases of hypertrophic neuropathy in man showing concentric Schwann cell proliferation may be due not only to the cellular proliferation, but to an increase in the extent of the endoneurial spaces. These become filled with a 'mucoid' material that stains metachromatically (Krücke, 1939). It has been suggested that this occurs as a result of alterations in vascular permeability (Thevenard, van Bogaert, Berdet & Rougerie, 1956), which is known to increase during Wallerian degeneration (Olsson, 1966; Mellick & Cavanagh, 1968). The nerves subjected to repeated crush injuries showed expanded endoneurial spaces, but these did not contain metachromatic material.

This study has not contributed to the question of the nature of the stimulus to Schwann cell multiplication during Wallerian degeneration. It is evident that the nuclear population continues to increase following repeated crushes over the range investigated, and, in view of the observations of Abercrombie & Santler (1957), it would be of interest to know how the magnitude of the increase correlated with the quantity of nerve fibres present at the time of each crush.

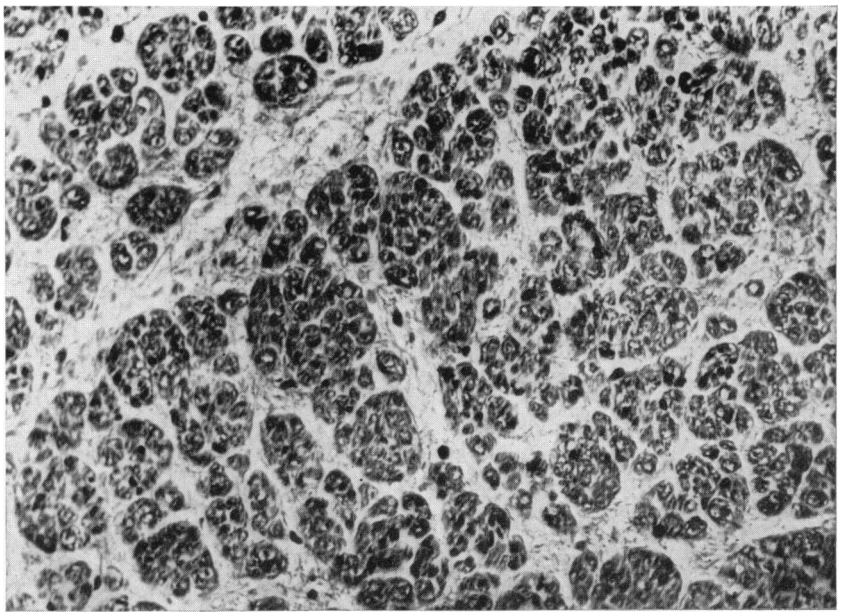

Fig. 4. Detail from Fig. 3, showing large groups of Schwann cells and expanded endoneurial spaces. Masson trichrome. × 370.

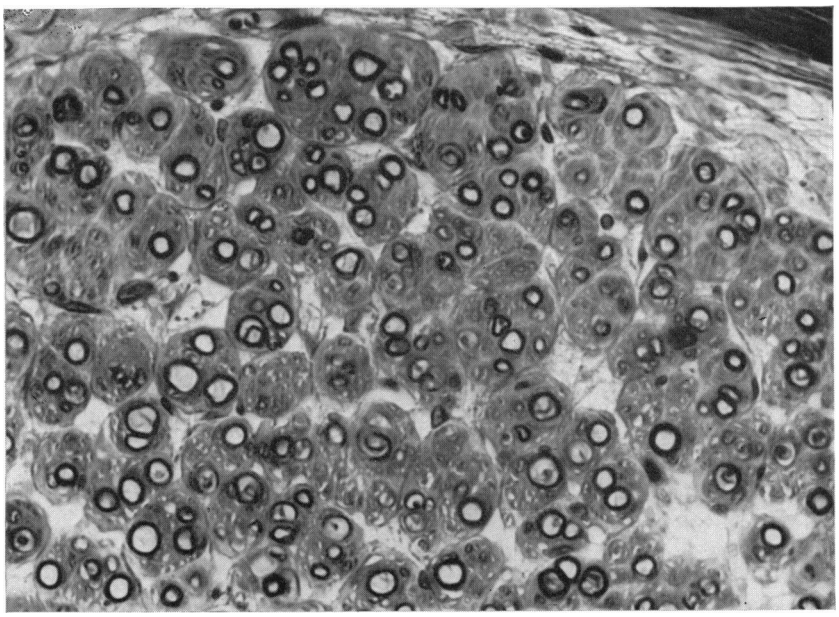

Fig. 5. Portion of transverse section through peroneal nerve following nine repeated crushes, biopsied at 10 months after final crush. The groups of Schwann cells seen in Fig. 4 are observed to be associated with multiple myelinated axons of varying diameter. Kultschitsky's haematoxylin-van Gieson. × 400.

SUMMARY

A quantitative assessment was made of nuclear population in the peroneal nerve of the rabbit following repeated localized crush injuries with intervening regeneration. This was performed up to a maximum of 9 times. The nerve distal to the site of the crushes showed a progressive increase in nuclear population. In the nerves subjected to multiple crushes, transverse sections showed large groups of Schwann cells associated with which were multiple myelinated axons. It is suggested that each of these groups is the product of a single Büngner band which has become progressively enlarged and subdivided by the repeated degeneration and regeneration of multiple axon sprouts.

I wish to thank Miss Ann Armstrong, Miss Theresa Tilley and Miss Jean Slatford for technical assistance, and Professor Michael Abercrombie, F.R.S., for helpful discussion. I am also indebted to Professor W. H. McMenemy for laboratory facilities at Maida Vale Hospital. This work was supported by a grant from the Muscular Dystrophy Group of Great Britain, which is gratefully acknowledged.

REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

ABERCROMBIE, M., EVANS, D. H. L. & MURRAY, J. G. (1959). Nuclear multiplication and cell migration in degenerating unmyelinated nerves. *J. Anat.* **93**, 9–14.

ABERCROMBIE, M. & JOHNSON, M. L. (1946). Quantitative histology of Wallerian degeneration. I. Nuclear population in rabbit sciatic nerve. *J. Anat.* **80**, 37–50.

ABERCROMBIE, M., JOHNSON, M. L. & THOMAS, G. A. (1949). The influence of nerve fibres on Schwann cell migration investigated in tissue culture. *Proc. Roy. Soc. Lond.* B **136**, 448–460.

ABERCROMBIE, M. & SANTLER, J. (1957). An analysis of growth in nuclear population during Wallerian degeneration. *J. cell. comp. Physiol.* **50**, 429–450.

BALLIN, R. H. M. & THOMAS, P. K. (1968). Hypertrophic changes in diabetic neuropathy. *Acta Neuropath.* **11**, 93–102.

CAVANAGH, J. B. (1967). On the pattern of change in peripheral nerves produced by isoniazid intoxication in rats. *J. Neurol. Neurosurg. Psychiat.* **30**, 26–33.

GUTMANN, E. & SANDERS, F. K. (1943). Recovery of fibre numbers and diameters in the regeneration of peripheral nerves. *J. Physiol. (Lond.)* **101**, 489–518.

JOSEPH, J. (1950). Further studies in changes of nuclear population in degenerating non-myelinated and finely myelinated nerves. *Acta anat.* **9**, 279–288.

KRÜCKE, W. (1939). Die mucoide Degeneration der peripheren Nerven. *Virchows Arch. path. Anat. Physiol.* **304**, 442–463.

MELLICK, R. S. & CAVANAGH, J. B. (1968). Changes in blood vessel permeability during degeneration and regeneration in peripheral nerves. *Brain* **91**, 141–160.

NICHOLS, P. C., DYCK, P. J. & MILLER, D. R. (1968). Experimental hypertrophic neuropathy: change in fascicular area and fiber spectrum after acute crush injury. *Proc. Staff Meet. Mayo Clin.* **43**, 297–305.

OLSSON, Y. (1966). Studies on vascular permeability in peripheral nerves. I. Distribution of circulating fluorescent serum albumin in normal, crushed and sectioned peripheral nerve. *Acta Neuropath.* **7**, 1–15.

SCHRÖDER, J. M. (1968). Die Hyperneurotisation Büngnerscher Bänder bei der experimentellen Isoniazid-Neuropathie: phasenkontrast und electronenmikroskopische Untersuchungen. *Virchows Arch. Zellpath., Abt.* B **1**, 131–156.

THEVENARD, A., VAN BOGAERT, L., BERDET, H. & ROUGERIE, J. (1956). Névrite hypertrophique progressive. *Rev. neurol.* **94**, 1–22.

THOMAS, G. A. (1948). Quantitative histology of Wallerian degeneration. II. Nuclear population in two nerves of different fibre spectrum. *J. Anat.* **82**, 135–145.

THOMAS, P. K. (1964). The deposition of collagen in relation to Schwann cell basement membrane during peripheral nerve regeneration. *J. Cell Biol.* **23**, 375–382.

THOMAS, P. K. (1968). The effect of repeated regenerative activity on the structure of peripheral nerve. In *Research in Muscular Dystrophy*, pp. 413–419. London: Pitman.

THOMAS, P. K. (1969*a*). The influence of repeated crush injuries on the nuclear population of peripheral nerve. *J. Physiol.* (*Lond.*), **201**, 63P.

THOMAS, P. K. (1969*b*). Schwann cell proliferation in chronic neuropathies. *J. Anat.* **105**, 212.

THOMAS, P. K. & LASCELLES, R. G. (1967). Hypertrophic neuropathy. *Q. Jl Med.* **36**, 223–238.

WEBSTER, H. DE F., SCHRÖDER, J. M., ASBURY, A. K. & ADAMS, R. D. (1967). The role of Schwann cells in the formation of 'onion bulbs' found in chronic neuropathies. *J. Neuropath. exp. Neurol.* **26**, 276–299.

WELLER, R. O. (1967). An electron microscope study of hypertrophic neuropathy of Dejerine and Sottas. *J. Neurol. Neurosurg. Psychiat.* **30**, 111–125.

YOUNG, J. Z. (1949). Factors influencing the regeneration of nerves. *Adv. Surg.* **1**, 165–220.

ZACKS, S. I., LIPSHUTZ, H. & ELLIOTT, F. (1968). Histochemical and electronmicroscopic observations on 'onion bulb' formations in a case of hypertrophic neuritis of 25 years duration with onset in childhood. *Acta Neuropath.* **11**, 157–256.