THE CENTRIFUGAL SPREAD OF STRUCTURAL CHANGE AT THE NODES IN DEGENERATING MAMMALIAN NERVES

By G. CAUSEY* and ELIZABETH PALMER

Department of Anatomy, University College, London

INTRODUCTION

During a histological study of the first changes in nerve degeneration it was found that the myelin retracts from the node at a very early stage (Causey & Palmer 1952). There were indications that this retraction of myelin at the nodes involved the nodes in an orderly sequence down the nerves from the crush to the periphery.

In view of the diversity of opinion as to the direction of onset of functional failure in a degenerating nerve (Rosenblueth & Dempsey, 1939; Rosenblueth & del Pozo, 1943; Parker, 1933; Titeca, 1935; Holobut & Jalowy, 1936; Erlanger & Schoepfle, 1946) and the possibility that increase in the naked axon area at the node might stop conduction in a myelinated fibre (Rushton 1951), we have made a careful investigation of the spread of this nodal involvement and have confirmed our previous findings.

METHOD

The nerve to the medial head of gastrocnemius (N.G.M.), and the phrenic nerve of rabbits were used.

With the rabbit under nembutal and ether anaesthesia, the N.G.M. was crushed in the upper part of the thigh with smooth-bladed watchmakers' forceps for 10 sec. After periods of 0–120 hr., the nerve was fixed *in situ* with a modified Flemming solution and embedded in paraffin and cut in serial sections at 5μ; staining was by the Weigert method. A more detailed description of the histological method may be found in Causey & Palmer (1952).

The phrenic nerve was crushed (in a few cases cut) in the neck. The site of the crush was marked by a loose cotton tie and at biopsy the whole nerve was excised. Pieces of the phrenic nerve 1 cm. in length (in this case in the fresh specimen) were fixed on cards in Flemming's solution and embedded as above. About 400 serial sections were cut from the middle of each centimetre piece.

The appearance of a normal node in successive serial sections at 5μ are shown in Pl. 1, figs. 1–4. The fibre marked '*A*' can be followed in the four successive sections; the narrowing of the axoplasmic canal is seen in figs. 2 and 3 but in none of these 5μ sections does the myelin fail to surround the axoplasm completely.

The method of fixation was kept constant throughout, and only four nodes were found in 160 normal nodes with absence of myelin in a 5μ section. This does not completely agree with the findings of Hess & Young (1952), who illustrate three nodes with a 5μ gap in the myelin at the node. Their method of fixation was similar

* Present address: Department of Anatomy, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2.

to ours but they do not report on measurements of a large number of normal nodes. Young (1952), using fresh teased fibres, obtained a mean figure for the myelin free gap of 0·5 μ. The length of the naked axon at the node in well-fixed Flemming material is probably much less than 5 μ.

In a normal nerve, therefore, cut at 5 μ thickness, there will be very rarely a section of a given node which is free from myelin. If, however, the naked part of the axon becomes larger than 5 μ there will be sections through a node where no myelin is stained.

Pl. 1, figs. 5–8, show four successive sections from a fibre in a nerve that had degenerated for 72 hr. Fig. 6 shows the narrow axoplasmic canal surrounded by deeply stained myelin. In fig. 8, however, the myelin is very faintly and diffusely shown. Fig. 8 shows the enormous increase in stainable myelin only 5 μ away.

In the serial sections of the N.G.M., nodes were examined and the number of sections without myelin staining was recorded. At least ten nodes taken at random were examined at each level, and the distance between the levels was calculated from the sections, so that the distances given in the results on the N.G.M. are of the nerve after fixation. With the phrenic nerve at least ten nodes from each piece of nerve were examined.

In the analysis of the data obtained, the hypothesis was set up that the length of axon free from myelin at the nodes varied as the time after crushing and the distance from the crush. That is to say,

$$y = ax_1 + bx_2 + c,$$

where y = length of axon free from myelin x_1 = time after crush (hr.), x_2 = distance from crush (mm.), and a, b and c are constants.

The evaluation of the constants a, b and c and their variance was carried out by Mr D. Sholl.

RESULTS

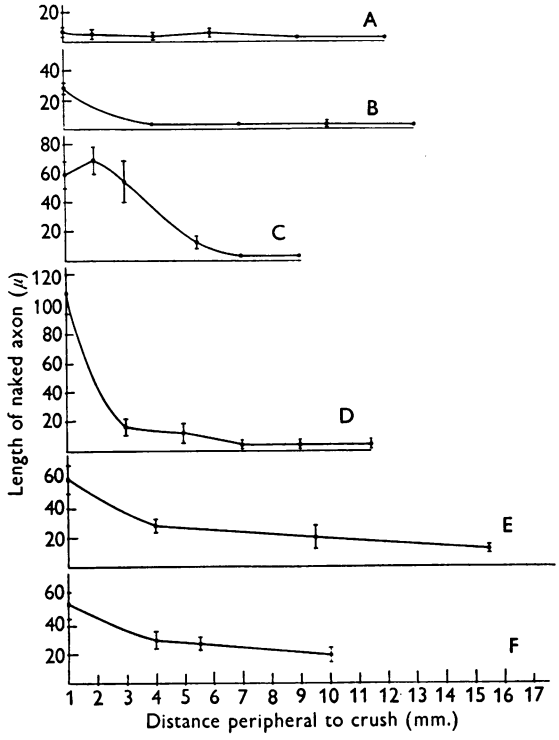
The appearance of the fibres at the neck level and diaphragmatic level of the phrenic nerve 72 and 96 hr. after a crush are shown in Pl. 2, figs. 10–13. It will be seen at the upper end of the nerve, just beyond the crush in both the 72 and 96 hr. specimens, that there are large distended fibres and that there are no normal nodes like those illustrated in Pl. 1, figs. 2 and 3. There are, of course, many nodes in different stages of retraction of the myelin but no node with the thick, crenated myelin and narrow axoplasmic canal. The sections from the distal ends of the specimens differ. The 96 hr. specimen, like the proximal ends of both specimens, shows no normal nodes and large distended fibres, but the distal end of the 72 hr. specimen shows normal nodes as '*n*', and is, in fact, indistinguishable from a transverse section of a normal phrenic nerve.

In examining these pictures, attention is drawn to the curious appearance of some of the fibres in the degenerating specimens such as '*s*', Pl. 2, figs. 10–13. The myelin is split and in-folded. This appearance has been observed regularly in mammalian nerves fixed with Flemming's solution when degeneration has gone on for 72 hr. or more. It has also been seen in 72 hr. degenerated fibres fixed in osmic acid and cut frozen, but not in formalin-fixed material. The effect seems to be due to rupture of distended parts of degenerating fibres when they harden in the fixative, and does

not occur in normal fibres. Pl. 1, fig. 9, shows a longitudinal segment of a teased fibre showing the split in the length of the ovoid of myelin stained with osmic acid.

The evidence of the photographs is suggestive of a peripheral spread of disorganization of the myelin, but for quantitative analysis the measurement of a large number of nodes at different levels is necessary.

The graphs in Text-fig. 1 show the pooled results of measurements of 600 nodes in the nerve to the gastrocnemius muscle.



Text-fig. 1. Graphs of the N.G.M. in rabbit at various times after crushing. The height of the ordinate represents the unmyelinated length of axon at the node, from the crush towards the periphery, and each ordinate represents the mean of at least ten readings. The abscissa is measured in mm. from the crush. A, 0–1 hr.; B, 1–10 hr.; C, 30 hr.; D, 48 hr.; E, 72 hr.; F, 96 hr. The standard error of the mean is shown.

Text-fig. 1A shows the pooled results of measurements in nerves between 0 and 1 hr. after crush. Within 1 mm. of the crush there are a few fibres with measurable increase in the naked axonal length, but at 3, 5, 7, 11 mm. below the crush there is no increase in the length of axon at the node free from myelin.

Text-fig. 1B is from three nerves at 1–10 hr. after crush. The amount of naked axon has increased very markedly close to the crush, and at the 1 mm. level one gets five or six sections of an individual nerve without any myelin, so that there is as much as 30 μ of axon which are free from any myelin.

Text-fig. 1C shows the pooled results round 30 hr. Close to the crush the number of sections free from myelin amounts to as many as twenty, giving a myelin-free gap of 100 μ.

Text-fig. 1D, E and F show plots of six nerves at later stages after crushing, the nodal retraction of myelin at the more distal level increases in relation to time until at 96 hr. the length of naked axon at the node is about the same at all the levels along the nerve.

After longer periods of degeneration, such as the 96 hr. specimen Text-fig. 1F, the mean gap may be considerably less than in the earlier specimen. There is a much greater variation in the size of the gaps in these nerves, and one cannot be certain in these later stages that it is only nodal gaps which are being measured. The gaps produced by the breaking-up of the internodal lengths of myelin into ovoids cannot be distinguished from nodal gaps. Erzholz granules can sometimes be seen in this type of break, and the spacing of the gaps in individual fibres becomes irregular.

The tables of crude data are too large for inclusion in this paper, but complete copies have been deposited in the Thane Library of University College, London. The standard error of the mean has been indicated for the readings at each level plotted in the graphs.

The analysis of these figures gives the following result:

$$y = 35{\cdot}74 + 0{\cdot}09x_1 - 2{\cdot}78x_2,$$

the coefficients of both x_1 and x_2 are highly significant ($P < 0{\cdot}01$). The relation of this analysis to the analysis of the similar observations on the phrenic nerve is discussed below.

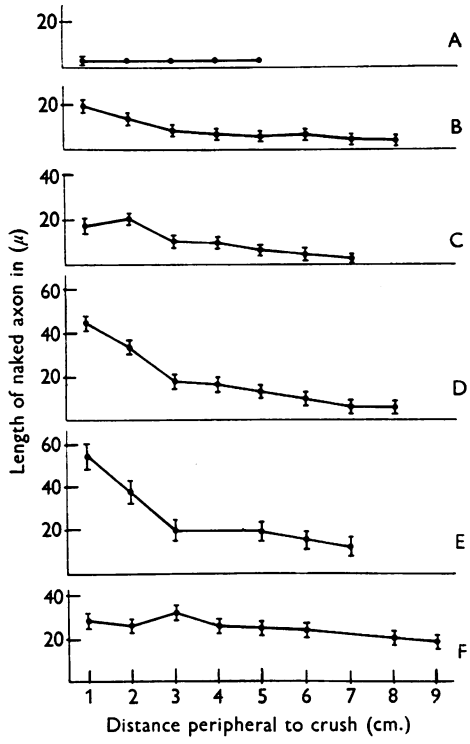
These measurements of the N.G.M. are over a short distance, and we have therefore examined the phrenic nerve to obtain a greater length of nerve. In dissecting out the phrenic nerve, a search was made for accessory branches entering the nerve in the upper part of the thorax which had been missed in the crush or cut in the neck. These were found on one occasion only, and in the resulting sections it was possible to trace a normal nerve bundle throughout the length of the nerve. This bundle has been disregarded in the subsequent measurement of nodes.

The normal phrenic nerve shows nodes all less than 5μ at five levels along an 8 cm. length of nerve. The pooled results are plotted in Text-fig. 2A.

The other plots in Text-fig. 2 show the distribution of retraction at 48, 60, 72 and 96 hr. after crushing; the steady progress of the involvement of nodes with increasing time of degeneration is clearly shown, until at 96 hr. the whole of the 8 cm. of nerve examined show long stretches of naked axon at the nodes amounting to some 50–60μ.

The four graphs B, C, D and F in Text-fig. 2 represent the pooled measurements of about 800 nodes, and the analysis of these figures gives the coefficient of x_1 as $0{\cdot}35$ and of x_2 as $-2{\cdot}63$, both of these are significant at $P < 0{\cdot}01$.

The analysis of the data on the phrenic nerves yields results similar to those found for the N.G.M. The hypothesis that the 'distraction' depends jointly on the time elapsed after the lesion and the 'distance', was tested by multiple regression analysis. The two regression coefficients were highly significant ($P < 0{\cdot}01$). This implies that after the lapse of a given time the 'distraction' decreases with the distance while, at a given distance, the distraction increases with the time after the lesion.



Text-fig. 2. Graphs of the phrenic nerve in rabbit at various times after crushing. The height of the ordinate represents the unmyelinated length of axon at the node, from the crush towards the periphery, and each ordinate represents the mean of at least ten readings. The abscissa is measured in cm. from the crush. A, normal nerve; B, 48 hr.; C, 53 hr.; D, 60 hr.; E, 72 hr.; F, 96 hr. The standard error of the mean is shown.

DISCUSSION

In considering the onset of failure of conduction in degenerating nerve fibres there is one aspect on which there is general agreement. Namely, that there is after a crush a latent period of about 48 hr., in mammalian nerve, during which conduction is 'normal'. In the histological examination of degenerating nerves the first changes are said to occur in the axon (Ramón y Cajal, 1928; Speidel, 1933; Weddell & Glees, 1941; Holobut & Jalowy, 1936), and these changes precede by many hours the onset of demonstrable failure of conduction. Erlanger & Schoepfle (1946), in view of this situation, wrote '...conduction ceases only with the disruption of the myelin sheath'. Whether actual disruption of the internodal myelin is a necessary precursor of failure of conduction has never been shown, and it seems more probable that if one accepts the saltatory nature of conduction in myelinated nerve fibres, then increase of the naked axon area at the node might in itself be sufficient to block conduction. According to Rushton (1951), the total nodal current is proportional to the axonal area at the node. It has been shown in these experiments that the naked axonal length at the node increases by a factor of more than ten times, before the break up of the internodal myelin column.

The observation that the retraction at the nodes proceeds after a latent period in a centrifugal direction, is of interest in indicating at least one structural change that has an orderly and not random progression. If the retraction at the nodes does in fact cause a conduction block, then it should be possible to obtain a nerve trunk in which at least some of the fibres had reached the stage of failure of conduction in the proximal part of the degenerating nerve, and not in the distal part. This possibility is being investigated.

As for the cause of peripheral progression of involvement of nodes, it is unlikely that this could be explained by the removal of a pressure exerted by the nerve cell along the length of the nerve fibre, because any loss of turgor pressure would be effective along the whole length of the fibre within a very short time, and it would be difficult to explain the delayed onset of the process.

It seems more likely that some substance or substances are being used up to maintain the form of the nerve—that this material is effective for some 48 hr., but with failure of replacement there is a final breakdown of the necessary metabolic processes. This view would not explain the sequence of involvement but only the delayed onset, and it is necessary on this hypothesis further to assume that either the current of injury or the local disorganization of the fine structure (see Causey & Palmer, 1952) act as a focal point, from which the changes spread in a manner analogous to the spread of crystallization in a supersaturated solution from the site of introduced crystal or foreign body.

SUMMARY

1. In the degeneration of myelinated mammalian nerve the myelin retracts from the node during the early stages of degeneration. The area of naked axon at the node is therefore increased.

2. The retraction first affects the nodes close to the lesion producing the degeneration and spreads in a peripheral direction.

We wish to thank Mr Sholl for the statistical analysis of the data. We thank Prof. J. Z. Young, F.R.S., for his interest throughout, and Mr J. Armstrong and Mr B. Whitton for photographic and technical assistance.

REFERENCES

Causey, G. & Palmer, E. (1952). Early changes in degenerating mammalian nerves. *Proc. Roy. Soc.* B, **139**, 597–609.

Erlanger, J. & Schoepfle, G. M. (1946). A study of nerve degeneration and regeneration. *Amer. J. Physiol.* **147**, 550–581.

Hess, A. & Young, J. Z. (1952). The nodes of Ranvier. *Proc. Roy. Soc.* B, **140**, 301–320.

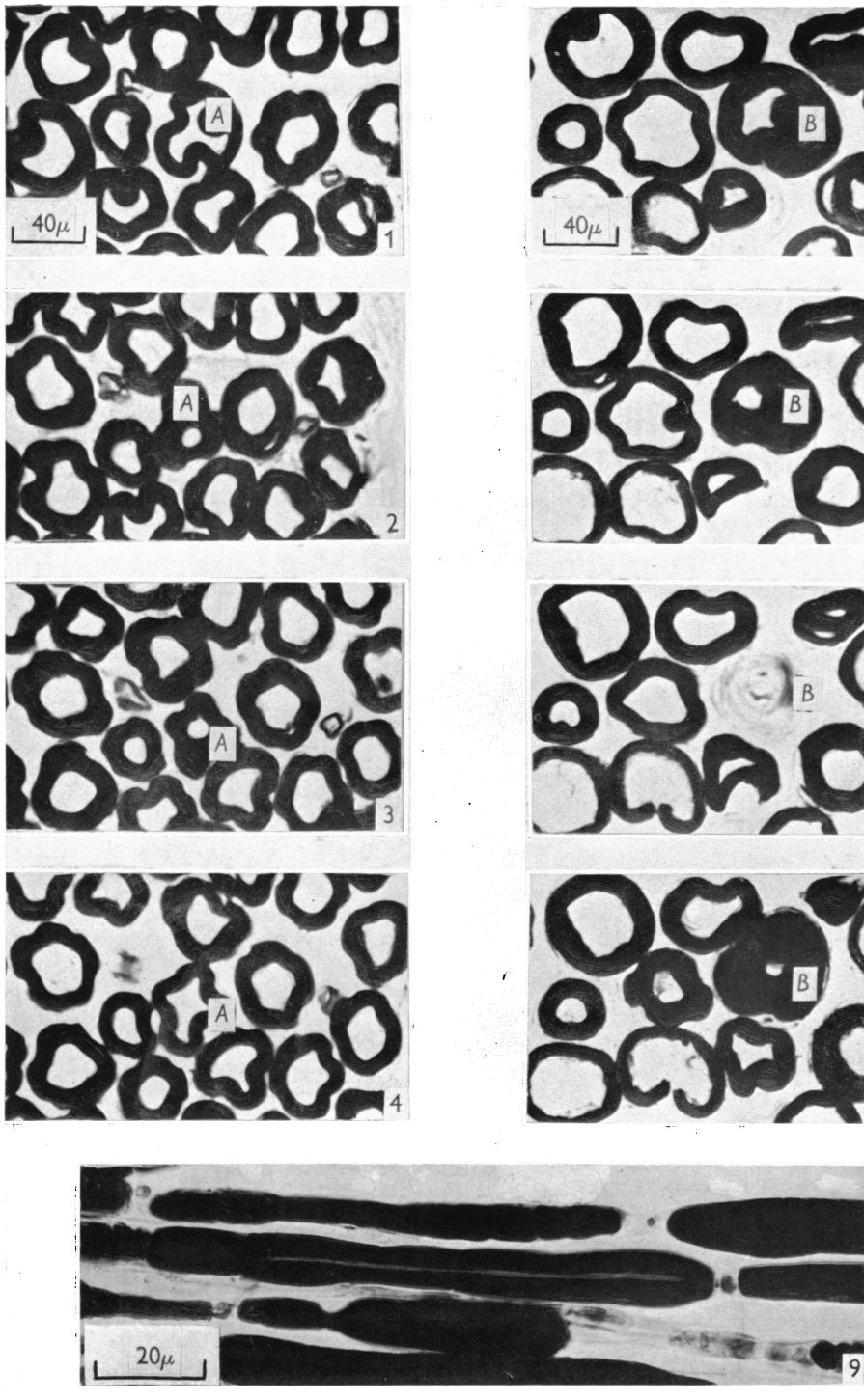
Holobut, W. S. & Jalowy, B. (1936). Die De- und Regeneration des peripheren motorischen Nervensystems auf einer morphologisch-funktionellen Grundlage. *Z. Zellforsch.* **25**, 541–564.

Parker, G. H. (1933). The progressive degeneration of frog nerve. *Amer. J. Physiol.* **106**, 398–403.

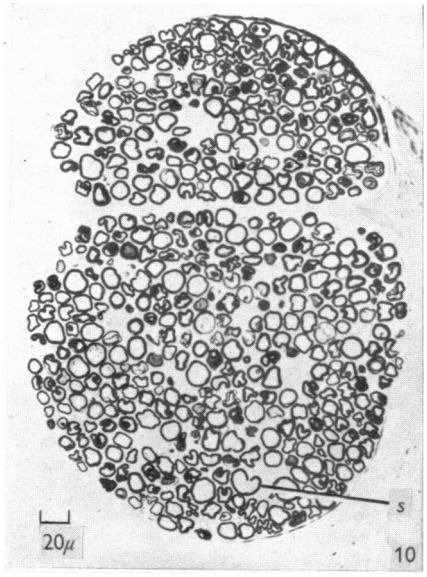
Ramón y Cajal, S. (1928). *Degeneration and Regeneration of the Nervous System.* Oxford: University Press.

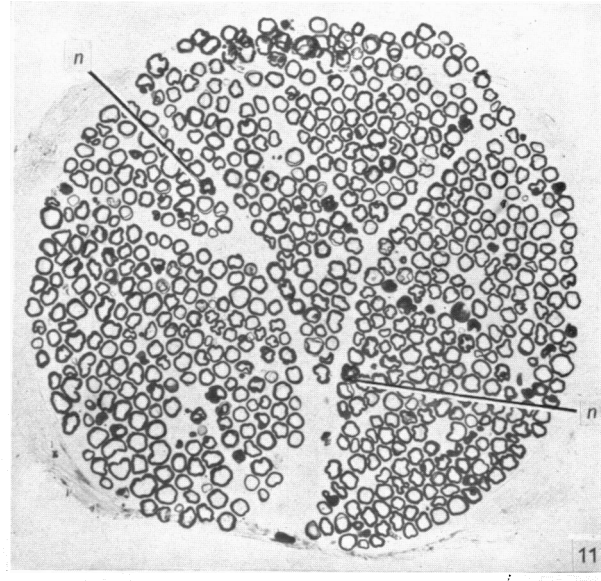
Rosenblueth, A. & Dempsey, E. W. (1939). A study of Wallerian degeneration. *Amer. J. Physiol.* **128**, 19–30.

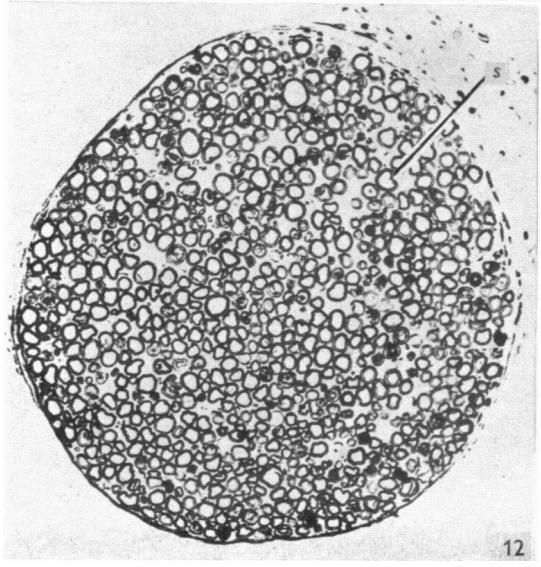
Rosenblueth, A. & del Pozo, E. C. (1943). The centrifugal course of Wallerian degeneration. *Amer. J. Physiol.* **139**, 247–254.

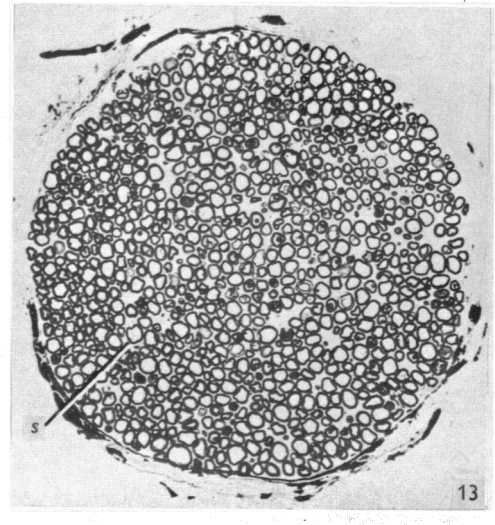


CAUSEY AND PALMER—STRUCTURAL CHANGE IN DEGENERATING MAMMALIAN NERVES









CAUSEY AND PALMER—STRUCTURAL CHANGE IN DEGENERATING MAMMALIAN NERVES

RUSHTON, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**, 101–122.

SPEIDEL, C. C. (1933). Studies of living nerves. *Amer. J. Anat.* **52**, 1–79.

TITECA, J. (1935). Étude des modifications fonctionelles du nerf au cours de sa dégénérescence Wallérienne. *Arch. int. Physiol.* **41**, 1–56.

WEDDELL, G. & GLEES, P. (1941). The early stages in the degeneration of cutaneous nerve fibres *J. Anat., Lond.*, **76**, 65–93.

YOUNG, J. Z. (1952). Dimensions at the nodes of Ranvier. *J. Anat., Lond.*, **86**, 476.

EXPLANATION OF PLATES

All nerves are fixed in Flemming solution and stained with the Weigert-Pal technique, except in Pl. I, fig. 9. The scale is indicated in the figures.

PLATE 1

Figs. 1–4. Successive serial transverse sections of a normal phrenic nerve. A typical normal node is seen in fibre 'A'.

Figs. 5–8. Successive serial transverse sections of a phrenic nerve after 72 hr. degeneration. Abnormal node in fibre 'B'. Note the almost complete absence of myelin in fig. 7.

Fig. 9. Teased fibres of N.G.M. after 72 hr. degeneration. The myelin is showing retraction and Erzholz granules can be seen. The longitudinal line in the central fibre is probably due to the splitting of the myelin discussed in the text.

PLATE 2

Fig. 10. Section of phrenic nerve after 72 hr. degeneration from the neck region, taken immediately below the crush. Note enlarged fibres and split fibres '*s*'. No normal nodes.

Fig. 11. Section of the same nerve as in fig. 10 at the diaphragmatic level, 7 cm. below the crush. Note normal nodes '*n*'.

Fig. 12. Section of phrenic nerve after 96 hr. degeneration taken from the neck level immediately below the crush. No normal nodes, note split '*s*' and enlarged fibres.

Fig. 13. Section through the same nerve as in fig. 12 at the diaphragmatic level, taken 8 cm. below the crush. The section presents the same absence of normal nodes with split and enlarged fibres as the neck level.