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A quantitative study of anterior root fibres during early myelination. II. Longitudinal variation in sheath thickness and axon circumference

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INTRODUCTION

The natural history of the maturation of peripheral nerve fibres has been relatively thoroughly documented and has been reviewed by Elfvin (1968) and by Peters & Vaughn (1970).

Both sheath thickness and axon circumference of immature fibres tend to vary from one level to another along a given internode (Robertson, 1962; Webster, 1971). The extent of such variation in sheath thickness can be studied by serial longitudinal sections, but it is exceedingly difficult to form an accurate estimate of variation in axon calibre by this method. In the present study the longitudinal variation in both parameters is measured using serial transverse sections at both light and electron microscopic levels, supplemented by the use of longitudinal electron microscopic sections to investigate variation in sheath thickness in the regions adjoining the nodes of Ranvier. This method also avoids the uncertainties as to the effects, especially on axon calibre, of the teasing apart of individual fibres.

It is generally accepted that there is a strong linear correlation between compact myelin sheath thickness and axon calibre in more mature nerve fibres (Friede & Samorajski, 1967; Samorajski & Friede, 1968; Williams & Wendell-Smith, 1971; Fraher, 1972). However, this correlation is considerably lower in immature fibres (Samorajski & Friede, 1968; Friede & Samorajski, 1970; Fraher, 1972). Such estimates of the correlation between sheath thickness and axon circumference, based on transverse sections of single levels of nerve fibres, do not take into account variations in either parameter within the internode, since the position within the internode at which a given fibre had been sectioned (subsequently referred to as the 'sheath level') cannot be identified except in the case of the region immediately adjoining the node. The uncertainty as to level of section can be minimized if the fibre is serially sectioned, for by this means an accurate measurement can be made of the number of turns of compact myelin at the level at which the sheath is thickest in any particular internode. This value represents the maximum myelin-forming activity attained by the Schwann cell in that internode. The arithmetic mean of all values for sheath thickness measured in serial sections of an internode is manifestly a much more satisfactory representation of the thickness of the sheath as a whole than a single value taken at an unknown internodal level. Similarly, the arithmetic mean of

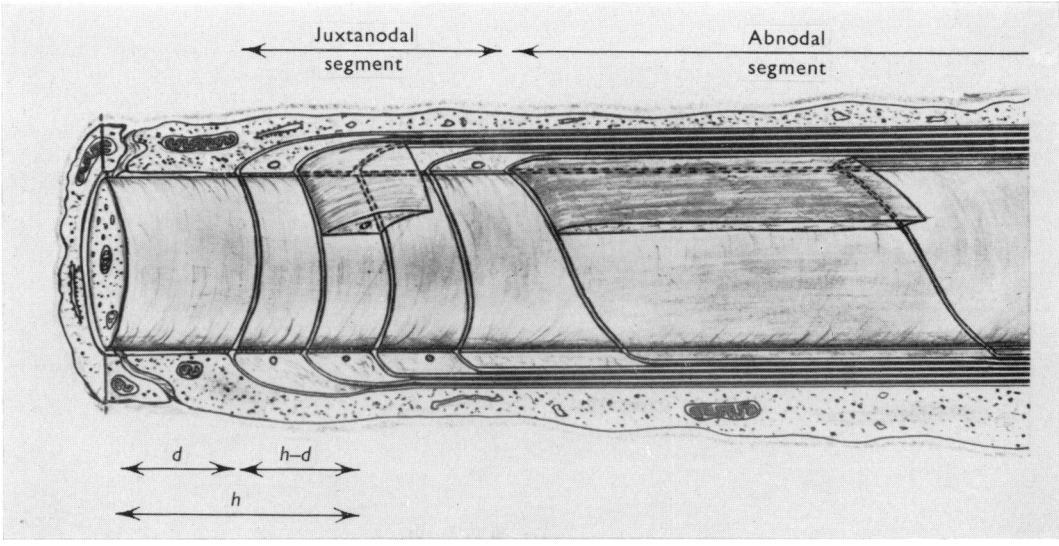


Fig. 1. Diagram of an immature myelinated fibre showing the broadening and flattening of successive turns of the inner Schwann cell cytoplasmic helix when traced away from the nodal region, facilitating the subdivision of the helix into juxtanodal and abnodal segments from a relatively early stage. The heminodal length (*h*) and its constituents parts *d* and *h–d* (see text) are also indicated.

several values for axon circumference is more representative of the internodal axon calibre as a whole than a single value taken at an unknown sheath level. The present study deals with the correlation between sheath thickness and axon circumference when such longitudinal variation of both parameters is taken into account.

For the purposes of the present investigation certain terms are defined as follows:

(i) *Midnodal level.* This is the centre of the zone of interdigitating Schwann cell processes or of microvilli in the case of more mature nodes.

(ii) *Heminodal length.* This is the distance between the midnodal level and the beginning of the compact myelin sheath (Fig. 1).

(iii) *Juxtanodal and abnodal segments.* The helix made up of that compartment of the Schwann cell cytoplasm which intervenes between the axon and the compact myelin sheath and the terminal cytoplasmic pockets (Fig. 1) tends to be much more tightly wound adjacent to the node (*juxtanodal* segment) than that part which is closer to the centre of the internode (*abnodal* segment). Except in the very earliest stages of myelination these two segments of the inner Schwann cell cytoplasmic helix can nearly always be clearly demarcated from one another (Fig. 1).

(iv) *Adnodal margins.* The unwound Schwann cell can clearly be roughly described as having four free margins (Fig. 16), two approximately parallel to the long axis of the axon, and two oblique to it. The latter pair will be referred to as the *adnodal margins* of the Schwann cell.

MATERIALS AND METHODS

Rats aged 1, 2, 4, 6, 12 and 17 days after birth were used. The method of fixation and embedding of tissue has been described already (Fraher, 1972). The animal was anaesthetized with ether and was then perfused through the left ventricle with a solution at 4 °C of 4% paraformaldehyde and 0·5% glutaraldehyde in phosphate buffer at a pH of 7·2. Following bilateral laminectomy, the 12 to 14 most rostral spinal medullary segments, together with the attached pairs of roots and the proximal parts of the spinal nerve trunks, were exposed by careful dissection under a dissecting microscope, and, having been removed *en bloc*, were osmicated and divided by transverse section into constituent segments. The curvature, shape, and orientation of each ventral root and its constituent rootlets were noted, and the segments were embedded in Araldite.

Transverse sections

The manner in which myelin sheath thickness varied along the entire length of an internode was studied in roots taken from rats 1, 2, 6, 12 and 17 days old. A single root was used at each age, and at 1 and 2 days was taken from the same animal as was used in the investigation of axon circumference/myelin sheath thickness relationships described previously (Fraher, 1972); at 17 days a litter mate was chosen instead. In each case ultrathin transverse sections of a lower cervical (C_6 or C_7) ventral root were made at each of 10 to 14 levels, the first level studied being placed about one-third of the way along the root between the attachment of its component rootlets to the spinal medulla and its point of fusion with the corresponding dorsal root. The sections were stained with 0·2% lead citrate for two minutes and then with uranyl acetate (saturated solution in 50% ethanol) for 10 minutes, and were examined in a Metropolitan-Vickers EM 6 electron microscope. The distance between adjacent levels was about 20 μm in all cases except the two day old, in which it varied from 4 to 20 μm. The total lengths of root studied varied from 180 to 300 μm. Thick (0·5 to 0·75 μm) sections were made of those portions of the roots between the levels at which ultrathin sections were taken and were mounted serially and stained for examination under the light microscope with a mixture of 0·8% toluidine blue and 0·2% pyronin B. Care was taken to ensure as far as possible that each root was sectioned transversely at all levels. All transverse sections were made using an automatic Porter–Blum ultramicrotome in order to obtain a more precise estimate of distances along the fibres examined.

In each root an area containing 70 to 100 myelinated fibres was chosen, photographed, and printed at $\times 2000$ optical magnification from the serial thick sections at intervals sufficiently close to one another (2–3 μm) to enable each fibre to be followed through the whole length of root studied. An electron micrographic montage of the corresponding area of the root was made at each of the levels at which ultrathin sections were cut. The electron optical magnification varied from one root to another, being approximately $\times 4000$, $\times 6000$ or $\times 8000$, whichever was the minimum sufficient to allow easy distinction between myelin lamellae when the plate was magnified $\times 20$. The exact magnification for each photographic session was calculated using a calibration grid. Light micrographs were made of the thick sections immedi-

ately preceding and following the ultrathin sections at each level, thereby allowing each fibre to be readily identified in the adjacent electron micrographic montage.

It is clearly possible to identify nodes of Ranvier in transverse section. The principal criteria for their identification by light microscopy from the serial sections were the disappearance of compact myelin over a short length of the fibre and the presence of dark-staining irregular Schwann cell processes around the axon, the axoplasm of which usually showed a considerable increase in density at nodal levels.

For the 1, 2, 6 and 12 day old roots those fibres for which both ends of one myelin segment were clearly identifiable on the light and/or electron micrographs were included in the study and each was given a serial number. Since the present part of the study was primarily concerned with the contour of the myelin sheath, and since the resolving power of the light microscope was in any case insufficient to enable identification of the midnodal level, distances along the internode were measured from the levels at which the compact myelin was seen to end. For each fibre both sheath thickness and axon circumference were measured at each of several sheath levels, i.e. from each of the electron micrographic montages which lay between the two ends of the compact sheath. Sheath thickness was measured by counting the *number of turns* of compact myelin from the negative electron micrographic plate, using a dissecting microscope in conjunction with a horizontal illuminated ground glass screen. Axon circumference was measured in mm from the electron micrographic print using a map meter and was expressed in μm, correct to the nearest 0·1 μm, using correction factors obtained as indicated previously (Fraher, 1972). If at any given level a fibre was judged to have been sectioned obliquely according to the criteria given previously (Fraher, 1972), its circumference measurement at that level was not taken into account.

The distance from both ends of the compact myelin sheath of each of the levels at which ultrathin sections were made was known with considerable precision for all roots studied except the 17 day old roots, in which the internodal lengths were for the most part greater than the distance covered by the present study. In the 17 day roots 25 fibres were selected at random from those for which the serial sections included one node of Ranvier, and each was given a serial number. Sheath thickness and axon circumference were measured at several levels, each at a known distance from one end of the sheath. From longitudinal sections it was evident that sudden marked variations in sheath thickness were very unusual. Accordingly, it was considered justifiable to represent sheath thickness variation by joining with straight lines the points plotted on the graphs expressing sheath thickness at various levels.

For each internode studied at each age as outlined above the following statistics were noted:

(i) in relation to sheath thickness (expressed as number of turns): (*a*) the maximum value (the probable maximum value at 17 days), (*b*) the arithmetic mean of all values, excluding those which were obviously paranodal;

(ii) in relation to axon circumference (expressed in μm): the arithmetic mean, standard deviation and coefficient of variation.

The values for maximum and mean sheath thickness and for mean axonal circumference were used in correlation and regression analyses performed at each of the five ages studied.

Longitudinal sections

Transverse sections separated by such distances as described above fail in most cases to give any clear indication of the manner in which sheath thickness varies in the juxtanodal region. This segment of the nerve fibre was studied from longitudinal ultrathin sections stained for the electron microscope as previously described. Magnification was approximately × 13000; calibration was carried out as described above at each photographic session. The number of roots examined was as follows: three in the newborn and at 1, 2 and 6 days after birth, two at 12 and 17 days after birth, and one at 4 days after birth. In the 1, 2, 6, 12 and 17 day old animals one of the roots examined in each case was either the other ventral root belonging to the same segment as the root from which the transverse sections were derived, or one member of the immediately rostral or caudal pair of ventral roots. Other roots were in each case taken from siblings.

In relation to each fibre examined, note was taken of each of the following, where the plane of section permitted (see Fig. 1):

(i) The heminodal length.

(ii) The sheath thickness (expressed where possible as the number of completed turns), measured at several levels along the internode, the distance of each from the midnodal level being recorded.

(iii) An indication of the pitch of the helix of periaxonal Schwann cell cytoplasm, obtained for all roots examined by measuring the distances from the midnodal level at which successive turns could be seen to terminate in the longitudinal sections.

It proved difficult to follow the thin myelin sheaths of newborn roots in transverse section under the light microscope between levels at which ultrathin sections were cut for the electron microscope. Accordingly, examination of sheath thickness variations along the internode was in this case restricted to the study of longitudinal ultrathin sections under the electron microscope as described above. For this and also for the 4 day old root the measurements made were of the same nature as those described above for longitudinal sections at the other ages studied. In addition, however, a study was made of the manner in which sheath thickness varied at known levels along segments of fibres which were not sectioned through a node and therefore at levels whose distance from the bounding nodes was unknown.

OBSERVATIONS

The numbers of fibres used to study the manner of variation of myelin sheath thickness along the internode were as shown in Table 1.

Variation in compact sheath thickness

Figs. 3*a*, 4*a*, 6*a*, 7*a* and 8*a* show sheath thickness at indicated levels along the length of typical compact myelin sheaths at various ages. The entire length of each sheath was studied by serial transverse sections. Tables 2 and 3 show mean and maximal compact sheath thickness, as well as mean, standard deviation and coefficient of variation of axon circumference for several such internodes.

Table 1. *The numbers of fibres studied by transverse and by longitudinal sections at each age*

Age (days)	Number	
	By serial transverse sections (l.m. and e.m.)	By longitudinal sections
Newborn	—	57
1	22	49
2	28	42
4	—	21
6	28	32
12	35	13
17	25	11

Table 2. *Examples of data derived from study of the entire length of internodes at one, two and six days after birth*

(For each internode the maximum internodal sheath thickness and the mean of all sheath thickness measurements are given (both expressed as number of turns) together with the mean of all axon circumference measurements ($\bar{x}$), its standard deviation (s.d.) and coefficient of variation (c.v.).)

Age (days)	Fibre no.	No. of turns		Axon circumference		
		Max.	Mean	$\bar{x}$ (μm)	s.d.	c.v.
1	1	11	9·0	5·4	0·8	14·8
	2	8	7·0	5·4	0·4	6·9
	3	5	4·8	6·2	1·4	21·7
	4	11	8·4	6·2	1·3	21·2
	5	13	10·8	7·0	1·0	14·7
	6	11	9·0	6·8	2·2	31·7
	7	12	10·8	5·5	0·3	5·8
2	1	9	8·3	5·0	0·9	18·0
	2	17	14·1	5·2	1·5	28·8
	3	18	15·7	6·1	1·0	15·6
	4	20	18·0	7·5	0·6	8·0
	5	13	11·1	6·0	0·9	15·0
	6	14	13·1	5·8	0·8	13·8
	7	7	6·0	5·8	1·4	24·1
6	1	27	25·9	6·5	0·7	10·9
	2	28	25·8	8·0	1·5	19·3
	3	30	28·0	9·0	1·1	12·3
	4	25	24·2	6·7	0·8	11·2
	5	20	20·0	8·0	1·6	20·5
	6	31	29·6	9·9	1·1	11·5
	7	25	23·9	6·6	1·0	15·3

Table 3. *Examples of data derived from study of the entire length of internodes at 12 days and from study of large proportions of the length of internodes at 17 days after birth*

(For each internode, the maximum internodal sheath thickness and the mean of all sheath thickness measurements are given (both expressed as number of turns), together with the mean of all axon circumference measurements ($\bar{x}$), its standard deviation (s.d.) and coefficient of variation (c.v.).)

		No. of turns		Axon circumference		
Age (days)	Fibre no.	Max.	Mean	$\bar{x}$ (μm)	s.d.	c.v.
12	1	24	22·2	6·9	0·7	9·9
	2	33	32·0	9·6	2·0	21·3
	3	29	28·2	8·1	1·1	14·0
	4	23	22·0	5·4	0·4	8·0
	5	33	30·9	7·8	1·4	18·1
	6	46	44·4	11·8	2·7	22·7
	7	29	26·6	7·2	1·1	15·1
	8	35	33·6	8·8	1·5	16·8
	9	41	38·7	10·6	1·7	15·9
17	1	55	52·8	14·6	1·7	11·5
	2	28	27·3	4·7	0·8	16·0
	3	59	56·9	13·9	1·1	8·2
	4	18	16·5	5·3	0·6	10·5
	5	57	55·0	13·2	0·9	7·0
	6	62	61·2	13·8	2·7	19·4
	7	17	16·8	4·6	1·8	40·1
	8	25	23·8	5·8	1·0	18·0

Newborn

Longitudinal sections through the newborn root (Fig. 2*a*, *b*) indicated that many compact sheaths of thickness close to the minimum observed (i.e. of 3 to 6 turns) consisted of a similar or unvarying number of turns over lengths of internode comprising a fraction of the total Schwann cell length which was considerable when compared with values estimated from serial transverse sections at one day after birth, and also with those given for this length of 60 to 70 μm by Webster (1971) for 7 day old rat sciatic nerve fibres at a similar degree of maturation.

1 Day

The thinner sheaths were similar to those in the newborn. Longitudinal sections of fibres probably belonging to the latter class (Fig. 3*b*) indicated that the number of turns changed little up to the zone of transformation from compact to non-compact myelin. Of the thicker sheaths about half were relatively symmetrical, i.e. the number of turns was maximal in the vicinity of the centre of the internode and diminished at a similar rate on either side of this when traced towards the bounding nodes (Fig. 3*a*, second example). This rate of decrease varied somewhat from one fibre to another; on average, at 10 μm from the level at which they became non-compact, such sheaths had decreased in thickness to a value which was 71 % of the maximum.

The remainder of the thicker sheaths were somewhat asymmetrical, a few of them

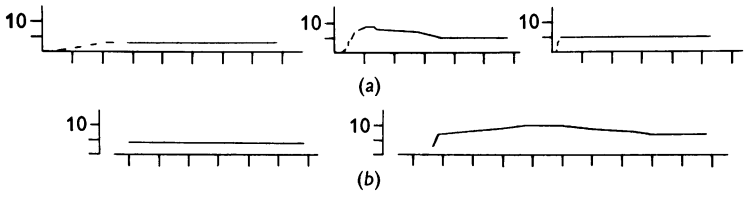


Fig. 2 Newborn

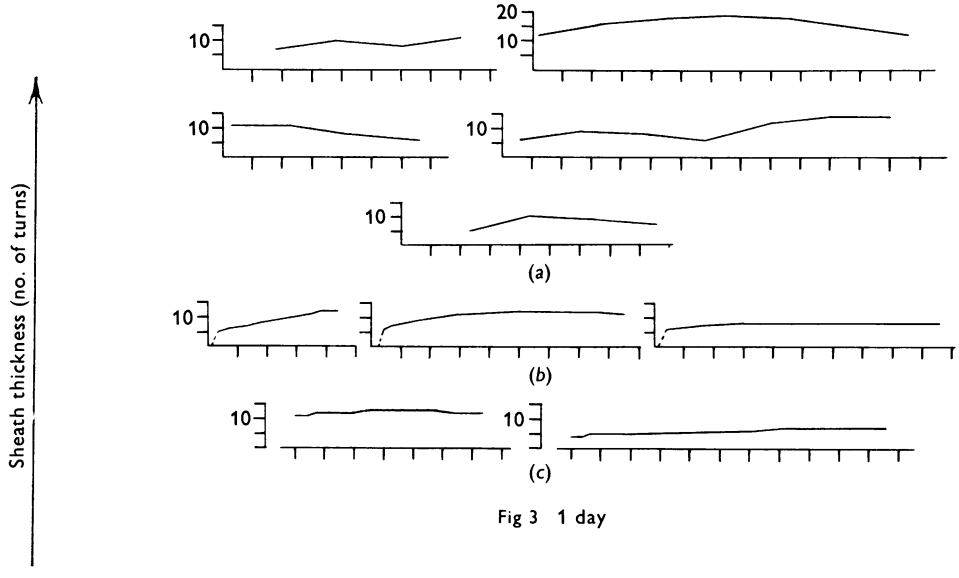


Fig 3 1 day

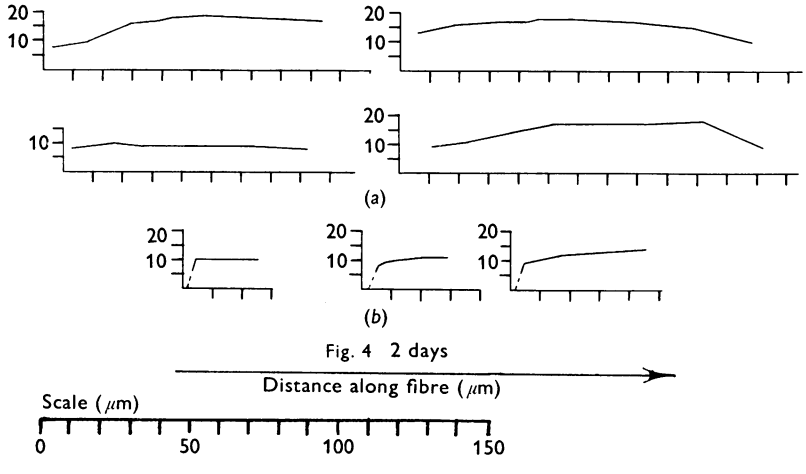
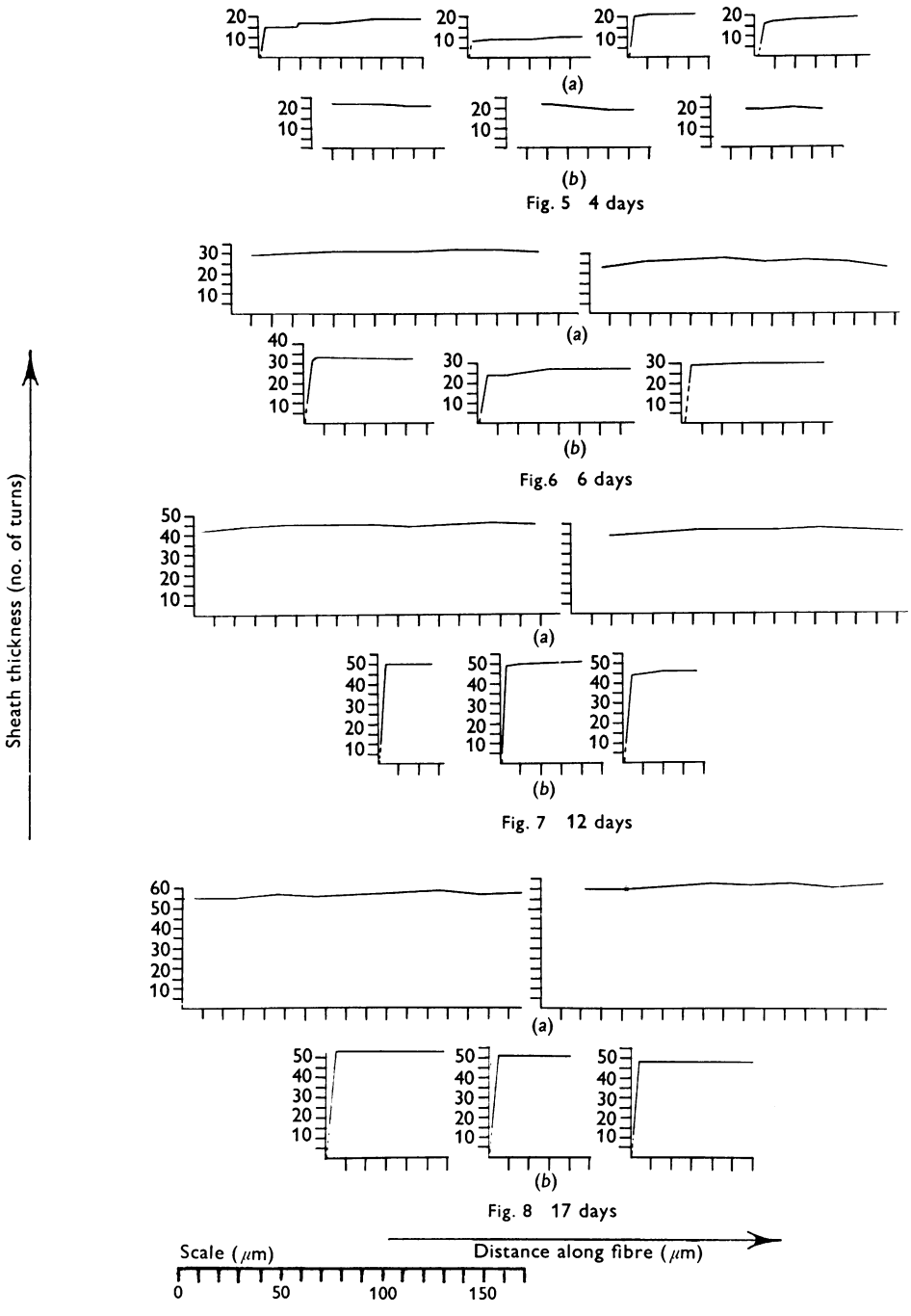


Fig. 4 2 days

Figs. 2–4. Graphs showing typical examples of myelin sheath thickness variation with distance along internode. Thickness is plotted against distance along entire internodes measured from one end of the compact myelin, using serial transverse sections, in the 1 day old (Fig. 3*a*) and 2 day old (Fig. 4*a*) roots. Thickness is plotted against distance from midnodal level, using longitudinal sections, in the newborn (Fig. 2*a*), 1 day old (Fig. 3*b*) and 2 day old (Fig. 4*b*) roots. Thickness is plotted against distance along longitudinal sections of fibres which did not include a bounding node in the newborn (Fig. 2*b*) and 1 day old (Fig. 3*c*) roots.

Continuous lines: compact myelin. Broken lines: non-compact myelin.



Figs. 5–8. Graphs showing typical examples of myelin sheath thickness variation with distance along internode. Thickness is plotted against distance measured from one end of the compact myelin, using serial transverse sections, along the entire internode in the 6 day old (Fig. 6*a*) and 12 day old (Fig. 7*a*) roots, and along a large fraction of the internode at 17 days (Fig. 8*a*).

Thickness is plotted against distance from midnodal level, using longitudinal sections, in the 4 day old (Fig. 5*a*), 6 day old (Fig. 6*b*), 12 day old (Fig. 7*b*) and 17 day old (Fig. 8*b*) roots. Thickness is plotted against distance along longitudinal sections of fibres which did not include a bounding node in the 4 day old root (Fig. 5*b*). Continuous lines: compact myelin. Broken lines: non-compact myelin.

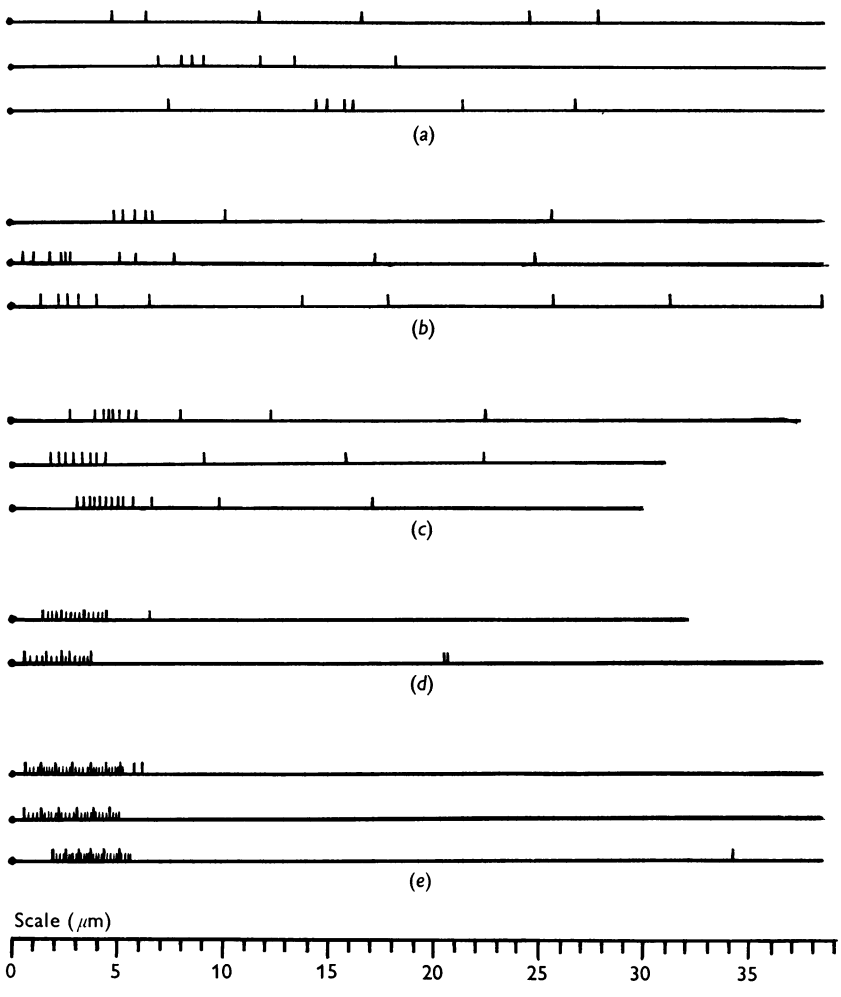


Fig. 9. Diagrams showing for typical individual fibres the mean distances in μm from the mid-nodal level (at origin) at which turns of the mesaxon terminated. Ages: (*a*) newborn, (*b*) 1 day, (*c*) 2 days, (*d*) 4 days and (*e*) 6 days old.

having their maxima close to a bounding node (Fig. 3*a*). A number showed a diminution in thickness near to the middle of the internode (Fig. 3*a*).

Longitudinal sections extending over sufficiently long segments of internodes were in accord with the above observations (Fig. 3*b*, *c*).

2 Days

Fewer fibres had asymmetrical sheaths at 2 days than at 1 day. Symmetrical sheaths resembled those at 1 day (Fig. 4*a*). The rate of diminution on either side of the maximum was slightly more gradual; at 10 μm from the end of the compact sheath the number of turns had fallen to an average of 77% of the maximum.

Longitudinal sections were again in accord with the above findings from transverse

sections. They indicated that the rapid juxtanodal diminution in sheath thickness nearly always began within about 10 μm of the end of the compact sheath and therefore within 15 μm of the adjacent midnodal level (Fig. 4*b*).

4 Days

Though all the 4 day old fibres were studied by longitudinal section, it seems very likely that sheath thickness generally diminished at a considerably slower rate than in younger animals up to a level about 5 μm or even less from the zone of transformation to non-compact myelin, and therefore at 7 μm or less from the midnodal level (Fig. 5*a*). It would seem probable from these graphs that a large proportion of sheaths consisted of a nearly uniform number of turns over most of the internode (Fig. 5*b*).

6 Days

Many sheaths showed little variation in thickness over a large fraction of the internodal length, there being a slight, generally symmetrical, decrease in the vicinity of the nodes (Fig. 6*a*). The rate of this decrease was much less than at 2 days; at a distance of 10 μm from the end of the compact sheath the number of turns was on the average 89 % of the internodal maximum. Longitudinal sections indicated that the rapid juxtanodal diminution in compact sheath thickness generally began less than 5 μm from the end of the sheath and therefore within 8 μm of the adjacent midnodal level (Fig. 6*b*).

12 Days

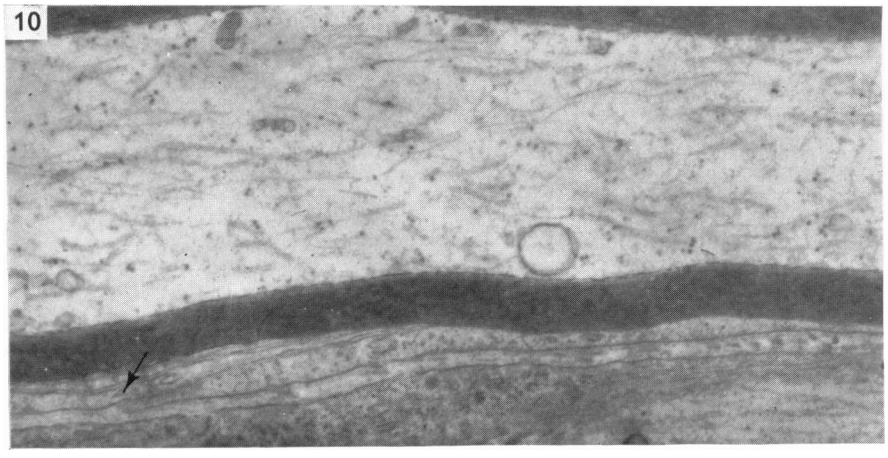
Apart from being considerably thicker, the sheaths at 12 days resembled closely those at 6 days (Fig. 7*a*). At 10 μm from the end of the compact sheath the average number of turns was found to be 89·5 % of the maximum for the internode. Longitudinal sections confirmed that sheath thickness almost certainly remained near the maximum up to a level closer to the adjacent midnodal level (about 5 μm) than at 6 days (Fig. 7*b*).

17 Days

At 17 days, with one exception, the segments of all fibres used in the study of sheath thickness variation included only one of the bounding nodes. However, a substantial proportion of the probable internodal length was included in each case. The features noted were those expected from observation of younger roots. Sheaths were on the whole thicker than at 12 days (Fig. 8*a*) and generally consisted up to a level within 5 μm of the adjacent midnodal level of a very uniform number of turns which was probably very close indeed to the maximum value for that internode (Fig. 8*b*).

General observations on longitudinal sections of compact sheaths

Compact myelin was found generally to appear first near the middle of the Schwann cell in the region of its nucleus, a finding in agreement with that of Webster (1971). All the sheaths of this sort which were observed consisted of three turns, except for one, which had two. In nearly all such cases the number of turns of the mesaxon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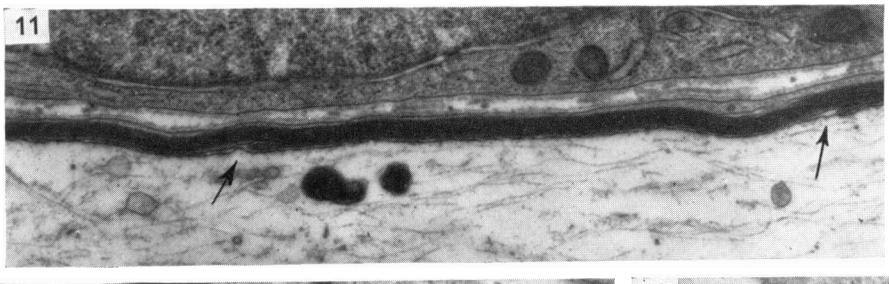
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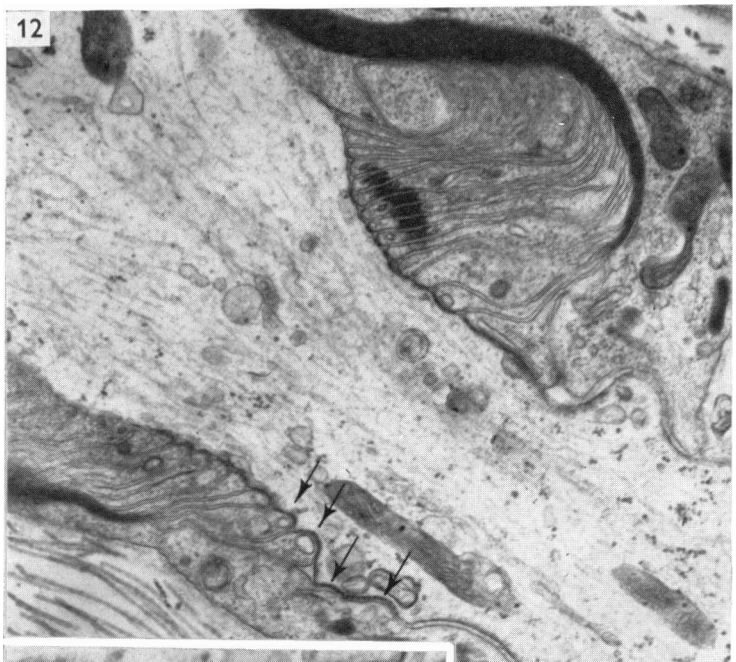
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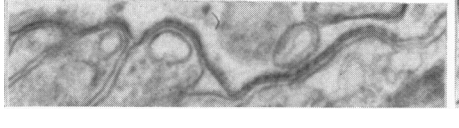

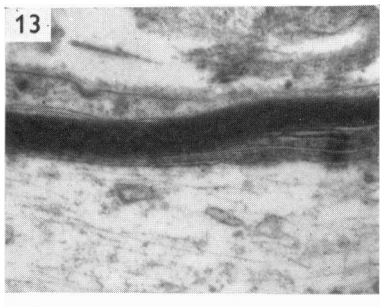
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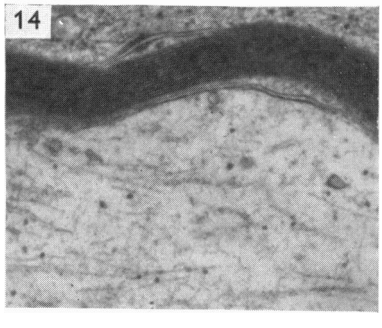
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remained unchanged, though non-compact, up to levels quite close to the presumptive nodes.

Before terminating, all turns become non-compact. In a minority of fibres, particularly those from younger animals, the decline in sheath thickness was accounted for through the plasma membranes of the outermost turn becoming continuous with the external plasma membrane of the Schwann cell (Fig. 10). Such appearances represent a section through a tongue of Schwann cell, perhaps in the process of adding a further turn to the sheath by wrapping itself around its external aspect. Such a mode of termination was rarely observed in the juxtanodal regions. However, in the abnodal regions, a substantial minority of turns ended in this way, though the majority ended by the innermost turns becoming continuous with the internal plasma membrane of the Schwann cell (Fig. 11).

Longitudinal sections showed that the compact sheath generally commenced by the condensation of several turns of the spiral mesaxon over a very short length (Fig. 12). The number of layers participating varied with age and maximum sheath thickness. In only a very small proportion of cases did the compact sheath consist, either at its commencement or at any other level, of only two turns for more than a fraction of a micrometre. This was despite the fact that two non-compact turns of the mesaxon frequently surrounded the axon over lengths of several micrometres. This finding is in agreement with previous observations on transverse sections (Fraher, 1972).

Observations on the juxtanodal region

The distance between the margin of the zone of condensation and the adjacent midnodal level tended to decrease with increasing sheath thickness as age advanced (see heminodal length, Table 4), and was quite short relative to the total Schwann cell length at all ages, even in the newborn. By the stage at which the fourth turn had been completed the heminodal length had nearly always decreased to 5 μm or less.

Both components of the heminodal length (*h*) also decreased with advancing age. The distance (*d*) between the midnodal level and the termination of the turn ending nearest to it (i.e. the length of axon enveloped only by the outer Schwann cell cytoplasm) decreased progressively with advancing age, as did the length of axon (*h–d*) surrounded by turns of non-compact myelin only (Table 4; Fig. 1).

Fig. 10. Longitudinal section of a 4 day old fibre showing a turn ending externally (arrow) near the centre of an internode. × 25000.

Fig. 11. Longitudinal section of 1 day old fibre showing turns ending (arrows) internally near the centre of the internode. × 25000.

Fig. 12. Longitudinal section through nodal and juxtanodal region of a 4 day old fibre showing (i) a stack of desmosome-like structures between adjacent terminal cytoplasmic pockets and (ii) periodic axolemmal densities (arrowed and inset). × 23400; inset × 53000.

Figs. 13 and 14. Longitudinal sections through 6 day old ventral root fibres showing a small stack of desmosome-like structures involving the innermost non-compact layers of the myelin sheath and seemingly also the axolemma (Fig. 13) and a single such structure on an outer non-compact layer of the sheath (Fig. 14). Both × 23800.

Table 4. *Mean* ($\bar{x}$), *standard deviation* (*s.d.*) *and range of values for heminodal length* (*h*), *together with the mean values of its constituent parts* (*see text*) *at the ages indicated*

(Calculated from data derived from longitudinal sections of all fibres studied at each age.)

		Heminodal length (*h*)					
				Range		*d*	*h–d*
Age (days)	No. of fibres	$\bar{x}$ (μm)	s.d.	Min.	Max.	Mean (μm)	Mean (μm)
Newborn	22	6·8	3·2	3·0	32·0	4·2	2·7
1	32	3·5	1·6	1·2	7·5	1·8	1·7
2	41	3·1	1·7	0·9	7·0	1·3	1·6
4	17	2·2	0·8	1·0	4·0	1·1	1·0
6	31	2·9	1·1	1·3	5·3	1·1	1·7
12	16	1·3	0·4	0·5	2·0	0·4	0·9
17	15	1·3	0·3	0·7	2·0	0·2	1·1

Observations on the internal Schwann cell cytoplasmic helix

The distances from the midnodal level to the level at which the turns of the mesaxon terminate, as measured on longitudinal sections taken from the newborn, 1, 2, 4 and 6-day specimens, are plotted in Fig. 9.

In the newborn fibres, the tightness of the internal Schwann cell cytoplasmic helix was quite variable, even between fibres having a similar probable maximum sheath thickness.

At 1 day, and to a lesser extent in the newborn, the pitch of the helix tended to decrease as the node was approached, the terminal cytoplasmic pockets generally being shaped like an elongated drop or a comma, but becoming progressively broader, flatter and more band-like when traced into the internode (Figs. 1, 9). However, as age advances it becomes progressively easier to identify a level of transition between more tightly and less tightly coiled segments of the helix – the juxtanodal and abnodal segments. Table 5 shows the mean pitches of these segments at the various stages studied. It is evident (Figs. 2–9) that with time an increasing proportion of the pockets belonging to internally ending turns comes to make up the juxtanodal segment of the helix. Concurrently the abnodal segment comes to consist of turns which become progressively broader and thinner as it becomes more loosely coiled. Eventually the abnodal segment takes the form of an attenuated sleeve of cytoplasm which is crossed by the longitudinally running mesaxon. These changes correlate with the finding that with advancing age a progressively increasing proportion of sheaths show a change in sheath thickness of no more than one lamella over considerable lengths (up to 130 μm) of the abnodal segment.

The mean length of the axon in relation to the juxtanodal helix was remarkably constant from 1 to 17 days after birth, despite a tenfold increase in the number of turns comprising that segment of the helix (Table 5). There was an increase of the order of 50 % in nodal and juxtanodal axon circumference when values at 1 day were compared with those at 17 days (e.g. from a mean value of 5·7 μm to one of 7·5 μm). Over the same time interval axonal circumference at other levels of the internode underwent a two to three-fold increase for thicker axons (Fraher, 1972).

Table 5. *Mean pitch of the abnodal and juxtanodal segments of the Schwann cell periaxonal cytoplasmic helix at the ages indicated, together with the mean length of the juxtanodal segment at each age*

(Calculated from data derived from longitudinal sections of all fibres studied at each age.)

Age (days)	Mean pitch (μm)		Mean length of axon in relation to juxtanodal segment (μm)
	Juxtanodal	Abnodal	
Newborn	0·97	—	6·8
1	0·35	5·05	3·3
2	0·30	4·35	3·25
6	0·16	11·23	4·1
12	0·11	10·21	3·5
17	0·08	28·34	3·3

General observations on longitudinally sectioned fibres

Desmosome-like structures were frequently seen in relation to the non-compact myelin of the juxtanodal region, where they were often arranged in stacks after 4 days (Fig. 12), as described by Harkin (1964), Gamble & Gossett (1966) and Berthold (1968). Those at opposite surfaces of a given terminal cytoplasmic pocket were in continuity with one another. In addition examples were seen in segments of non-compact myelin on both the internal and external aspects of the compact sheath at all ages included in the study (Figs. 13, 14), often at levels close to the middle of the internode. A smaller number apparently also involved the apposed axolemma and inner plasma membrane of the Schwann cell (Fig. 13).

Periodic axolemmal densities (see Peters & Vaughn, 1970), which may be transverse sections of helical thickenings of the outer leaflet of the axolemma (Hirano & Dembitzer, 1967), were observed in only one of the juxtanodal regions examined in the newborn animals. Their frequency increased with advancing age (Fig. 12, inset).

The general ultrastructural features of the developing myelin sheaths, axons, Schwann cells and nodes of Ranvier were in close agreement with the descriptions of Berthold (1968), Berthold & Skoglund (1968) and Peters & Vaughn (1970).

Statistical treatment of data derived from longitudinal study

For all the internodes examined by serial transverse sections in each of the five roots included in the present study, correlation and regression analyses were performed using mean internodal sheath thickness (x) and mean internodal axon circumference (y) (Table 6, r_{mean}). The results were very similar to those of analyses using maximum internodal sheath thickness and mean internodal axon circumference (Table 6, r_{max}). The correlation between maximum and mean sheath thickness was very high indeed, being greater than 0·97 at each age. For the root examined at each age, apart from that at 6 days, the correlation between mean (or maximum) internodal sheath thickness and mean internodal axon circumference was stronger than that found between sheath thickness and axon circumference measured at one cross-sectional level. This is illustrated graphically in Fig. 15. Some of the values

Table 6. *Statistical data calculated for the indicated numbers of myelinated internodes from one cervical ventral root at each of the indicated ages*

((i) Regression equation of mean internodal axon circumference (y) on mean internodal sheath thickness (x). (ii) Coefficient of linear correlation between mean internodal axon circumference and mean internodal sheath thickness (r_{mean}). (iii) r^2_{mean}. (iv) Coefficient of linear correlation between mean internodal axon circumference and maximum internodal sheath thickness (r_{max}).)

Age (days)	No. of fibres	Regression equation of y on x	Correlation coefficient r_{mean}	r^2_{mean}	Correlation coefficient r_{max}
1	22	$y = 4{\cdot}9698 + 0{\cdot}1255\,x$	0·6038	0·3646	0·6007
2	28	$y = 4{\cdot}6931 + 0{\cdot}0995\,x$	0·4157	0·1728	0·5460
6	28	$y = 5{\cdot}0996 + 0{\cdot}1397\,x$	0·4408	0·1943	0·6221
12	35	$y = 1{\cdot}9336 + 0{\cdot}2175\,x$	0·9034	0·8161	0·8822
17	25	$y = 1{\cdot}2930 + 0{\cdot}2104\,x$	0·9436	0·8904	0·9469

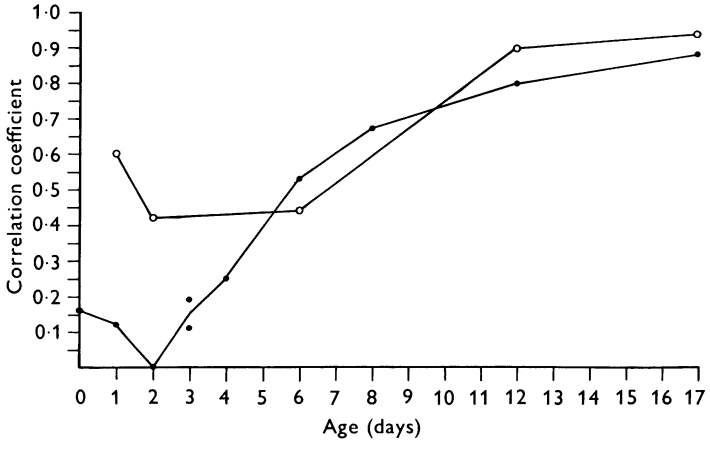

Fig. 15. Graphs illustrating the manner in which the correlation coefficient between sheath thickness and axon circumference varied with age (i) when allowance was made for longitudinal variation in both parameters, i.e. as found in the study based on serial transverse sections through several internodes (open circles), and (ii) when such allowance was not made, i.e. as found from measurements made on a single transverse section of one root (dots).

calculated for a single cross-sectional level have been previously published (Fraher, 1972); those at 6 and 12 days were calculated from a random sample of 120 fibres at the most proximal level in the present study. From this figure it is also evident that both sets of correlation coefficients remain at relatively uniform, though different, levels over the first 4 to 6 days after birth, after which all have risen to a very similar level by 12 days, continuing to rise at a slower rate up to the 17th day at least.

The proportion of the variance of y that can be attributed to its linear regression on x is given by r^2. From Table 6 it is evident that the proportion of the variance of axon circumference attributable to its linear regression on sheath thickness (r^2_{mean}) is between 17 % and 36 % up to and including 6 days after birth, when allowance is made for longitudinal variation in the dimensions of both. The same measure has risen to 81 % at 12 days and is 89 % at 17 days.

Table 7. *Pooled estimates of the variance of all axon circumferences at ages indicated*

(Value for each age calculated from variance of axon circumference based on measurements at different levels along all internodes.)

Age (days)	Variance
1	1·49
2	1·14
6	1·98
12	2·18
17	2·27

Table 7 indicates that the pooled estimates of the variance of all axon circumferences measured at each age in the present study show an upward trend with the passage of time.

Relative position of ends of mesaxon

It was found (Fraher, 1972) that, in the peripheral nervous system, the points of continuity of the internal and external ends of the mesaxon with the internal and external Schwann cell plasma membranes were within the same quadrant for 72·5 % of all myelinated fibres at 1 day after birth. The proportion was found to be higher for thinner sheaths than for thicker ones. The present investigation showed that at 4 and 12 days the ends of the mesaxon lay in the same quadrant in remarkably similar proportions (71 % and 73 % respectively) of cases, and that these proportions were relatively unaffected by sheath thickness.

DISCUSSION

Longitudinal changes in sheaths during maturation

If it is assumed that the stages in development of the myelin sheath are similar for ventral root fibres, whatever the eventual sheath thickness, then the different fibres in the younger roots can be considered to exemplify different stages in the development of mature sheaths. Assuming this to be so, and using the findings described above, it is possible to trace in outline the manner in which the morphology of the sheath, and therefore of the unrolled Schwann cell as a whole, changes with time (Fig. 16).

Thinner sheaths tend to show remarkably little variation along the internode in the number of constituent turns of myelin, whether compact or non-compact, implying that initially mesaxonal elongation in a circumferential direction occurs at a similar rate over much of the internode. The high proportion of cases in which the inner and outer ends of the mesaxon are within the same quadrant of thinly myelinated ventral root sheaths (Fraher, 1972) is consistent with the total circumferential elongation of such a mesaxon amounting almost exactly to an integral number of turns during the stage at which the sheath consists of 3–5 turns. It is thus evident that in the earliest stages of its maturation the uncoiled Schwann cell has approximately the form of a trapezium whose longer edge is external (Fig. 16*a*).

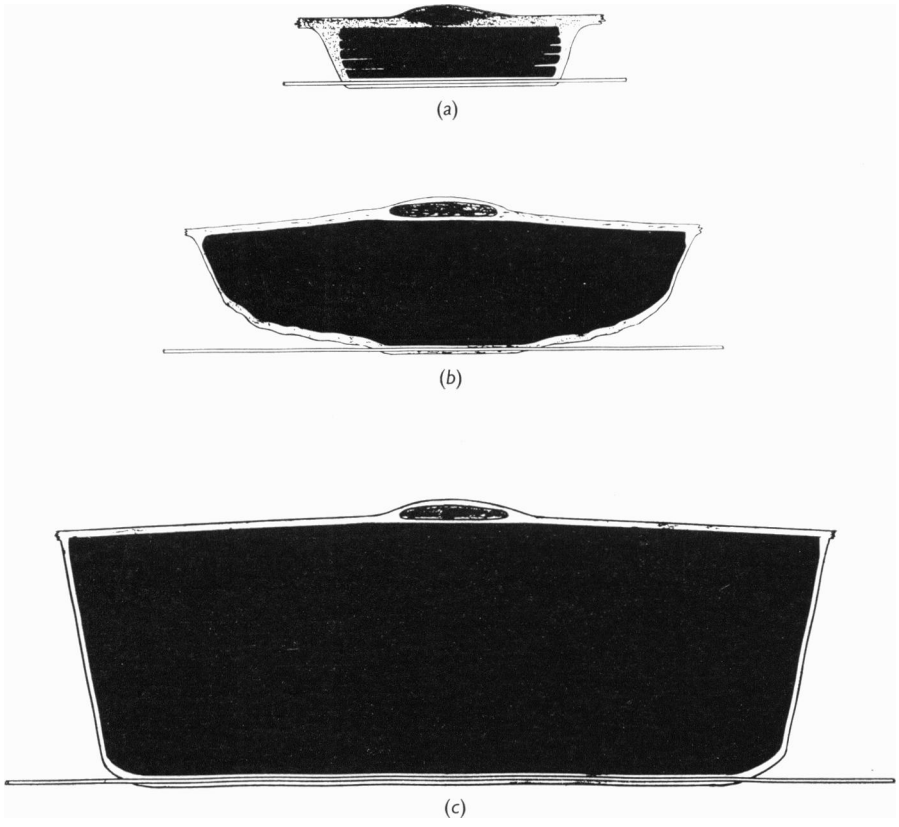

Fig. 16. Diagrams showing the form of the unrolled Schwann cell and compact sheath (*a*) in the earliest stages of compact sheath maturation, (*b*) shortly afterwards and (*c*) towards the fully mature stage (see text).

The present findings also imply that once condensation to form compact myelin has been initiated by the disappearance of the cytoplasm between the turns of the mesaxon (generally when about three turns are present), the process spreads rapidly to involve a large part of the sheath, usually extending over two-thirds or more of the circumference (Fraher, 1972) and longitudinally to within a few micrometres of the ending of the outermost of the three or more constituent turns. These findings are in agreement with Speidel's (1964) observation, on living amphibian material, that the myelin sheath in some instances increases in length very rapidly; he noted by time-lapse cinematography that a myelin sheath could increase its length at one end by 8 to 13 μm over a period of only 2 hours.

Further increase in mesaxonal area, beyond the stage at which it describes 6–8 turns about the axon, takes place over its whole length, but not at the same rate in all parts. Sometimes the sheath thickness increases more rapidly at two levels along the sheath than in the intervening segment, but generally the greatest rate of increase in sheath thickness occurs at a single sheath level. From an early stage this level may involve the central portion of the internode, the rate diminishing progressively

on either side. It may, however, involve an eccentric segment relatively close to one of the bounding nodes, but eventually the central portion of the sheath usually comes to consist of a greater thickness of myelin with the number of turns gradually diminishing in a symmetrical fashion on either side. The unwound Schwann cell then tends towards having a flattened diamond shape (Fig. 16*b*), both longitudinally running edges of the cell being convex, the convexities facing away from one another.

Since the increase in compact sheath thickness as the central part of such an internode is approached occurs through the addition of extra turns both internally (Fig. 11) and externally (Fig. 10), the former to a considerably greater extent than the latter, it follows that the positions of both the inner and, to a lesser extent, the outer ends of the mesaxon change in opposite directions about the axon when traced towards the level of greater sheath thickness, each in such a way as to increase the number of turns in the sheath. This is in agreement with the observations of Webster & O'Connell (1970) and of Webster (1971), but differs somewhat from those of Robertson (1962) who found that the outer end of the mesaxon remained relatively constant in position.

Further increase in sheath thickness involves the whole length of the sheath from one end of the internode to the other, but the portions closer to the bounding nodes undergo a greater net increase relative to central portions. The result is that all levels of the sheath, apart from the juxtanodal, progressively come to consist of a more nearly equal number of turns. Subsequently, despite the approximately twofold increase in sheath thickness which occurs between 6 and 17 days, progressively fewer turns end internally or externally at abnodal levels, as the juxtanodal helix becomes more and more tightly coiled. Thus, during this period, all levels of the abnodal sheath increase in thickness at virtually the same rate, each additional net turn being added over almost the whole of a considerably greater internodal length in a shorter average time than at earlier stages. These changes are correlated with a marked increase in the pitch of the abnodal segment of the inner Schwann cell cytoplasmic helix. This could be consequent upon the removal of the innermost turns, or upon the migration of the *adnodal* margins of each individual turn away from one another along the axon towards the bounding nodes, so that their ends approach and in most cases come into close apposition with the juxtanodal segment, thereby adding to the number of terminal cytoplasmic pockets comprising the latter.

The tendency is therefore for the shape of the unwound sheath to approach and again approximate to that of a trapezium, the external edge of which is slightly longer than the internal (Fig. 16*c*).

The variations noted in sheath thickness at different levels of each of several internodes, especially in the earlier stages, could have come about in a variety of ways. In the 1 and 2 day old animals the decrease in sheath thickness from the level at which it is greatest is largely the result of turns ending internally at all levels along the internode (Fig. 11). This is compatible with the hypothesis that a significant proportion of the turns of the sheath are added by a spiral extension of the inner lip of Schwann cell cytoplasm around the inner aspect of the compact sheath. If this occurs, it indicates a 'high point' of mesaxonal elongation and myelin formation, perhaps located mainly in the inner Schwann cell cytoplasmic compartment, such activity falling off on either side.

However, the decrease of sheath thickness on either side of the maximum could also be explained by the inner turns being removed, the process beginning near the nodes and progressing away from them. From the shape of the unwound Schwann cell (Fig. 16*b*), it is clear that such a process would involve several turns at any one time at this stage of development. If such unwinding were to continue to take place (perhaps to permit increase in axon calibre) throughout the period of increase of sheath thickness, then, after 4 to 6 days, all parts of a given turn must be removed more nearly simultaneously, since differences in number of turns along the internode tend to be much less for thicker sheaths.

Growth in length of the sheath could contribute to the finding of turns ending internally at all internodal levels, due to the outer turns elongating parallel to the long axis of the axon at a more rapid rate than the inner ones. Against this hypothesis is the finding that at birth, at 1 day, and at 2 days, the lengths for which the thinner sheaths tend to consist of a uniform number of turns are greater than the corresponding lengths for the thicker sheaths; this finding, however, is not universal.

Thus the internal ending of turns at all internodal levels is less likely to arise solely from the lagging behind of the inner turns during growth in length of the sheath than from spiral extension of the inner cytoplasmic lip around the axon, or from a removal of the innermost turns.

The tendency for opposite ends of the mesaxon to lie within the same quadrant (Peters, 1964; Fraher, 1972) was clear for those sheaths whose thickness varied least with internodal length, and whose longitudinal edges when unwound would therefore have been closer to being parallel with one another and with the long axis of the axon.

It is possible that the compact sheath adapts to the increasing calibre of the enveloped axon by slippage of adjacent lamellae relative to one another. The occurrence of such a mechanism was suggested by Hirano & Dembitzer (1967) in relation to developing central nervous system sheaths. Evidence in favour of the occurrence of slippage has been adduced from abnormal material – in relation to sheaths of transected nerve fibres, both proximal and distal to the level of section (Friede & Martinez, 1970*a*, 1970*b*), and in swollen nerve fibres proximal to a ligature (Friede & Miyagishi, 1972).

In relation to the possibility of slippage it may be pointed out that in the present study stacks of desmosome-like structures were first evident at 4 days in the juxtanodal region. If these structures do represent firm attachments between adjacent turns of the juxtanodal helix, then they, together with the presumably firm attachment of the plasma membranes of the juxtanodal helix to the underlying axolemma by 7-layered complexes (Elfvin, 1961; Hildebrand, 1971) and possibly also by the periodic axolemmal densities (Fig. 12), could provide mechanisms by which the helix resists the suggested unwinding. Furthermore, unwinding of the helix, while it retained its firm attachment to the axolemma, would exert severe tangential stresses on the latter.

While the earliest turns of the juxtanodal helix are being formed, axon calibre does not show any constant pattern of variation between nodal, juxtanodal or abnodal regions. Subsequent turns are laid down over an axon whose calibre is progressively increasing. The postulated resistance to unwinding provided by the juxtanodal cytoplasmic helix could be a causative factor explaining why, with ageing, increase in calibre of the nodal and juxtanodal segments lags progressively behind

that of other parts of the internode. The observations of Williams & Hall (1970) on living sciatic nerve fibres suggest that such a constriction exists at living nodes. However, the diminution in cross-sectional area of the terminal cytoplasmic pockets which occurs as the fibre ages would allow a little dilatation of the juxtanodal axon to occur.

Juxtanodes and paranodes

If the paranodes are taken to include the terminal cytoplasmic processes of all turns of the sheath then it is clear that, at 2 days of age and less, a very considerable fraction of the length of each internode (in many cases more than one-half, in some cases almost the whole) is made up by its pair of paranodes. However, by 17 days the two paranodes have become virtually synonymous with the juxtanodal segments.

If Berthold (1968), Conradi & Skoglund (1969), and Hildebrand (1971) take what they term the 'decrement zone' of the fibre to refer to the length of axon overlaid by the tightly coiled juxtanodal segment, then the mean values for this length found in the present observations are in close agreement with their findings for maturing cat ventral root, anterior horn motoneuron axon, and lateral white funiculus internodes respectively. The one exception found in the present study was that of the newborn, in which it was possible in only a small proportion of cases to distinguish between the two segments of the inner Schwann cell helix. In those cases in which this could be done juxtanodal lengths were very variable.

Statistical findings

Comparison of the two sets of correlation coefficients in Fig. 15 makes it clear that a proportion of the low correlation found using measurements at a single level over the first 6 days or so can be accounted for by the failure to take note of the variation in sheath thickness and in axon calibre from one level to another of the same internode.

At 1 and 2 days the variation in axon calibre from one level to another was relatively small, the values for standard deviation and coefficient of variation of circumference of individual axons, as well as the pooled estimates of the variance and standard deviation of axon circumference for all fibres examined, being relatively low (Tables 2, 3, 7). This implies that the mean circumference was a relatively good estimate of axon calibre. There was some variation in sheath thickness at different internodal levels in the majority of fibres at this stage. Nevertheless, when this was allowed for by calculating the correlation coefficient using mean or maximum sheath thickness and mean axon circumference, only 17 to 36 % of the variance of axon circumference attributable to its linear regression on sheath thickness was accounted for in terms of a linear regression. However, the correlation was considerably stronger than that previously reported for the study based on single cross-sections at similar ages (Fraher, 1972).

At 4 days after birth, though the form of the curve showing sheath thickness at different levels along the internode was much closer to the plateau than at younger stages, there was nevertheless little evidence of an increase in the correlation with axon calibre, as judged from a single cross-sectional level (Fig. 15).

By the stage when most of the curves showing sheath thickness alteration along

the internode were of an approximately plateau form, the disadvantages of using a single cross-sectional level for the calculation of correlation coefficients were greatly lessened. At 6 days, when sheath thickness was close to uniform over a large fraction of almost all internodes, the correlation between sheath thickness and axon circumference, taking into account longitudinal variation in both parameters, showed no tendency to rise relative to comparable values at 1 and 2 days. The proportion of the variance of axon circumference attributable to its linear regression on sheath thickness remained less than 40%. The axon circumferences tended to be a little more variable between different internodal levels than in the younger examples (Table 7). While this might tend to lower the value of the correlation coefficient slightly, the increase in axon calibre variance was part of a continuing trend which occurred throughout all the time intervals between 2 and 17 days (Table 7).

Between 6 and 12 days, the general form of the curves of sheath thickness changed very little apart from an increase in the number of turns, the degree of variation between the number of turns at different sheath levels being very similar in most cases. Over this period, circumference variance showed an overall rise (Table 7). Paradoxically, instead of falling, as might be expected from the above evidence, the correlation coefficient rose markedly and the proportion of the variance accountable for in terms of a linear regression doubled in this period (Table 6).

These trends were continued up to 17 days, when the correlation coefficient had come close to unity and 90% of the variance was accounted for in terms of a linear regression.

The progressive increase in the intensity of association between axon circumference and sheath thickness seems clearly to lag behind the progression of the increasing uniformity of sheath thickness along the internode which follows on the stage when there is a symmetrical decrease on either side of the maximum. Also, the changes noted in axon calibre variance would tend to decrease rather than increase the correlation. Thus in the early stages of myelination the correlation seems to vary in a manner which is not fully accounted for by concurrent changes in axon calibre and sheath thickness, even when account is taken of the variation of both parameters from one level to another of the internode.

Whether or not a given axon becomes myelinated very probably depends primarily on the nature of the axon itself. Evidence in support of this is provided by the experiments on regeneration into cross-anastomoses of nerves carried out by Simpson & Young (1945) on rabbits and by Hillarp & Olivecrona (1946) on rats. One way in which an axon could influence the myelinating activity of its associated Schwann cells would be by means of its calibre. If this were to determine sheath thickness a positive statistical correlation would be found between the two parameters. However, such a correlation could be found in the absence of any casual relationship between axon circumference and sheath thickness. This could arise simply from a concurrent increase in both parameters during fibre maturation. Since all fibres do not mature in phase with one another, this would result in points on a scatter diagram relating these parameters to one another coming to stretch upwards and to the right from the origin (see, for example, Fraher, 1972), thereby giving rise to a positive correlation. If, on the other hand, axon circumference were to determine sheath thickness, the present evidence suggests that the determining influence would be quite

weak during early myelination but would seem to be stronger after the stage when compact sheath thickness finally comes to be relatively uniform over most of the length of the internode.

SUMMARY

1. A study has been made of longitudinal variation in myelin sheath thickness with distance along the internode during myelination.

2. From the findings it is suggested that mesaxonal elongation in a circumferential direction at first occurs at a similar rate over much of the internode. Further elongation tends for a time to occur more rapidly near the middle of the internode, but when sheath thickness has reached about half of its final value the number of turns comprising the sheath is again about the same over most of its length. This remains the case during further increase.

3. It is suggested that the variation in internodal sheath thickness in the earlier stages can be explained on the basis of a spiral extension of the inner Schwann cell lip around the axon, or on the basis of a removal of the innermost turns; it is less likely to be due to growth in length of the sheath.

4. It is found that the inner and outer ends of the mesaxon tend to lie in the same quadrant when the outer and inner edges of the unrolled Schwann cell are closest to being parallel.

5. When account is taken of longitudinal variation in axon circumference and sheath thickness the value of the correlation coefficient between the two is greater than that obtained when no account is taken of these factors. However, in either case this correlation rises considerably after about 6 days after birth, and has reached a value near to unity by 17 days. A statistical correlation could exist between axon calibre and sheath thickness in the absence of any causal relationship between these parameters. However, if axon calibre influences sheath thickness then the realization of this influence is low in the early stages of myelination.

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