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X-RAY DIFFRACTION STUDY OF MYELIN STRUCTURE IN IMMATURE AND MUTANT MICE

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SUMMARY

X-ray diffraction patterns were obtained from freshly dissected central and peripheral nerves of quaking, myelin synthesis deficiency (*msd*), and trembler mutants, as well as immature and adult normal mice. The patterns were compared with respect to strength of myelin diffraction, background scatter level, repeat period, and intensity and linewidth of Bragg reflections. The deficiency of myelin in optic nerves was found to be (in decreasing severity): quaking > immature > trembler ≃ normal adult; and in sciatic nerves: trembler > immature > quaking > *msd* ≃ normal adult. Repeat periods about 3 Å less than that for normal adult sciatic myelin were detected in corresponding nerves from immature, quaking, and trembler mice. In some trembler sciatic nerves a second phase having a 190–200 Å period and accounting for about 60 % of the total ordered myelin was also evident. Comparison of electron density profiles of membrane units calculated from the repeat periods and diffracted intensities for sciatic myelins indicate structural differences at the molecular level. The main findings are: (1) quaking myelin shows a significant elevation of density in the external protein-water layer between membrane bilayers; (2) the membrane bilayer of immature myelin is ≈ 2 Å thinner than that for normal adult; (3) the membrane bilayer of the more compact phase in trembler myelin is ≈ 5 Å thinner than for normal; and (4) the difference in repeat periods for the two phases present in some of the trembler nerves can be accounted for predominantly by distinct membrane bilayer separations at the external boundary.

INTRODUCTION

Light and electron microscope studies of nervous tissue from mutant mice reveal severe myelin deficiencies in the central nervous systems of jimpy, quaking [1] and myelin synthesis deficiency (*msd*) mice [2], and in the peripheral nervous system of trembler mice [3]. Less severe hypomyelination in the peripheral nervous system of quaking mice has also been observed [4]. The present study using X-ray diffraction was undertaken to determine whether the membrane structure of the morphologically

recognizable myelin formed in immature and myelin-deficient mice (quaking, *msd*, trembler) can be distinguished from that of normal, mature animals.

X-ray diffraction has been previously used to examine molecular organization [5] and membrane structure [6, 7] in the normal myelin sheath; and recent X-ray measurement of central nervous system and peripheral nervous system myelin from quaking and jimpy mice [8] confirms the severe lack of myelin in these mutants. The present study, in addition, finds differences in membrane structure at the molecular level among the myelins from quaking and trembler mutants, and immature and adult normal mice.

MATERIALS AND METHODS

Specimens. Optic, trigeminal and sciatic nerves were dissected with minimal manipulation from five quaking and eight control mice (equally divided between normal Balb/cJ, C3H/HeJ, C57BL/6J(+/+ at the *qk* locus) and C57BL/6J littermates of quaking mice which may be +/+ or +/*qk*) ranging in age from 39–226 days old. Sciatic nerves were dissected from 16 and 20 day old male C57BL/10J mice bearing the sex-linked mutation myelin synthesis deficiency, jp^{msd}; from one 9-day old C57BL/6J and one 10-day old non-inbred normal mouse; and from one 11-month old, one 14-month old and one 20-month old non-inbred mouse heterozygous for the dominant (*Tr*) mutation. The optic nerve of the trembler mouse and the lateral olfactory tract, trapezoid body, and optic nerves of the 10-day old mouse were also examined.

X-ray diffraction. The freshly dissected nerves were tied off at each end, and mounted in contact with phosphate-buffered saline in thin-walled quartz capillaries which were then sealed. X-ray diffraction patterns were obtained from specimens at room temperature on 80–200 mm cameras using the mirror-focused copper K_α radiation from the fine-line source of a Philips X-ray tube operated at 40 kV, 15 mA, or using a point-focused beam produced by a double-mirror or mirror-monochromator assembly on an Elliott rotating anode run at 40 kV, 18 mA. The large difference in intensity between the strongest and weakest observable reflections necessitated the use of multiple films (Ilford Industrial G) and exposures of up to 3 days. Over this period of time, postmortem deterioration in the specimen did not generally affect the myelin sheath, judging from the stability of its diffraction pattern.

Measurement of X-ray films. The spacings of the diffraction orders were measured on a Nikon microcomparator having 10× magnification. For each type of myelin (e.g. quaking, littermate control, normal, etc.) the periodicities measured for different specimens varied by about ±2 Å; and the individual measurements on sharp diffraction spectra for films exposed on the 200 mm camera were accurate to ≈ 0.3% (±0.5 Å). Intensities of the diffraction spectra were measured with an Optronics Photoscan, and plotted on a Calcomp Plotter. Integrated peak intensities were calculated numerically or measured using a planimeter to determine the area under each peak after background subtraction; and integrated line-widths were calculated by dividing the integrated peak intensities by their respective peak heights. Intensity data from successive films in a given exposure were scaled by comparing the relative intensities of reflections common to the films. The intensity measurements were made from twelve X-ray exposures on sciatic nerves from five different quaking mice, six exposures on nerves from four different control littermates, five exposures

from four normals, and one exposure each from the *msd* mice and their controls, from the immature mice, and from the trembler mouse sciatic nerves.

Comparison of diffracted intensities. For each type of myelin the measured intensities from different X-ray exposures were averaged for each diffraction order h. The significance of differences between corresponding mean intensities $\bar{I}(h)$ from different myelin types was evaluated using the Student t-test for samples with equal variances, or using the method of Aspin and Welch [9] for samples with unequal variances.

Calculation of electron density profiles. Since the radial scattering density through the array of myelin membrane pairs is periodic, it can be represented by a summation of density harmonics:

$$\rho(r) = 2/d\sum_h \pm|\bar{F}(h)|\cos(2\pi hr/d)$$

where d is the measured lamellar repeat, and the squares of the structure factors $\bar{F}^2(h) = h\bar{I}(h)$ for line collimation, or $\bar{F}^2(h) = h^2\bar{I}(h)$ for point-focus geometry. Each structure factor represents the amplitude $|F(h)|$ of the h^{th} electron density harmonic of frequency $(2\pi h)$ through the lattice. Different density profiles $\rho(r)$ were scaled by setting $\sum_h \bar{F}^2(h)/d =$ constant. The similarity in the membrane bilayer structures of the myelin from the central and peripheral nervous systems of different species [7, 10] provided a basis for assigning phases [10] to the mouse myelin diffraction patterns.

RESULTS

Comparison of diffraction patterns (Table I). Compared to patterns of normal adult nerves from the peripheral nervous system, the diffraction patterns from corresponding nerves of myelin-deficient mice (except for the *msd*) showed weaker coherent scattering and higher small-angle background levels. These anomalies in myelin diffraction from peripheral nerves were found to increase in severity in the order: quaking < immature < trembler (Figs. 1 and 2). Linewidths of the diffraction spectra from immature and trembler sciatic nerves were visibly broadened; and measurements on the strong, low order reflections ($h = 2$–5) from the peripheral nervous system nerves of normal, quaking, and *msd* mice revealed a less noticeable but significant increase of $\approx 10\,\%(P < 0.01)$ in linewidth for quaking myelin reflections. Weak, higher order reflections to spacings about 15 Å were detected in patterns from normal, quaking, and 10-day-old sciatic nerves; but no discrete higher orders were apparent from trembler sciatic myelin. All the reflections in the myelin diffraction patterns from normal adult, *msd*, quaking, 11 month old trembler and immature mice clearly belonged to single lattices; but the multiple, low-angle equatorial reflections recorded from the 14 and 20 month old trembler mice sciatic nerves suggested more than one repeat period for myelin in this mutant at these ages. In addition, sciatic nerves from the older trembler mice showed a strong series of meridional reflections (Fig. 2B, top) which were orders of a 660 Å collagen periodicity.

Optic nerves from the trembler mutants (Fig. 3D) showed discrete myelin diffraction spectra to spacings about 15 Å typical of normal optic nerve. Comparable X-ray exposures of central nerve tissue from the immature mouse (Fig. 3C) showed very weak myelin diffraction which consisted only of the discrete second and

TABLE I

SUMMARY OF KEY OBSERVATIONS FROM DIFFRACTION PATTERNS

	Sciatic nerves		
	Normal adult	*msd*	Quaking
Strength of coherent scattering*			
From myelin	0	0	
From collagen**	0		0
Small angle background level*	0	0	
Linewidths of diffraction spectra*†	0	0	
Spacing of highest Bragg order (h) detected in long exposures	≈ 12 Å (h = 15)		≈ 12 Å (h = 15)
Repeat periods	177 Å	177 Å	174 Å
Intensity distribution	2 > 4 > 3 > 5 > (1) > 11 > 8 > 10 > 7 > 6 > 9	–	8 ≃ 7 > 10 > 6 > (9)
Statistical significance of differences in relative intensities	0	0	I_4/I_2 $P < 0.01$ I_8/I_{11} $P < 0.05$ I_{10}/I_{11} $P < 0.10$

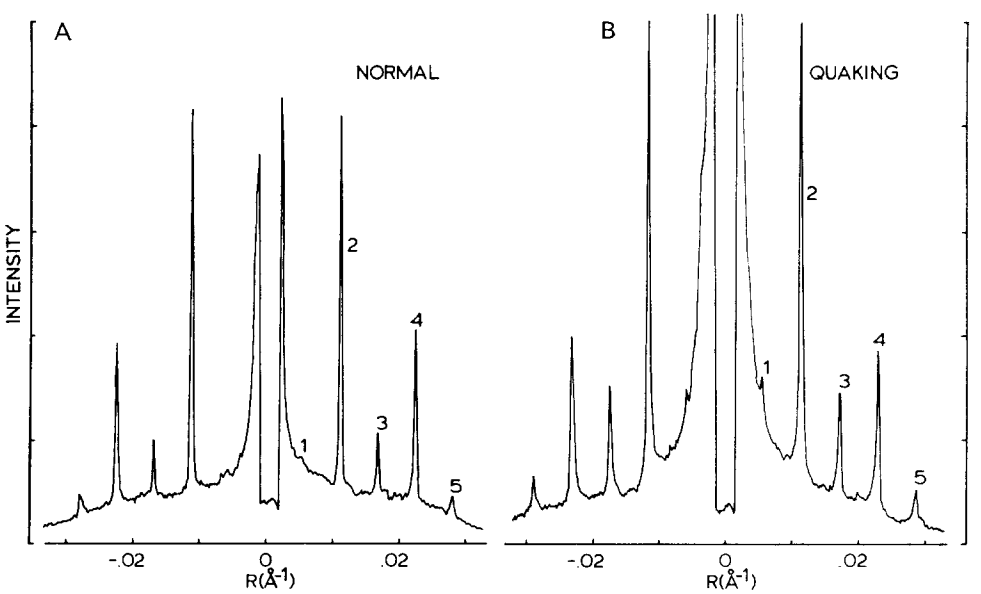

Fig. 1. Densitometer tracings on a linear scale of diffracted intensity vs. diffraction angle ($R = 2\sin\theta/\lambda$) for sciatic nerves from (A) a symptom-free littermate of a quaking mouse, and (B) a quaking mouse. Successive diffraction orders h = 1–5 are indicated in the figure. The exposure times for these patterns, which were recorded on the 200 mm line-collimation camera, were ≈ 0.5 h for the control, and ≈ 1.8 h for the quaking. Repeat periods for these two specimens are 177 Å and 173 Å, respectively. The patterns show increased linewidths for the reflections from quaking myelin; elevated small-angle scattering from quaking nerve; and enhancement of the low order reflections for quaking myelin relative to the fourth order intensity which is similar in these two exposures.

Sciatic nerves		Central nervous system tissue			
Immature	Trembler (14-month-old)	Normal adult	Trembler	Immature	Quaking
− −	− − −	0	0	−	− − −
n.o.	+++	n.p.	n.p.	n.p.	n.p.
+	+++	0	0	0	++
++	+++	0	0	0+	+++
≈ 16 Å	≈ 35 Å	≈ 16 Å	≈ 16 Å	≈ 40 Å	≈ 40 Å
($h = 11$)	($h = 5$)	($h = 10$)	($h = 10$)	($h = 4$)	($h = 4$)
174 Å	173 and 190 Å	160 Å	155 Å	158 Å	≈ 160 Å
	$2 > 4 \simeq 3 > 5$	$2 > 4 > 3 \geqq 10 > 6 > 5 >$ (8)		$2 > 4$	
$6 \simeq 8 > 10$	and $3 > 5 \simeq 2 > 4$				
−	−	−	−	−	−

* For the qualitative comparisons indicated; 0, represents the norm; +, more than the norm; −, less than the norm; ×, not examined; n.o., not observed; n.p., not present.

** In long exposures using point-focus geometry, the 5th and 9th orders of the collagen periodicity are detected, and are comparable in intensity to the weak higher order myelin reflections; in the trembler mutant, however, the collagen diffraction spectra are comparable in intensity to the low-angle myelin reflections.

† The low order reflections from quaking are 10 % greater in linewidth compared to normal ($P < 0.01$).

fourth order reflections. Quaking mouse optic nerve (Fig. 3B) also gave very weak myelin diffraction, but the second and fourth order reflections were very diffuse, and were difficult to distinguish over the strong, small angle background scatter.

Repeat periods. Repeat periods of 174±2 Å were measured for trigeminal and sciatic nerve myelin from the quaking mouse, and for sciatic nerve myelin from immature mice. These values were about 3 Å less than the periods measured for corresponding nerves from littermate controls and normal adults, and from *msd* mice and their littermate controls. The multiple, low-angle equatorial reflections recorded from trembler mouse sciatic nerves indexed as orders $h = 2$–5 from repeat periods about 173 Å and 190–200 Å. The 160 Å period measured for normal mouse optic nerve myelin was ≈ 5 Å greater than that measured for trembler optic nerves, but was within the uncertainty in the period measured from the diffuse reflections for quaking optic myelin. Repeat periods measured from immature central nerve myelin were 158±1 Å.

Diffraction intensities. The diffraction patterns (Figs. 1 and 2) generally showed an alternation of weak odd and strong even orders at low angles, and significant scattering in the 15–16 Å region. These features are typical of patterns obtained from central and peripheral nerve myelin of different species [5, 7, 10, 11]. In contrast, the diffraction patterns from sciatic nerves of the older trembler mice were atypical: the 2nd–5th order reflections were of comparable intensity, and the 3rd order was the

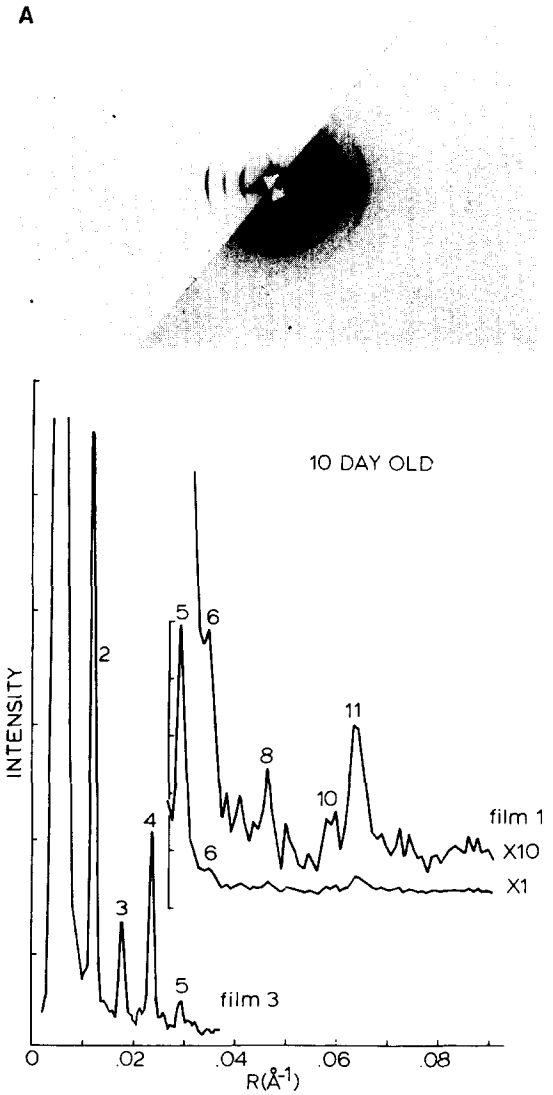

Fig. 2. For legend see oppsite page.

dominant reflection from the slightly expanded phase. The lamellar repeat periods of quaking and control or normal peripheral nerve myelin were slightly different; and there were statistically significant differences between the intensities of corresponding reflections (Tables I and II). No significant differences between corresponding intensities were found, however, in comparing data between littermate controls and normal myelin, or between *msd* mutants and their controls. Measurement of diffraction intensities from immature sciatic myelin indicated that in comparison with mature myelin the magnitude of the second order was weaker relative to neighboring low-angle reflections and the higher order Bragg reflections ($h = 6, 8, 10, 11$) were stronger relative to low orders.

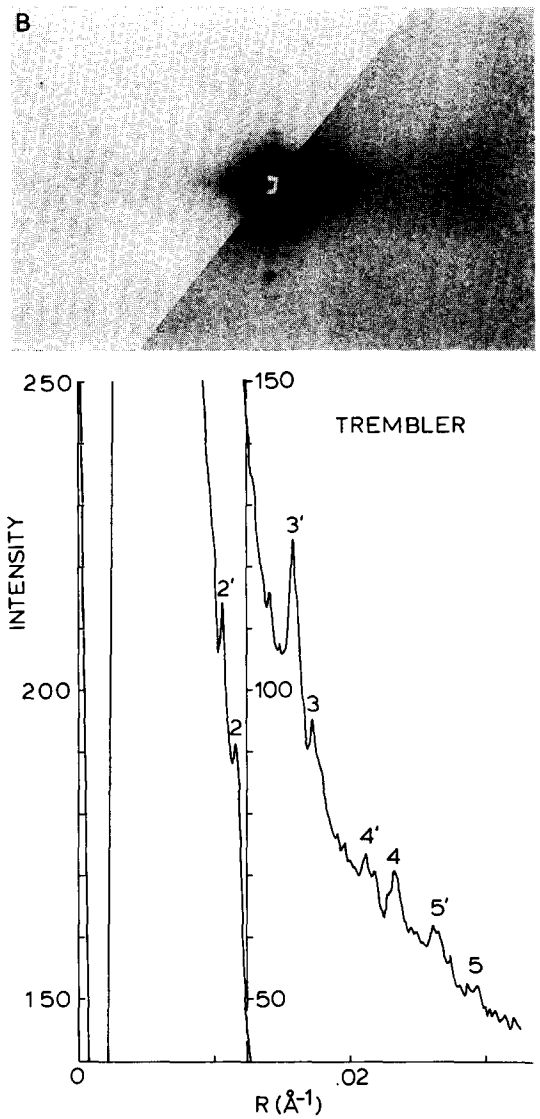

Fig. 2. Diffraction patterns (top) and densitometer tracings (bottom) for sciatic nerves from (A) a 10-day-old normal and (B) a 14-month-old trembler mouse. Exposure times were 70 h using the 80 mm point-focus double mirror camera. Both patterns, particularly that from trembler sciatic, show strong scatter at small angles. Only one set of lamellar reflections, indexing as orders $h = 2, 3, 4, 5, 6, 8, 10, 11$ from a repeat period 173 Å, is visible on the pattern from immature myelin, and no meridional collagen reflections are apparent. For the trembler nerve, two sets of equatorial reflections are visible, indexing as $h = 2, 3, 4$, and 5 from repeats of 173 Å (unprimed orders) and 190 Å (primed orders), as well as diffuse equatorial scatter at higher angles. Strong meridional reflections from collagen are present, including orders $h = 5, 8, 9, 12, 20$ and 25 from a 660 Å periodicity.

Comparison of electron density profiles. The density profiles for quaking and normal sciatic myelins (Fig. 4) calculated using diffraction data to $\approx$ 15 Å spacing ($h = 1$–11), show that their membrane bilayers are very similar. However, while the

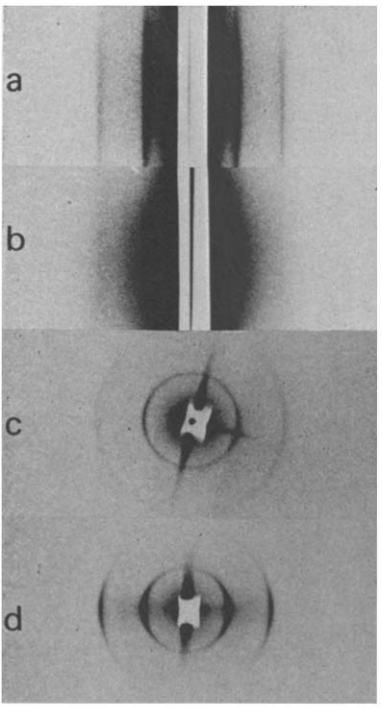

Fig. 3. Diffraction patterns from optic nerves of (a) normal, (b) quaking, and (d) trembler adult mice, and (c) trapezoid body of 10-day-old normal mouse. Exposure times were ≈ 3 h for the quaking pattern recorded on 122 mm line-collimation camera, and ≈ 1 h for the normal on an 80 mm line-collimation camera. Second and fourth order reflections from repeat periods ≈ 160 Å are visible in both patterns; but there is considerably more small-angle scatter from quaking optic nerve. The point-focus patterns (c, d) were recorded on a 155 mm mirror-monochromator camera, with exposure times of ≈ 15 h for the trembler optic nerve pattern, and ≈ 20 h for the pattern from trapezoid body. In addition to the discrete second and fourth order reflections observed in both patterns, a weak first order is detected in the trembler pattern. The vertical streak arises from the monochromator.

center-to-center separation of the high density lipid polar group peaks in the normal membrane unit is 48 Å, that in the quaking appears to be 1 Å narrower. A more distinctive difference between the density profiles is that the average electron density level in the external protein-water layer between membrane bilayers (for $r \geqslant 75$ Å) is greater in quaking than in normal myelin.

The membrane unit density profiles for immature and adult sciatic myelin (Fig. 5) are compared for two scalings of the bilayer profile for immature myelin. With one scaling (Fig. 5A) there is superposition of corresponding high density lipid polar group layers, low density disordered hydrocarbon layers, and mean electron density levels, or baselines. The main differences between the profiles are the decrease in density of the shoulders in the hydrocarbon layer, and the increase in density outside the bilayer in adult myelin. With the second scaling (Fig. 5B), there is superposition of the high density peaks and of the shoulders in the bilayers. Compared to immature myelin, the adult membrane unit shows a higher mean electron density, as well as elevated density levels at the center of the bilayer and outside the bilayer. For either

TABLE II

REPEAT PERIODS AND STRUCTURE FACTORS FOR MOUSE SCIATIC NERVE MYELINS

	Normal adult		Quaking		10-day-old		*msd*		*msd* littermate		Trembler (14-months-old)	
d(Å)	177		174		174		177		177		173	190
h	$F(h)$	($\delta F(h)$)	$F(h)$	($\delta F(h)$)	$F(h)$	($\delta F(h)$)	$F(h)$	($\delta F(h)$)	$F(h)$	($\delta F(h)$)	$F(h)$	$F(h)$
1	−0.13	(0.01)	−0.21	(0.01)	−0.02	(0.02)	–		–		−0.16	−0.12
2	1.41	(0.02)	1.46	(0.01)	1.23	(0.02)	1.38	(0.02)	1.36	(0.02)	0.83	0.50
3	0.78	(0.03)	0.79	(0.02)	0.74	(0.03)	0.72	(0.02)	0.85	(0.02)	1.07	±1.71
4	−1.44	(0.03)	−1.32	(0.02)	−1.46	(0.05)	−1.49	(0.04)	−1.40	(0.03)	−1.46	−0.91
5	−0.71	(0.04)	−0.72	(0.01)	−0.76	(0.02)	−0.75	(0.02)	−0.83	(0.02)	−1.10	±1.30
6	0.06	(0.06)	0.07	(0.07)	0.20	(0.01)						
7	−0.08	(0.01)	−0.11	(0.01)	0							
8	0.14	(0.02)	0.12	(0.02)	0.24	(0.03)						
9	±0.09	(0.03)	0		0							
10	0.13	(0.03)	0.10	(0.03)	0.22	(0.03)						
11	0.31	(0.03)	0.41	(0.04)	0.54	(0.03)						

d, repeat period; h, diffraction order; $F(h)$, mean structure factor with phase; $\delta F(h)$, uncertainty in structure factor calculated from standard error (S.E.M.) of the intensity measurement. The tabulated structure factors have been scaled on the origin peak of their Patterson functions (i.e., $1/d\Sigma F^2(h)$ = constant). To compare relative diffracting powers for the two myelin phases of trembler sciatic nerve, the tabulated structure factors for the 190 Å phase must be scaled up by 1.3. First order reflections were not detected from immature and trembler sciatics due to high background scatter around the beam stop, so their first order intensities were taken as half the minimum detectable intensity with an uncertainty of ±100 %; and the short, line-collimation camera used for the *msd* sciatics and their controls, had insufficient first order resolution.

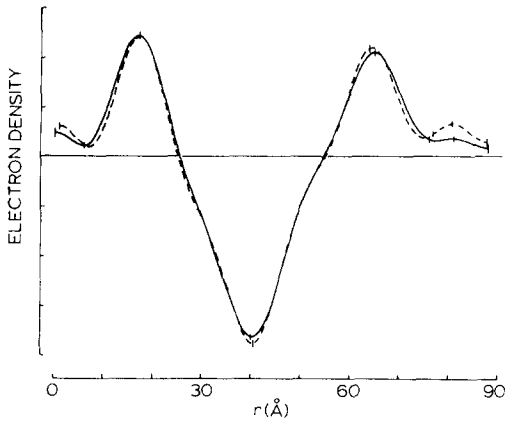

Fig. 4. Calculated electron density profiles of sciatic myelin membrane units for normal/control (solid) and quaking (dashed) mice as a function of distance r from the cytoplasmic boundary. The external boundary between membrane units is on the right. Note that the membrane unit shown is half the lamellar repeating unit which consists of a pair of membranes with their cytoplasmic surfaces apposed. The profiles have been calculated using diffraction data to 15–16 Å spacing (h = 1–11). The short vertical bars on the density profiles are representative uncertainties, calculated from $\pm[\sum_h \delta F^2(h)\cos^2(2\pi hr/d)]^{\frac{1}{2}}$, where $\delta F(h)$ is the uncertainty in the structure factor $F(h)$ (Makowski, L., personal communication). The membrane units, which were superimposed to minimize the differences in their membrane bilayer regions, are judged to be distinct from one another at corresponding positions where their density levels lie beyond the measured uncertainties at that position. On this criterion, the profiles differ in density slightly at the outer portions of the high density lipid polar group peaks (6 Å $\leq r \leq$ 14 Å and 68 Å $\leq r \leq$ 72 Å), and more significantly in the external protein-water layer (76 Å $\leq r \leq$ 85 Å).

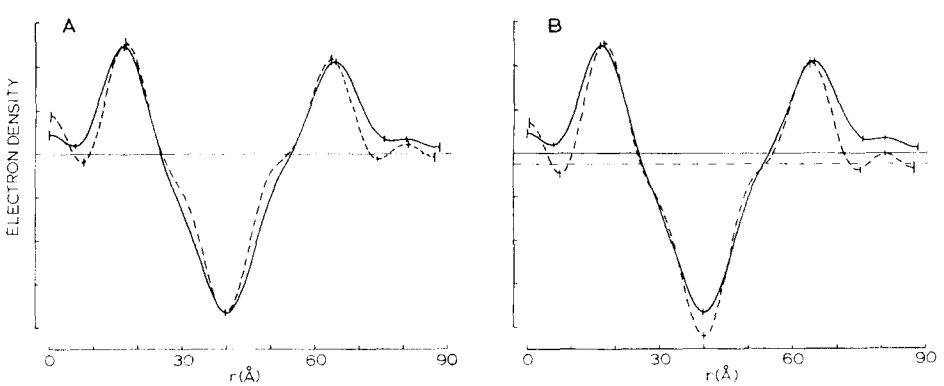

Fig. 5. Comparison of electron density profiles calculated from diffraction data to 15–16 Å spacing for adult (solid) and 10 day old (dashed) mouse sciatic myelins for two scalings of the bilayer profiles.

scaling, it appears that with maturation the center-to-center separation of the high density lipid polar group layers increases ≈ 2 Å from 46 Å in immature to 48 Å in the adult.

Comparisons involving membrane unit density profiles for the other sciatic myelins examined are limited to low resolution since their diffraction patterns show discrete reflections only to ≈ 35 Å spacing. The uncertainties in the measurements of the similar, low resolution, patterns obtained from sciatic myelins of *msd* mice and their littermate controls do not allow making structural distinctions between their density profiles until additional and higher-resolution diffraction data are collected from these myelins.

Sciatic nerve myelin from the 11-month-old trembler mouse revealed a repeat period of 174 Å which is similar to the 173 Å periods detected in the older trembler mice. The calculated membrane bilayer profile, however, was indistinguishable from that of the normal mouse.

Membrane unit density profiles for the distinct lattices observed in sciatic myelin from the older trembler mice are shown in Fig. 6 compared with the low resolution profile for normal adult myelin. Sign relations for the diffraction spectra from the 173 Å and 190 Å phases were assigned so that the density profiles would correspond with membrane bilayer profiles of other peripheral nerve myelins [6, 7]. For the 190 Å phase, two structures are consistent with this constraint, and they correspond to opposite sign choices for the odd order reflections. In one structure, the membrane bilayers are closer together at their cytoplasmic than at their external boundaries; in the other structure, the closer apposition is at the external boundary. The published electron micrographs of trembler sciatic myelin [3] are unsatisfactory for distinguishing between these two structures, as are our own electron micrographs of sciatic nerve from an 18-month-old trembler mouse which showed a single, predominant myelin

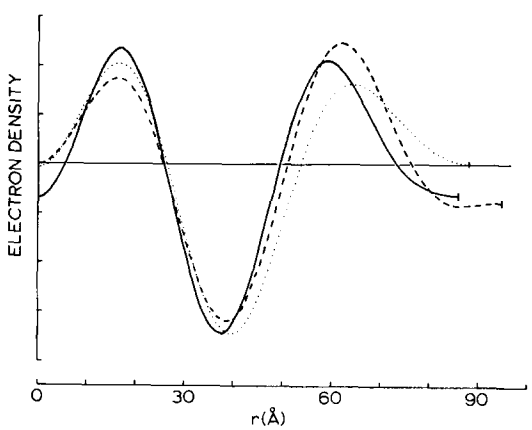

Fig. 6. Trembler sciatic myelin density profiles for the 173 Å (solid) and the 190 Å (dashed) period membrane arrays compared with the density profile for normal mouse sciatic myelin at the same resolution (dotted). The low resolution profiles indicate a similar separation of high density lipid polar group layers across the cytoplasmic boundaries, and thinner membrane bilayers in the mutant compared to the normal myelin. The density profile calculated from the alternative phasing of the diffraction data for the 190 Å period structure would show no similarity to the other structures in packing of membrane bilayers.

periodicity of 124 Å. The structure having closer apposition of bilayers at cytoplasmic boundaries shows a membrane unit separation at this boundary which is similar to those of the normal and 173 Å period trembler myelin lattices (Fig. 6). In all three lattices, the center of the high density layer near the cytoplasmic boundary is 16–17 Å from this boundary, while the other high density layer is variably located relative to the external boundary. The difference in repeat periods between the two phases present in some of the trembler sciatic myelins, therefore, can be accounted for mainly by a corresponding difference in membrane bilayer separation at external boundaries. The alternative structural solution for the 190 Å phase would require differences in membrane-membrane interaction at both cytoplasmic and external boundaries. The center-to-center separations of the high density peaks measured from the low resolution profiles for normal and trembler (173 and 190 Å) membrane units are 48, 43 and 46 Å, respectively. Examination of the apparent differences in density levels among the membrane unit profiles would require diffraction data extending to higher resolution.

In myelin which shows multiple lamellar phases, the fraction of ordered myelin in each phase is proportional to the relative diffracting power of that membrane array, which is calculated from the intensity of the reflections belonging to that phase [12]. The relative diffracting powers of the membrane arrays in the 14-month-old trembler mouse sciatic nerve indicate there is about 1.5 times as much myelin in the structure having the 190 Å repeat period as in the 173 Å array.

DISCUSSION

Detection of structural anomalies at the cytological level. The X-ray patterns obtained from nerves of myelin-deficient mice are consistent with light and electron microscope studies which indicate that for quaking [1, 4] and *msd* [2] mice the deficiency is more severe in central than in peripheral nerves, and that for the trembler mouse the deficiency may be confined to peripheral nerves [3, 13]. The increase in linewidth of reflections from quaking sciatic nerve myelin, also observed by Appeldoorn et al. [8], can be accounted for by the reduced number of myelin layers per axon measured from electron micrographs [4], but could arise as well from increased lattice disorder and greater myelin sheath disorientation in the nerve. Quantitative measurements of linewidths and arcwidths of reflections on point-focus diffraction patterns of quaking and control myelin would resolve this question.

In point-focus patterns from trembler and immature sciatic nerves the diffraction spectra are visibly broadened but without increased arcing, which indicates there is variability in membrane pair separation in the myelin sheaths, and that the predominant orientation of the membrane arrays is normal to the axis of the nerve fibers. In trembler sciatic myelin the membrane disorder is sufficiently great so that only continuous diffraction is observed in the 15–16 Å region. In contrast, discrete Bragg reflections are still observed in this region of the diffraction pattern from immature sciatic myelin indicating there is considerable molecular order in the thin sheaths of myelinating fibers.

Myelin repeat periods measured by X-ray diffraction from quaking and immature sciatic nerves, from the compacted phase of trembler sciatic nerve, from trembler optic nerves and from immature central nervous system myelin are a few Ångstroms less than those measured from corresponding nerves of normal adult mice.

A previous X-ray diffraction study of myelin from mutant mice did not distinguish any difference in repeat periods between quaking and normal mouse sciatic myelin [8]. A comparison of conventional, thin-section electron micrographs, and electron micrographs of freeze-etch replicas of cross-fractured myelin from immature and adult human sural nerve found that the myelin membrane pair is thinner in immature myelin [14]. Extensive measurements on electron micrographs of glutaraldehyde: carbohydrazide embedded tissue indicate that the myelin periodicity is smaller in immature compared to adult for both the central and the peripheral nervous systems [26].

Only a single, compacted membrane array is detected in diffraction patterns from quaking and immature sciatic nerves. This is consistent with electron microscope observations on corresponding nerves [4, 15] which show that any unfused or loose membrane layers present do not form ordered, expanded arrays. The X-rays scattered from these disordered regions of the myelin sheath likely contribute to the increased small-angle background levels from these nerves. In diffraction patterns from sciatic nerves of the older trembler mice two membrane arrays are observed; and intensity measurements indicate about 1.5 times as much myelin is in the slightly expanded as in the compacted phase. The calculated electron density profiles indicate that the 17 Å greater repeat period of the expanded lattice can be accounted for by a comparable increase in the width of the protein-water layers between membrane units. Since these boundary layers are comprised of 80–90 % water by volume [7, 16], then the distinctive membrane lattices must differ significantly in their water content. The removal of this water during conventional tissue preparation for electron microscopy (which includes alcohol dehydration and Epon embedding) would remove the major distinguishing feature of the lattices, and a single collapsed myelin periodicity would predominate, in agreement with our measurements on the electron micrographs.

Correlation of chemical with structural molecular data. In order to make specific correlations with a high degree of confidence, physical, chemical and X-ray structural data should be collected for the same myelin specimen; however, while diffraction analysis ordinarily treats the ordered myelin of intact nerve it is purified myelin which is used for chemical analysis. Most of these chemical data are from measurements on central nerve myelin, with only a few reports for purified peripheral nerve myelin. It is possible that the isolated material used for physical and chemical characterization is not representative of the ordered membrane arrays detected by diffraction of intact nervous tissue; however, electron micrographs of fractionated material show well-defined multilamellar myelin having similar period and staining properties as intact internodal myelin [17–19], and X-ray diffraction patterns to 15 Å spacing from purified central nervous system myelin reveal a period and intensity distribution similar to intact central nervous system myelin (Melchior, V., Kirschner, D. A. and Caspar, D. L. D. to be published). In view of the paucity of data for making detailed correlations, we are presently limited to offering qualitative rather than quantitative conclusions about the chemical basis of structural differences we have observed among the different myelins.

In the electron density profile for sciatic nerve myelin from the quaking mouse, the elevation of density in the external protein-water space between membrane bilayers might be accounted for by a protein conformational or compositional anomaly which may be related to the hypomyelination observed in these nerves [4].

The 1 Å narrower bilayer in quaking myelin compared to normal arises from slightly decreased density levels at the outer surfaces of both lipid polar group layers, rather than from any thinning of the lipid hydrocarbon layer itself which might have been expected from the lowered levels of total lipid and cerebrosides [20] and the deficiency of long-chain fatty acids [21] found in myelin isolated from quaking sciatic nerve. Fig. 4 and 5 indicate that the sciatic myelin membrane unit of quaking mouse is more similar to that of normal adult than to that of immature mouse. In the central nervous system, however, morphological observations and chemical measurements show that myelin from the quaking mouse is more similar to that from the immature mouse [22].

The 2 Å increase in membrane bilayer thickness for adult compared to immature mouse sciatic myelin can be variously accounted for: e.g., an increase in the mean hydrocarbon chain length with age; the inclusion of some molecular component(s) into the lipid bilayer which would decrease hydrocarbon chain mobility thereby increasing chain extension; the addition of electron dense material to the outer surfaces of the high density lipid polar group layers which would shift the centers of these peaks outward. These possibilities are consistent with chemical measurements on myelin isolated from developing brain which indicate changes in lipid composition, including increased proportions of galactolipids in the rat [23] and of long-chain fatty acids in the mouse [24]. Changes in myelin protein composition of mouse brain, including increased proportion of proteolipid protein relative to basic protein [17] have also been measured; but developmental changes in protein composition of rat sciatic myelin have been found to be much less marked than in the central nervous system tissue [25].

The variability in clinical symptoms and in myelin diffraction of the trembler mice suggests that what we are observing is probably not a primary expression of the genetic disorder in this mutant. The 11-month-old trembler mouse exhibited more motor control and less dragging of hindlimbs compared to the 14- and 20-month-old mice. The diffraction from collagen was less marked in the 11-month-old. A single myelin phase having a 174 Å periodicity was detected in the 11-month-old, but two coexisting myelin arrays having periods 173 Å and 190–200 Å were detected in sciatic nerves from the older mice. The membrane bilayer structure in the 11-month-old appeared normal, but that in the corresponding phase of the older mice appeared significantly thinner. Judging from this heterogeneity in myelin arrays and in bilayer structures, abnormalities in both protein and lipid composition of trembler mouse peripheral myelin can be expected. According to electron microscope evidence, the lesion in the trembler mouse is characterized by hypomyelination, segmental demyelination with remyelination, and proliferation of connective tissue [3]. An X-ray diffraction study of trembler sciatic nerve as a function of postnatal age would likely provide information on the dynamic, developmental aspects of this neurological disorder which appears to affect myelin packing, stability and membrane structure.

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