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Anatomical changes in peripheral nerves compressed by a pneumatic tourniquet

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INTRODUCTION

Local compression is known to damage peripheral nerves. Severe compression may actually crush the fibres and lead to Wallerian degeneration; the excitability of the nerve distal to the lesion is lost and recovery may take many months (Seddon, 1943; Denny-Brown & Brenner, 1944*a*). Mild compression produces a physiological block which is reversed as soon as the pressure is released. On the basis of their studies with pneumatic cuffs or clamps in man, Lewis, Pickering & Rothschild (1931) concluded that this physiological block was due to local asphyxia.

There is a lesion, distinct from either of the two described above, which follows compression of intermediate severity. In such a lesion there is a local conduction block with preservation of distal excitability; this may take several weeks to recover (Erb, 1876; Déjérine & Bernheim, 1899; Seddon, 1943). Using a tourniquet or a spring clip to produce compression, Denny-Brown & Brenner (1944*a*, *b*), found in cats that the anatomical basis for this was demyelination with preservation of axonal continuity at the site of compression. Denny-Brown & Brenner thought that this lesion, like that produced by Lewis *et al.* (1931), was due to local ischaemia of the nerve and that direct mechanical pressure was an unlikely explanation. In support of this they cited the experiments of Grundfest (1936) who had shown that enormous pressures were necessary to abolish conduction in excised frog nerve enclosed in an oxygenated pressure chamber.

During the course of recent experiments in which a tourniquet was used to produce long-standing conduction block in the peripheral nerves of baboons (Fowler, Danta & Gilliatt, 1972), we have found a characteristic lesion which precedes paranodal demyelination. The anatomical features and distribution of this lesion suggest that the damage to the nerve fibres is a direct result of the applied pressure, and not a consequence of secondary ischaemia. A brief preliminary account of our findings has been reported (Ochoa, Danta, Fowler & Gilliatt, 1971).

MATERIAL AND METHODS

Female baboons (*Papio papio*) weighing 9–12 kg were used. Their dates of birth were unknown, but from their dentition and the presence of regular menstrual cycles they were thought to be not less than 5 years old. All procedures were carried out

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under intravenous pentobarbitone sodium (60–120 mg) after preliminary tranquillization with intramuscular phencyclidine (2 mg/kg) and promazine (1 mg/kg).

A pneumatic cuff consisting of a rubber bag measuring 10×5 cm in a specially reinforced sleeve was placed round the knee, with the bag lying over the medial popliteal nerve. When the cuff was inflated the length of nerve compressed was approximately 5·5 cm. In most experiments a cuff pressure of 1000 mm Hg was maintained for 1–3 hours. This produced a local conduction block in the medial and lateral popliteal nerves with weakness of the leg and foot muscles. Both the clinical weakness and the conduction block then lasted for several weeks or months. In a few experiments a cuff pressure of 500 mm Hg was used, but this produced neither clinical paralysis nor a conduction block, although the conduction studies indicated that some nerve damage had occurred, resulting in a persistent conduction delay. Further details of the clinical state of the animals and of the nerve conduction studies are given by Fowler *et al.* (1972).

Animals were killed for anatomical study at intervals which varied from 2 to 3 minutes to six months after release of the pressure cuff. In all, 16 animals were examined. The abdominal aorta was cannulated prior to death and the lower half of the animal was perfused with 4 % glutaraldehyde in phosphate buffer at pH 7·4. Perfusion was maintained for 25–30 minutes at pressures of 150–200 mm Hg. Following this, the medial popliteal nerves were carefully exposed. Under a mark tattooed on the skin, where the upper edge of the cuff had been, a stitch was placed in the epineurium. Nerve samples were taken for three different techniques:

1. Some long stretches of nerve, including the portion under the cuff, were removed and immersed in 1 % osmium tetroxide; loose bundles were then teased in glycerine. This allowed the study of the distribution of the lesion in relation to the cuff.

2. Small samples for longitudinal and transverse sections were taken at measured distances from the stitch in the epineurium. They were post-fixed in 1 % osmium tetroxide in Dalton's solution, and were then dehydrated and embedded in Epon. 1 μm sections were made at different levels, stained with toluidine blue, and examined by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds method) and examined under a Siemens Elmiskop I electron microscope.

3. To relate the ultrastructural changes in individual fibres to the positions of the nodes of Ranvier, single teased fibres were prepared for electron microscopy. Transverse sections had previously been prepared from single fibres by Spencer & Thomas (1970) and by Dyck & Lais (1970). In order to obtain longitudinal sections a modified method was developed which can be summarized as follows. Samples from the nerve trunks, 2–3 cm in length, were post-fixed in Dalton's fluid, dehydrated, and impregnated with fluid Epon. From these, single fibres were teased with sharpened forceps, examined under the light microscope and photographed. The fibres were then embedded strictly in one plane and the Epon hardened. Ultrathin longitudinal sections were made from selected parts of each fibre and were then stained and examined under the electron microscope. A detailed description of the technique is given by Ochoa (1972).

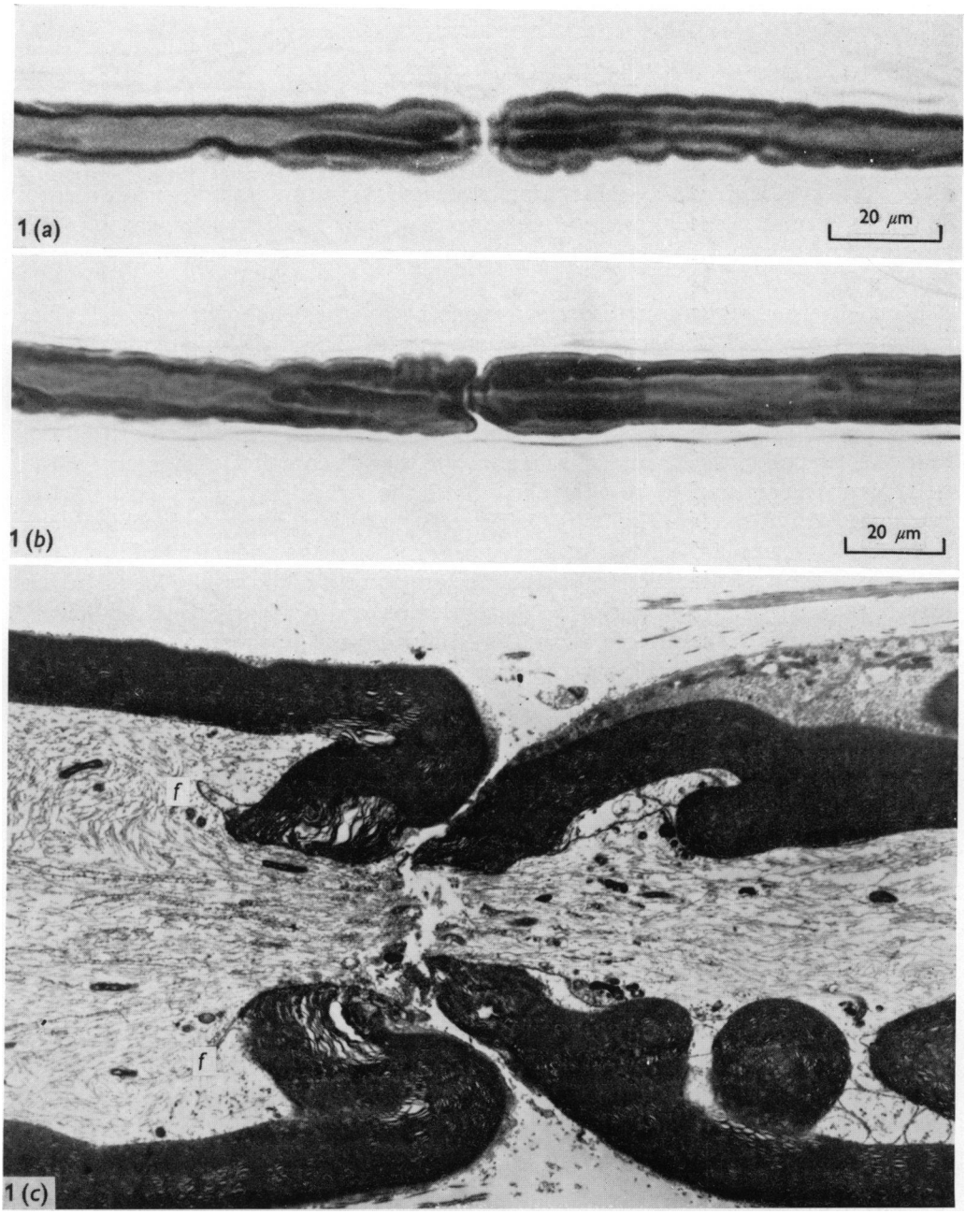


Fig. 1 (*a*). Normal node of Ranvier showing a nodal gap 1·2 μm wide.

(*b*) Abnormal node of Ranvier, 4 days after compression. There is minimal invagination of the paranode on the left by the one on the right, with obliteration of the nodal gap.

(*c*) Low power electron micrograph of node shown in 1 *b*. The axolemma of the invaginated paranode shows redundant folds (*f*). The discontinuity of nodal axoplasm is due to artefact. × 4900.

RESULTS

Displacement of the nodes of Ranvier

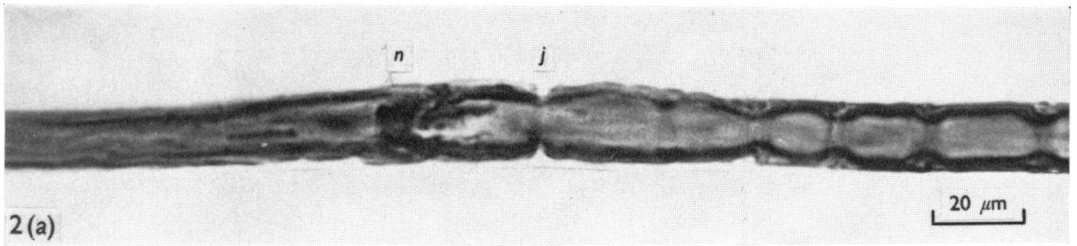
When single teased fibres were examined during the first week after compression, a characteristic abnormality of the nodes of Ranvier was seen in the large myelinated fibres. In its mildest form this consisted of a slight invagination of one paranode by an adjacent one, with partial obliteration of the nodal gap. An example can be seen in Fig. 1, in which a normal node is also shown (Fig. 1*a*). The light micrograph of the abnormal node is shown in Fig. 1*b* and a low power electron micrograph of the same node in Fig. 1*c*.

More severe invagination of one paranode by another is shown in Fig. 2. The general appearances of the nodal region are shown in the light micrograph above (Fig. 2*a*) and the detailed changes in the electron micrograph of the same region below (Fig. 2*b*). It can be seen that the paranode on the left has been invaginated by the one on the right to a depth of approximately 35 μm. The myelin sheath of the latter lies partially inside that of the former, and there is complete obliteration of the nodal gap. The terminal loops of myelin of both the ensheathed and the ensheathing paranodes can be seen in Fig. 2*b* (*t.l.*). These show their normal relationship to the nodal axolemma in both cases, thus indicating the site of the node. Part of Fig. 2*b* is shown at higher magnification in Fig. 2*c*. The attachment of the myelin loops (in this case of the ensheathing paranode) to the axolemma can be clearly seen. Additional evidence that this is the site of the node is obtained from the presence of transverse bands between the terminal loops and the axolemma. In Fig. 2*c* a redundant fold of axolemma (*f*) can be seen to the left of the terminal loops. Similar folding of the axolemma can be seen in the same position in this fibre shown in Fig. 1*c*.

The narrowing of the axon at the node, which is normally present in large myelinated fibres, is exaggerated under the folds of myelin which overlie the region in abnormal fibres (Figs. 1*c*, 2*b*). Axoplasmic organelles and particles may accumulate in relation to these local strictures. Past the stricture, towards the invaginated paranode, the neurofilaments appear distorted and may form curly patterns towards the surface of the axon (Fig. 1*c*).

In Figs. 2*a* and 2*b* there is a constriction of the fibre to the right of the node. This constriction underlies the junction between the two Schwann cells and is labelled *j*. Such junctions can be identified by the discontinuity of the Schwann cell cytoplasm (here containing abundant mitochondria) and by the presence of microvilli. Fig. 3*a* is taken from another fibre to illustrate this point. It shows the basement membrane, the cytoplasm of the adjacent Schwann cells, their microvilli, and the indented myelin underlying them. By conventional light microscopy such an indentation could easily be mistaken for a node of Ranvier, but closer inspection shows that there is no nodal gap; electron micrographs make it clear there has in fact been displacement of the node with respect to the Schwann cell junction.

Fibres showing varying degrees of invagination are illustrated in Fig. 4. In each case the new position of the node of Ranvier (*n*) and its original position under the Schwann cell junction (*j*) are shown. In these fibres the positions were checked by electron microscopy but it was possible to identify their site by light microscopy alone, the constriction indicating the Schwann cell junction being clearly visible, and



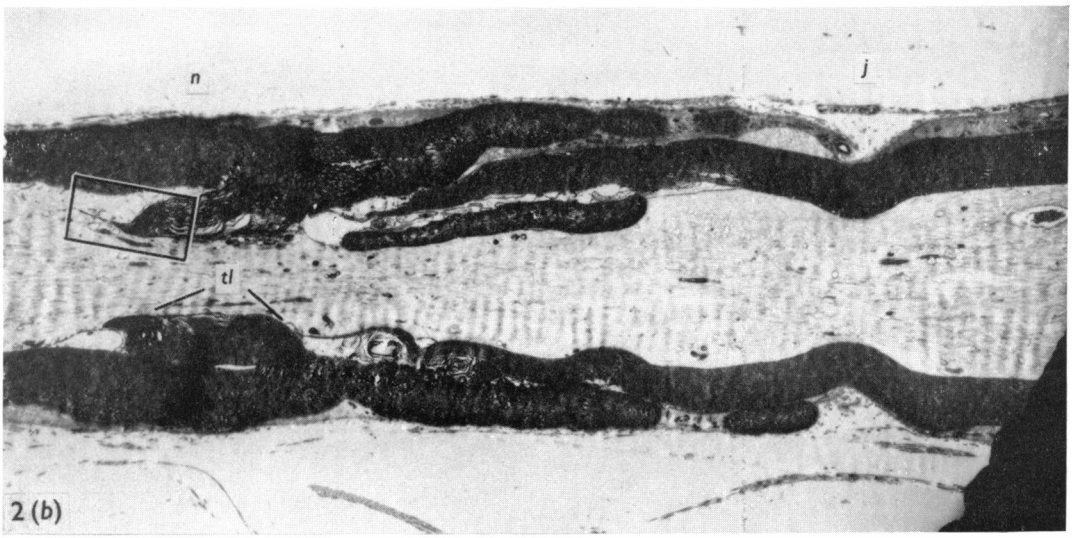


Fig. 2(*a*). Abnormal node of Ranvier, 4 days after compression. At the expected site of the nodal gap there is indented myelin (*j*). To the left there is a dark area (*n*) marking the new position of the node. Note Schmidt–Lanterman clefts opened on the right.

(*b*) Low power electron micrograph of the nodal region shown in (*a*). *j*, Schwann cell junction; *n*, node of Ranvier covered by infolded myelin; *t.l.*, terminal loops of myelin lamellae. × 2850.

(*c*) Enlargement of area contained in rectangle in 2*b*, to show typical arrangement of terminal loops of myelin lamallae (*t.l.*) in relation to axolemma. Folded axolemma (*f*) is also shown. × 26000.

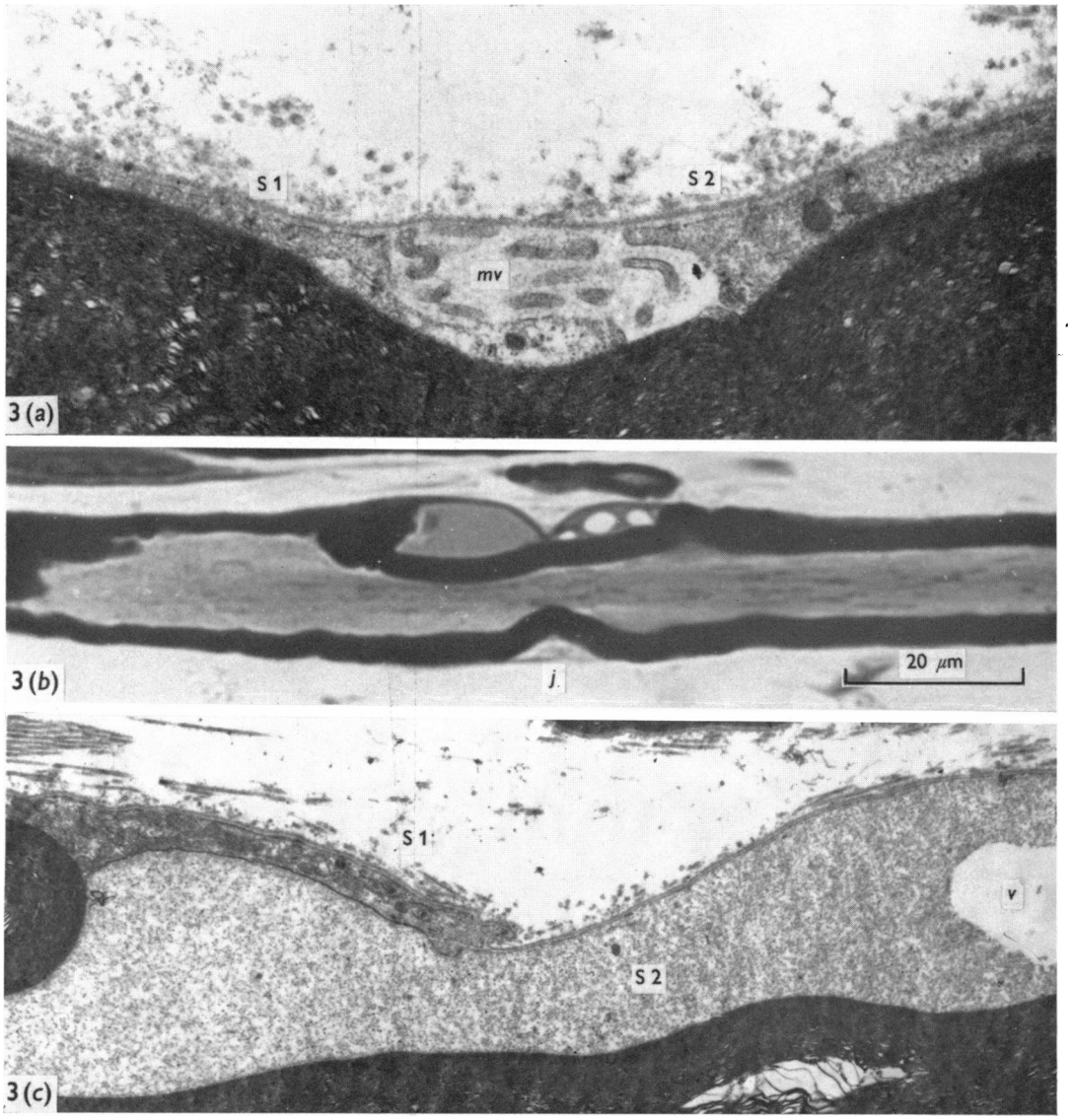


Fig. 3(*a*). Schwann cell junction, 18 hours after compression, overlies myelin instead of node of Ranvier. Microvilli (*mv*) from the two Schwann cells S1 and S2, are seen within the basement membrane. × 15800.

(*b*) Longitudinal section of fibre 4 days after compression. It shows indented myelin at the Schwann cell junction (*j*) and swollen Schwann cell cytoplasm containing vacuoles. Epon-toluidine blue.

(*c*) Electron micrograph showing detail of the fibre in 3*b*. The thin tongue of paranodal Schwann cell cytoplasm on the left (S1) has been dissected from the myelin by a swollen cell process intruding from the Schwann cell on the right (S2). In the latter, cytoplasmic differentiation is lost and there is a vacuole (*v*). × 8700.

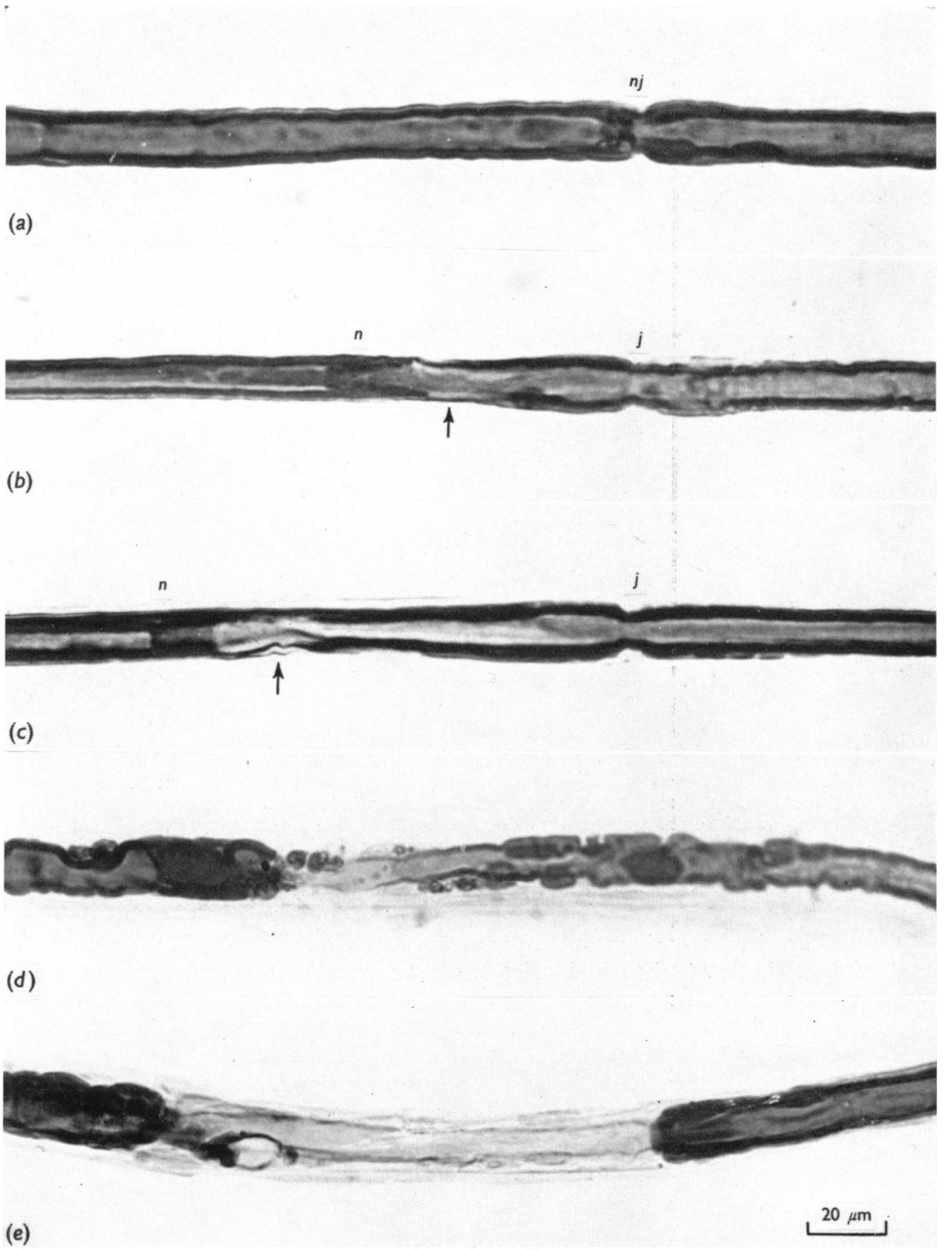


Fig. 4(*a–c*). Abnormal fibres, 4 days after compression, showing different degrees of nodal displacement (reaching 120 μm in *c*). *j*, Schwann cell junction; *n*, new position of node. Note thinning of myelin at arrows.

(*d*) A fibre, 15 days after compression, undergoing demyelination of the paranodal region. There is tapering of the myelin of the paranode on the right.

(*e*) A thinly myelinated intercalated segment, 61 days after compression.

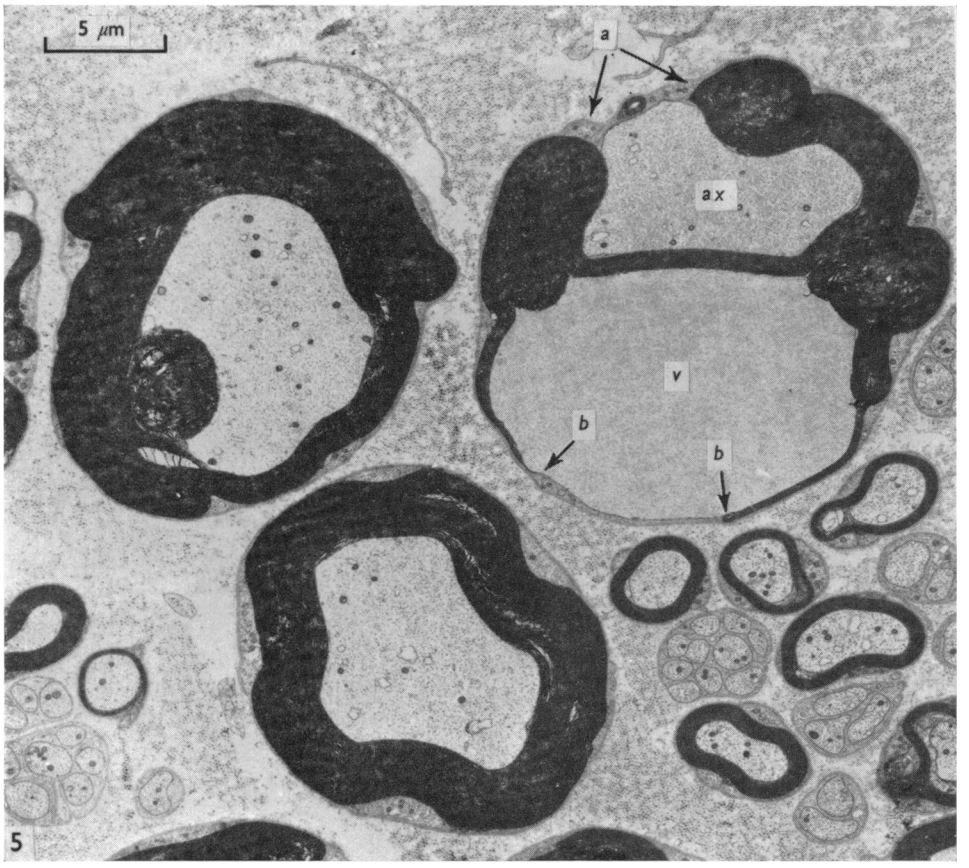


Fig. 5. Transverse section from a nerve, 18 hours after compression, to show myelin rupture and oedema. The fibre on the left shows partial rupture of myelin lamellae. The fibre on the right shows rupture of the whole thickness of the myelin sheath at *a*, the axon (*ax*) being separated from the basement membrane by a thin Schwann cell process. A collection of homogeneous material (*v*) has split the myelin sheath with rupture of the outer lamellae at *b*. Small myelinated and unmyelinated fibres appear normal.

the nodal region appearing as a dark zone within the fibre (see also Fig. 2*a*). In the fibre shown in Fig. 4*c* the nodal region is 120 μm from the constriction marking the site of the Schwann cell junction. The maximal separation of the node from the Schwann cell junction in our material has been approximately 300 μm. In the fibres shown in Fig. 4*b* and 4*c* partial rupture of the myelin has occurred (arrows). This was a common finding when the distance between the node and the Schwann cell junction was greater than 50 μm. It will be discussed in more detail below.

Early changes in Schwann cells and myelin

There were early changes in the cytoplasm of the Schwann cells. The normal cytoplasmic differentiation was sometimes lost and the contents were replaced by homogeneous granular material, this change being accompanied by swelling. An example is

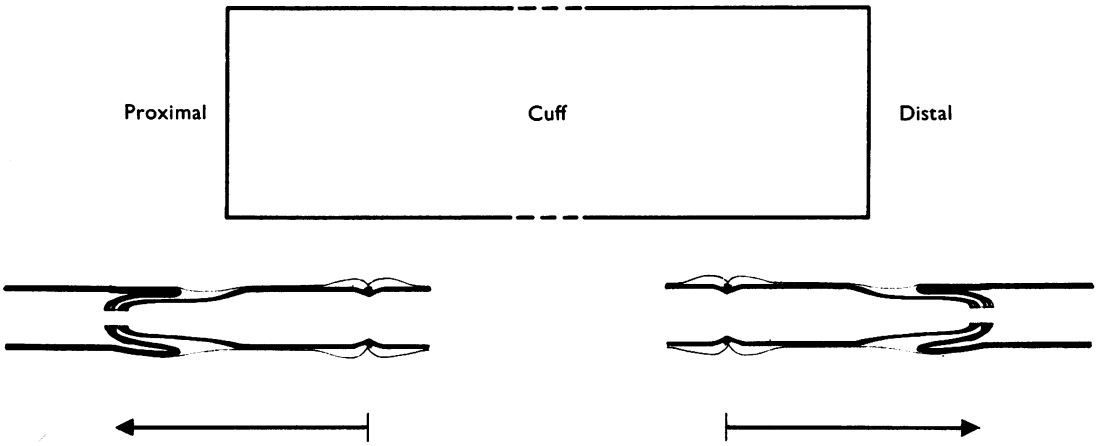


Fig. 6. Diagram to show the direction of displacement of the nodes of Ranvier in relation to the cuff.

shown in Fig. 3*b* and 3*c*. The site of the Schwann cell junction can be identified by the indentation marked *j* in Fig. 3*b*; above it there is swollen Schwann cell cytoplasm containing vacuoles. In the electron micrograph of the same region (Fig. 3*c*) it can be seen that the cytoplasm of Schwann cell S1 has an abnormal homogeneous appearance; it is swollen and lies between a tongue of normal cytoplasm (S2) and the underlying myelin. One vacuole (*v*) is present in the plane of section. In other sections the Schwann cell cytoplasm contained swollen mitochondria, and it seems possible that vacuoles such as those seen in Fig. 3, either membrane-bound or without a membrane (as in Fig. 3*c*), are the result of mitochondrial disintegration.

The cytoplasmic and mitochondrial changes described above were seen in nerves examined during the first week after compression but had disappeared after 2 weeks. A more persistent change occurred in the paranodal myelin itself. Transverse sections of nerves taken within 24 hours of compression showed not only rupture of myelin lamellae but also their separation by homogeneous or finely particulate material, presumably fluid. These changes might then persist for many weeks. Examples are shown in Fig. 5. The fibre on the left shows partial rupture of the myelin with coiling back of the free ends. In the fibre on the right the whole thickness of the myelin is ruptured at the points marked (*a*) and the axolemma is in direct contact with Schwann cell plasma membrane. This fibre also shows a large intra-myelin vacuole (*v*) which is distended with homogeneous material; the outer lamellae appear to have been ruptured at (*b*) by the distension. Such appearances, suggesting intra-myelin oedemain addition to rupture of myelin lamellae, were common in the first few weeks after compression, and were seen to a diminishing extent in later specimens.

Fibre size and susceptibility to damage

A consistent finding in all the nerves examined was the sparing of small myelinated and non-myelinated fibres. In Fig. 5 it can be seen that none of the small myelinated fibres is abnormal. In our teased preparations as well as in transverse sections, the characteristic early lesion, consisting of invagination of one paranode by another,

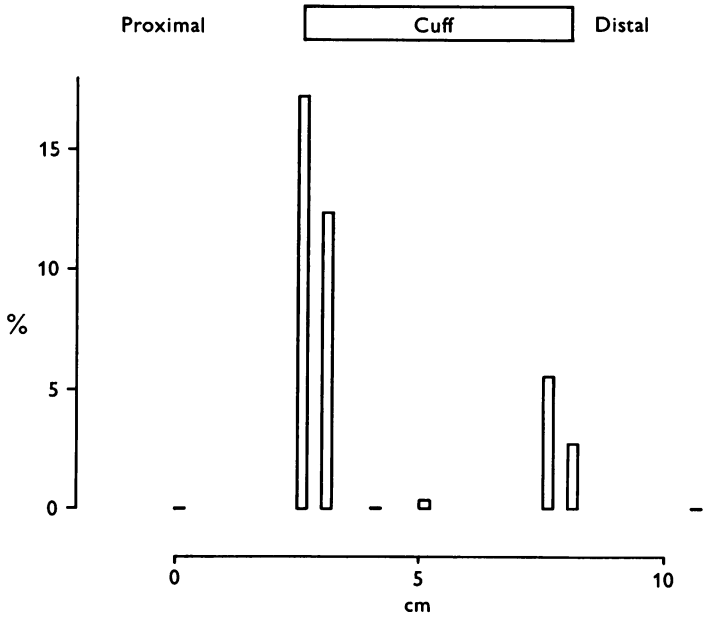


Fig. 7. Medial popliteal nerve, 4 days after compression at 1000 mm Hg for 90 minutes. The histogram shows the proportion of abnormal fibres in transverse sections at different levels under the cuff, the abnormal fibres being expressed as a percentage of the large myelinated fibre population at each level. For further details see text.

was largely restricted to fibres with an axon diameter greater than 5 μm. The only pathological change to be found in small myelinated fibres was Wallerian degeneration, which was sometimes seen in severely affected nerves, in which it also affected a proportion of the large myelinated fibres. In the non-myelinated fibres some early cytoplasmic and mitochondrial changes were seen in the Schwann cells, but actual degeneration of axons was rare.

These results are of interest in relation to the previous observation of Fowler *et al.* (1972) that pain sensation had always preserved in the affected limbs of these animals, even when compression had been sufficiently severe to produce a complete conduction block in the high velocity efferent and afferent fibres.

Distribution of lesions under the cuff

Displacement of the nodes of Ranvier was maximal under the edges of the cuff, with relative or complete sparing under its centre. This distribution was found both in teased fibres and in transverse sections. The teased fibres showed an additional feature; the nodes of Ranvier were displaced in the same direction in all the fibres at any one level under the cuff, the direction in each case being towards uncompressed tissue. Thus the displacement was in one direction under the proximal edge of the cuff and in the opposite direction under its distal edge. This is shown diagrammatically in Fig. 6.

While it was difficult to tease apart long enough lengths to show the complete distribution of lesions in a single fibre, a picture could be built up by dividing the

nerve into 2 cm portions which were teased separately, their orientation being carefully preserved. From these it appeared that successive nodes over a distance of 2–3 cm might be damaged under either edge of the cuff, these regions being separated by a central portion, 1·5–3·0 cm in length, which was largely or completely spared. In each of the affected regions, the depth of invagination of individual nodes gradually increased towards the centre of the damaged zone and then decreased again. There were thus, as judged from the depth of invagination of individual nodes, two points of maximal nerve damage, which were under the two edges of the cuff and separated by approximately 5 cm.

An additional feature was revealed when transverse sections of the whole nerve were cut at different levels under the cuff. Areas containing not less than 800 large myelinated fibres (axon diameter 5 μm or over) were selected at each level for quantitative study. Within these areas the abnormal fibres (showing changes similar to those shown in Fig. 5) were counted and their number expressed as a percentage of the total number of large myelinated fibres in the sample. A histogram based on such an analysis is shown in Fig. 7.

From this it can be seen that the proportion of abnormal fibres was greater under the proximal than under the distal edge of the cuff. At the proximal level approximately 15 % of the fibres in the transverse section were abnormal. It must be remembered, of course, that in each fibre the abnormality would not occupy the whole of the internode but only the part adjacent to the node. If only 15 % of each internode were occupied by the lesion, the result shown in Fig. 7 would indicate that all the large myelinated fibres were affected in the proximal part of the lesion. When histograms showing the proportion of remyelinating fibres at different levels were subsequently prepared from recovering nerves, a similar result was obtained (Ochoa *et al.* 1971; Fowler, 1973). These findings confirm the impression gained from teasing that all the nodes on the large myelinated fibres were abnormal in the most severely affected parts of compressed nerves.

Demyelination

In nerves examined between 7 and 14 days after the application of a tourniquet, some fibres still showed the changes described in the previous sections. In others, the damaged paranodal myelin was clearly undergoing degeneration, droplets and debris being present within the Schwann cell cytoplasm (Fig. 4*d*). Later, the remnants were removed by macrophages which had invaded the region. This process usually affected the myelin of both the ensheathed and the ensheathing paranodes.

After 15 days, no invagination was identified, the most prominent change at this stage being the appearance of paranodal demyelination. An example is shown in Fig. 8*a*. Immediately to the left of the demyelinated zone there is osmiophilic myelin debris. On the right, the myelin appears to taper gradually from its normal thickness as the demyelinated zone is approached. An electron micrograph of the same region (Fig. 8*b*) shows that this tapering occurs in a step-wise fashion, abrupt decreases in myelin thickness occurring at the points marked by arrows, the intervals between them being 10 and 15 μm. These points may correspond to the original sites of Schmidt–Lanterman incisures. One of them is shown at higher magnification in Fig. 8*c*, and it can be seen that the torn lamellae have formed loops with dark

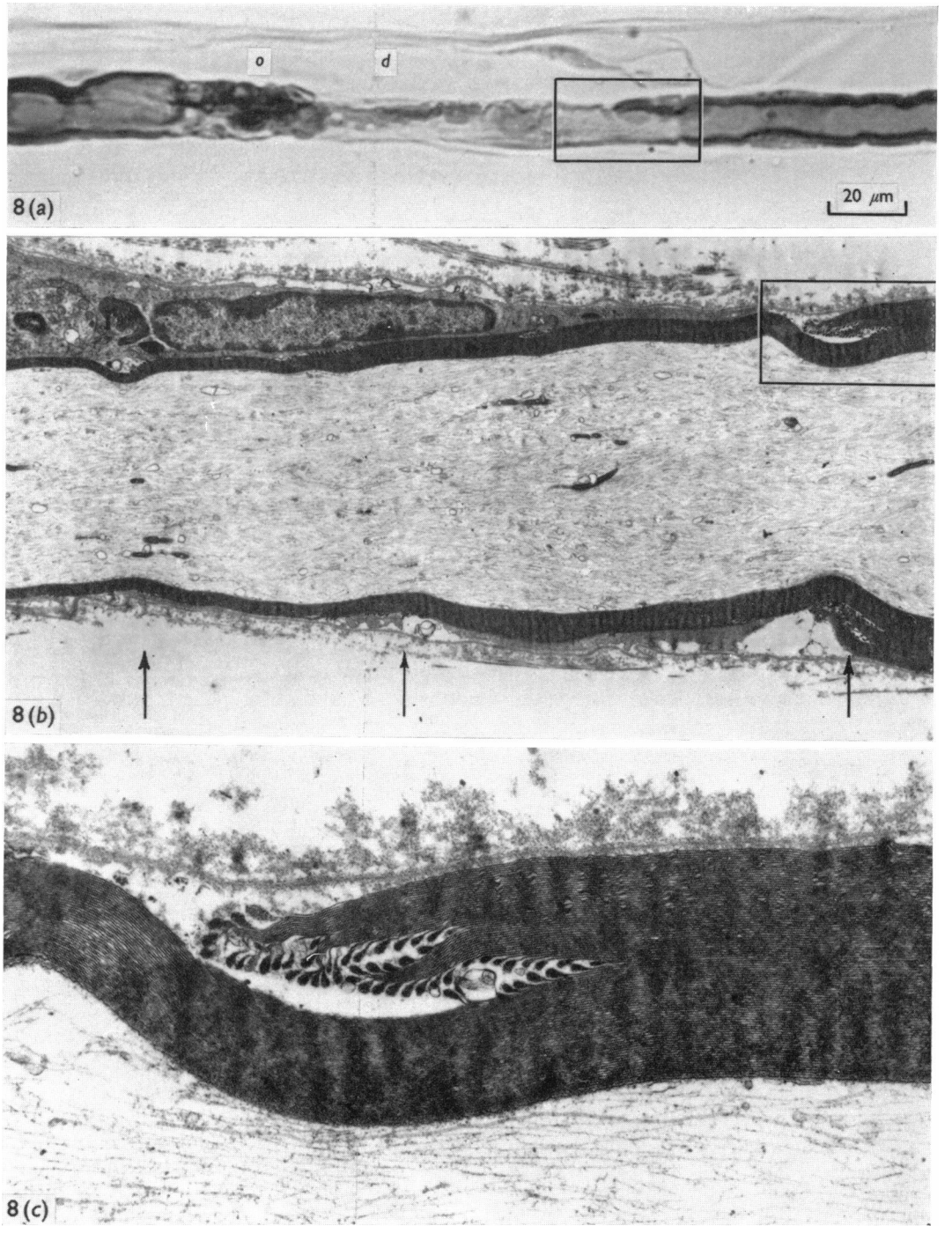


Fig. 8(*a*). Abnormal nodal region, 43 days after compression. A demyelinated stretch of axon (*d*) is limited on the left by osmiophilic debris (*o*) and by tapering myelin on the right.

(*b*) Low power electron micrograph shows detail of area enclosed in rectangle in 8*a*. Tapering results from stepwise interruption of several adjacent myelin lamellae: their free ends occur in clusters at points arrowed. Note mononuclear cell between myelin and basement membrane. × 4000.

(*c*) Enlargement of area enclosed in rectangle in 8*b* to show club-like ends of amputated lamellae. × 19200.

homogeneous contents. Amputation of the myelin lamellae at the Schmidt–Lanterman clefts might cause the appearance shown in Fig. 8*c*, but an alternative explanation would be that these are terminal loops of myelin stripped from the juxta-nodal axolemma.

In any one fibre, does the length which is demyelinated match the distance separating the node of Ranvier and the Schwann cell junction after nodal displacement? This is difficult to answer, as one cannot examine the same fibre at different stages of the same process. We have seen under the electron microscope a Schwann cell junction overlying myelin which gradually tapered towards the edge of a completely demyelinated zone. This suggests that myelin at the edge of an invaginating lesion can survive, so that the length of completely demyelinated axon would be less than the distance by which the node had been displaced. In other fibres, myelin debris was present beyond the zone of invagination, suggesting that the length of axon finally demyelinated would be slightly greater than the distance of nodal displacement. In general, however, the maximal displacement of the node in early lesions (approximately 300 μm) was comparable to the maximal length of axon subsequently demyelinated. Throughout our material demyelination of more than 300 μm has been rare. In contrast to Denny-Brown & Brenner's findings in the cat, we saw very few completely demyelinated internodal segments. When these were found, they usually occurred in severely affected nerves which had been subjected to the maximum compression used.

Severity of the lesion in relation to cuff pressure and duration

The changes described in the preceding sections resulted from compression at 1000 mm Hg. When a pressure of 500 mm Hg was used, the pathological changes were similar but milder in degree. Thus, the depth of invagination and displacement of individual nodes was less, and few fibres were seen in which the myelin was torn. In addition, the length of the affected zones under the edges of the cuff was less. This difference is of some interest in relation to our previous physiological findings, since compressions at 500 mm Hg resulted in slowing of impulse conduction without complete block (Fowler *et al.* 1972).

In the group of nerves compressed at 1000 mm Hg physiological studies showed a significant correlation between the duration of compression and the severity and duration of the subsequent conduction block. One might therefore expect that nerves compressed for 180 minutes would show more severe histological changes than those compressed for 60 or 75 minutes. However, a difference in the extent of displacement of individual nodes of Ranvier was difficult to demonstrate. In one animal, cuffs at 1000 mm Hg were inflated round both knees, one cuff being released after 1 hour and the other after 3 hours. The animal was then killed and the nerves examined. Nodal

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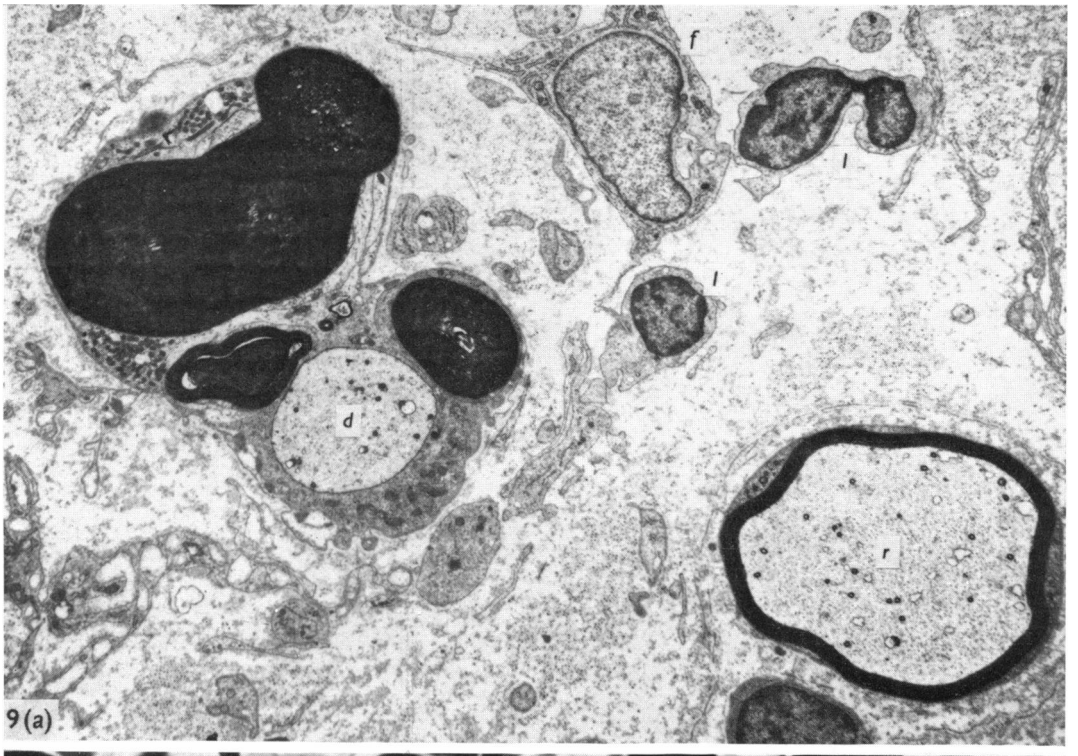
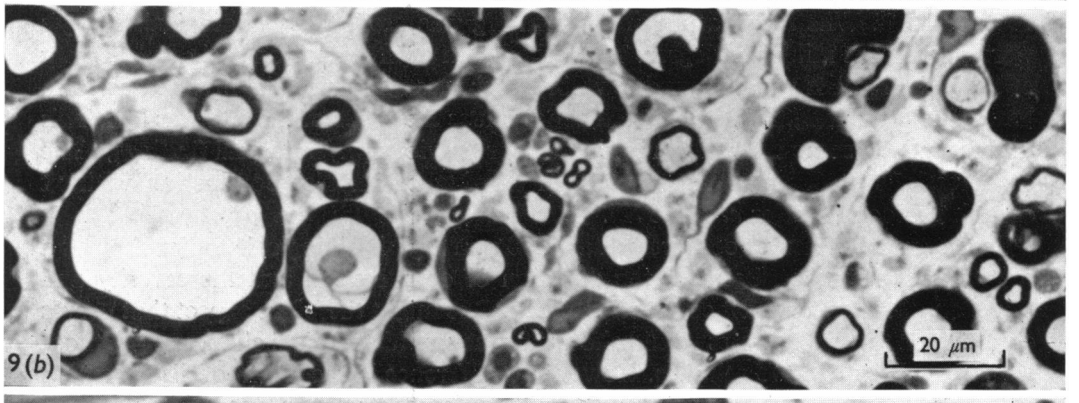
Fig. 9(*a*). Low power electron micrograph of nerve 14 weeks after compression. *d*, demyelinated axon; *r*, remyelinating axon; *f*, fibroblast; *l*, lymphocytes. × 3300.

(*b*) Light micrograph of area adjacent to that shown in 9*a*. There are two swollen fibres with distended myelin and shrunken axons. Epon-toluidine blue.

(*c*) Longitudinal section of another nerve, 6 weeks after compression, to show paranodal situation of swellings. Epon-toluidine blue.



9(a)



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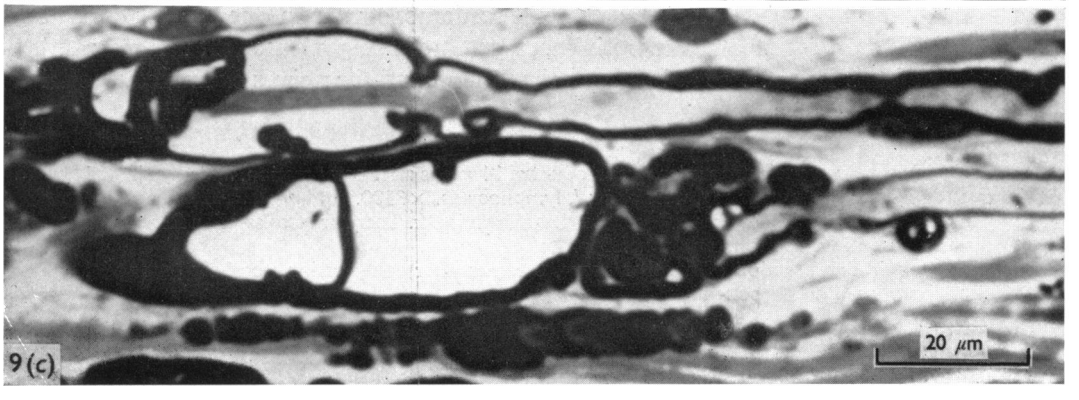


9(c)

displacements of up to 300 μm were seen on both sides but the total number of affected nodes was less after 1 hour of compression than after 3 hours.

There were additional features in nerves subjected to the longer periods of compression. One of these was demyelination of complete internodal segments, which was not seen after compression for 60 to 75 minutes. Even after compression for 180 minutes this was an uncommon finding. When segmental demyelination did occur, its distribution was similar to that of the paranodal demyelination, that is, under the edges of the cuff but not under its centre.

A second feature which distinguished the longer periods of compression was Wallerian degeneration. This was more conspicuous in nerves compressed for 150 or 180 minutes than in those compressed for 90–120 minutes. It was not seen in nerves compressed for only 60 or 75 minutes at 1000 mm Hg or in any of the nerves compressed at 500 mm Hg. No quantitative studies of the amount of Wallerian degeneration in individual nerves were carried out, but an estimate could be made from the electrophysiological findings. In eleven nerves subjected to repeated nerve conduction studies before and after compression, it was estimated by Fowler *et al.* that the proportion of alpha motor fibres undergoing degeneration varied from zero to 30 % with a mean for the whole group of 11 %.

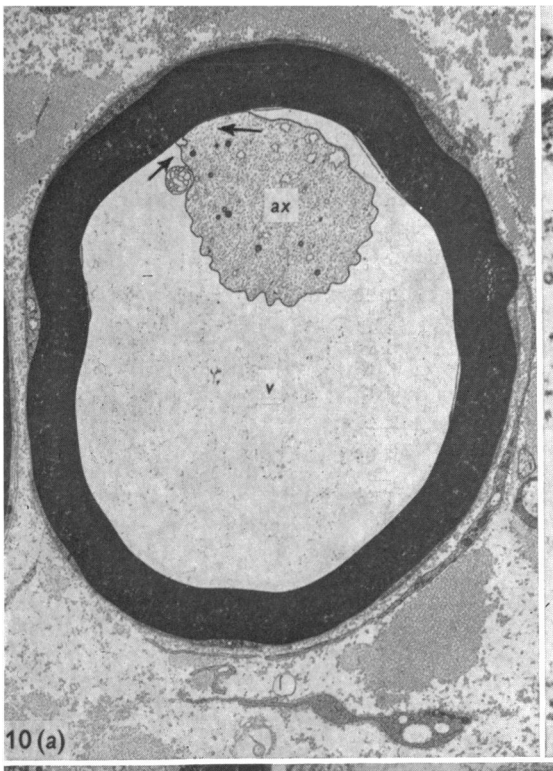
Although repeatedly looked for, no examples were seen of either paranodal or segmental demyelination in nerve fibres in the distal part of the limb. Even in nerves which had been subjected to compression for 180 minutes, and which showed Wallerian changes in a proportion of the fibres at the ankle, demyelination was absent.

Remyelination

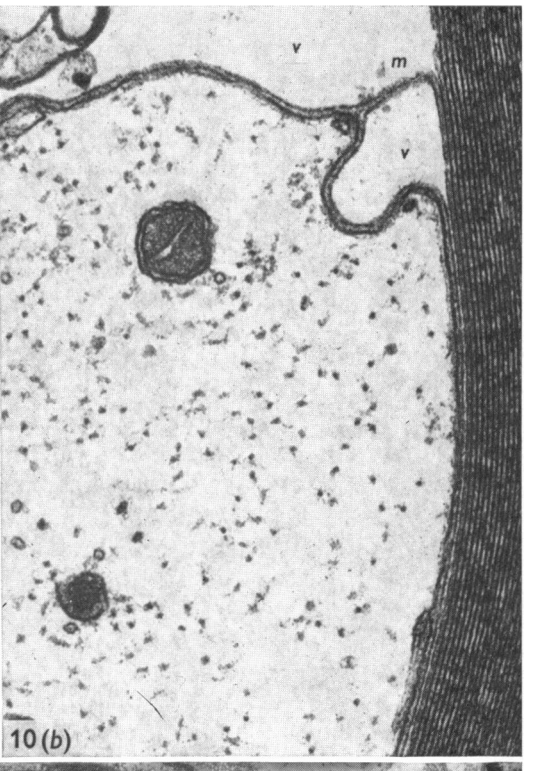
The final stage of the pathological process in nerves affected by compression was usually the intercalation of Schwann cells with the production of short, thinly remyelinated segments (Fig. 4*e*). These were generally similar to the intercalated segments originally described by Renaut (1881) and Lubińska (1958), and subsequently identified in many different forms of peripheral neuropathy (Thomas & Lascelles, 1965; Fullerton, 1966; Hopkins, 1970). At some nodes the appearances during recovery were rather different, no intercalated segment being present, but the paranodal myelin being thin, and tapering gradually towards the node. We were uncertain whether this indicated remyelination by lateral extension of myelin from an intact region as proposed by Allt (1969), or whether the thin tapered myelin was due to the persistence of partial demyelination similar to that shown in Fig. 8.

There was considerable variation in the time-course of remyelination in different fibres of the same nerve. An example is shown in Fig. 9*a*, which was taken from a nerve 14 weeks after compression. On the left there is a demyelinated axon 6 μm in diameter, ensheathed by Schwann cell processes containing myelin remnants. To its right there is a remyelinated axon, 9·5 μm in diameter, which already shows a well-formed sheath of compact myelin.

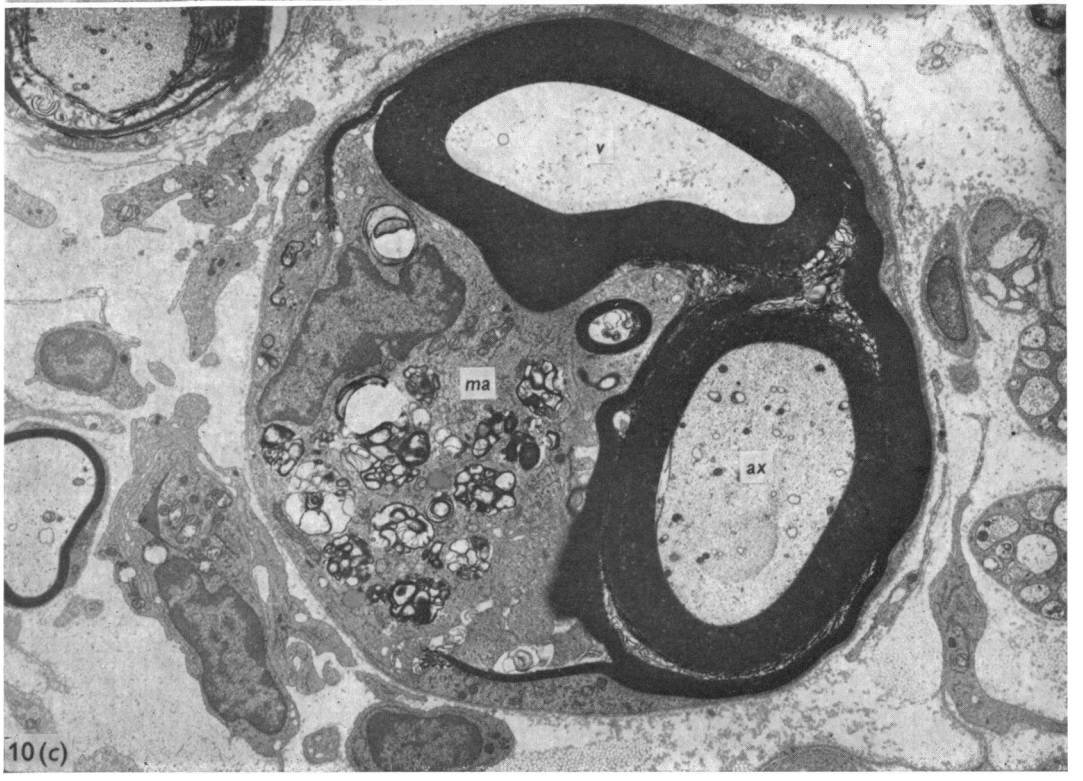
Why should some fibres still be demyelinated more than 3 months after injury? This may be related to a phenomenon seen to some extent in all recovering animals, namely the presence of swollen fibres showing evidence of intra-myelin and peri-axonal oedema. Fig. 9*b* is from the same nerve as in Fig. 9*a*, and shows a mixture of normal, demyelinated and thinly remyelinated fibres. In addition there are two



10 (a)



10 (b)



10 (c)

grossly swollen fibres, each showing a distended myelin sheath separated from a small shrunken axon. Two further examples from a longitudinal section of a nerve 6 weeks after compression are shown in Fig. 9*c*. In one of these the plane of section was such that the axon is not shown and the swelling appears empty. In the other, a shrunken central axon can be seen.

From these and other sections it was clear that these were localized swellings up to 120 μm in length and up to 30 μm in diameter, situated in the paranodal region adjacent to a demyelinated or remyelinated portion of the axon. An electron micrograph of a paranodal swelling is shown in Fig. 10*a*. The axon is shrunken and its fibrous contents are densely packed. It is suspended in a finely granular matrix, suggestive of oedema fluid, which almost completely separates it from the myelin sheath, to which it is still linked by its mesaxon. In this case the fluid accumulated in the inner cytoplasmic tongue of the Schwann cell (Fig. 10*b*). In other fibres the fluid split the compact myelin, leaving several layers covering a shrunken axon. In these fibres also, the fluid was assumed to be intracellular since it split the major dense line.

What is the fate of the paranodal swellings? It appears that they are eventually invaded by macrophages which remove the distended myelin, as in the example shown in Fig. 10*c*. Such delayed demyelination could explain the demyelinated axons and the myelin debris occasionally seen in nerves many months after compression (for example see Fig. 9*a*).

In considering the functional significance of the swellings, two points should be emphasized. In the first place many were present in nerves which had been compressed for long periods at 1000 mm Hg but relatively few were seen after shorter periods at the same pressure; they were rare after compression at 500 mm Hg. Furthermore, the swellings were numerous at a relatively early stage of recovery and had mostly disappeared by the time recovery was complete.

The relationship of these changes to the prolonged conduction blocks seen in our physiological experiments is dealt with in the discussion.

DISCUSSION

The characteristic lesion we have described involves displacement of the node of Ranvier from its normal site; this implies stretching of the paranodal myelin on one side of the node and invagination of the paranodal myelin on the other. Since there is no similar displacement of the Schwann cell junction, the result is lateral movement of the axon and paranodal myelin relative to the Schwann cells on either side of the node; this must cause local rupture of the Schwann cell membrane (mesaxon). The invaginated paranodal myelin remains enclosed within its Schwann cell but is

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Fig. 10(*a*). Low power electron micrograph of a swelling 6 weeks after compression. The swollen inner tongue of Schwann cell cytoplasm (*v*) separates most of the surface of the axon (*ax*) from the myelin sheath. A sector remains attached to the sheath. × 3000.

(*b*) Enlargement of area arrowed in 10*a*. The mesaxon (*m*) is seen with swollen Schwann cell cytoplasm (*v*) on either side of it. × 44000.

(*c*) A myelinated fibre from the same nerve as above, showing axon (*ax*) and a pocket of oedema (*v*). A macrophage (*ma*) has penetrated the basement membrane and broken into the myelin sheath. × 4600.

effectively separated from it, and the invaginating paranodal myelin is extracellular with respect to its own Schwann cell. In both cases degeneration of the myelin follows. Although the Schwann cells show some reactive changes, they retain their viability and it is rare for demyelination to involve complete internodal segments.

Why should nodal displacement occur during compression? We suggest that the primary event is displacement of axoplasm from under the cuff and that the axoplasmic movement meets resistance at the nodes of Ranvier where two factors may operate. In the first place there may be an increase in the viscosity of the axoplasm at the node, this region acting as a plug which is then forced in the direction of axoplasmic movement. Alternatively, the normal narrowing of the axon which occurs at the node (Hess & Young, 1952; Berthold, 1968) could form an obstacle to the free movement of axoplasm. In both cases the result would be displacement of the nodal axolemma and of the myelin attached to it.

The normal narrowing of the axon at the node would provide a possible explanation for the selective effect of compression on the large myelinated fibres, with sparing of small myelinated fibres. In the latter, the axons are not narrowed at the nodes, so that movement of axoplasm along the fibre could occur without nodal displacement. In suggesting this we have assumed that movement of axoplasm along small fibres does occur with the cuff pressures we have used. There is, however, no proof that this is so. Since the force required to displace fluid within a tube is inversely related to the diameter, it is possible that the pressures we used were sufficient to produce axoplasmic movement in large fibres but not in small ones. Direct measurement of axoplasmic movement in small fibres would be necessary to answer this question.

Why are the lesions concentrated under the edges of the cuff? This could be explained by the pressure gradient in the tissues between the parts under the cuff and those beyond its edge. With the relatively wide cuff we have used, the gradient would be maximal under the edges of the cuff and least under its centre. Without such a gradient one would not expect axoplasmic movement or displacement of the nodes of Ranvier to occur, even if the absolute pressure in the tissues were high. As an example of the ability of a peripheral nerve to withstand high pressures provided that there is no gradient between one part and another, one may cite the experiments of Grundfest (1936), who showed that when an isolated portion of frog nerve was wholly enclosed in an oxygenated pressure chamber, pressures of the order of 1000 atmospheres were necessary to abolish conduction. It is interesting that both Edwards & Cattell (1928) and Bentley & Schlapp (1943) considered that the effect of pressure on peripheral nerves was to produce distortion which was maximal at the edges of the compressed part. The latter authors found that when exposed cat sciatic nerve was compressed between two pneumatic cushions, most of the fibres appeared to be blocked at one or other edge of the compressed zone rather than at the centre.

In our own experiments we do not know whether the conduction block which follows a tourniquet is due to obliteration of the nodal gap itself, or whether changes in membrane permeability and impedance elsewhere along the internode might be responsible. From the physiological studies of Fowler *et al.* (1972) it seems that mild compression produces slowing of conduction, whereas more severe compression results in conduction block. Using diphtheria toxin in the rat, Rasminsky & Sears

(1972) have studied the effect of demyelination on conduction in single fibres. Their conclusion, which may be relevant to the present results, was that myelin damage first resulted in a reduced current flow at the node and a delay in the activation of adjacent nodes. There was thus an overall fall in conduction velocity before the myelin damage was sufficient to block conduction completely. Further studies of velocity changes in single fibres and in different regions under the cuff in our tourniquet lesion are clearly required to investigate this question further.

It was shown by Fowler *et al.* (1972) that the conduction block which follows compression at 1000 mm Hg might last for many weeks or months, and that there was a significant correlation between the duration of compression and that of the subsequent block. In these experiments conduction was studied in the nerve fibres supplying a small foot muscle, the motor fibres being stimulated above and below the cuff and the muscle response being recorded from abductor hallucis. Using needle electrodes inserted through the skin, this procedure could be carried out repeatedly under anaesthesia without damage to the nerve or muscle. It should be emphasized that these physiological experiments were concerned with conduction in a selected group of motor fibres passing under the cuff, whereas the anatomical studies were carried out on the whole medial popliteal nerve trunk at that level. For this reason an exact correlation cannot be made between the degree of physiological block and the pathological appearances of the particular fibres responsible for it.

It is, however, possible to make some general comments concerning the long delay which often occurred before conduction in damaged fibres was resumed. In this context we attach considerable importance to the paranodal swellings described above. These swellings are apparently due to the distension of the myelin sheath by oedema fluid; the axon itself is shrunken and perhaps compressed. Such swellings may be found in decreasing numbers in nerves for several months after compression and it seems reasonable to suggest that they are responsible for the conduction block which persists in some fibres for a similar period. Their late breakdown would account for the occasional demyelinated axon seen during the later stages of recovery (Fig. 9*a*), and it seems unnecessary to postulate an additional defect of remyelination to explain the presence of such axons.

In addition to the variation in the rate of recovery we have shown to exist between one fibre and another, it must be remembered that there may be a similar variation between different nodal lesions on the same fibre. If as many as 30 nodes of Ranvier on a single fibre were damaged initially, one must envisage a series of separate conduction blocks along the fibre, each one of which must recover before conduction could be resumed through the whole region. In such a situation the physiological deficit would be more severe than the histological appearances might suggest.

The origin of the paranodal swellings requires special comment. In some ways their appearance is reminiscent of the distended fibres seen in cerebral oedema due to tri-ethyl tin (Aleu, Katzman & Terry, 1963; Hirano, Zimmerman & Levine, 1968). Similar appearances were described by Spencer & Thomas (1970) in peripheral nerve fibres proximal to nerve section. In both cases, however, there were differences. For example, the swelling seen after tri-ethyl tin appeared to be generalized rather than localized to the paranodal regions. In the case of nerve section the swellings were localized but occurred in thinly myelinated fibres which were thought to be

remyelinating. In contrast to this, the swellings seen in the present study always occurred in the terminal portions of the original internodes, although they were adjacent to those parts of the axon which were demyelinated or thinly remyelinated (that is, the sites of previous invagination). In some cases the distended myelin was of normal thickness, indicating that partial myelin rupture of the type shown in Fig. 5 was not a pre-requisite for the formation of swellings. There was also a difference in the distribution of the oedema fluid seen in the first few days after compression and that seen at a much later stage. In the former it was never limited to the inner tongue of Schwann cell cytoplasm surrounding the axon. In the late paranodal swellings, however, oedema fluid in this situation was a common finding. In spite of these differences, it seems reasonable to conclude that the late swellings are the result of the intra-myelin oedema which is first seen within a few hours of compression, and which can, apparently, persist and alter its distribution with the passage of time. In contrast to this, oedema of the Schwann cell cytoplasm external to the myelin sheath was only seen during the first few days, after which it disappeared.

There has in the past been considerable discussion as to the role of ischaemia in relation to the local demyelination which results from compression. It was argued by Denny-Brown & Brenner (1944*a*) that the pressure was itself insufficient to produce direct mechanical damage, and that it acted by producing secondary ischaemia of the compressed region. This was similar to the mechanism postulated by Lewis, Pickering & Rothschild (1931) for the reversible physiological block which is produced in man by a sphygmomanometer cuff inflated to a pressure slightly greater than that of the systolic blood pressure. While we accept that ischaemia may be responsible for the reversible physiological block described by Lewis *et al.*, it is clear that the anatomical changes which we have described must be a direct result of the applied pressure. It could not be argued that a lesion such as this one, involving as it does the displacement of anatomical structures, could be caused by ischaemia alone.

It may be added that ischaemia alone did not produce demyelination in our experiments. Distal to a cuff at 1000 mm Hg conduction in motor fibres failed within 40–50 minutes, but continuation of the procedure for 2–3 hour periods did not result in demyelination distal to the site of compression. Ischaemia combined with minimal compression was no more effective in producing demyelination. This was tested by using a cuff pressure of 250 mm Hg (Ochoa, Rudge & Gilliatt, unpublished observations). The systolic blood pressure in an anaesthetized baboon is approximately 120 mm Hg, and our standard cuff at 250 mm Hg produced complete circulatory arrest, the reversible physiological block in fibres distal to the cuff having the same time-course as that produced by a cuff pressure of 1000 mm Hg. However, when the cuff at 250 mm Hg was maintained for 2 hours, there was no persistent conduction block or conduction delay, and subsequent anatomical examination of the compressed nerve revealed no demyelination.

Although ischaemia alone or in combination with compression at 250 mm Hg did not produce demyelination in our experiments, it is possible that in combination with compression at 500 or 1000 mm Hg it could contribute to the severity of the result. We know that compression at 1000 mm Hg for 3 hours produces a longer-lasting conduction block than a similar pressure applied for 1 hour. The results

described in the present paper suggest that this may be related to the greater number of paranodal swellings seen after prolonged compression. Could these latter changes be the result of compression combined with prolonged ischaemia? In the case of the rapidly reversible cytoplasmic and mitochondrial changes we have seen in the Schwann cells external to the myelin, the appearances are in some ways similar to those described by Webster & Ames (1965) and McGee-Russell, Brown & Brierly (1970) after anoxia or hypoglycaemia. While we are not aware of any published reports of intra-myelin oedema resulting from ischaemia, its appearance after cyanide administration in mice (S. Hall, personal communication) suggests that it can occur. The possible contribution of ischaemia to the intra-myelin oedema and thus to the paranodal swellings seen in the present study clearly requires further investigation.

Finally, one may ask whether displacement of the nodes of Ranvier occurs in pressure lesions other than those caused by tourniquet. While it seems probable that a similar basic lesion is present in a variety of human pressure palsies of the type described by Seddon as due to 'neurapraxia' (Seddon, 1943), there is at the present time no direct evidence that this is so, and further experiments are in progress to establish this point.

There are, however, three other situations which require mention. The first of these is the nerve crush in which, as Lubińska has shown, there is paranodal demyelination involving two or three nodes proximal to the level of the crush (Lubińska, 1959, 1961). It seems possible that the paranodal demyelination might be the result of axonal movement caused by the crush and, indeed, appearances suggesting invagination of myelin are shown in Figs. 18 and 19 of Lubińska (1959). The other situation in which this lesion may occur is in the much older experiment originally described by Ranvier in 1878. In this, an isolated segment of frog nerve was compressed with a spring clip *in vitro*; Ranvier's drawings and his description of the resulting changes strongly suggest that paranodal invagination had occurred, although the nerve had already been placed in fixative at the time of compression.

These latter experiments are cited by Hess & Young (1952), who studied single teased nerve fibres which had been placed in silver nitrate and nitric acid for several hours (to stain the cross of Ranvier at the nodes) and then washed and mounted in glycerin. They found that slight pressure on the coverslip of these preparations caused the axon to 'move within the sheath leaving stained neurilemma at the node'. In this way they produced displacement of the nodal axoplasm; from their illustrations it is clear that the cementing disk remained in its original position, but it cannot be seen whether invagination of the paranodal myelin occurred.

SUMMARY

A pneumatic cuff inflated to 1000 mm Hg round the knee for 1–3 hours has been used to produce pressure neuropathy in the baboon. A previous study has shown that after this procedure a conduction block in the medial popliteal nerve may persist for several weeks or months.

An electron microscope study of single teased fibres has shown that the primary lesion caused by compression of the large myelinated fibres is a displacement of the node of Ranvier from its usual position under the Schwann cell junction. This is

accompanied by stretching of the paranodal myelin on one side of the node and invagination of the paranodal myelin on the other.The nodal axolemma can sometimes be identified as far as 300 μm from its original position under the Schwann cell junction. In such cases there is partial or complete rupture of the stretched paranodal myelin.

The nodal displacement is maximal under the edges of the cuff, with relative or complete sparing under its centre, the direction of displacement always being away from the cuff towards uncompressed tissue. These findings suggest that the pressure gradient within the nerve between its compressed and its uncompressed parts is the factor responsible for the movement.

This nodal lesion is followed by breakdown of the paranodal myelin. Remyelination subsequently occurs with the formation of short intercalated segments. Repair may be delayed by intramyelin and periaxonal oedema, which results in localized swellings in which a shrunken axon is separated by oedema fluid from a distended myelin sheath. Such local swellings can be found in diminishing numbers for several months after compression. It is suggested that their persistence could be responsible for the delay in functional recovery.

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