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X-RAY DIFFRACTION ANALYSIS OF DEHYDRATED MYELIN

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Improved X-ray diffraction data from dry nerve myelin are presented. In addition to the spacings of approx. 150 Å, 60 Å, 44 Å and 34.6 Å, which have been previously reported, we identify a 14 Å series. The data suggests that the hydrocarbon chains in the single bilayer (≈60 Å) is ordered, whereas in the double bilayer (≈150 Å) and in the fluid phase (≈44 Å) it is disordered. It is shown that cholesterol (≈34.6 Å) exists as a bilayer, and the 14 Å series is probably another cholesterol phase.

Introduction

The structure of myelin in sciatic nerve has been extensively studied (Refs. 1–5, and references therein). Diffraction patterns from normal and swollen nerves [6], and from nerves modified by other physical treatments such as air drying [7,8], rehydration [9], freezing and thawing [10] have been recorded and analyzed. Extreme dehydration of myelin leads to irreversible segregation of various components. The diffraction patterns from such mixed structures are difficult to unscramble [7,8]. X-ray diffraction patterns from pure lipids, lipid extracts, and mixtures of lipids [11], and the investigation of the effects of various solvents on the structure of myelin [12] have provided a basis for understanding the nature of various lipid-protein mixtures that give rise to the different repeat periods in the X-ray diffraction photographs of dry nerves. The 150 Å series has been attributed to a double bilayer with protein in the interlayer space [13]; it has been implied that the 60 Å and the 44 Å spacings might arise from free lipid components [12,14]; the 34.6 Å reflection has been assigned to cholesterol [1]. In this report we present better quality X-ray diffraction photographs from rabbit and frog sciatic nerves. An effort is made to analyze the nature of the particles which give rise to cholesterol and cholesterol-like reflections. Relationships among these phases and the process of drying will be discussed.

Materials and Methods

X-ray diffraction patterns were obtained from rabbit and frog sciatic nerves. Freshly dissected nerves were dried by suspending under light tension in ambient atmosphere. X-ray patterns were recorded at room temperature and humidity on Ilford-G films using an optically focussing X-ray camera and nickel filtered copper radiation from an Elliott rotating anode micro-focus X-ray generator. The samples were held in place with soft wax in a 1 mm size groove in the sample holder. Slit collimation photographs were obtained using only one mirror; pinhole photographs were obtained using a pair of slits of width ≈0.1 mm at right angles to the plane of the mirror. Sample-to-film distance was usually about 6 cm. Several (3 to 6) films were placed in the film cassette to record an

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adequate range of intensities. Densitometer tracings were obtained using a Joyce-Loebl microdensitometer (Model MK IIIC). The background scattering curves were drawn empirically. Integrated intensities, $I(h)$ where h is the order of Bragg reflection, were measured by integrating the areas under the diffraction peaks. The scaling factor between the films was calculated by using well resolved reflections appearing on two successive films. Same scale factor was applied to all the films in the pack and was usually 2.8. Corrected intensities, $J(h)$, for the low angle reflections were calculated by using the expression $J(h) = hI(h)$ for slit collimation photographs [3], and by using $J(h) = h^2 I(h)$ for pinhole photographs [15]. This takes into account both the Lorentz factor and the disorientation correction. The intensities were normalized by setting

$$\sum_h J(h) = 1000$$

since the repeat period for the samples whose intensities were averaged was equal [15].

Results and Interpretation

Table I shows a typical set of repeat periods and d-spacings (d) obtained from an X-ray diffraction photograph of a dry nerve (Fig. 1). Such

TABLE I

d SPACINGS (IN Å) OF THE VARIOUS REFLECTIONS FROM DRIED RABBIT SCIATIC NERVE AND THEIR SOURCE

Orders of the reflections which have been indexed are given in parentheses. Other symbols used in the parentheses to describe the reflections are: M, meridional; U, almost uniform azimuthal intensity distribution; C, crossed pattern with arcs in each of the four quadrants; all the other reflections are equatorial.

Double bilayer	Single bilayer	Fluid phase	Cholesterol	
161.5 (1) [a]				
80.9 (2)				
	64.4 (1) [a]			
		44.1		
40.0 (4)				
			34.6 (1)	
	32.1 (2)			
	21.5 (3)			
			17.3 (2)	
	16.1 (4)			
				14.4
	12.8 (5)			
			11.6 (3)	
	10.8 (6)			
	9.3 (7)			
			8.6 (4)	
	7.2 (9)			
			6.9 (5)	
	6.4 (10)			
				5.8 (C)
			5.3 (M)	
			5.2 (M)	
			5.0 (M)	
				4.65 (M)
	4.2 (M)			
				3.95 (U)
				3.80 (U)

[a] Typical corresponding repeat periods for the frog sciatic nerve are: double bilayer, 149 Å; single bilayer, 61 Å. There are no systematic differences between frog and rabbit sciatic nerves in other d-spacings.

Fig. 1. X-ray diffraction (XRD) photograph of a dried rabbit nerve. The axes of the nerve in all the photographs in this paper are vertical; equatorial reflections are on the horizontal axis, and the meridional reflections are along the vertical axis. The insets in this and the following figures are cut-outs of the bottom films (usually the third) in the film pack. Typical set of d spacings determined from such photographs are listed in Table I.

patterns, as pointed out by Bear Finean and their coworkers [1,7,11–14], arise from the different repeat periods corresponding to the various species of lipid-protein mixtures present in dried myelin. In arriving at these assigments we made use of the photographs in which there was no contribution from some of the phases (Fig. 2). We also compared the degree of orientation of the various reflections; for instance, in Fig. 1 the 161 Å series shows more orientation than the 64 Å series, and the 14 Å series is least oriented. Furthermore, by comparing the data from rabbit and frog sciatic nerves, which show different repeats for both the single and the double bilayer, and by using photographs such as Fig. 3b in which only the single bilayer is hydrated while the double bilayer remained unchanged, we were able to conclusively identify the low angle spacings corresponding to the single and the double bilayer.

The series of lines with $d \approx 60$ Å, attributable to an array of single bilayers (which will be referred to as single bilayers for brevity), were seen in all the photographs [8]. Since there are at least 8 reflections in a wide angle diffraction pattern (Fig. 1), it is difficult to isolate the wide angle

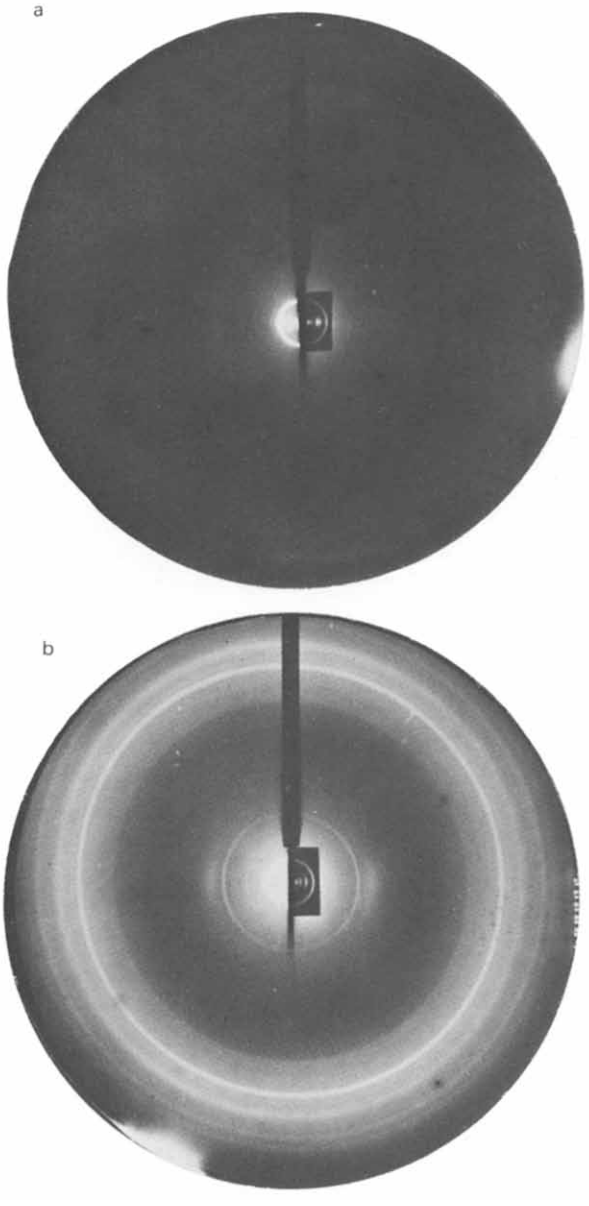

Fig. 2. X-ray diffraction photograph of dried nerves to indicate the source of the 4.2 Å reflection. (a) Dried frog sciatic nerve; d spacings (in Å) visible in the photograph are: (D, double bilayer; S, single bilayer): 146 (D1), 73 (D2), 61 (S1), 42, 30.5 (S2), 20.3 (S3) and 4.2 (b) Redried rabbit sciatic nerve: d spacings (in Å) of the reflections visible in the photograph are: 100, 63 (S1), 42, 21 (S3), 14, 4.6, 4.5, 4.2, 3.9, and 3.8.

spacings which are associated with this single bilayer. We accomplished this by analyzing photographs such as the ones shown in Fig. 2. In Fig. 2a, the 4.2 Å reflection is seen in the absence of the ≈ 14 Å reflection and the 34.6 Å series from cholesterol. In Fig. 2b, the 4.2 Å reflection is seen in the absence of the ≈ 150 Å series. If the 4.2 Å reflection were due to the ≈ 44 Å fluid phase, then it would not have been well oriented. Thus, eventhough in Fig. 1 the 4.2 Å reflection is seen along with 150 Å, 60 Å, 44 Å, 36.4 Å and 14 Å lines, it is due only to the ≈ 60 Å series. This 4.2 Å reflection from the single bilayer is in contrast to the 4.7 Å reflection obtained from a fresh nerve [2], and suggests that the hydrocarbon chains in the single bilayer are highly ordered. Because of the closer packing of the hydrocarbon chains this phase can be referred to as the condensed phase.

On rehydration the condensed phase transforms into a new phase with a repeat period of about 100 Å [8]. This can be seen in Fig. 3b which shows the 60 Å spacing of the dry nerve (Fig. 3a) being replaced after partial rehydration by a ≈ 100 Å spacing. The 60 Å reflections always reappear on redrying. These results indicate that a part of the myelin double bilayer irreversibly dissociates into single bilayers.

A series of lines with $d \approx 150$ Å were seen in most of the photographs. This has been assigned to a phase in which the repeat units is a double bilayer composed of lipids and proteins [13]. During our rehydration experiments we observed that just as the 60 Å band gives rise to the 100 Å spacing, the 150 Å series (which will be referred to as a double bilayer) transforms into an hydrated species with $d \approx 200$ Å (Fig. 3c).

An intense reflection with a Bragg spacing of ≈ 44 Å was seen in all the photographs of dry nerves. This reflection is highly disoriented; no other reflection, low or wide angle, could be associated with this reflection. The 44 Å reflection, therefore, is probably due to a fluid-like arrangement of myelin lipids or of lipid and lipid-protein mixture. If the nerve is dried in tension,then the ring splits into six discrete spots as shown in Fig. 4. The angle between the spots is approx. 60°. Such a pattern has also been reported by Finean et al. [16].

An interesting aspects of our X-ray photographs is the series of reflections with a repeat of 34.6 Å and a multitude of lines from 14 Å to 3 Å (Figs. 1 and 2b). It has been shown that the lines with a repeat period of 34.6 Å indicate the separa-

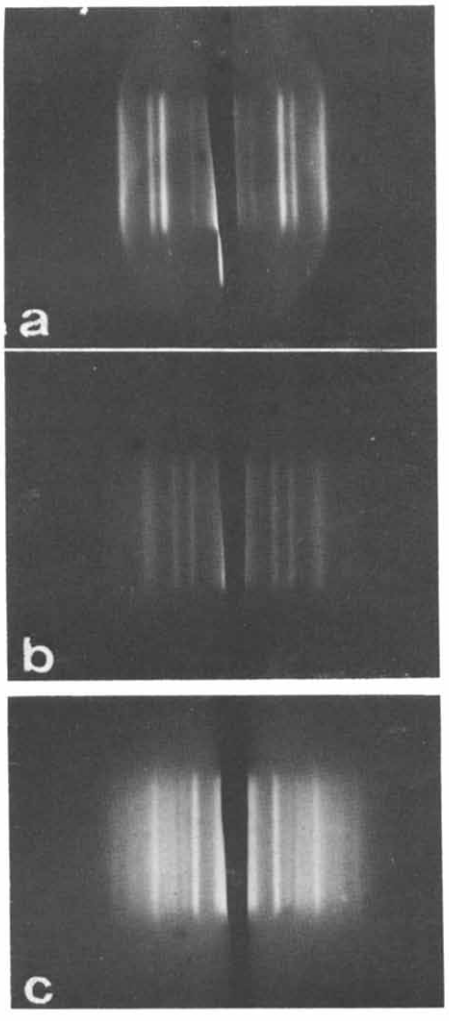

Fig. 3. X-ray diffraction photographs of a dried frog sciatic nerve during rehydration. (a) Dry nerve; *d* spacings of the three clearly visible reflections are: 74 Å (2nd order of the double bilayer), 61 Å (1st order of the single bilayer), and 42 Å (fluid phase). (b) Partially hydrated nerve; reflections with repeats of 106 Å and 53 Å (1st and 2nd order of the hydrated single bilayers) and 74 Å (2nd order of the double bilayer) are visible in the photograph. (c) Completely hydrated nerve; this pattern was obtained immediately after the second photograph; 1st and 2nd orders of the single bilayer (112 Å and 56 Å) are clearly visible in the photograph. The lines in between these two reflections can be resolved and attributed to the hydrated double bilayer with a *d* spacing of approx. 250 Å.

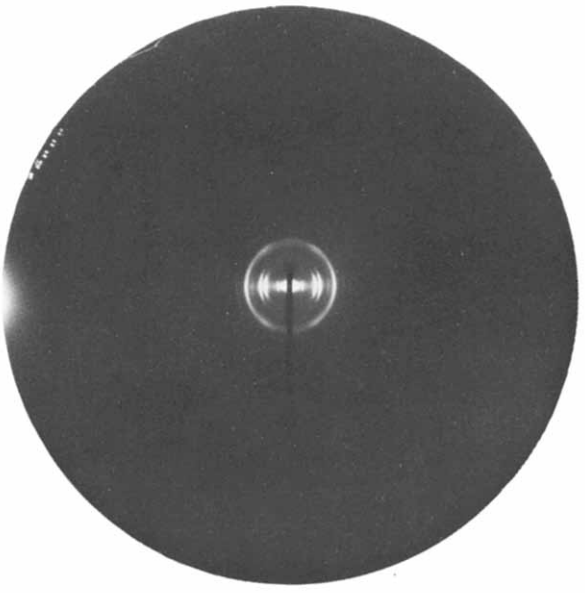

Fig. 4. X-ray diffraction photograph of a dried frog sciatic nerve showing the ≈44 Å ring splitting into six spots. Reflections with d spacings of 146 Å, 73 Å and 49 Å (1st, 2nd, 3rd order of the double bilayer) and 61 Å (1st order of the single bilayer) are also visible in the photograph.

tion of cholesterol in dehydrated myelin [7,8]. This assignment agrees with the X-ray diffraction results obtained from pure lipids, mixtures of lipids, and lipid extracts [11]. To understand the nature of this cholesterol phase, we compared the intensities of 34.6 Å spacing reflections with single crystal and powder diffraction data from cholesterol (Table II). It can be seen that there is no satisfactory agreement between our data and those from crystals. Therefore, the arrangement of cholesterol molecules in dry nerves is probably different from that in crystals. It is possible that the cholesterol molecules giving rise to 34.6 Å series are arranged in the form of bilayers.

TABLE II

COMPARISON OF UNCORRECTED INTENSITIES FROM CHOLESTEROL PHASE IN NERVE MYELIN WITH POWDER AND SINGLE CRYSTAL DATA OF CHOLESTEROL

h	Present work	Single [a] crystal data	Powder [b] data
1	170531	– [c]	800
2	59755	59755	120
3	29243	3739	4
4	36861	6860	16
5	88344	4257	13
6	0	4158	97

[a] Personal communication from Dr. B.M. Craven, University of Pittsburgh.
[b] Ref. [17].
[c] Not recorded.

By examining diffraction photographs from several dried nerves, we found that one set of lines (14.4, 5.8, 4.65, 3.95, 3.80, all in Å; Table I) occur independently and are not related to the 34.6 Å series (Fig. 2b). This was confirmed by studying the degree of orientation of the reflections in several X-ray diffraction photographs of dry nerves. We therefore conclude that the 14 Å series represents a separate phase. This phase cannot be attributed to lipid or lipid-cholesterol or lipid-protein complexes, since such particles always give rise to an intense reflection with a Bragg spacing >45 Å [11]. However, cholesterol, either in pure form or when incorporated in the form of a domain into some lipids can give rise to the 14 Å reflection. This suggests the possibility that the 14 Å series may be due to a form of cholesterol.

Discussion

It is clear from the data presented thus far that the dehydration of peripheral nerves brings about several changes in the interactions among the fatty acids, cholesterol, and proteins present in myelin. These changes, which might involve fractionation of lipids, preferential binding of myelin proteins to specific lipids, and a rearrangement of the hydrocarbon chains, manifest themselves in the formation of many different phases.

Formation of a condensed state, as evidenced by the meridional 4.2 Å reflection, probably leads to the formation of cholesterol bilayers. This conjecture is supported by observations such as the occurrence of separate thermal transitions of cholesterol and phospholipid molecules when the water content of myelin is reduced below a critical value of 20% [18]. since cholesterol molecules are held together by weak forces, mainly by dispersion forces and an average of one hydrogen bond per

molecule [19], it is unlikely that the cholesterol molecules will be precisely ordered. This might account for the occasional splitting of a few cholesterol reflections (e.g., 14.0 Å into 14.1 Å and 14.4 Å).

Since cholesterol in the dry state forms a separate phase one can assume that the double bilayer, the condensed phase, and the fluid phase are not rich in cholesterol. Selective solvation experiments have infact shown that the condensed phase is relatively free of cholesterol [12]. Because of the large number of reflections (ten) from the condensed phase, this single bilayer probably represents a homogeneous phase within which the hydrocarbon chains are highly ordered.

In contrast to the single bilayer, the double bilayer with a repeat period of ≈ 150 Å is asymmetric. This follows from the electron microscope studies of Elkes and Finean [12]. Since we never observed any reflection beyond the fourth order, the double bilayer probably represents a non-homogeneous phase. The observation by Elkes and Finean [10] that lipid extracts give 34.6 Å, 44 Å, and 60 Å, but not the 150 Å, indicates that the 150 Å repeat period represents a separate lipid-protein complex.

Our X-ray diffraction results on the 44 Å band suggest that the hydrocarbon chains in this fluid phase is disordered. Since the fluid phase and the condensed phase are interconvertible by appropriate changes in the temperature, the 44 Å band being dominant at temperatures $>20°C$ [10,16], it is probable that there is no spatial correlation in the arrangement of the lipids in this phase.

We have thus presented X-ray diffraction photographs in which the reflections from various phases are well resolved, and also discussed the presence of single and double bilayers, fluid phase and the cholesterol phases.

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