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The development of alpha and gamma motoneuron fibres in the rat

II. A comparative ultrastructural study of their central and peripheral myelination

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INTRODUCTION

This paper examines the growth of the myelin sheath in central and peripheral segments of alpha and gamma ventral motoneuron fibres. Using the axon calibre data (Kaar & Fraher, 1985), sheath thickness–axon calibre relationships are examined and compared between the two fibre groups. It has generally been assumed that a single statistical relationship exists between myelin sheath thickness and axon circumference both during development and in the adult (Friede & Samorajski, 1967; Williams & Wendell-Smith, 1971; Boyd & Kalu, 1973; Sima, 1974; Fraher, 1972, 1976; Berthold & Carlstedt, 1977; Berthold, 1978; Hildebrand & Hahn, 1978; Friede & Bischausen, 1980; Biscoe, Nickels & Stirling, 1982; Berthold, Nilsson & Rydmark, 1983). In this paper the relationship is investigated separately in both alpha and gamma fibre groups during development and at maturity.

MATERIALS AND METHODS

The material used in this paper was the same as that in the two previous papers of this series (Fraher & Kaar, 1984; Kaar & Fraher, 1985). Myelination was studied at the 6 day stage and subsequently. Fibres were divided on the basis of axon circumference (Kaar & Fraher, 1985) into alpha and gamma groups. Myelin sheath thickness (number of lamellae) was measured at several levels, 5 μm apart, centrally and peripherally over distances of 20–30 μm from the paranode, of between 100 and 400 fibres of each class at each age and was plotted against distance from the node (Fig. 1). The mean sheath thickness was calculated for the central and peripheral abnodal segment of each fibre. Percentage frequency histograms were derived from these values for alpha and gamma fibres at each age (Fig. 2). Using the mean values for pooled fibres in each class, growth rates for myelin sheath thickness were calculated (Fig. 3).

Scatter diagrams were plotted relating the mean abnodal values for sheath thickness and axon circumference (Kaar & Fraher, 1985) for each fibre. Separate plots were made for the alpha and gamma groups and both groups combined at each age, for peripheral (Fig. 4) and central (Fig. 5) fibre segments. Correlation and regression analyses (Table 2; Fig. 6) were carried out on the data. The regression lines for alpha and gamma fibre groups were compared with one another using the Indicator Variable technique (Table 4).

The overall mean g-ratio of axon to fibre circumference was calculated for central

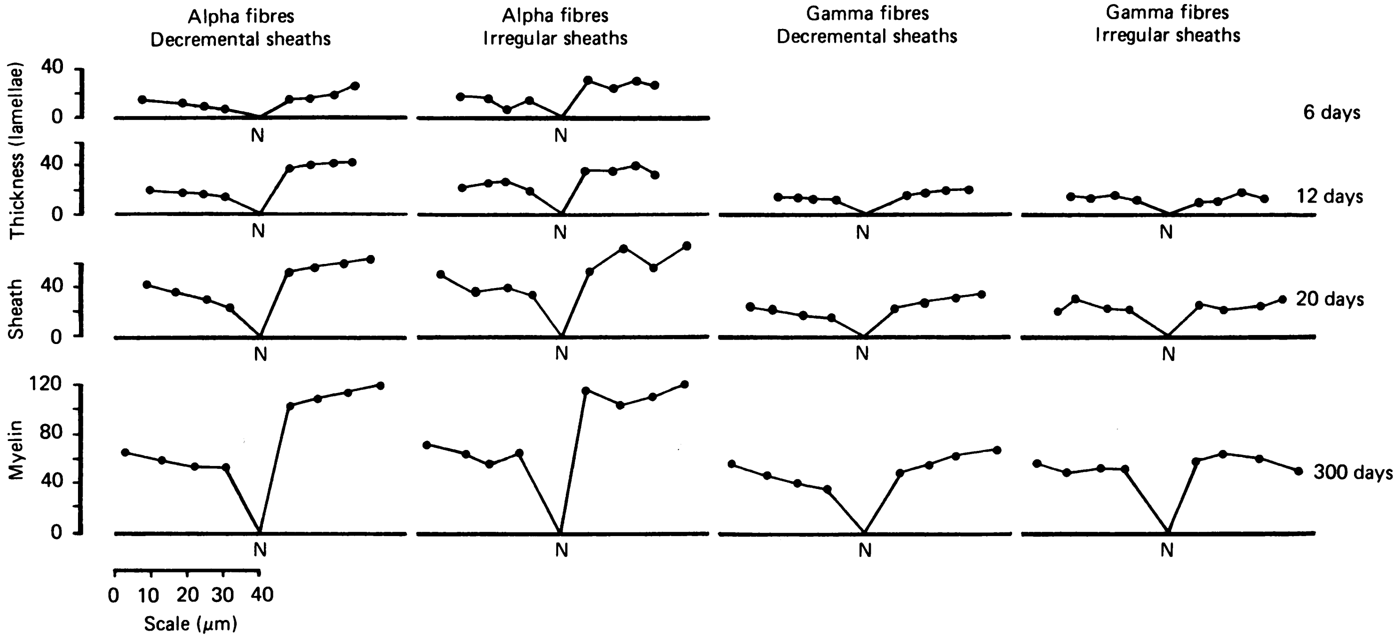

Fig. 1. The patterns of myelin sheath thickness variation central (left) and peripheral (right) to the transitional node (N) for alpha and gamma fibre groups.

and peripheral segments of fibres in both groups (Table 3). Fibre circumference was calculated by a simple geometric method, using known values for axon circumference (Kaar & Fraher, 1985), number of lamellae and the myelin period.

The *in vivo* *g*-ratio was also estimated for alpha and gamma fibres (Table 3). In calculating this, account was taken of the decrease in myelin periodicity which occurs with fixation both centrally (Finean, 1961; Hildebrand & Muller, 1974) and peripherally (Karlsson, 1966; Berthold, Corneliuson & Rydmark, 1982), and of the differential swelling of alpha and gamma axons during fixation (Berthold *et al.* 1982).

OBSERVATIONS

Longitudinal variation in sheath thickness

In both alpha and gamma fibre groups, longitudinal sheath thickness variation presented three patterns at each age: more than one half of all sheaths decreased progressively in thickness in the direction of the paranode (Fig. 1). These will be referred to as *decremental sheaths*. In about one quarter of all cases sheath thickness remained constant. The remaining sheaths varied irregularly in thickness. These proportions were similar for central and peripheral segments. There was no relationship between the type of sheath enveloping a given axon centrally and peripherally.

Among decremental sheaths of alpha and gamma fibres, the number of turns lost increased with age (Table 1). It was comparable for central and peripheral sheaths at each age. This represented a greater proportional decrease for central than for peripheral sheaths, since the former were the thinner of the two. For both fibre groups the mean thickness of the level furthest from the paranode was close to mean internodal values for cervical ventral motoneuron axons (Fraher, 1976, 1978*a*) both centrally and peripherally at similar ages.

Sheath thickness–axon circumference relationships of alpha fibres

At each age the abnodal sheath thickness of alpha fibres was unimodally distributed (Fig. 2) and was greater peripherally than centrally by a relatively constant proportion (Table 1). It increased with age in both locations. The daily increase was greater peripherally than centrally over each interval examined. It was greatest peripherally between 1 and 6 days and centrally between 12 and 20 days (Fig. 3*a*). There was no significant correlation between central and peripheral sheath thickness on individual fibres at 6 days. At all other ages it was significant and the correlation coefficient varied from 0·41 to 0·62.

The scatter diagrams relating sheath thickness to axon circumference give an overview of the combined effects of age changes in the two parameters. The plot points had an elliptical distribution (Figs. 4, 5) which shifted upwards and to the right with maturation as both parameters increased. The long axis of the 300 day peripheral distribution was almost parallel to the X-axis. Central distributions showed a greater degree of scatter than peripheral distributions. This was reflected in their lower correlation coefficients (Table 2).

The slopes of the peripheral regression lines relating the parameters tended to decrease with age, especially between 20 and 300 days (Table 2; Fig. 6). Central regression lines had similar slopes at 6, 12 and 300 days. In both locations the slope was greatest at 20 days. Regression lines were considerably steeper peripherally than centrally, especially at 6 days, indicating a thinner central sheath for any given axon

Table 1. *Mean percentage decrease in the thickness of decremental abnodal myelin sheaths bordering definitive transitional nodes. The most 'nodal' abnodal level lay immediately adjacent to the transitional paranode.*

		Central				Peripheral				
Fibre type	Age (days)	n at most 'nodal' level	n at most 'internodal' level	Mean difference	Decrease (%)	n at most 'nodal' level	n at most 'internodal' level	Mean difference	Decrease (%)	Ratio C/P
Alpha	6	6	10	4	40·0	16	23	7	30·4	0·44
	12	13	19	6	31·6	35	41	6	14·6	0·42
	20	26	41	15	36·6	50	61	11	18·0	0·57
	300	50	61	11	18·0	98	115	17	14·5	0·49
Gamma	12	9	12	3	25·0	15	19	4	21·0	0·64
	20	15	23	8	34·8	23	29	6	20·7	0·64
	300	37	53	16	30·2	53	67	14	20·9	0·66

n, Mean number of myelin lamellae calculated for all decremental sheaths.
C/P, Overall mean ratio of central to peripheral myelin sheath thickness.

Table 2. *Linear correlation and regression statistics relating myelin sheath thickness to axon circumference*

		Central abnodes					Peripheral abnodes				
Type of transitional node	Age (days)	n	P	r	a	b	n	P	r	a	b
Presumptive (myelinated)	3α	24	NS	—	—	—	160	**	0·33	−0·8	1·2
	6α	17	*	43	0·8	0·8	94	**	0·71	−7·9	3·5
	12γ	31	NS	—	—	—	50	**	0·67	−0·2	0·4
Definitive (alpha)	6	85	*	0·19	4·8	0·4	95	**	0·73	−6·4	3·3
	12	344	**	0·15	12·1	0·5	201	**	0·35	21·9	1·5
	20	233	**	0·35	12·4	1·3	98	**	0·65	19·9	2·4
	300	180	**	0·23	38·4	0·5	137	**	0·62	73·9	1·0
Definitive (gamma)	12	63	**	0·40	3·9	1·4	40	**	0·83	−8·6	4·4
	20	144	**	0·54	2·1	2·2	76	**	0·85	0·7	3·4
	300	101	**	0·56	12·1	2·5	76	**	0·69	21·5	3·2

n, Number of pairs. *P*, Statistical significance of relationship: NS, not significant ($P \geqslant 0·05$). *, Moderately significant ($0·05 > P \geqslant 0·01$). **, Highly significant ($P < 0·01$). *r*, Correlation coefficient. *a*, Intercept of regression line with *Y*- (myelin sheath) axis. *b*, Slope of regression line. γ, Gamma fibres mainly or exclusively. α, Alpha fibres mainly or exclusively.

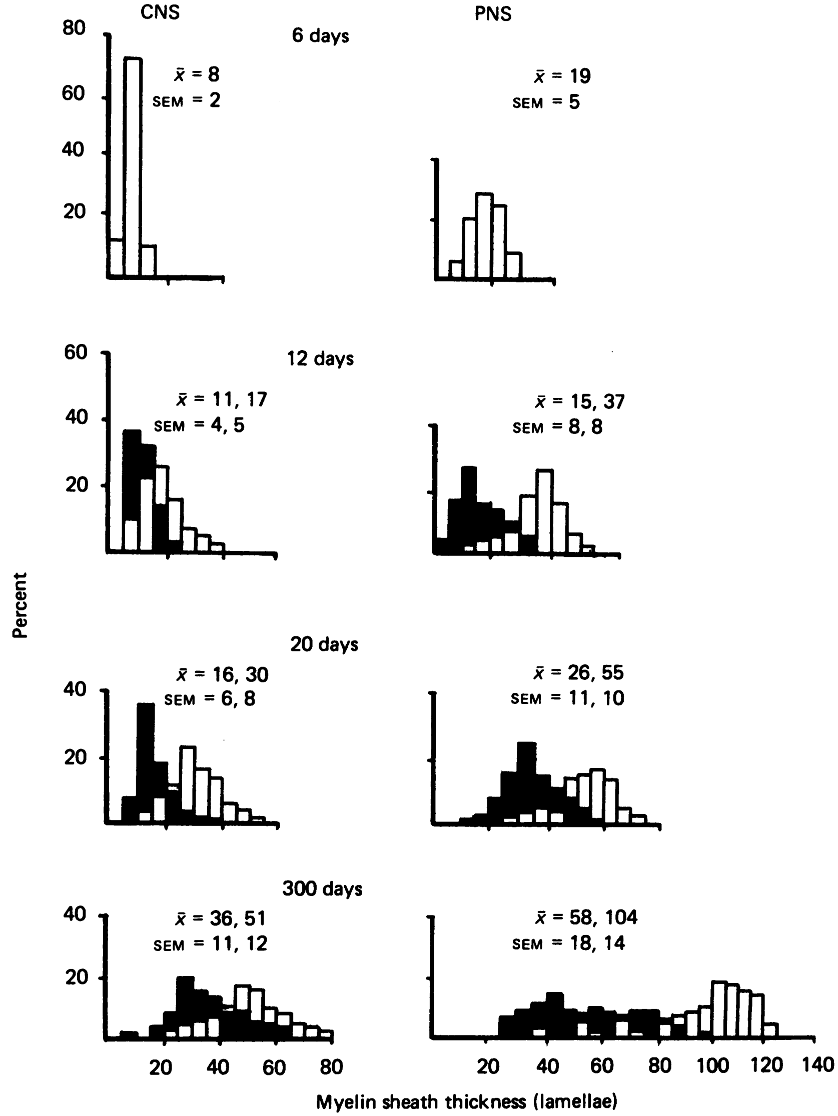

Fig. 2. Superimposed percentage frequency histograms for central and peripheral myelin sheath thickness of gamma (■) and (□) alpha fibre groups. The mean ($\bar{x}$) and its standard error (S.E.M.) are given for each. Each pair of values consists of that for gamma fibres first and for alpha fibres second.

calibre. At 6 days, soon after onset of myelination in alpha fibres, the linear regression line was similar peripherally ($P = 0{\cdot}6$) for fibres possessing presumptive and for those possessing definitive nodes (Table 2).

The calculated mean *g*-ratios in the fixed and embedded state were 10 % greater than the estimated *g*-ratios *in vivo*, though both showed similar age changes (Table 3). Values decreased between 6 and 12 days and changed little subsequently. Central values were about 10 % greater than peripheral.

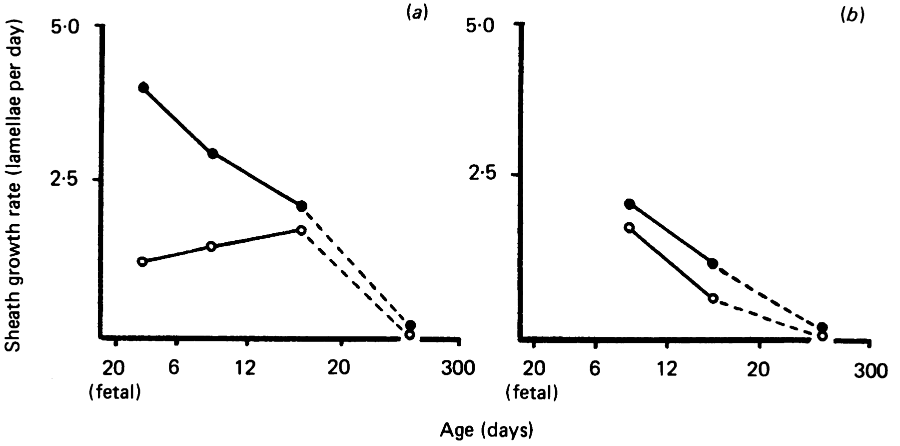

Fig. 3(*a*–*b*): The growth rate (daily increase in number of lamellae) for central (○—○) and peripheral (●—●) myelin sheaths on (*a*) alpha and (*b*) gamma fibres over the intervals shown.

Table 3. *The ratio* g *of axon to fibre circumference*

Age (days)	*g*-ratios in fixed and embedded state				Estimated *in vivo g*-ratios			
	Central		Peripheral		Central		Peripheral	
	Gamma	Alpha	Gamma	Alpha	Gamma	Alpha	Gamma	Alpha
6	—	0·94	—	0·84	—	0·91	—	0·78
12	0·88	0·90	0·83	0·78	0·79	0·85	0·74	0·70
20	0·86	0·87	0·80	0·78	0·78	0·82	0·70	0·70
300	0·80	0·88	0·73	0·80	0·70	0·83	0·61	0·72

Sheath thickness–axon circumference relationships of gamma fibres

The abnodal sheath thickness of gamma fibres had a unimodal distribution at all ages (Fig. 2). The mean central thickness was about two thirds of the peripheral value at each age (Table 1). The daily rate of increase in sheath thickness varied in a similar fashion centrally and peripherally (Fig. 3*b*). It was greatest between 6 and 12 days in both regions. The correlation between central and peripheral sheath thickness values for individual fibres was highly significant at all ages and varied from 0·63 to 0·77.

In all cases, the scatter diagrams relating sheath thickness to axon circumference formed an elliptical cluster which moved upward and to the right with age, reflecting growth in both parameters (Figs. 4, 5). At 300 days the orientation of the cluster resembled that for previous ages. These features were reflected in the regression lines (Fig. 6; Table 2). The slopes of these changed relatively little with age. Those for peripheral fibre segments decreased slightly, while those for central segments increased progressively. They were steeper peripherally than centrally at each age.

Comparison of sheath–axon relationships in alpha and gamma fibres

The mean central sheath thickness was a significantly greater proportion of the peripheral value for gamma than for alpha fibres. Also, the correlation between the two was stronger for the gamma than for the alpha group. As age advanced, the

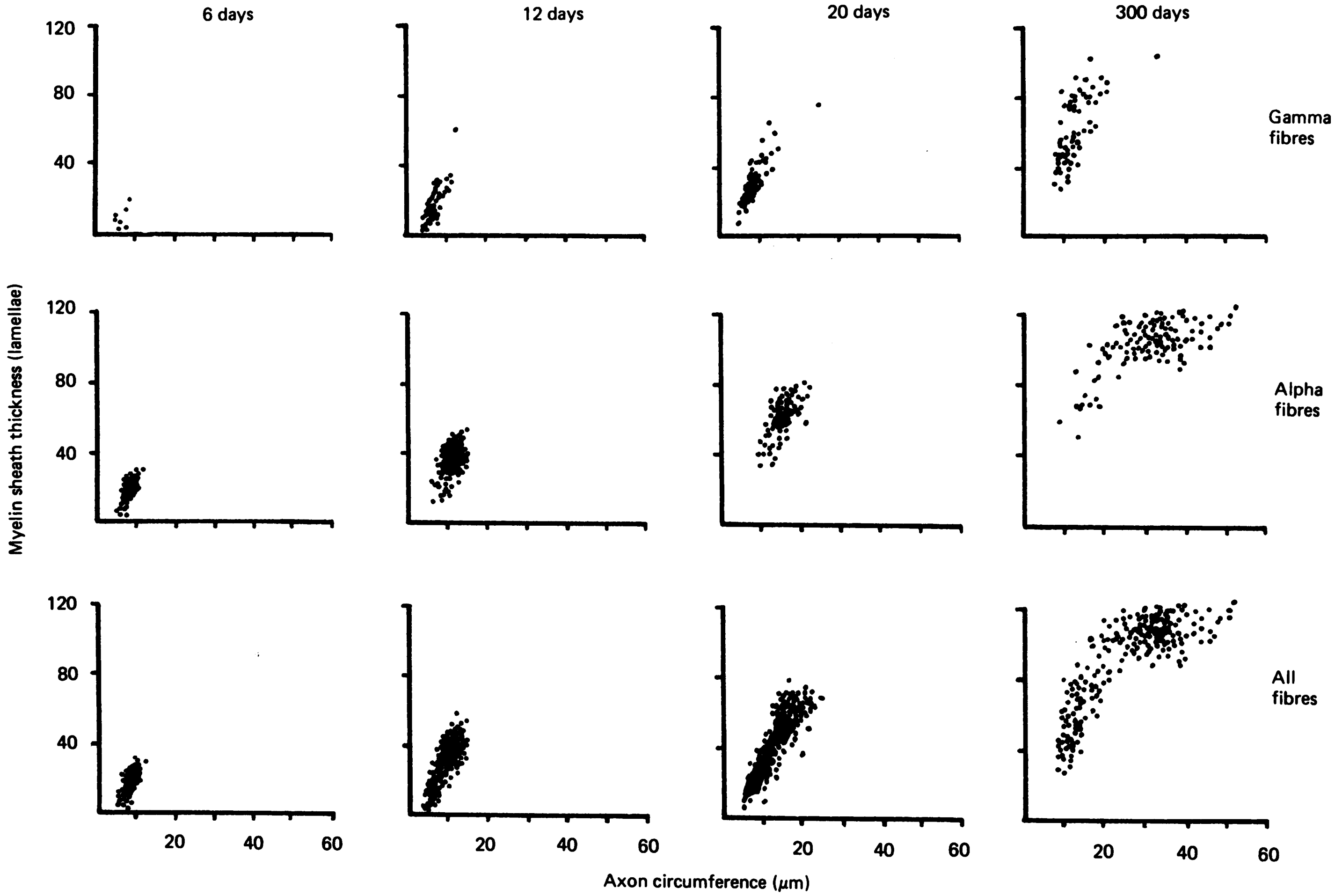

Fig. 4. Scatter diagrams relating mean abnodal sheath thickness to mean abnodal axon circumference for peripheral segments of alpha and gamma fibres and of both groups combined.

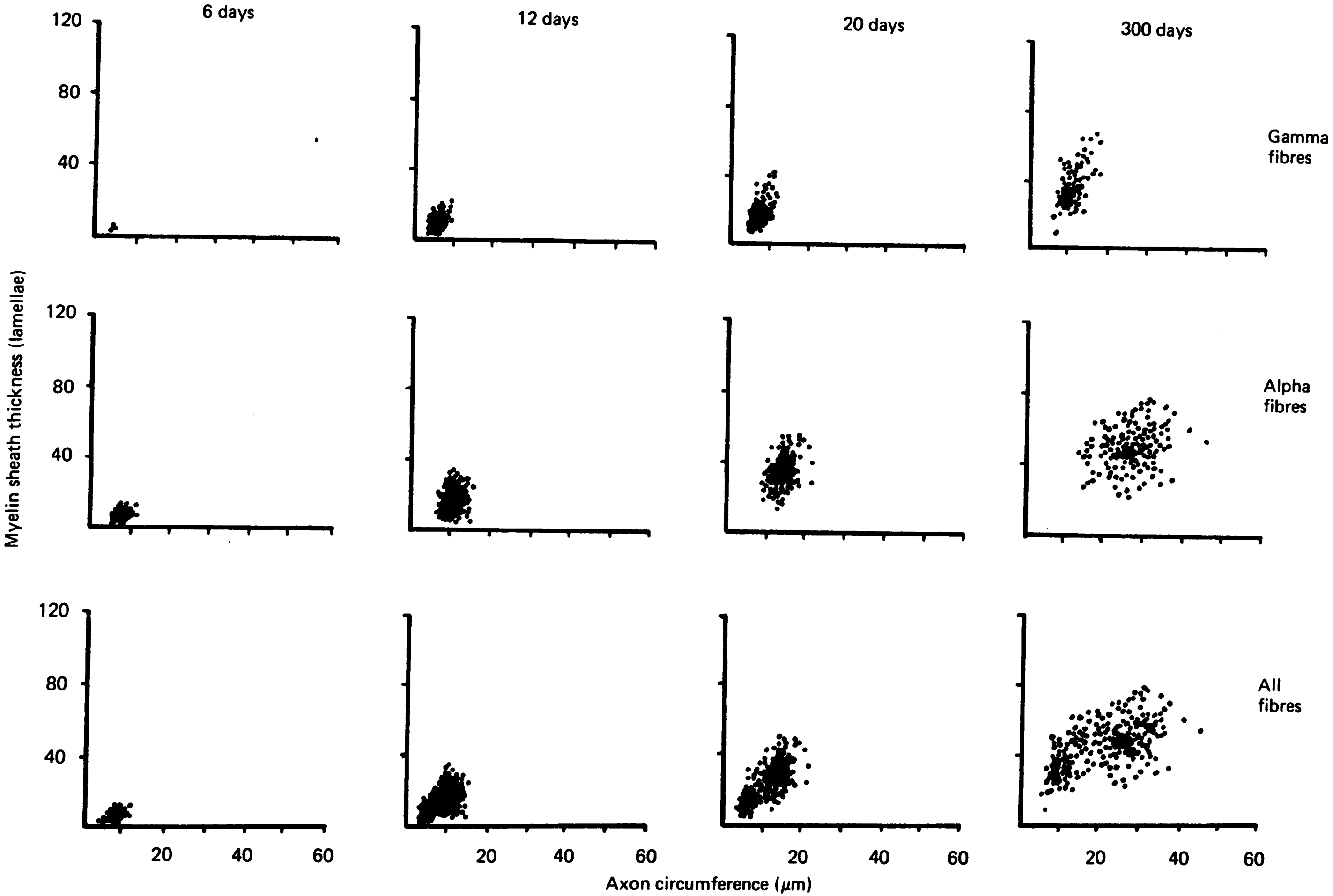

Fig. 5. Scatter diagrams relating mean abnodal sheath thickness to mean abnodal axon circumference for central segments of alpha and gamma fibres and of both groups combined.

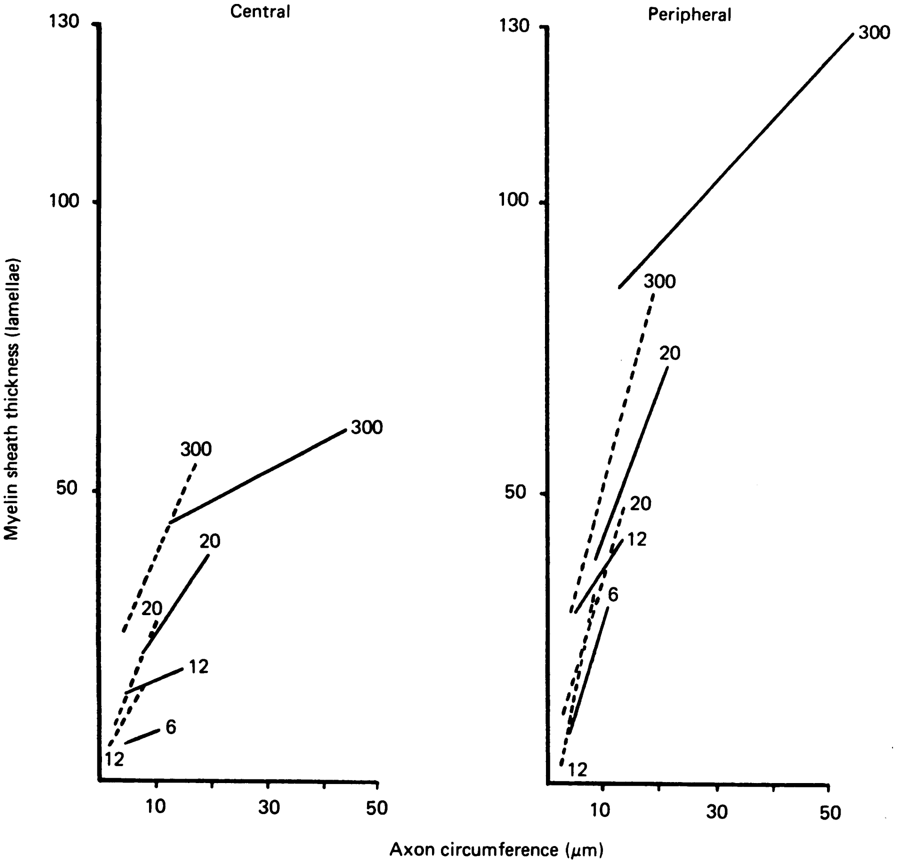

Fig. 6. Regression lines relating myelin sheath thickness to axon circumference for central and peripheral segments of alpha (——) and gamma (– – – –) fibres at the ages indicated.

clusters of plot points representing alpha and gamma fibres tended to separate from one another. The process remained incomplete, however, even at 300 days (Figs. 4, 5). The cluster for gamma fibres tended to lie below and to the left of the corresponding cluster for alpha fibres at each age, centrally and peripherally. At 300 days, centrally and peripherally, the alpha fibre cluster had its long axis approximately parallel to the X-axis while that for the gamma fibre cluster remained similar to previous ages. These differences became more evident on examination of the regression lines which were steeper for gamma than for alpha fibres at each age (Fig. 6). The differences were tested by comparing the linear regression equations relating the two variables for alpha and gamma fibres at each age, using the Indicator Variable technique (Table 4). This showed that the regression lines were statistically significantly different from one another in all cases. The same technique showed that there was no significant difference between the peripheral regression lines at *equivalent stages* of maturation. Thus, at stages following shortly after the onset of myelination in both fibre groups, the regression equation for gamma fibres at 12 days resembled that for alpha fibres at 6 days (Fig. 6; Table 4). The regression lines for gamma fibres tended to lie to the left of those for alpha fibres at equivalent stages, indicating that myelin sheaths of

Table 4. *Comparison of the slopes of the linear regression lines relating myelin sheath thickness to axon circumference at abnodes bordering the definitive transitional nodes of different groups of fibres, using the Indicator Variable technique*

	Age	Central					Peripheral				
	(days)	β	SEβ	t	n-4	P	β	SEβ	t	n-4	P
Gamma versus alpha fibres	12	1·41	0·33	4·3	403	**	−2·07	0·54	3·8	237	**
	20	−0·96	0·42	2·3	373	*	−1·18	0·39	3·0	170	**
	300	−2·01	0·44	4·6	277	**	−2·16	0·36	6·0	209	**
Gamma fibres at 12 and 20 days versus alpha fibres at 6 days	12	1·63	0·65	2·5	76	*	0·29	0·81	0·4	130	NS
	20	0·87	0·17	5·1	157	**	0·62	0·31	2·0	166	*

β, Coefficient of the interaction term. SEβ, Standard error of β. t, Value of t statistic ($= \beta/\mathrm{SE}\beta$). n, Number of observations. n-4, Degree of freedom of t.P, Two-tailed statistical probability of a difference: *, moderately significant ($0·01 \leqslant P < 0·05$). **, highly significant ($P < 0·01$).

alpha fibres tended to be thinner than those of gamma fibres when the axons had a similar calibre.

At first, during the presumptive node stage, there was a loose correlation between sheath thickness and axon circumference in some cases but this strengthened after a short period for all classes of fibre (Table 2). This occurred earlier among alpha than among gamma fibres. It tended to be stronger among gamma than among alpha fibres and to be greater peripherally than centrally. Within both fibre groups the correlation showed no general tendency to increase with age. The *g*-ratios for gamma fibres decreased progressively with age (Table 3), unlike those for alpha fibres. At 300 days, they were about 10 % less than those for alpha fibres.

DISCUSSION

Decremental sheath segment

The persistence throughout life of substantial longitudinal variation in thickness of the abnodal segments of most sheaths central and peripheral to the transitional node is noteworthy. In contrast to myelin sheaths generally, where longitudinal sheath thickness variation decreases with maturation (Fraher, 1973, 1978*a*) the degree of unwinding over the decremental segment increases with age centrally and peripherally. This may represent a further sustained morphological immaturity in the region of the transitional zone in addition to those features already documented (Carlstedt, 1980; Fraher & Rossiter, 1983*a*, *b*).

At all stages, the decremental segment has a length of about 20 to 30 μm. It may be readily distinguished in a number of ways on morphological grounds from the paranode. Firstly, at the junction of the two the spiral becomes much less tightly wound towards the abnodal side. Secondly, the developing and mature transitional paranode can be clearly identified (Fraher & Kaar, 1984) since it possesses numerous features in common with paranodes described by, for example, Schnapp & Mugnaini (1978), Wiley-Livingston & Ellisman (1980) and Rosenbluth (1983). Thirdly, in the decremental segment the pitch of the spiral changes little along its length whereas it tends to become more tightly wound towards the nodal end of the paranode.

Different circumference–sheath thickness relationships for alpha and for gamma fibres

The previous paper (Kaar & Fraher, 1985) shows that two populations of axons (alpha and gamma) can be statistically separated from one another even before the onset of myelination, that the proportions of the two populations are relatively fixed at 6 days, and that both follow different maturation paths subsequently. The present study shows that there is an additional difference between the two groups, namely, each has a different linear sheath thickness to axon circumference relationship at each age up to and including maturity. Thus, when developing axons of similar calibre are compared, gamma axons tend to have thicker myelin sheaths than alpha axons, the regression line being steeper for the former group. The cluster of plot points for the former lies to the left of that for the latter, especially centrally. Thus, gamma axons seem to be more myelinogenic than alpha axons. They induce myelination at a smaller circumference (Kaar & Fraher, 1985) and possess relatively thicker myelin sheaths at all stages of development. These features suggest that both the initiation and progress of myelination are closely related phenomena and so may be controlled by the same mechanism. This mechanism appears, however, to be intrinsically different within alpha and gamma fibres, in so far as the settings for the sheath thickness to axon calibre relationships remain different. These extensive differences suggest that alpha and gamma fibre populations are distinct from one another throughout myelination. It is therefore unlikely that any flux of fibres takes place from the gamma to the alpha groups, as suggested by Berthold *et al.* (1983).

Recent evidence has suggested that in adult nerves the axon calibre–myelin sheath thickness relationship varies with the size of the fibre. Many authors have found a curvilinear or hemi-sigmoidal distribution of plot points in the adult peripheral (Boyd & Kalu, 1973; Sima, 1974; Berthold & Carlstedt, 1977; Berthold, 1978; Arbuthnott, Boyd & Kalu, 1980; Biscoe *et al.* 1982; Berthold *et al.* 1983) and central nervous systems (Berthold & Carlstedt, 1977; Hildebrand & Hahn, 1978). Some authors have attempted to straighten the distribution of plot points by the use of logarithms. Previous quantitative studies have also generally assumed that there is a single relationship between sheath thickness and axon circumference for all fibres during development, both peripherally (Friede & Samorajski, 1968; Fraher, 1976) and centrally (Fraher, 1976). These approaches may obscure real differences which, as the present study shows, come to light only when both groups are examined separately.

The decline in the g-ratio with decreasing axon calibre in peripheral axons is in agreement with the findings of Gasser & Grundfest (1939), Taylor (1942) and Williams & Wendell-Smith (1971). Estimated overall *in vivo* g-ratios for both gamma and alpha fibres agree well with the values of Berthold *et al.* (1983) for cat lumbar ventral root fibres and lie within the optimal ranges calculated by Rushton (1951) and Smith & Koles (1970). For central axons, the g-ratio also decreases with increasing fibre size, as found by Hildebrand & Hahn (1978). However, the g-ratios in the present study tend to be larger than those of the last mentioned authors for both alpha and gamma fibres.

Different axon circumference–sheath thickness relationships for central and peripheral segments

The regression lines relating sheath thickness to axon circumference centrally and peripherally are in different positions and have statistically significantly different slopes at each age. The overall mean g-ratios in the two locations are also different. These findings are true for both alpha and gamma fibre groups. A previous developmental study (Fraher, 1978*b*) found no significant difference between the regression lines for intramedullary and peripheral parts of cervical ventral root fibres. That study, however, did not distinguish between alpha and gamma fibre groups, a factor which would tend to increase the amount of variation in the data pairs and obscure differences between central and peripheral segments.

Age changes in sheath thickness–axon calibre relationship

The present study offers insights into the progression of the different relationships between sheath thickness and axon circumference in adult alpha and gamma fibres. Both relationships change with age centrally and peripherally. For *alpha* fibres, the regression lines tend to become less steep with age. However, this does not take place smoothly. The 20 day line is considerably steeper than that at preceding and succeeding stages. Sheath thickness increases more for each unit increase in axon circumference over the 12–20 day interval than over any other. In accordance with this is the finding that, at 20 days, overall mean peripheral sheath thickness is 53 %, while overall mean circumference is only 47 % of the 300 day value. Equivalent central values are 59 % and 52 %, respectively. Thus there may be a general increase in the rate of myelin production centrally and peripherally in relation to alpha fibres 2–3 weeks after its onset. Between 20 and 300 days, the slopes of the lines decrease because the relative increase in sheath thickness is less than that in axon circumference. By contrast, among gamma fibres, increase in sheath thickness tends to keep pace with that in axon circumference. Age changes in the slopes of the regression lines are relatively minor, there being a slight increase centrally and a decrease peripherally.

Two further points emerge from study of temporal changes in sheath thickness to axon circumference relationships. Firstly, many of the regulating mechanisms for myelin production by oligodendrocytes and Schwann cells may be similar, since within each class of fibre there is a close relationship between changes centrally and peripherally in the position and slope of the regression line and in the value of the g-ratio. Secondly, because the sheath thickness–axon circumference relationship changes during maturation, it is not possible to deduce from adult values what the relationship is like during development. Despite this, several authors (Friede & Samorajski, 1967; Arbuthnott *et al.* 1980; Biscoe *et al.* 1982) have attempted to do so. Because of the different axon–myelin relationships in the alpha and gamma fibre groups, it is not acceptable to base extrapolations regarding the onset of myelination principally or exclusively on data from gamma fibres or on data to which logarithmic curves have been fitted. The present findings indicate that such extrapolations are valid only at very early ages.

Strength of correlation between sheath thickness and axon circumference

The correlation between sheath thickness and axon circumference is stronger peripherally than centrally and in gamma than in alpha fibres in each location. For both fibre groups it decreases with age peripherally and remains low centrally. Thus peripheral myelin sheath thickness appears to be most closely related to axon circumference around the time of onset of myelination. Other studies (Friede & Samorajski, 1967; Fraher, 1973, 1976; Low, 1976) have found that the strength of association increases with age in all fibres together. This may be a consequence of the larger increase in the ranges of values for both sheath thickness and axon circumference in pooled fibres, compared with separated alpha and gamma fibre groups. In any event, in interpreting correlation coefficients it must be borne in mind that correlation analysis should properly be applied only to bivariate normal distributions (Sokal & Rohlf, 1969; Bronson, Bishop & Hedley-White, 1978) as is done in the present study.

Functional implications

The unwinding of the sheath over the decremental segment may allow current leakage into the interstitial fluid space, especially close to the paranode. Because of the observed differences in sheath thickness, any such leakage is likely to be greater in absolute terms centrally than peripherally. It is likely to affect gamma fibres relatively more than alpha fibres. This is because the longitudinal distance over which unwinding occurs is similar in both groups, but internodal lengths are short on gamma fibres centrally (Hess & Young, 1949, 1952; McDonald & Olrich, 1971; Murray & Blakemore, 1980) and peripherally (Vizoso & Young, 1948; Fullerton & Barnes, 1966). Consequently the proportion of the internode over which the sheath unwinds is greater for gamma than for alpha fibres.

There is likely to be a large increase in the conduction velocity of nerve impulses in all fibres on passing from the central into the peripheral nervous system, for three reasons. Firstly, sheath thickness and axon circumference are both greater peripherally than centrally. Secondly, the *g*-ratio of some gamma and many alpha fibres centrally is likely to be greater than the optimum physiological value as calculated on theoretical grounds (Rushton, 1951). By contrast, the majority of peripheral segments appear to have values within the optimum range. Thirdly, internodal length is likely to be considerably greater peripherally than centrally (Fraher, 1978*c*; Carlstedt, 1980; Murray & Blakemore, 1980). All these features are positively associated with greater conduction velocity along nerve fibres (Paintal, 1978; Waxman, 1980). The difference may be somewhat greater for alpha than for gamma fibres since the relative increase in sheath thickness peripherally is greater for the former. A different pattern of variation occurs in dorsal lumbosacral roots in the adult cat in which sheaths are thicker centrally than peripherally and axon circumference does not change between the two locations (Berthold & Carlstedt, 1977, 1982). Consequently conduction velocity is likely to be lower peripherally than centrally. During development however, the pattern of differences in conduction velocity in the cat may resemble that occurring throughout life in the rat; the above authors found that developing central sheaths are relatively shorter and thinner than peripheral sheaths.

SUMMARY

The abnodal myelin sheaths of the internodes immediately central and peripheral to the transitional node possess a decremental segment over which sheath thickness gradually decreases in the direction of the paranode. This may represent a sustained morphological immaturity of the sheath. Alpha and gamma fibre groups have different sheath thickness to axon circumference relationships at each age during development and at maturity. Gamma fibres have relatively thicker sheaths than alpha fibres. In both groups the relationship is different for central and peripheral fibre segments and also changes during maturation. It is therefore not permissible to make inferences from the adult relationship regarding the relationship during development. Changes in the relationship follow similar patterns for central and

peripheral fibre segments, suggesting that the control mechanisms are closely linked in both locations. Sheath thickness growth both peripherally and centrally lags behind axon calibre growth for alpha fibres. The two keep pace for gamma fibres. The strength of the correlation between sheath thickness and axon circumference changes little with maturation in either group. The morphological differences between central and peripheral segments of ventral motoneuron fibres suggest that conduction velocity increases when the impulse enters the peripheral nervous system.

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REFERENCES

ARBUTHNOTT, E. R., BOYD, I. A. & KALU, K. U. (1980). Ultrastructural dimensions of myelinated peripheral nerve fibres in the cat and their relation to conduction velocity. *Journal of Physiology* **308**, 125–157.

BERTHOLD, C. H. (1978). Morphology of normal peripheral axons. In *Physiology and Pathobiology of Axons* (ed. S. G. Waxman), pp. 3–63. New York: Raven Press.

BERTHOLD, C. H. & CARLSTEDT, T. (1977). Observations on the morphology at the transition between the peripheral and central nervous system in the cat. III, Myelinated fibres in S_1 dorsal rootlets. *Acta physiologica scandinavica, Suppl.* **446**, pp. 43–60.

BERTHOLD, C. H. & CARLSTEDT, T. (1982). Myelination of S_1 dorsal root axons in the cat. *Journal of Comparative Neurology* **209**, 225–232.

BERTHOLD, C. H., CORNELIUSON, O. & RYDMARK, M. (1982). Changes in shape and size of cat spinal root myelinated nerve fibres during fixation and Vestopal-W embedding for electron microscopy. *Journal of Ultrastructure Research* **80** 23–41.

BERTHOLD, C. H., NILSSON, I. & RYDMARK, M. (1983). Axon diameter and myelin sheath thickness in nerve fibres of the ventral root of the seventh lumbar nerve in the adult and developing cat. *Journal of Anatomy* **136**, 483–508.

BISCOE, T. J., NICKELS, S. M. & STIRLING, C. A. (1982). Numbers and sizes of nerve fibres in mouse spinal roots. *Quarterly Journal of Experimental Physiology* **67**, 473–494.

BOYD, J. A. & KALU, K. U. (1973). The relation between axon size and number of lamellae in the myelin sheath for afferent fibres in groups I, II and III in the cat. *Journal of Physiology* **232**, 31–33.

BRONSON, R. T., BISHOP, Y. & HEDLEY-WHITE, E. T. (1978). A contribution to the electron microscopic morphometric analysis of peripheral nerve. *Journal of Comparative Neurology* **178**, 177–186.

CARLSTEDT, T. (1980). Internodal length of nerve fibres in dorsal roots of cat spinal cord. *Neuroscience Letters* **19**, 251–256.

FINEAN, J. B. (1961). Nature and stability of nerve myelin. *International Review of Cytology* **12**, 303–336.

FRAHER, J. P. (1972). A quantitative study of anterior root fibres during early myelination. *Journal of Anatomy* **112**, 99–124.

FRAHER, J. P. (1973). A quantitative study of anterior root fibres during early myelination. II. Longitudinal variation in sheath thickness and axon circumference. *Journal of Anatomy* **115**, 421–444.

FRAHER, J. P. (1976). The growth and myelination of central and peripheral segments of ventral motoneurone axons. A quantitative ultrastructural study. *Brain Research* **105**, 193–211.

FRAHER, J. P. (1978*a*). The maturation of the ventral root-spinal cord transitional zone. *Journal of the Neurological Sciences* **36**, 427–449.

FRAHER, J. P. (1978*b*). Quantitative studies on the maturation of central and peripheral parts of individual ventral motoneuron axons. I. Myelin sheath and axon calibre. *Journal of Anatomy* **126**, 509–533.

FRAHER, J. P. (1978*c*). Quantitative studies on the maturation of central and peripheral parts of individual ventral motoneuron axons. II. Internodal length. *Journal of Anatomy* **127**, 1–15.

FRAHER, J. P. & KAAR, G. F. (1984). The transitional node of Ranvier at the junction of the central and peripheral nervous systems: An ultrastructural study of its development and mature form. *Journal of Anatomy* **139**, 215–238.

FRAHER, J. P. & ROSSITER, J. P. (1983*a*). Cell clusters on fetal rat ventral root: Prenatal development. *Journal of Anatomy* **136**, 111–128.

FRAHER, J. P. & ROSSITER, J. P. (1983*b*). Cell clusters on rat ventral roots: Postnatal development. *Journal of Anatomy* **137**, 555–571.

Friede, R. L. & Bischausen, R. (1980). The precise geometry of large internodes. *Journal of the Neurological Sciences* **48**, 367–381.

Friede, R. L. & Samorajaski, T. (1967). Relation between the number of myelin lamellae and axon circumference in fibres of vagus and sciatic nerve of mice. *Journal of Comparative Neurology* **130**, 223–232.

Friede, R. L. & Samorajski, T. (1968). Myelin formation in the sciatic nerve of the rat. A quantitative electron microscopic, histochemical and radioactive study. *Journal of Neuropathology and Experimental Neurology* **27**, 546–571.

Fullerton, P. M. & Barnes, J. M. (1966). Peripheral neuropathy in rats produced by acrylamide. *British Journal of Industrial Medicine* **23**, 210–221.

Gasser, H. S. & Grundfest, H. (1939). Axon diameters in relation to the spike dimensions and the conduction velocity in mammalian A fibres. *American Journal of Physiology* **127**, 393–414.

Hess, A. & Young, J. Z. (1949). Correlation of internodal length and fibre diameter in the central nervous system. *Nature* **164**, 490–491.

Hess, A. & Young, J. Z. (1952). The nodes of Ranvier. *Proceedings of the Royal Society*, **B 140**, 301–320.

Hildebrand, C. & Hahn, R. (1978). Relation between myelin sheath thickness and axon size in spinal cord white matter of some vertebrate species. *Journal of the Neurological Sciences* **38**, 421–434.

Hildebrand, C. & Muller, H. (1974). Low angle X-ray diffraction studies on the period of central myelin sheaths during preparation for electron microscopy – a comparison between different anatomical areas. *Neurobiology* **4**, 71–81.

Kaar, G. F. & Fraher, J. P. (1985). The development of alpha and gamma motoneuron fibres in the rat. I. A comparative ultrastructural study of their central and peripheral axon growth. *Journal of Anatomy* (in press).

Karlsson, U. (1966). Comparison of myelin period of peripheral and central origin by electron microscopy. *Journal of Ultrastructure Research* **15**, 451–468.

Low, P. A. (1976). Hereditary hypertrophic neuropathy in the trembler mouse. Part 2. Histopathological studies: electron microscopy. *Journal of the Neurological Sciences* **30**, 343–368.

McDonald, W. I. & Ohlrich, G. D. (1971). Quantitative anatomical measurements on single isolated fibres from the cat spinal cord. *Journal of Anatomy* **110**, 191–202.

Murray, J. A. & Blakemore, W. F. (1980). The relationship between internodal length and fibre diameter in the spinal cord of the cat. *Journal of the Neurological Sciences* **45**, 29–41.

Paintal, A. S. (1978). Conduction properties of normal peripheral mammalian axons. In *Physiology and Pathobiology of Axons* (ed. S. G. Waxman), pp. 131–144. New York: Raven Press.

Rosenbluth, J. (1983). Electrophysiology and morphology of myelinated nerve fibres. V. Intramembranous particle distribution in nerve fibre membranes. *Experientia* **39**, 953–963.

Rushton, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *Journal of Physiology* **115**, 101–122.

Schnapp, B. & Mugnaini, E. (1978). Membrane architecture of myelinated fibers as seen by freeze-fracture. In *Physiology and Pathobiology of Axons* (ed. S. G. Waxman). New York: Raven Press.

Sima, A. (1974). Relation between the number of myelin lamellae and axon circumference in fibres of ventral and dorsal roots and optic nerve in normal, undernourished and rehabilitated rats. *Acta physiologica scandinavica Suppl.* **410**, 1–38.

Smith, R. S. & Koles, Z. J. (1970). Myelinated nerve fibres – computed effect of myelin thickness conduction velocity. *American Journal of Physiology* **219**, 1256–1258.

Sokal, R. R. & Rohlf, F. J. (1969). *Biometry, the Principles and Practice of Statistics in Biological Research*. San Francisco: W. H. Freeman & Co.

Taylor, G. W. (1942). The correlation between sheath birefringence and conduction velocity with special reference to cat nerve fibres. *Journal of Cellular and Comparative Physiology* **20**, 359–372.

Vizoso, A. D. & Young, J. Z. (1948). Internode length and fibre diameter in developing and regenerating nerves. *Journal of Anatomy* **82**, 110–134.

Waxman, S. G. (1980). Determinants of conduction velocity in myelinated nerve fibres. *Muscle and Nerve* **3**, 141–150.

Wiley-Livingston, C. A. & Ellisman, M. H. (1980). Development of axonal specialisations defines nodes of Ranvier and precedes Schwann cell myelin elaboration. *Developmental Biology* **79**, 334–355.

Williams, P. L. & Wendell-Smith, C. P. (1971). Some additional parametric variations between peripheral nerve fibre populations. *Journal of Anatomy* **109**, 505–526.