Studies of brain myelin in the "quaking mouse"

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ABSTRACT Myelin was isolated from the brains of "quaking" and littermate control animals and its composition was determined. The brains of quaking animals contained approximately one-fourth as much myelin as the control animals. There were qualitative as well as quantitative differences between the myelin from the two groups. By continuous cesium chloride gradient flotation it was shown that the myelin from the quaking animals consisted solely of a band corresponding to the heavier and smaller of the two bands found in normal controls. Cholesterol and glycolipids were lower and phospholipids (mainly phosphatidylcholine) and protein were higher in quaking animals than in controls. Also, phosphatidalethanolamine was decreased, and several consistent differences in the fatty acids (both unsubstituted and hydroxy) and aldehydes of the component lipids were found. In general there were smaller amounts of monounsaturated fatty acids in quaking animals. We suggest from these findings that myelin in the quaking mouse has certain compositional similarities with juvenile myelin, but it may be an abnormal type of myelin.

SUPPLEMENTARY KEY WORDS phospholipids · glycolipids · cholesterol · fatty acids · protein · hydroxy acids · gas–liquid chromatography · thin-layer chromatography

THE QUAKING MOUSE is a mutant of the C57BL/6J strain with a neurologic disease characterized pathologically by a general deficiency of myelin in the central nervous system (1, 2). Neurologically, the animals have tremors and seizures that develop early in life, but the life span is close to normal. There have been several recent analyses of lipids of the whole brains of animals with this disease[1] (3–6) as well as the closely related, more severely afflicted "jimpy" mouse (7–9).

In the present study, we have isolated myelin from the brains of quaking mice 42–60 days old and have shown by direct measurement that they have approximately 25% of the total quantity of myelin in control animals. In addition to this quantitative difference we found several compositional differences, both in lipid classes and fatty acids, as well as differences in the flotation characteristics between myelin isolated from diseased and normal animals.

MATERIALS AND METHODS

Materials

Phospholipids, cerebrosides, and sulfatide were purchased from Applied Science Laboratories, State College, Pa.; fatty acids were from the Hormel Institute, Austin, Minn. and Applied Science Laboratories; and hydroxy fatty acids were from Analabs, North Haven, Conn. and Applied Science Laboratories. All other chemicals and solvents were of the highest purity available commercially. Precoated stationary phases for GLC were obtained from Applied Science Laboratories or Analabs. Sil-Prep for preparing TMS derivatives of hydroxy acids was obtained from Applied Science Laboratories. It contained hexamethyldisilazane–trimethylchlorosilane–pyridine 3:1:9 (v/v/v).

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; Sph, sphingomyelin; LPE, lysophosphatidylethanolamine; CN, cerebroside with unsubstituted acid; CH, cerebroside with hydroxy acids; SN, sulfatide with unsubstituted acid; SH, sulfatide with hydroxy acids; Chl, cholesterol; O, origin; TMS, trimethylsilyl derivative.

[1] Singh, H., and N. Spritz. Unpublished results.

Preparation of Myelin

Preparation of Brain Dispersions. The mutant and littermate control animals of strain C57BL/6J-qk were obtained from Jackson Memorial Laboratories, Bar Harbor, Me. The mice, all females, in groups of 4–6 were decapitated and the brains were immediately removed, dispersed in 0.25 M sucrose at 4°C, and homogenized in a Tri-R tissue grinder with a Teflon pestle (Tri-R Instruments, Inc., Rockville Centre, N.Y.).

Isolation of Myelin. A fraction with properties of myelin was prepared, with slight modification, according to the procedure of Autilio, Norton, and Terry (10) using a Beckman L2-65B ultracentrifuge.

(*i*). 4–6 brains were dispersed in 20 ml of 0.25 M sucrose and layered over 0.88 M sucrose in three tubes. The tubes were centrifuged at 40,000 rpm (198,000 *g*) for 45 min in a SW-40 (Spinco) swinging bucket rotor.

(*ii*). The "myelin" separating at the interphase as a white fluffy precipitate was collected after removing the sucrose solution over it. The "myelin" in 0.88 M sucrose was diluted with water to give a 0.25 M solution of sucrose; this was layered over 0.88 M sucrose and step *i* was repeated.

(*iii*). Myelin separating at the interphase was collected as in step *ii*, suspended in distilled water (25 ml), and centrifuged at 40,000 rpm (105,000 *g*) for 20 min using a 50 TI angle rotor.

(*iv*). The supernatant solution in step *iii* was discarded, the myelin pellet was dispersed in water, and step *iii* was repeated.

(*v*). The pellet obtained after step *iv* is designated as "myelin." For analysis at this step, the myelin pellet was washed two times with distilled water to remove sucrose. Each time the myelin was suspended in the centrifuge tube and centrifuged at 40,000 rpm (105,000 *g*) for 20 min. The myelin pellet was then dispersed in 5 ml of water and stored. Aliquots of this aqueous suspension were used for analysis.

In some experiments, myelin obtained from the five steps described above was subjected to further flotation using a cesium chloride gradient.[2] The myelin equivalent to one brain from a control mouse was washed once with water and layered on each tube containing, in order from top down, 3 ml of 0.3 M cesium chloride, 8 ml of a continuous gradient of 0.3 M cesium chloride increasing to 1.3 M cesium chloride, and finally 2 ml of 1.3 M cesium chloride. The gradient was made using a universal density gradient mixer (Buchler Instruments Inc., Fort Lee, N.J.). The tube was centrifuged for 1 hr at 40,000 rpm (198,000 *g*). With control animals two distinct layers were observed, a large white layer near the middle of the tube and another layer near the bottom of the gradient. Similar results as described above were obtained when the ultracentrifuge was operated at 25,000 rpm (75,000 *g*) in steps *i* and *ii*, at 35,000 rpm (80,800 *g*) for step *iii*, and at 29,000 rpm (100,000 *g*) when the cesium chloride gradient was used. The two layers of cesium chloride gradient are designated, respectively, "light myelin" and "heavy myelin." With the quaking group, only the band corresponding to "heavy myelin" was observed. The appearance of the tube in this purification step was reproducible, and there was no pellet at the bottom or top of the tube in any experiment. The two fractions from the control group were collected separately, washed with distilled water, and centrifuged to obtain a well-packed pellet. The pellets were suspended in water and aliquots of this suspension were used for analysis.

[2] Norton, W. T., and A. N. Davison. Personal communication.

Extraction of Lipids

The lipids from aqueous myelin suspension were extracted essentially by the procedure of Folch, Lees, and Sloane Stanley (11). 20 ml of chloroform–methanol 2:1 was added to 1 ml of myelin suspension and a clear to a faintly opalescent single phase solution was obtained. This was washed with 0.1 M sodium citrate. The lower $CHCl_3$ layer was washed with pure solvent "upper phase" and the lipid was recovered from the chloroform solution.

Analytical Procedures

Protein was determined on an aliquot of myelin suspension by the procedure of Lowry, Rosebrough, Farr, and Randall (12), using bovine serum albumin as a standard. Cholesterol was determined by the use of a Technicon AutoAnalyzer (13). In some instances cholesterol was estimated after isolation by TLC, and values were comparable to those obtained on an aliquot from the total lipid extract. Lipid phosphorus was determined by Bartlett's method (14). Galactose was determined by a ferricyanide procedure (15), using cerebroside as a standard. Phosphatides and galactolipids were separated by two-dimensional TLC (see below), and after visualization with iodine vapor, appropriate areas of the plate were scraped and the lipids were eluted with methanol and estimated by methods described above.

Chromatographic Separations

Thin-layer chromatography was done on layers of silica gel H, 500 μ thick and activated at 110°C for 1 hr. All plates were prewashed with methanol and reactivated before use. Lipids for quantitative analysis were separated by two-dimensional TLC using the solvents described by Rouser, Kritchevsky, and Yamamoto (16), with the following modification (see Fig. 2). The

chromatograms were developed in the first dimension with chloroform–methanol–28% aqueous ammonia 65:35:5, and the strip containing the lipids was sprayed with 12% by weight of HCl in methanol (17). This procedure hydrolyzes the alkenyl linkages so that the material isolated as LPE after development in the second solvent represents the original alk-1-enyl compounds. After 5 min the HCl was removed under a stream of nitrogen. The plate was then developed in the second dimension with chloroform–acetone–methanol–acetic acid–water 5:2:1:1:0.5. In some experiments cholesterol was first separated from other lipids by TLC, using petroleum ether (bp 30–60°C)–ethyl ether–acetic acid 85:15:1 as the solvent.

For fatty acid analysis, the lipids were first fractionated into cholesterol, glycolipids, and phospholipids according to Rouser, Kritchevsky, Simon, and Nelson (18), on a Unisil column (0.5 × 10 cm). Cholesterol was eluted with 40 ml of chloroform and the glycolipids were removed with 20 ml of chloroform–acetone 1:1 and 100 ml of acetone. Finally, phospholipids were eluted with 75 ml of methanol. The separations were monitored by TLC for various components as described above.

The glycolipids were fractionated into cerebrosides and sulfatides by TLC, using chloroform–acetone–methanol–acetic acid–water 5:2:1:1:0.5. The components were visualized by spraying the plates with 0.5% rhodamine 6G in methanol and viewing under ultraviolet light. The two components were scraped off and interesterified with 14% BF_3 in methanol at 100°C for 90 min (19). Methyl esters were extracted with ether (3 × 3 ml). Hydroxy acids and unsubstituted acids were separated by TLC, using benzene as a solvent system, and after visualization as described above the acids were extracted from the silica gel with ether. Unsubstituted acids and hydroxy acids after conversion to trimethylsilyl derivatives (20) were analyzed by GLC. Trimethylsilyl derivatives were prepared at room temperature for 30 min with Sil-Prep (0.5 ml) added to the dry hydroxy acid ester. The solvent was removed under a stream of N_2 and the TMS derivative was dissolved in carbon disulfide and analyzed by GLC.

The phospholipids were separated by TLC using chloroform–methanol–28% ammonia 65:35:5 containing 0.05% 2,6-di-*tert*-butyl-*p*-cresol as an antioxidant. The individual phospholipids were interesterified as described above and analyzed by GLC, using a Packard 805 gas chromatograph with a U-shaped glass column (6 ft × 1/4 inch I.D.) and equipped with a flame ionization detector. The stationary phase was 10% EGSS-X or 10% EGS on Chromosorb W. Some separations were also done on a column containing 10% SE-30 on Anakrom ABS. Fatty acids from phospholipids were analyzed by temperature programming from 150 to 190°C. TMS derivatives of hydroxy acids and unsubstituted acids from glycolipids were analyzed at 195°C. The area under the peaks was estimated by integration, using an electronic integrator (Infotronics, Inc., Houston, Tex.). The peaks were identified by comparison with standard esters or by equivalent chain length, and the linearity of the detector response was checked by NIH mixtures D and F.

RESULTS

Composition of Mouse Brain Myelin

The weight of brains, yield of myelin, and data on control and quaking animals used in this study are given in Table 1. There was a marked decrease in the amount of mylein isolated per brain in quaking animals as compared with controls ($P < 0.001$). Some of the myelin preparations were examined by electron microscopy and, in both quaking and control preparations, lamellar structures uncontaminated by fragmentary or intact nerve fibers were found. A typical sedimentation pattern of myelin isolated by discontinuous sucrose gradient on a continuous cesium chloride gradient is shown in Fig. 1.

The average composition of mouse brain myelin is shown in Table 2. There was more protein in myelin from the quaking mice ($P < 0.002$) and a decrease in total lipid ($P < 0.002$) in comparison with the control group. The data on lipid class composition are expressed as percentage of weight in Table 3, and as molar composition in Table 4. A typical two-dimensional

TABLE 1 DATA ON ANIMALS OF STRAIN C57BL/6J-qk

	Controls (20)*	Quaking (20)*
Age of animals	42–60 days	42–60 days
Sex	Female	Female
Body weight, *g*	19.3 ± 0.72†	16.0 ± 0.58
Brain weight, *mg*	385 ± 11.04	361 ± 5.06
Myelin (total lipid + protein) *mg/brain*	17.77 ± 1.92	4.88 ± 0.37

* Number of animals in each group.
† Mean ± SE.

TABLE 2 AVERAGE COMPOSITION OF MOUSE BRAIN MYELIN

	Control	Quaking
	%	%
Total protein	31.5 ± 0.69*	36.1 ± 0.29
Total lipids	68.5 ± 0.69	63.9 ± 0.29
Cholesterol	15.6 ± 0.57	12.1 ± 0.61
Glycolipids	20.1 ± 1.78	15.7 ± 2.78
Phospholipid	32.8 ± 0.81	36.1 ± 2.75

* The values are means ± SE of four different preparations for control group and three for quaking group.

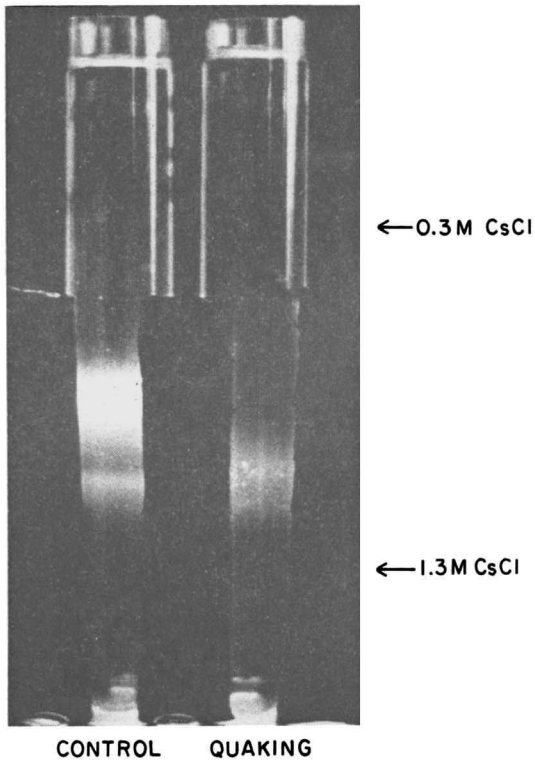

FIG. 1. Ultracentrifugal pattern on a continuous cesium chloride gradient of control and quaking myelin isolated by a sucrose discontinuous gradient. The arrows indicate the upper and lower concentration limits of continuous gradient.

thin-layer separation of lipids is shown in Fig. 2. Phosphatidylcholine was significantly ($P < 0.001$) higher in the quaking animals, with reciprocal decrease in cholesterol and glycolipids when expressed as percentage of total lipid. Also, the plasmalogen form of phosphatidylethanolamine (phosphatidalethanolamine) was significantly lower in the quaking group ($P < 0.001$).

TABLE 4 AVERAGE LIPID COMPOSITION OF MOUSE BRAIN MYELIN

	Control	Quaking
	mole % of total lipid	
Cholesterol	37.9	32.3
Total glycolipid	22.4	19.6
Cerebroside (unsubstituted acid)	5.9	4.9
Cerebroside (hydroxy acid)	8.2	6.2
Sulfatide (unsubstituted acid)	4.6	4.6
Sulfatide (hydroxy acid)	3.7	3.9
Total phospholipid	39.7	48.1
Phosphatidylethanolamine	7.7	7.9
Phosphatidylcholine	14.7	26.2
Phosphatidylserine	4.5	4.8
Sphingomyelin	2.5	1.7
Phosphatidalethanolamine	10.2	7.3
Eng–Smith ratio*	1.13	1.13

* (PE + sphingolipids)/cholesterol. The values for individual preparations of controls (four preparations) were 1.35, 0.94, 0.98, and 1.17, and for quaking (three preparations), 1.18, 1.24, and 0.90.

Fatty Acid Composition of Phosphatides

The fatty acid compositions of total phosphatides, PE, PC, and all "other" phosphatides (containing mainly Sph, PS, and PI) are given in Table 5. The major saturated fatty acids in total phosphatides of both control and quaking groups were 16:0 and 18:0; the amount of 16:0 in the control animals was lower than in the quaking animals. The main monounsaturated fatty acids were 18:1 and 20:1 in both groups, but the amount in the quaking group was lower than in the normals. The main polyunsaturated fatty acids were the same in both groups, but the amount of 22:6 in control animals was less than in the quaking group.

The differences in fatty acid composition indicated above are more pronounced when phosphatidylethanol-

TABLE 3 LIPID COMPOSITION OF MOUSE BRAIN MYELIN

	Control	Quaking
	% of total lipid	
Cholesterol	22.8	19.0
Total glycolipid	29.4	24.5
Cerebroside (unsubstituted acids)	7.5 ± 0.24*	6.0 ± 0.95
Cerebroside (hydroxy acids)	10.3 ± 1.36	7.6 ± 0.61
Sulfatide (unsubstituted acids)	6.4 ± 0.33	5.6 ± 0.09
Sulfatide (hydroxy acid)	5.2 ± 1.05	5.3 ± 0.69
Total phospholipid	47.8	56.4
Phosphatidylethanolamine	9.3 (19.4 ± 1.14)†	9.4 (16.6 ± 1.56)
Phosphatidalethanolamine	12.3 (25.6 ± 0.12)	8.6 (15.3 ± 0.55)
Phosphatidylcholine	17.7 (37.0 ± 1.88)	30.8 (54.6 ± 1.57)
Phosphatidylserine‡	5.5 (11.5 ± 0.77)	5.6 (9.9 ± 1.32)
Sphingomyelin	3.1 (6.4 ± 0.67)	2.1 (3.7 ± 0.85)

* The values are means ± SE of eight determinations on four preparations for controls and four determinations on three preparations for quaking group.

† Results in parentheses are expressed as percentage of total phosphorus. The values are means ± SE of eight determinations on four preparations for controls and five determinations on three preparations for quaking group.

‡ All samples contained small amounts of phosphatidylinositol.

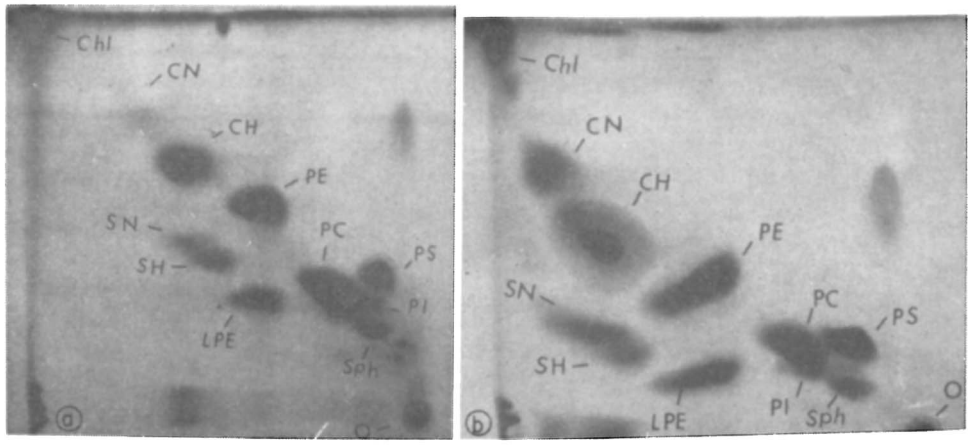

FIG. 2. Two-dimensional TLC of myelin lipids; (*a*), quaking group and (*b*), controls. Components were visualized by charring after spraying with $K_2Cr_2O_7$:H_2SO_4. TLC was done on layers of silica gel H, 500 μ thick and activated at 110°C for 1 hr. The chromatograms were developed in the vertical direction with chloroform–methanol–28% (by weight) aqueous ammonia 65:35:5 followed by drying for 10 min under nitrogen. The strip containing the lipids was sprayed with HCl in methanol (12% by weight) (17). The HCl was then removed with dry nitrogen and the plate was developed in the second dimension (right to left) with chloroform–acetone–methanol–acetic acid–water 5:2:1:1:0.5. As indicated in Table 3, the CN fraction was lightly stained, but when measured by galactose content, the CN approximated 40% of the total cerebrosides in both groups.

amine and phosphatidylcholine are considered alone. Similar differences were found for other phosphatides, which consisted mainly of phosphatidylinositol, phosphatidylserine, and sphingomyelin.

Fatty Aldehyde Composition of Phosphatides

In two experiments, methyl esters and dimethyl acetals, obtained after interesterification of total phosphatides, were separated by TLC and analyzed by GLC. The fatty aldehyde composition (average of the two), expressed as percentage of total aldehyde, was for the controls and quaking animals, respectively, 16:0, 19.4 and 29.6%; 16:1, 2.0 and 1.3%; 18:0, 43.0 and 59.2%; and 18:1, 36.3 and 9.6%. Small amounts of 14:0, 17:0, and chain lengths longer than 18:0 were also present.

Fatty Acid Composition of Glycolipids

Hydroxy Acids. The compositions of hydroxy acids from cerebrosides and sulfatides are given in Table 6, and a

TABLE 5 FATTY ACIDS OF PHOSPHATIDES FROM CONTROL AND QUAKING MOUSE BRAIN MYELIN

Fatty Acid‖	Total Phosphatide				Phosphatidylethanolamine*			Phosphatidylcholine			"Other" Phosphatidies		
	Control†		Quaking†		Control‡			Control‡			Control‡		
	Mean	Range	Mean	Range	Mean	Range	Quaking§	Mean	Range	Quaking§	Mean	Range	Quaking§
16:0	19.5	18.1–20.1	28.5	27.7–29.1	5.9	5.5–6.2	8.9	37.2	36.3–38.4	43.0	5.7	4.7–6.6	14.0
16:1	0.7	0.3–1.7	1.1	0.7–1.5	0.5	0.3–0.7	2.4	1.1	0.7–1.5	1.6	0.8	0.5–1.2	t¶
18:0	24.6	23.7–25.0	27.8	24.0–31.6	22.5	21.0–25.1	24.0	17.1	15.3–18.9	13.4	39.3	37.3–41.3	42.2
18:1	27.6	26.3–28.4	17.2	15.9–19.9	27.7	27.1–28.1	17.8	34.0	33.3–34.8	27.7	27.5	27.3–27.6	14.1
18:2	0.8	0.3–1.5	2.2	2.0–2.9	3.4	2.7–4.0	5.8	2.8	2.5–3.0	3.4	3.4	2.7–4.1	3.3
20:0	0.5	0.3–0.6	0.3	0.2–0.3	0.8	0.7–0.8	t	0.4	0.4–0.4	0.3	0.8	0.4–1.1	0.9
20:1	5.0	4.5–5.4	1.1	1.1–1.2	7.9	7.9–7.9	2.1	2.3	2.1–2.4	1.0	3.2	2.9–3.4	1.0
20:2	0.8	0.6–1.3	0.6	0.3–0.9	0.3	0.1–0.3	1.8	0.3	0.3–0.3	0.3	1.2	0.5–1.9	0.8
22:0	0.2	0.1–0.2	0.4	0.4–0.4	0.2	0.1–0.2	0.8	t			t		0.7
20:4	7.7	7.5–8.0	7.7	6.8–8.1	11.5	10.9–12.2	11.2	4.3	3.1–5.4	4.0	4.9	3.6–6.1	6.8
24:0	t										1.5		
22:5**	3.9	3.5–4.2	2.0	1.6–2.3	6.4	6.4–6.5	4.4			0.5	1.3		1.9
22:6	8.1	7.1–9.3	12.0	11.2–13.8	13.1	12.7–13.5	20.1			3.2	9.5	9.2–9.7	12.9

* The fatty acids represent total phosphatidylethanolamine fraction, diacyl and alkyl acyl type of PE.
† Average of at least two determinations on three separate preparations.
‡ Average of at least two determinations on two separate preparations.
§ Average of at least two determinations on one preparation.
‖ In the notation used the first two digits give the number of carbon atoms and the third digit the number of double bonds.
¶ t, trace component.
** Tentatively identified.

typical illustration of GLC separations is shown in Fig. 3. Longer-chain (above C_{20}) hydroxy acids predominate in myelin from both control and quaking groups. However, there are significant differences in the relative amounts of various acids. The amount of 24:1 hydroxy acid in the quaking group is 16% of that for the controls, while 23:0 hydroxy acid in controls is 48% of that in the quaking group. Similar differences in fatty acid composition were present in sulfatides.

Unsubstituted Acids. The unsubstituted acid composition is given in Table 7. As in the case of hydroxy acids, unsubstituted acids in cerebroside resemble those in sulfatide. Although the level of unsubstituted acids was slightly lower in the quaking group than in the controls, essentially the same fatty acids were present in both groups. However, the relative amounts of various individual components differed greatly. There were, in general, less longer-chain fatty acids in the

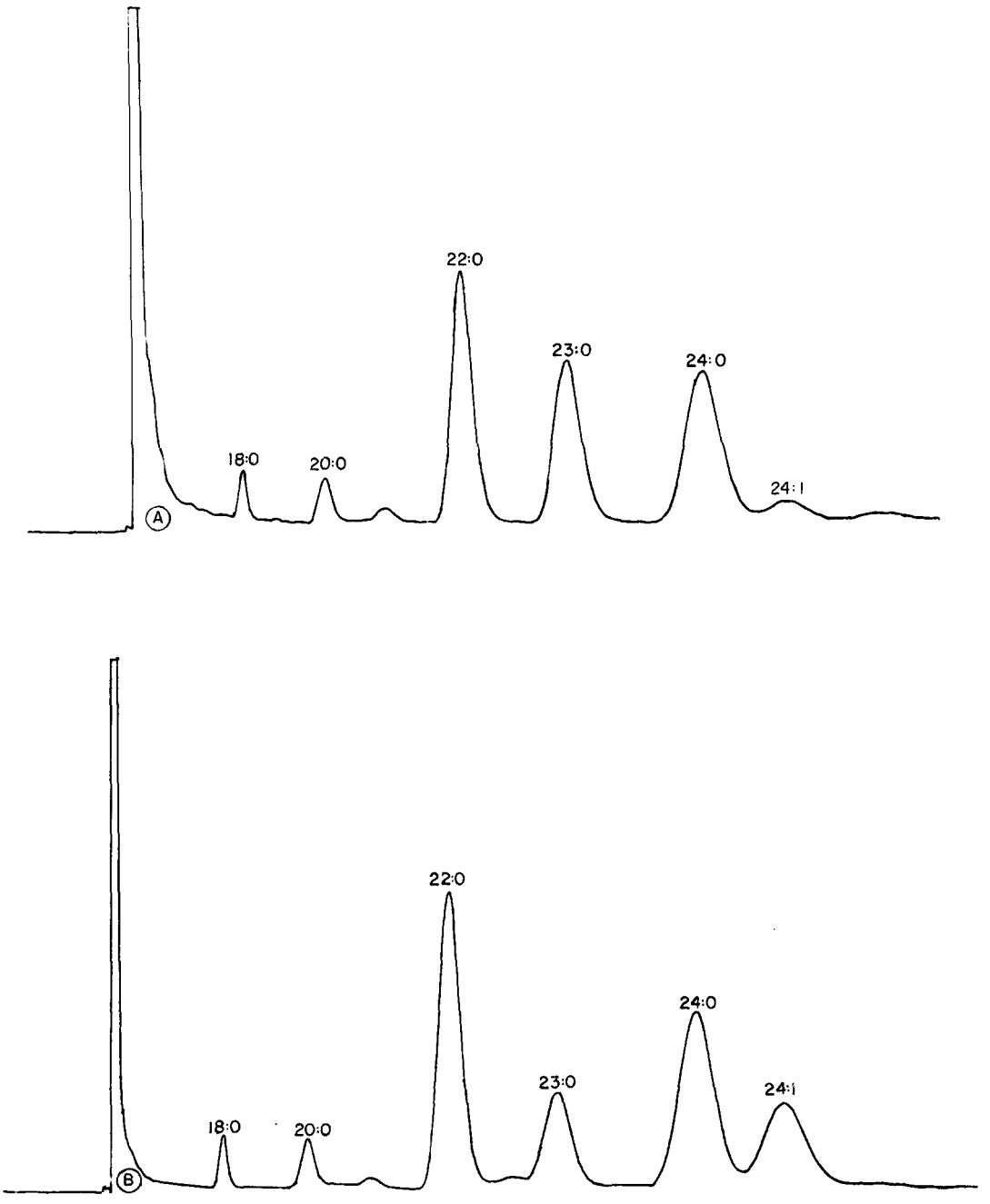

FIG. 3. Gas–liquid chromatogram of hydroxy acids from cerebrosides as their TMS derivatives. (*A*), "quaking" mice and (*B*), controls Conditions: column, 10% EGSS-X on Chromosorb W (6 ft × 1/4 inch I.D.); temperature, 195°C. The various peaks are identified on the chart.

TABLE 6 HYDROXY FATTY ACID COMPOSITION OF SPHINGOLIPIDS

Fatty Acid	Cerebroside				Sulfatide		
	Control*		Quaking*		Control*		
	Mean	Range	Mean	Range	Mean	Range	Quaking†
	%		%		%		%
16:0 h	trace		trace				
18:0 h	3.5	3.4–4.5	3.3	3.2–4.4	4.3	3.1–5.5	2.1
20:0 h	3.3	3.3–3.3	2.8	2.3–3.3	3.1	2.7–3.6	2.5
20:1 h	0.5		1.4	1.1–1.7	0.7	0.4–0.9	0.1
22:0 h	33.3	30.9–34.8	32.8	31.2–34.2	31.6	31.4–31.6	25.0
22:1 h	0.4	t–1.1					
23:0 h	12.1	11.4–13.3	25.5	25.0–26.0	9.5	9.1–10.1	29.3
24:0 h	33.7	30.9–36.0	31.3	31.3–32.8	38.9	38.5–39.7	37.7
24:1 h	12.9	11.2–16.0	2.1	1.4–3.6	10.9	10.3–11.5	2.6
25:0 h‡							

* Average of at least two determinations on two separate preparations.
† Average of two determinations on one preparation.
‡ In some analyses, traces of longer-chain acids were also detected.

TABLE 7 UNSUBSTITUTED FATTY ACID COMPOSITION OF GLYCOLIPIDS

Fatty Acid	Cerebroside			Sulfatide	
	Control*				
	Mean*	Range	Quaking	Control†	Quaking†
	%			%	
14:0	trace		trace	trace	trace
16:0	1.6	1.0–2.1	9.4	1.3	12.4
18:0	5.5	4.4–6.4	18.8	8.6	22.6
18:1	4.9	1.5–8.3	8.4	2.7	10.5
20:0	1.6	1.2–1.9	2.4	2.0	1.1
20:1	0.6	0.6–1.0		0.3	
22:0	9.7	9.4–9.9	16.7	8.1	9.1
22:1	1.8	1.6–2.1		2.2	
23:0	4.3	4.3–4.3	12.3	2.6	7.5
23:1	0.7	0.5–0.8		0.6	
24:0	21.0	19.9–22.1	24.3	18.7	22.7
24:1	46.5	40.0–52.9	7.0	51.4	12.3
25:0‡	1.6	1.4–1.9		1.9	

* Mean of at least two determinations on two separate preparations.
† Mean of at least two determinations on one preparation.
‡ Traces of longer-chain fatty acids, presumably 26:0, were also detected.

quaking group than in the control group. Although the amount of longer-chain fatty acids varied between control and quaking groups, the most striking differences in composition were observed in the case of 24:1. Its amount was 15% and 24% of controls in quaking myelin cerebrosides and sulfatides, respectively.

There was a relatively large amount of 16 and 18 carbon fatty acids in the quaking group, especially 16:0, 18:0, and 18:1. These were present to only a small extent in the control group.

DISCUSSION

Previous studies of brain lipids in the quaking mouse (2–5) have suggested a deficiency in myelin on the basis of histological findings and a decreased content of compounds such as sphingolipids and plasmalogen, considered to reflect myelin content in whole brain homogenates. We have been able to verify and quantify this deficiency in the present study by direct isolation and measurement and have found myelin to be reduced approximately fourfold to an average of 4.8 mg/brain.

In addition to this quantitative abnormality of myelin in this disorder, important qualitative differences were found between the myelin of normal and quaking mice. The sedimentation pattern of the myelin isolated by discontinuous sucrose gradient on a continuous cesium chloride gradient showed two bands in the case of control animals, a pattern similar to that recently described by Banik and Davison (21) for rat brain

myelin. In contrast, the quaking group showed only one band corresponding in density to the heavier band in controls. This observation is consistent with the higher protein content that we found in myelin from the quaking group. Also, the lipid classes were distributed differently, such that the cholesterol and glycolipid were lower and phospholipid (reflecting the difference in phosphatidylcholine) was higher in the quaking group. Phosphatidalethanolamine was decreased considerably in the quaking group, and several consistent differences between the fatty acids (both normal and hydroxy) of the component lipids were found.

The difference in phospholipid fatty acid reflected in most lipid classes a decrease in monounsaturated acid, particularly 18:1 and 20:1, in the myelin from the quaking group. This finding was also true in the case of aldehydes of total phospholipids as well as unsubstituted and hydroxy acids of galactolipids, where 24:1 chain length was markedly depleted compared to 24:0.

On the basis of metabolic turnover rates of myelin lipids, Eng and Smith (22) proposed a 1:1 molar relationship for the sum of noncholesterol lipids with slower turnover rates (PE and sphingolipids, called "stable lipids") to cholesterol. This relationship has been shown to be valid for a number of species and for myelin from the peripheral nervous system (23, 24). The value for the Eng–Smith ratio ranges from 0.90 to 1.08 for various species. In the present study an average value of 1.13 was observed for both the control and quaking animals. This is in agreement with the value of 0.95 for mouse brain myelin reported by Horrocks (25) for animals of the same age group. However, the molar ratios of cholesterol–lipid phosphorus and galactose–lipid phosphorus differed from those reported by Horrocks. Whether the differences between our data and those of Horrocks represent a methodological difference or variations in our experimental material remains uncertain. It is of interest to note that although control and quaking animals have different lipid compositions of myelin, they give the same value for the Eng–Smith ratio, suggesting that this value reflects some fundamental structural property of the membrane.

Several findings suggest that the membrane fractions isolated in these studies were uncontaminated myelin in both control and quaking animals: (*a*) solubility in chloroform–methanol 2:1; (*b*) absence of nonlamellar structures as judged by electron microscopy; (*c*) cholesterol, galactolipid, and protein concentrations generally in the range described only for myelin; (*d*) microsome membranes, the most likely contaminant, were isolated and they remained in the 1.3 M cesium chloride portion of continuous gradient, clearly separate from either myelin fraction; and (*e*) in the quaking animals, a single band was obtained in the continuous cesium chloride gradient. Since these animals have been shown to have myelin, although in decreased amount (1, 2), it is difficult to accept that this single band with flotation properties like that of myelin does not correspond to that identified as myelin by microscopy.

There are some differences between the analytical findings of this study and some previously reported data for fatty acid composition of normal adult mouse brain myelin. Our data on cerebroside fatty acids differ substantially from those of Blass (26) and this may reflect differences in technique, since he analyzed nonderivatized hydroxy and unsubstituted acids together and utilized correction factors for variations in detector response. Our data for these acids do, however, closely resemble those of O'Brien and Sampson (27) for human myelin where 24:1 exceeded 24:0.

Although our findings do not provide an explanation for the basic defect in the quaking mouse, they suggest that maturation of myelin is impaired in the mutant animals. This explanation is consistent with the quantitative and qualitative abnormality as well as with the appearance of symptoms at an early age. We have carried out preliminary studies on 18-day-old quaking and littermate animals and have shown a sedimentation pattern on cesium chloride gradient similar to that shown in Fig. 1. This suggests that during development the parts of brain which are necessary for the synthesis of adult myelin are defective in mutant mice. Myelin obtained from even younger animals might support the contention that myelin from adult quaking mice is the same as that found very early in life in normals.

Recently it has been shown that the intramitochondrial chain elongation system is very active during the period of rapid myelination in rat brain (28). During this period the sphingolipids with long-chain fatty acids are accumulated at a fast rate in brain, especially in myelin. Thus, it is likely that there is a defect in the desaturation or chain elongation system or in the factors controlling the synthesis of molecular species of lipids with these alkyl moieties, resulting in a pattern like that found in these studies for the quaking animals. The marked abnormality in lipid class composition could reflect structural requirements related to an altered fatty acid pattern, although we cannot rule out a primary defect in the synthesis of complex lipids or even in the protein moiety which then produces a secondary abnormality in lipid content (29).

It is a pleasure to thank Dr. Dorothea Zucker Franklin for the electron microscopy. We are also grateful to Dr. William Norton for his valuable advice concerning this work. This work was supported in part by grants from the Veterans Administration and the Public Health Service (AM-13525-01).

Manuscript received 17 July 1970; accepted 9 March 1971.

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