J. Anat. (1971), **110**, 2, pp. 191–202
With 6 figures
Printed in Great Britain

Quantitative anatomical measurements on single isolated fibres from the cat spinal cord

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(*Accepted* 26 *July* 1971)

INTRODUCTION

Conduction in single, central nerve fibres under normal and pathological conditions has been described in several recent reports (BeMent & Ranck, 1969; McDonald & Sears, 1970*b*). Although it is now established that central nerve fibres show regular interruptions in the myelin sheath which in many respects resemble the nodes of Ranvier in the peripheral nervous system (Allison & Feindel, 1949; Hess & Young, 1949, 1952; Bodian, 1951; Nakai, 1954; Pease, 1955; Metuzals, 1960; Peters, 1960*b*, 1966; Uzman & Villegas, 1960), the lack of precise information about the dimensions of central nerve fibres has hampered the interpretation of these physiological experiments.

Reliable measurements of internodal length and fibre diameter are best obtained from single fibres isolated by teasing. Although teasing is relatively easy in the peripheral nervous system, it is much more difficult in the central nervous system, presumably because, in central fibres, the outermost lamellae of adjacent sheaths fuse to form intraperiod lines where they come into contact (Peters, 1960*a*). Hess & Young (1949, 1952), using intravital staining with methylene blue followed by fixation with ammonium molybdate, were able to demonstrate in the rabbit central nervous system a direct relationship between internodal length and fibre diameter. But the myelin in their preparations was inevitably poorly preserved by modern standards. Because of the importance of a knowledge of the dimensions of central, myelinated fibres for physiological and pathological studies, we have re-examined this question using a different technique which ensures good fixation and is adaptable to the study of experimental, pathological material (Harrison *et al.* 1970). The observations have been made on the cat spinal cord because of the frequency with which this species is used in physiological studies of normal and pathological nerve fibres.

METHODS

Four normal adult cats were used. Initial fixation was achieved by retrograde perfusion through the abdominal aorta with 600 ml of 3½ % glutaraldehyde in M/15 phosphate buffer, followed by 400 ml of Dalton's fluid (1 % chrome solution (pH 7·2), 1 % osmic acid and 0·85 % NaCl; Dalton, 1955) according to the method of Harrison *et al.* (1970). The fixed thoracolumbar cord, approximately T10–L4, was then removed from the animal and divided transversely into blocks about 1 cm in length. These blocks were further divided into dorsal and ventral halves. Blocks which were incompletely blackened were post-fixed in Dalton's fluid for 24 hours at 4 °C. The dorsal half of the cord was used for the present study; the data thus refer to fibres

obtained from the posterior columns and dorsal half of the lateral columns. Longitudinal sections approximately 60 μm in thickness were cut on a freezing microtome and the sections were then taken through increasing concentrations of glycerin, from 7½ to 60 %, over a period of one to two hours to prevent abrupt dehydration and subsequent distortion of the fibres (Lubińska, 1960). The sections were left in 60 % glycerin for at least 24 hours. Longer periods of immersion sometimes appeared to increase the ease with which the fibres could be teased. Sections were teased in 60 % glycerin with fine mounted needles under a dissecting microscope, using a similar technique to that for teasing peripheral nerve fibres (Vizoso & Young, 1948; Thomas & Young, 1949). Great care was taken to minimize stretching and damage to the fibres. Measurements were made on the isolated fibres with the light microscope, using an ocular micrometer, at magnifications of × 25 for internodal length and × 400 for external fibre diameter and nodal gap. In determining fibre diameter we have adopted the procedure of Williams & Kashef (1968) and taken the mean of diameters measured at 5 equidistant points in each internode. The measurements of internodal length were taken to the nearest 10 μm while the readings for the external fibre diameter were taken to the nearest 1 μm.

It is customary with peripheral nerve fibres to make measurements after passage through creosote and permanent mounting in DPX or Canada balsam. This method proved unsatisfactory for central nerve fibres. Comparison of observations made on 25 fibres in the teasing medium with observations made on the same fibres after conventional mounting showed an average shrinkage in diameter of 8 %, which was highly significant ($P < 0{\cdot}0025$). Although there was no significant change in internodal length ($P > 0{\cdot}45$), mounting introduced distortions to the myelin and led to a loss of detail. For example incisures visible in unmounted fibres were often obliterated after mounting. For this reason we routinely measured the fibres in 60 % glycerin before mounting and used a different method, which minimized distortion, for preserving the fibre permanently. The fibre was placed in a pool of warmed glycerol-gelatin (Sigma Chemical Company). It was then manœuvred so that one end stuck to the slide just beyond the edge of the drop. The fibre was then straightened, care being taken to touch it as little as possible and to exert a minimum of tension on it. Despite this, recognizable artefactual breaks in the myelin sometimes occurred (see below). Excess glycerol was removed from around the fibre and the remainder allowed to harden by leaving at room temperature. After about an hour, the slide on which the fibre had been placed was inverted and lowered carefully on to a coverslip on which a further drop of warmed glycerol-gelatin had been placed. After 24 hours at room temperature, the coverslip was ringed with microscopic cement white.

RESULTS

The perfusion technique regularly resulted in good fixation, as judged by uniform hardness of grey and white matter. The penetration of osmium was somewhat variable. Although some sections were uniformly blackened, others showed areas of relative pallor. It was more difficult to isolate fibres in the paler areas and measurements were made only on fibres from the black regions. Such fibres showed good preservation of fibre outline and well-rounded nodes, the appearances being similar to those

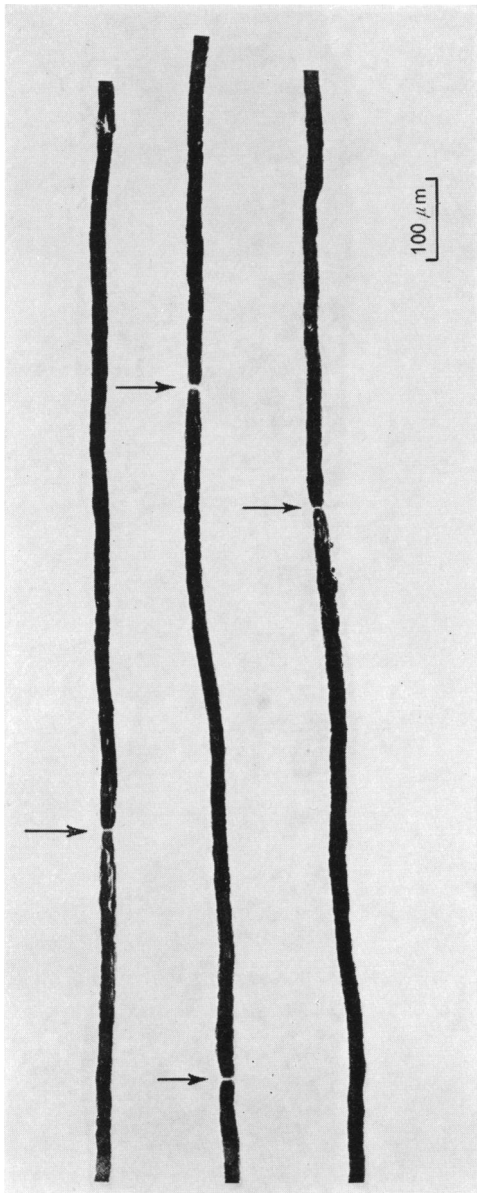

Fig. 1. Normal single, myelinated nerve fibre from the spinal cord of the adult cat, fixed by perfusion with 3½ % glutaraldehyde and Dalton's fluid. Photographs of consecutive lengths of the same fibre are mounted one below the other for ease of presentation. The fibre is approximately 4·3 mm long and about 20 μm in diameter. Internodal lengths are 1·03 mm, 0·91 mm and 1·29 mm.

of peripheral fibres. Although it was relatively easy to isolate single complete internodes, it was much more difficult to isolate longer lengths. In the present experiments, we have isolated 73 fibres with single complete internodes, 12 fibres with two consecutive internodes and 2 fibres with three consecutive internodes.

Fig. 1 shows a single fibre isolated for approximately 4·3 mm, photographs of consecutive lengths of the same fibre being mounted one below the other for convenience. The myelin is uniformly blackened and the outline smooth. Nodes, marked by arrows, are approximately 1 mm apart. Examination of a nodal region under high power (Fig. 2, A) shows a rounded myelin contour on either side and a bare axon in between. The appearances resemble a node of Ranvier in the peripheral nervous system. Further examples of typical nodes from other fibres are shown in Fig. 2, B–D. It was common to observe an osmiophilic zone in the nodal gap resembling the cementing disc of Cajal in the peripheral nervous system (Fig. 2, B). During teasing artefactual breaks in the myelin sometimes occurred (arrowed in Fig. 2, D). These were readily distinguishable from nodes by the sharpness and irregularity of the myelin margins in the artefacts; such appearances could be produced easily by deliberately damaging a fibre. Moreover the position and form of nodes could usually be established before manipulation of the individual fibre, and any new interruptions were thus obvious. Finally in some fibres the presence of a 'cementing disc' gave added certainty to the identification of nodes.

It can be seen from Fig. 1 that there are variations in diameter within a single internode. In determining the relationship between internodal length and fibre diameter, we have taken the diameter of each internode as the mean of five measurements (see method). The maximum variation in diameter measurements within an internode was 24 %; 85 % of the internodes had all measurements within 15 % of the mean. This variation is less than that reported for peripheral fibres (Sunderland & Roche, 1958).

A second kind of interruption in myelin was frequently seen within the internodes (Fig. 3). The oblique slit-like appearance in the photograph and the funnel-like appearance seen on focusing up and down on the specimen resembles the Schmidt–Lantermann incisures of the peripheral nervous system. Electron microscopic evidence for incisures in the central nervous system has been obtained recently (Blakemore, 1969; Harrison & Ochoa, 1971).

Fig. 4 shows the relationship between internodal length and external fibre diameter. Although there is scatter in the results, it can be seen that, in general, smaller diameter fibres have shorter internodal lengths, and large diameter fibres longer internodal lengths. The relationship is linear, the calculated regression line having a highly significant slope ($y = 0{\cdot}387 + 0{\cdot}037x$; $P < 0{\cdot}0005$). First inspection of the data suggested that there might be some flattening at the upper end of the curve. However, fitting a quadratic regression curve showed that this did not provide a significantly better fit than a straight line ($y = a + b_1x + b_2x^2$; t for $b_1 = 2{\cdot}48$ ($P < 0{\cdot}01$); t for $b_2 = -1{\cdot}19$).

The pooled data for Fig. 4 show considerable variation in internodal length for a given fibre diameter. Fig. 5 shows the variations in internodal length found within individual fibres isolated with consecutive internodes. In these fibres, measurement showed a maximum variation in mean internodal diameter for a given fibre of 2 μm;

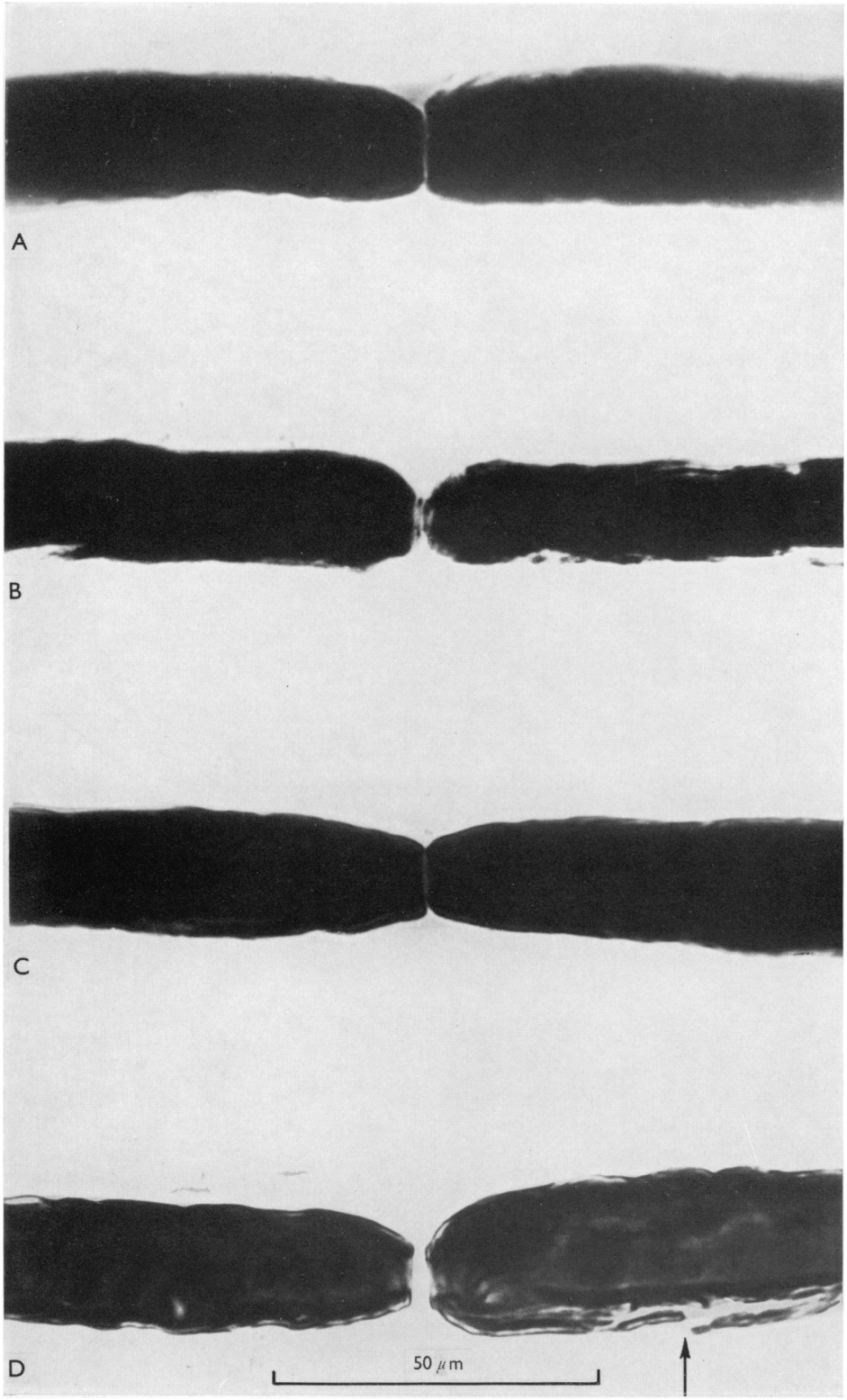

Fig. 2. A–D. High-power photomicrographs of nodes in the central nervous system. A higher power view of the second node in Fig. 1 is shown in A. Nodes from other fibres are shown in B–D. The arrow in D points to an artefactual break in the sheath of the fibre introduced during teasing.

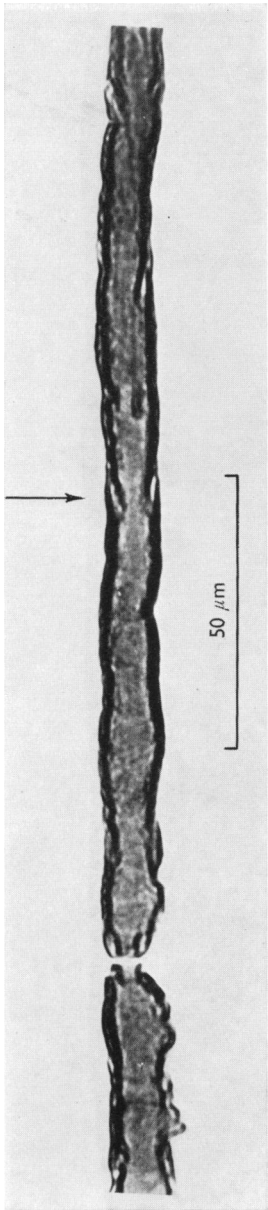

Fig. 3. Photomicrograph of another central nerve fibre, showing a node and also an incisure (arrow) resembling the Schmidt–Lantermann incisures of the peripheral nervous system.

in five fibres the diameters were the same. The data are presented according to the convention adopted by Fullerton *et al.* (1965), i.e. a single value for diameter is given for each fibre; this is the diameter of the widest internodal segment measured in the fibre concerned. Values for different internodal lengths in the same fibre are joined by a line. The number of observations is too small to draw definite conclusions,

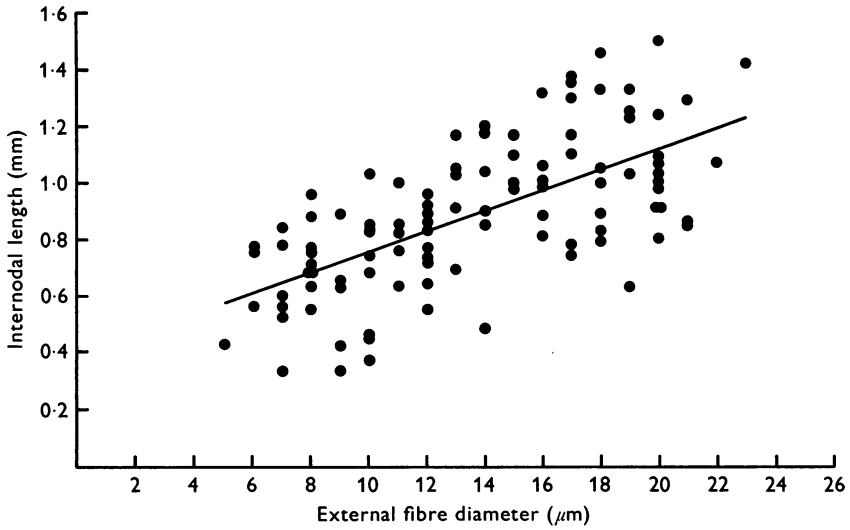

Fig. 4. Relationship between internodal length and external fibre diameter for 103 internodes of fibres isolated from the spinal cord of the adult cat. The figure shows the calculated regression line for the data.

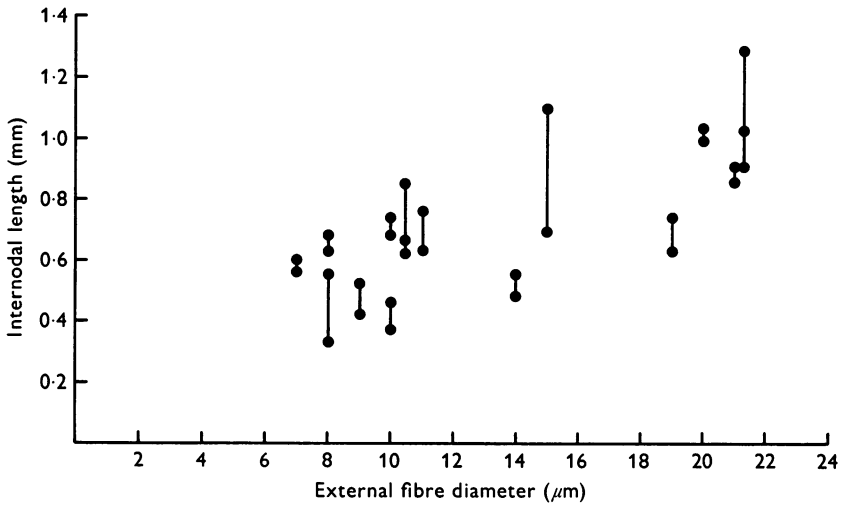

Fig. 5. Distribution of internodal length in fibres with consecutive internodes isolated from the spinal cord of the adult cat. A single value for diameter has been given for each fibre; this is the diameter of the widest internodal segment measured in the fibre concerned. Values for different internodal lengths in the same fibre are joined by a line.

but it appears that the range of internodal length for any individual fibre is less than the range for the pooled data for fibres of the same diameter.

Hess & Young (1949) commented that, in their material from the rabbit spinal cord, they saw occasional fibres with half or twice the expected internodal length. While the scatter of the results in our material from the cat is such that some small-diameter fibres had only half the internodal length predicted from the regression line, this was not the case for larger-diameter fibres, and no fibre with twice the predicted internodal length was seen. Although it is possible that this may represent a species difference, it seems more likely that it is due to the greater ease of identification of nodes allowed by the present fixation method.

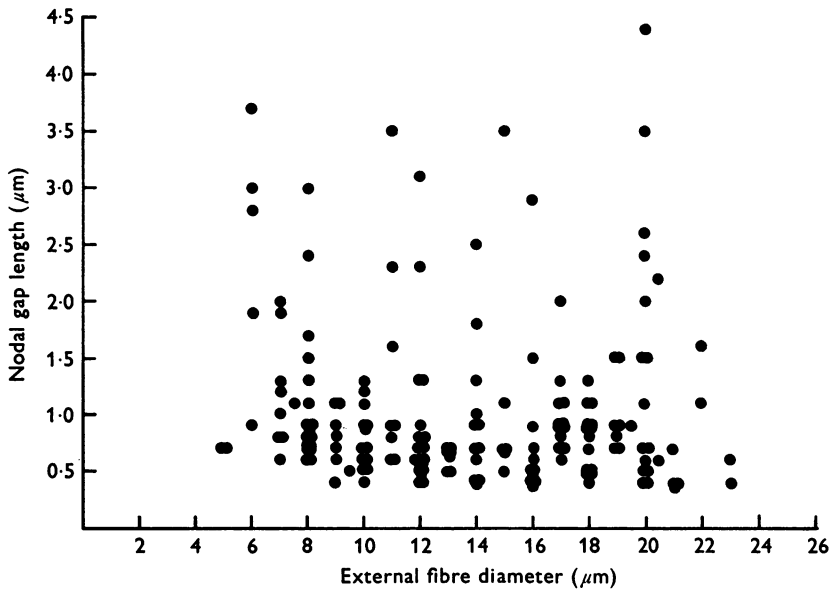

Fig. 6. Relationship between nodal gap length and external fibre diameter for 177 nodes from the spinal cord of the adult cat. Where the nodes were bounded on either side by complete internodes, a single fibre diameter was derived from the mean of the diameters for each internode. Otherwise the diameter of the complete internode adjacent to the node was taken as the appropriate diameter for that node.

We have attempted to obtain some measure of nodal gap length. Precise measurement is not possible for several reasons. The gap length is close to the limits of resolution at the highest magnification we could use for unmounted fibres in glycerin (× 400). Even within these limits it was sometimes impossible to obtain a single meaningful measurement because the myelin terminated at different levels in different focal planes. What we did was to measure the distance between the rounded ends of the compact myelin (Cavanagh & Jacobs, 1964), making allowance for any irregularities by eye. The results are shown in Fig. 6. Despite the difficulties in measurement, it was quite clear that variations in size were real. The range 0·4 μm–4·4 μm is greater than that found in the peripheral nervous system of the rabbit by Hess & Young (1952), who reported a range of 0·25 μm–0·5 μm using osmium fixation *in situ*. Because the method

of fixation and staining has a considerable effect on nodal gap size (Cavanagh & Jacobs, 1964), other published data for peripheral nerve fibres are not strictly comparable. No other quantitative data are available for the central nervous system, although Cajal (1909), Hess & Young (1952) and Pease (1955) have noted that the nodal gap appears to be wider in central than in peripheral fibres. Our measurements were made on fibres from the posterior and lateral columns and should not be applied to myelinated fibres in the grey matter. Here, nodes may be specialized as presynaptic varicosities (Peters, Palay & Webster, 1970) and a wider variation in nodal length might be expected. Kashef (1966) and Jacobs (1967) found an inverse relationship between nodal gap length and fibre diameter in peripheral fibres, but no such relationship emerges for central fibres (Fig. 6).

DISCUSSION

Our data confirm the report by Hess & Young (1949, 1952) that in central, as in peripheral, nerve fibres there is a linear relationship between internodal length and fibre diameter. The slope of the regression line for our data for the cat is less than that for Hess & Young's data for the adult rabbit. While this may reflect a species difference, it is possible that differences in technique might contribute.

Table 1. *Predicted internodal length for nerve fibres of 10 μm diameter in the peripheral nervous system in various species. Data derived from the literature*

Species	Nerve	Internodal length	Author	Fixation
Adult cat	Peroneal	1·11 mm	Lubińska (1960)	1 % buffered osmium tetroxide (preceded by trypsin digestion)
Adult cat	Peroneal	0·95 mm	Hursh (1939)	1 % osmium tetroxide
Adult rabbit	Peroneal	0·85 mm	Vizoso & Young (1948)	Formol saline followed by 1 % osmium tetroxide
Adult guinea-pig	Peroneal	0·97 mm	Fullerton *et al.* (1965)	Formol saline followed by 1 % osmium tetroxide
Adult rat	Posterior tibial	1·46 mm	Fullerton & Barnes (1966)	Formol saline followed by 1 % osmium tetroxide
Adult human	Anterior tibial	0·84 mm	Vizoso (1950)	Formol saline followed by 1 % osmium tetroxide
Adult human	Sural	0·85 mm	Lascelles & Thomas (1966)	Formol saline followed by 1 % osmium tetroxide

How do the present results compare with results for the peripheral nervous system? This question is conveniently answered by comparing the internodal lengths derived from the regression lines in different studies, for fibres of 10 μm diameter. In the present experiments, a fibre of 10 μm should have an internodal length of 0·75 mm. Table 1 (compiled from the literature) shows the predicted internodal length in peripheral nerves of a variety of species. In all cases, the measurements were made after osmium fixation, although a variety of treatments preceded this in the different studies. Where regression lines are not given by the original authors, we have drawn

a line by eye through their data. In every case the predicted internodal length is greater than we have found in the central nervous system. As previously mentioned, differences in technique may contribute to this, but it seems likely that Cajal's (1909) original suggestion that nodes are closer together in the central nervous system is correct.

What relationship do the data presented have to the living state of the nerve fibre? At the outset it must be emphasized that there is no single state of nerve fibres which can be regarded as 'normal'. Considerable variations in tension on the fibres in the spinal cord occur as the spine moves through full extension to full flexion (Breig, 1960), and these will presumably produce variations in fibre diameter and internodal length. In addition to the uncertainty deriving from the varying states of the fibre in the living animal, various artefacts are introduced by fixation and teasing. Changes in the size of cellular elements are produced by glutaraldehyde and osmium tetroxide fixation (Van Harreveld & Khattab, 1968). Teasing must introduce variations both in diameter (where the fused sheaths of adjacent fibres are separated) and in internodal length (by variable stretching). Although every effort was made to minimize errors introduced by teasing, some distortion was inevitable. In spite of these remarks and the inevitably approximate nature of the relationships between various anatomical measurements of nerve fibres, it is important to have some such quantitative information, both for the interpretation of electrophysiological studies, and for the analysis of pathological mechanisms.

BeMent & Ranck (1969), for example, have recently studied conduction in single fibres in the posterior columns of the normal cat. It is interesting to compare their physiological measurements and our anatomical results. On the basis of periodic, longitudinal variations in stimulating current threshold in six single fibres, they predicted internodal lengths of 500–1125 μm. These values are in good agreement with those shown in Fig. 4. They did not observe any relationship between the periodicity and conduction velocity, but their sample was small and, as our data show, the range of internodal lengths for each diameter (and therefore conduction velocity) class is wide. They found a variation in internodal length of less than 25 % over 3–4 internodes; this is rather less than that shown in Fig. 5 (up to 40 %). We have confirmed their expectation that, as in the peripheral nervous system, there are variations in fibre diameter within a single internode. We cannot comment on their conclusion (based on the application of the Hursh factor of 6 derived from the peripheral nervous system) that there are few fibres in the posterior columns with diameters of greater than 9–10 μm. Their observations were made in the low cervical and high thoracic regions, and ours include measurements on fibres from the whole dorsal half of the cord at a somewhat lower level.

Recent ultrastructural investigations have shed light on the cellular mechanisms of demyelination in the central nervous system (Lampert, 1965; Prineas *et al.* 1969) but many questions remain. Does central demyelination occur as in the peripheral nervous system, in a truly segmental pattern corresponding with whole internodes? Does demyelination commence as in peripheral fibres, in the paranodal regions? Remyelination can occur under certain circumstances in the central nervous system (Bunge, 1968) but we do not know whether it can take place over whole internodes, or whether only partial demyelination can be repaired. Nor do we know whether,

if remyelination of whole internodes can occur, the adult internodal length appropriate to the fibre diameter can be restored or not. Answers to such morphological questions are fundamental to the interpretation of electrophysiological data from experimental demyelinating lesions (McDonald & Sears, 1970*a*, *b*) and to the understanding of the mechanism of remission in human demyelinating diseases such as multiple sclerosis. The present work provides the technique and normal information necessary as a basis for an experimental study of these problems.

SUMMARY

A technique is described for isolating single, well-fixed, myelinated fibres from the spinal cord of the cat. Measurements on 103 complete internodes confirm a direct relationship between internodal length and fibre diameter. It appears that there is no relationship between the length of the nodal gap and the diameter of the fibre. The importance of this work in the analysis of physiological and pathological mechanisms is discussed.

This work was supported by a grant from the Medical Research Council and was carried out while Dr Ohlrich was an M.R.C. Research Fellow. The perfusions were carried out in collaboration with Dr J. Ochoa and Mr B. M. Harrison. We wish to thank Professor R. W. Gilliatt and Dr R. G. Willison for helpful discussion and Professor Armitage of the London School of Hygiene and Tropical Medicine for advice on statistics. Mr K. Yogendran and Miss Carol Aldridge gave valuable technical assistance.

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