Fatty acid and fatty aldehyde composition of the major brain lipids in normal human gray matter, white matter, and myelin*

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SUMMARY Gray matter, white matter, and myelin were isolated from the frontal lobes of the brains of humans aged 10 months, 6 yr, 9 yr, and 55 yr. The major lipids, including ethanolamine glycerophosphatides (EGP), serine glycerophosphatides (SGP), choline glycerophosphatides (CGP), sphingomyelin, cerebroside, cerebroside sulfate, and ceramide were isolated by column chromatography and their fatty acid and fatty aldehyde compositions were determined by gas–liquid chromatography.

EGP and SGP from myelin had a fatty aldehyde composition which differed from that of EGP and SGP from gray matter; octadecenaldehydes were present in much higher proportions in these lipids from myelin than in those from gray matter. EGP and SGP also contained high proportions of 20- and 22-carbon polyunsaturated fatty acids, whereas CGP contained small proportions of these acids. Each glycerophosphatide from gray matter contained approximately 3- to 6-fold higher proportions of polyunsaturated fatty acids than did the same glycerophosphatide from myelin. Sphingomyelin, cerebroside, cerebroside sulfate, and ceramide also differed in their fatty acid compositions depending upon their tissue source; each sphingolipid from myelin in the younger subjects contained 5- to 9-fold higher proportions of long-chain fatty acids (C_{19}–C_{26}) than did the same sphingolipid from gray matter.

The lipids from myelin in the baby (10 months) were very similar to those from myelin in the adult, both with respect to their content of polyunsaturated fatty acids and to their content of long-chain fatty acids. These findings suggest that myelin in the baby is "chemically mature" in its lipid composition at an early age.

KEY WORDS brain · lipid fractions · fatty acids · fatty aldehydes · gray matter · white matter · myelin · man · age

One very important aspect of the lipid composition of any tissue is the fatty acid composition of each lipid. In the past, several groups of investigators (1–6) have analyzed the fatty acid compositions of pure lipids isolated from cerebral tissue, and other investigators (7–10) have analyzed the fatty acids of total lipids, total glycerophosphatides, and total sphingolipids of the brain. However, no studies have been performed in which each individual major lipid class has been isolated from the brain and its fatty acid composition defined. The preceding paper (11) dealt with the isolation and quantification of each of the major lipid classes in human brain using column chromatographic procedures. The present report deals with the analyses of the fatty acid and fatty aldehyde compositions of these lipids from gray matter, from white matter, and from myelin of four human subjects free from cerebral pathology, aged 10 months, 6 yr, 9 yr, and 55 yr.

MATERIALS AND METHODS

The procedures involved in the extraction of tissue, the isolation of myelin, the column chromatographic isolation of each lipid, the paper chromatographic determination of the purity of each lipid, and the clinical

Abbreviations: Fatty acids are denoted by chain length: number of double bonds. ω6 denotes a double bond six carbons from the methyl end of the fatty acid chain. Ald, aldehyde; h, hydroxy fatty acid; EGP, ethanolamine glycerophosphatides; SGP, serine glycerophosphatides; CGP, choline glycerophosphatides; DMA, dimethyl acetals; GLC, gas–liquid chromatography.

* Brain Lipids IV. For previous papers in this series see J. S. O'Brien, D. L. Fillerup, and J. F. Mead, *J. Lipid Res.* **5:** 109, 1964 and **5:** 329, and J. S. O'Brien and E. L. Sampson, *J. Lipid Res.* **6:** 537, 1965.

descriptions of each subject, are described in detail in the accompanying article (11).

Isolation of Fatty Acids and Fatty Aldehydes

Fatty acids and fatty aldehydes were released from each glycerophosphatide by heating the lipid in 5% methanolic HCl for 1 hr at 60° as described previously (4). Fatty acid methyl esters and fatty aldehyde DMA were extracted together into petroleum ether. The DMA were then selectively isolated by preferential extraction, after saponification of the fatty esters in methanolic KOH, according to Farquhar (12, 4). The quantities of total DMA present in each glycerophosphatide were determined by weighing the isolated DMA or by gas–liquid chromatography (4). The GLC method and the weighing method for determining DMA content agreed within ±4% in 14 determinations.

Each sphingolipid was hydrolyzed in 2 N HCl for 2 hr at 110° in a sealed tube at a concentration of 2 mg of lipid per ml of aqueous acid. These conditions have previously been found to give complete release of fatty acids (5). The isolation and quantification of unsubstituted fatty acids, hydroxy fatty acids, and sphingosine were accomplished using the method described by O'Brien and Rouser (13). An attempt was also made to detect positional isomers other than 2-hydroxy derivatives among the hydroxy fatty acids by GLC (13). In the present study each hydroxy acid which was present in concentrations >1% of the total hydroxy fatty acids in cerebroside and cerebroside sulfate in each subject could be accounted for as a 2-monohydroxy derivative.

Gas–Liquid Chromatography

Fatty acid methyl esters, acetoxy methyl esters of hydroxy acids, and fatty aldehyde DMA were chromatographed both on polar and nonpolar columns prepared as previously described (3, 4, 13). The separation of the glycerophosphatide fatty esters was satisfactorily achieved on a 10% diethylene glycol succinate polyester column (4) while the separation of the much longer-chain sphingolipid fatty esters and acetoxy fatty esters was best achieved on a 3% Apiezon L column (3, 5, 13). Purified fatty esters and fatty aldehydes were then used as reference standards for identification and quantification. Those fatty esters for which standards were unavailable were tentatively identified by their carbon number (14). Fatty esters were also chromatographed after palladium-catalyzed hydrogenation (13) to confirm the identity of certain unsaturated derivatives.

The error in quantification of fatty esters was determined by analyzing a fatty ester standard made up of pure 14:0, 16:0, 16:1, 18:0, 18:1, 20:0, 21:0, 23:0 and 24:0, and NHI Standard F from the National Institutes of Health. It was found that quantitative results with both standards agreed with the stated composition with a relative error of less than 5% for major components (>10% of the total mixture) and less than 7% for minor components (<10% of the total mixture). The quantitative standardization of fatty acids was begun in our laboratory in October 1963 and the later values (obtained on all samples from Subjects 1 and 2 and on myelin from all four subjects) are more accurate than earlier values. The fatty acid compositions of glycerophosphatides and sphingolipids from the 55 yr old man, reported earlier (3, 4) were redetermined using more rigorous fatty acid standardization. Standards were not available for the 20- and 22-carbon polyunsaturated fatty acids and values for these acids may be in error because of differences in the argon-ionization detector response for these fatty acids. Quantification was by triangulation.

All the major fatty esters (greater than 1% of the total) had retention times identical with those of known fatty esters except for two unsaturated fatty esters (evidenced by their disappearance after hydrogenation) present in EGP and SGP. These fatty acid chromatographed in the region expected for 22:3, 22:4 and 22:5. Mohrhauer and Holman (15) isolated 22:5ω6 from the brain and reported a retention time on a polyester column for this fatty acid which was very similar to that of one of the major unknown polyunsaturated fatty acids in EGP and SGP. The relative proportions of 22:5ω6 given by them (15, 16) were also similar to those we found for this acid. We have therefore designated the major unknown polyunsaturate in EGP and SGP as 22:5ω6. The remaining polyunsaturates are given tentative identifications based on their carbon numbers compared to those given for standard polyunsaturated fatty acids by Hofstetter, Sen, and Holman,[1] and by comparison with known standards of 22:6ω3 and 20:5ω3 from the Hormel Institute.

RESULTS

Although there were certain consistent changes in the chain length and/or degree of unsaturation of the fatty acids with age or with tissue source, several inconsistencies were present. It is not known whether such inconsistencies reflect procedural difficulties during isolation, nutritional differences, individual variations, or other causes. At the present time, we will concentrate upon those changes which are of large magnitude and which are consistently present from one subject to another.

[1] Unpublished data.

TABLE 1 FATTY ALDEHYDE COMPOSITION OF BRAIN GLYCEROPHOSPHATIDES

Fatty Aldehyde	Ethanolamine Glycerophosphatides												Serine Glycerophosphatides								
	10-month old			6-yr old			9-yr old			55-yr old			10-month old			6-yr old			55-yr old		
	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M
14:0ald	tr.	0.7	tr.	tr.	0.5	tr.	4.1	5.3	0.4	0.4	tr.	1.4	0.5	1.0	1.8	tr.	2.4	0.3	tr.	0.5	0.6
15:0ald	tr.	0.2	tr.	tr.	0.2	0.2	1.2	0.9	tr.	tr.	1.4	tr.	tr.	1.4	—	tr.	0.8	0.1	tr.	1.3	tr.
16:br*ald	1.9	3.1	0.9	2.5	3.0	tr.	3.1	3.7	tr.	2.0	2.0	3.4	1.4	3.9	tr.	3.8	4.5	0.1	tr.	tr.	tr.
16:0ald	19.2	43.0	45.2	23.7	32.5	38.0	17.8	21.5	38.6	13.5	23.0	29.0	22.4	41.1	42.5	25.0	32.5	37.4	18.7	33.0	28.5
16:1ald	1.6	tr.	tr.	tr.	1.2	0.6	3.8	tr.	tr.	tr.	tr.	tr.	1.3	1.0	tr.	tr.	1.0	0.9	—	tr.	2.1
17:0ald	3.3	2.1	2.2	2.3	1.8	0.8	1.5	1.9	1.2	1.3	1.3	2.2	3.6	2.0	2.4	3.2	2.4	1.1	—	1.8	3.2
18:br*ald	2.9	1.1	0.5	4.5	1.0	0.5	5.9	1.2	1.2	2.3	0.4	1.3	2.0	2.3	0.8	3.3	2.5	0.9	tr.	0.9	tr.
18:0ald	63.7	27.9	27.5	54.4	21.6	21.8	47.9	26.5	14.0	56.7	22.0	21.0	59.3	31.3	29.2	49.8	19.9	20.0	53.0	19.6	17.5
18:1ald	7.0	21.9	23.7	12.6	38.0	38.0	14.0	37.6	44.6	23.1	49.9	41.7	9.2	15.9	20.7	15.0	33.5	39.3	28.2	42.0	43.0

Each aldehyde is expressed as a percentage of the total aldehydes present in each glycerophosphatide. Small amounts of minor aldehydes including 12:0 ald 13:0 ald, 13:1 ald, 14:1 ald, 15:1 ald, and 16:1 ald were quantified but are not included in the table.

GM, gray matter; WM, white matter; M, myelin.

* br denotes a branched chain aldehyde (tentative identification only).

Fatty Aldehyde Composition of Glycerophosphatides

The fatty aldehyde compositions of EGP and SGP are given in Table 1. The major aldehydes were palmitaldehyde, stearaldehyde, and octadecenaldehydes [olealdehyde and *cis*-vaccenaldehyde (17)] with smaller proportions of odd-chain and branched aldehydes, consistent with the earlier reports of Debuch (18). Peaks corresponding to DMA longer than 18 carbons were occasionally detected, but in trace amounts only. Consistent with earlier results in adult humans (4), the fatty aldehyde composition of EGP was very similar to that of SGP in each tissue. However, the fatty aldehyde composition of EGP and SGP from gray matter differed from that of EGP or SGP from white matter or from myelin. In each subject octadecenaldehyde was present in very low proportions in EGP and SGP from gray matter but was present in much higher proportions in EGP or SGP from white matter or from myelin. The proportions of octadecenaldehyde also increased in EGP and SGP with age accompanied by a concomitant diminution of stearaldehyde.

Fatty Acid Composition of Glycerophosphatides

The fatty acid compositions of EGP, SGP, and CGP are given in Table 2. EGP and SGP contained high proportions of 20- and 22-carbon polyunsaturated fatty acids, the major ones being 20:4, 22:5ω6, and 22:6. Consistent with earlier results (4), CGP contained only very small proportions of these acids. EGP, SGP, and CGP also differed markedly in their content of palmitic acid; CGP acids contained 31–55%, EGP contained 5–12%, and SGP contained the smallest proportions of 16:0 (1–3%). Each glycerophosphatide from gray matter contained much higher proportions of polyunsaturated fatty acids than the same glycerophosphatide from white matter, in accordance with previous results in adult humans (4).

The myelin glycerophosphatides contained the smallest proportions of polyunsaturated fatty acids of all: the glycerophosphatide from myelin contained approximately one-third to one-sixth the proportions of polyunsaturated fatty acids as the same glycerophosphatide from gray matter. Another tissue difference was noted for 18:1 and 20:1. Both fatty acids were present in higher proportions in each glycerophosphatide from myelin than from gray matter.

The age-dependent changes in the fatty acid compositions of glycerophosphatides were small. There was a tendency for the proportions of 18:1 and 20:1 to increase with increasing age in gray matter. In general, however, the absence of age-dependent change was more significant. Polyunsaturated fatty acids were present in nearly the same proportions in EGP and SGP from gray matter of the 10 month old child as in EGP and SGP from gray matter of the 55 yr old man.

Fatty Acid Composition of Sphingolipids

Hydroxy Acid Content. The α-hydroxy fatty acid content of cerebroside and cerebroside sulfate is given in Table 3. Cerebroside, cerebroside sulfate, and ceramide contained hydroxy fatty acids while sphingomyelin did not. Cerebroside contained higher proportions of hydroxy fatty acids (24–80% of the total fatty acids) than cerebroside sulfate (25%), or ceramide (approximately 10%). The hydroxy fatty acid content of cerebroside increased with age, in accordance with the findings of Kishimoto and Radin (19) while that of cerebroside sulfate remained approximately the same at all ages studied. Cerebroside sulfate contained nearly the same content of hydroxy acids in each tissue at all ages.

Unsubstituted Acid Composition. The unsubstituted fatty acid compositions of these sphingolipids are given in Table 4. A wide variety of fatty acids was present, rang-

TABLE 2 FATTY ACID COMPOSITION

Fatty Acid	Carbon Number	Ethanolamine Glycerophosphatides												Serine		
		10-month old			6-yr old			9-yr old			55-yr old			10-month old		
		GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M
14:0	14.0	0.3	0.3	0.7	0.2	0.1	0.8	0.4	0.6	0.8	0.2	0.5	0.4	0.3	0.3	0.5
16:0	16.0	5.0	11.5	6.0	10.2	6.3	13.8	6.7	4.7	7.1	6.7	6.7	6.5	1.7	1.0	1.4
16:1	16.4	0.4	2.9	1.3	0.9	1.1	1.5	0.7	1.0	1.4	0.4	1.4	0.4	0.4	0.5	0.6
18:0	18.0	25.3	16.0	28.2	26.7	6.8	11.9	28.0	12.6	7.0	26.0	9.0	7.7	33.0	21.5	47.7
18:1	18.4	4.8	44.0	38.1	10.9	43.0	57.1	8.0	34.3	43.9	11.9	42.4	72.5	9.6	46.5	30.5
18:2	18.9	0.2	1.0	1.4	0.2	0.2	tr.	0.7	0.5	0.3	tr.	tr.	tr.	0.2	0.5	0.4
20:1	20.5	0.3	4.3	2.9	0.8	4.7	1.7	0.9	2.9	3.6	1.5	7.9	3.9	0.2	2.0	0.2
20:2*	20.8	0.3	2.1	2.0	tr.	2.3	tr.	tr.	1.8	1.3	tr.	2.4	tr.	0.2	2.0	1.4
20:3$_{\omega}$9*	21.1	0.5	1.3	2.0	1.6	1.3	tr.	tr.	1.1	0.5	0.5	1.6	tr.	tr.	1.8	0.8
20:3$_{\omega}$6*	21.5	0.8	tr.	1.5	tr.	1.0	tr.	1.3	2.1	1.4	tr.	tr.	0.8	1.0	1.5	0.9
20:4	21.9	19.7	3.5	3.1	18.2	9.0	2.8	18.5	8.3	9.6	13.8	6.4	1.6	3.2	3.6	2.2
22:4$_{\omega}$6*	23.3	0.5	tr.	—	0.6	0.4	tr.	tr.	1.1	1.1	tr.	tr.	tr.	tr.	1.2	tr.
22:5$_{\omega}$6	23.8	12.3	4.0	2.8	9.1	18.7	9.2	7.0	15.3	15.9	14.3	13.7	4.6	6.0	4.5	1.6
22:5$_{\omega}$3*	24.1	4.4	2.0	1.9	2.3	0.6	tr.	2.8	2.1	1.0	tr.	0.5	0.5	8.6	2.3	4.5
22:6	25.2	23.3	5.0	4.5	16.7	3.2	1.3	22.2	9.4	4.0	24.3	3.4	0.6	33.5	10.3	4.3
% Polyunsaturated acid		62.0	18.9	22.2	48.7	36.7	13.3	52.5	41.7	35.1	52.9	28.0	8.1	50.3	27.7	16.1

Each fatty acid is expressed as percentage of total fatty acids in each glycerophosphatide.
GM, gray matter; WM, white matter; M, myelin.
* Tentative identifications only. Carbon numbers obtained on a diethylene glycol succinate polyester column at 187°. Small amounts of odd-chain fatty acids and those with carbon numbers of 22.5 (22:3$_{\omega}$9?), 24.5 (24:1?) and 25.7 (24:4$_{\omega}$6?) were quantified but are not included in the table.

ing in chain length from 14 to 26 carbon atoms, and including odd-chain fatty acids and both saturated and monounsaturated derivatives, as reported previously (1, 3, 5). The proportions of polyunsaturates, if present, were too small for reliable quantification.

In general, the sphingolipids contained two groups of fatty acids, the medium-chain fatty acids (14–18 carbon atoms) and the long-chain fatty acids (19–26 carbon atoms). In the younger subjects the sphingolipids from gray matter contained predominantly *medium* chain fatty acids while those from myelin or from white matter contained predominantly *long* chain fatty acids. The quantitative differences between these tissues were large; in the younger subjects each sphingolipid from myelin contained 5- to 9-fold higher proportions of long-chain fatty acids than the same sphingolipid from gray matter. Large differences were also noted when the sphingolipids from the three younger subjects were compared to those from the adult. In the adult, cerebroside and cerebroside sulfate contained predominantly (70–90%) long-chain fatty acids from gray matter, from white matter, and from myelin, in accordance with previous results (1, 3). However, in the three younger subjects, cerebroside, cerebroside sulfate, and sphingomyelin from gray matter contained predominantly medium-chain fatty acids while these lipids from myelin or from white matter contained greater proportions of long-chain fatty acids (especially cerebrosides and cerebroside sulfates). Thus, there was a tendency for the fatty acids of each sphingolipid to increase progressively in chain length with age in each tissue. However, the apparent rate of fatty acid chain elongation was not the same in each tissue; the sphingolipids from *myelin* or *white matter* contained high proportions of long-chain fatty acids at a very early age (10 months) while the sphingolipids from *gray matter* achieved the same high levels of long-chain fatty acids at a much later age (some time between 9 and 55 yr).

Although there was a general tendency for each sphingolipid to contain progressively longer fatty acids with age, the proportion of long-chain fatty acids was not the same in each sphingolipid. Sphingomyelin and ceramide contained much lower proportions of long-chain fatty acids than cerebroside and cerebroside sulfate did, and although cerebroside and cerebroside sulfate contained similar proportions (85–90%) of long-chain fatty acids in the adult, in the two younger subjects cerebroside sulfate contained somewhat higher

TABLE 3 HYDROXY FATTY ACID CONTENT OF SPHINGOLIPIDS

	10-month old			6-yr old			9-yr old			55-yr old		
	Gray Matter	White Matter	Myelin	Gray Matter	White Matter	Myelin	Gray Matter	White Matter	Myelin	Gray Matter	White Matter	Myelin
Cerebroside	24	53	45	34	34	38	41	38	36	57	75	82
Cerebroside sulfate	29	26	—	25	23	29	27	27	25	25	22	25

Hydroxy acids are expressed as percentages of the total fatty acids present in each sphingolipid.

Glycerophosphatides									Choline Glycerophosphatides									
6-yr old			9-yr old		55-yr old			10-month old		6-yr old			9-yr old			55-yr old		
GM	WM	M	GM	WM	GM	WM	M	GM	WM	GM	WM	M	GM	WM	M	GM	WM	M
0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.3	2.1	0.5	0.9	0.1	1.3	0.7	1.0	2.9	1.3	0.4
2.8	1.3	2.7	2.0	1.8	2.3	1.7	2.6	46.4	35.1	42.5	42.8	32.0	54.7	31.0	29.9	45.0	34.3	40.1
0.6	0.4	0.4	0.7	0.5	0.3	0.4	0.6	1.2	2.5	3.5	2.5	0.7	2.4	4.3	2.6	3.1	1.0	0.8
46.0	49.5	44.4	49.5	42.4	25.4	35.8	40.0	11.0	14.6	11.7	12.1	17.3	9.8	13.2	14.7	9.3	13.4	6.1
7.7	24.9	36.9	7.8	33.1	21.5	39.7	43.3	29.2	40.1	30.6	38.1	49.8	26.8	42.1	45.6	31.4	45.2	51.6
tr.	0.3	tr.	0.4	tr.	tr.	0.3	tr.	0.2	0.3	tr.	0.6	tr.	tr.	0.4	0.6	0.4	0.4	0.6
0.8	3.2	3.7	0.6	2.2	1.0	5.3	3.6	0.5	0.9	1.2	tr.	tr.	tr.	1.0	—	0.7	1.1	—
1.4	1.8	1.7	tr.	1.7	tr.	1.4	tr.	tr.	tr.	tr.	—	—	—	0.6	—	—	—	—
1.5	tr.	0.8	tr.	1.4	tr.	tr.	tr.	tr.	tr.	tr.	—	—	—	0.5	0.5	tr.	tr.	—
tr.	1.2	tr.	0.4	1.3	0.7	0.6	tr.	0.8	0.2	3.2	—	—	tr.	tr.	—	tr.	—	—
5.4	2.3	1.4	2.8	2.0	1.6	2.0	4.7	8.1	3.0	5.3	2.6	tr.	4.5	3.3	2.0	4.1	1.3	0.4
tr.	1.4	tr.	tr.	1.5	tr.	tr.	tr.	tr.	tr.	—	—	—	—	—	—	—	—	—
5.4	4.4	2.6	5.4	3.7	5.0	4.8	2.3	tr.	tr.	—	—	—	—	—	—	—	—	—
4.6	1.8	tr.	3.6	1.7	3.3	0.9	tr.	tr.	tr.	—	—	—	—	tr.	—	—	0.3	—
23.5	4.6	1.1	25.4	4.1	36.6	5.6	2.3	tr.	tr.	1.0	tr.	tr.	tr.	0.9	0.2	3.1	0.1	tr.
41.8	17.8	7.6	38.0	17.4	47.2	15.6	9.3	9.1	3.5	9.5	3.2	tr.	4.5	5.7	3.3	7.6	2.1	1.0

proportions of long-chain fatty acids than did cerebroside.

Composition of Hydroxy Acids. The average over-all chain length of the hydroxy acids progressively increased with age (Table 5), as in the unsubstituted acids. Cerebroside and cerebroside sulfate from white matter and from myelin in the younger subjects contained higher proportions of long-chain hydroxy fatty acids than those from gray matter. In addition, an interesting difference was noted between the hydroxy fatty acids and the unsubstituted acids. In each tissue and in both glycosphingolipids, the proportions of long-chain fatty acids were higher in the hydroxy series than in the unsubstituted fatty acid series.

DISCUSSION

Comparison of Fatty Acids from Each Tissue

It was evident from the data in the preceding report (11) that the brain is not homogeneous in its lipid composition. The present study demonstrates that, depending upon the tissue source, the lipids in the brain differ not only in their class composition but in their fatty acid composition as well. That is, the fatty acid (and fatty aldehyde) composition of each lipid class is strikingly different depending upon whether it is isolated from gray matter, white matter, or myelin. It is apparent, then, that lipids in each of these tissues are not structurally identical, as has been assumed in the past, but must be considered as different molecular species. Since the fatty acid composition of a lipid can exert a great influence upon its metabolic activity, the recognition of the tissue differences in fatty acid composition of lipids is especially pertinent to metabolic and enzyme studies of brain lipids.

Anatomical Comparisons

The differences in lipid and fatty acid composition between gray matter, white matter, and myelin may be explained on an anatomical basis. Gray matter is rich in cells having an abundance of cytoplasm while myelin is a multilayered membrane structure formed by a spiral wrapping of the plasma membrane of the oligodendrocyte around the nerve axon (20–22). White matter, being rich in myelin, has a lipid composition resembling that of pure myelin. The high proportions of polyunsaturated glycerophosphatides in gray matter may be related to a high content of mitochondria in this "cytoplasmic" tissue, since mitochondria from the brain are known to contain high proportions of polyunsaturated fatty acids, e.g., approximately 25% of the total fatty acids (23). The high proportions of saturated glycerophosphatides and long-chain sphingolipids in myelin may be explained on the basis of the ability of such lipids to form stable bimolecular lipid leaflets. The theoretical implications of this phenomenon have been discussed in detail elsewhere (24).

Myelin at Different Ages

In the previous study, the lipid composition of myelin was found to change but little between the ages of 10 months and 55 yr (11). The present study demonstrates that the fatty acid compositions of myelin lipids also were quite similar in the 10-month old baby and in the

TABLE 4 UNSUBSTITUTED FATTY ACID

Fatty Acid	Ceramide				Sphingomyelin												Cerebroside		
	10-month old		9-yr old		10-month old			6-yr old			9-yr old			55-yr old			10-month old		
	GM	WM	GM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M
14:0	0.5	3.3	3.3	tr.	0.4	0.5	0.3	0.7	0.7	0.3	1.4	0.8	0.4	1.0	tr.	0.4	4.3	1.3	1.9
16:0	19.0	18.1	43.1	12.8	5.9	6.0	9.0	12.5	9.0	3.7	9.4	12.0	7.0	6.8	10.2	5.4	40.1	8.3	9.3
18:1	9.2	8.4	10.5	5.8	1.0	1.2	2.3	2.8	3.6	0.4	2.2	4.3	2.0	2.6	6.5	0.4	11.9	14.4	6.6
18:0	68.2	37.8	38.6	23.7	89.5	71.6	62.8	77.0	35.7	41.1	78.6	44.5	43.1	61.9	20.1	33.6	32.4	24.8	14.6
20:0	0.5	1.0	0.5	1.3	0.6	0.8	0.8	0.7	0.7	0.9	1.2	0.9	0.4	2.7	1.1	0.5	0.6	2.1	1.2
22:1	tr.	0.4	tr.	1.3	tr.	0.6	0.5	tr.	0.6	0.6	—	0.4	0.4	0.1	1.3	0.2	0.2	0.4	1.0
22:0	0.3	1.2	0.3	2.2	0.2	1.3	1.3	0.5	1.6	1.6	0.5	1.4	0.9	1.1	1.7	0.8	0.9	2.6	4.3
23:1	0.2	0.6	0.3	1.4	0.1	0.3	0.4	0.3	0.7	0.9	tr.	0.5	0.5	0.5	1.3	0.6	tr.	1.0	0.6
23:0	0.3	1.6	0.2	3.7	0.2	0.5	0.5	0.7	1.4	1.6	tr.	1.6	1.3	1.5	2.8	1.4	0.5	2.1	1.2
24:1	0.7	13.9	1.3	25.8	0.7	10.8	10.0	2.1	27.1	32.1	2.9	23.1	32.3	12.0	30.2	40.0	3.0	17.5	27.4
24:0	0.3	4.3	0.6	11.9	0.5	3.3	3.7	1.1	7.3	9.9	1.5	3.5	4.5	1.9	6.9	8.0	2.8	13.4	15.2
25:1	tr.	1.6	tr.	3.1	0.3	0.8	1.6	tr.	3.4	0.4	0.8	2.2	2.6	2.5	8.3	3.6	tr.	2.4	3.6
25:0	tr.	1.0	tr.	1.6	tr.	0.4	0.5	tr.	1.8	1.8	tr.	1.4	1.2	0.4	3.4	1.8	tr.	2.0	2.0
26:1	tr.	2.3	tr.	2.8	tr.	1.5	2.6	tr.	4.0	3.1	tr.	2.0	2.5	2.4	5.5	2.4	tr.	5.8	7.3
26:0	tr.	2.2	tr.	tr.	tr.	0.4	1.0	tr.	1.1	tr.	tr.	1.0	tr.	tr.	tr.	tr.	tr.	1.3	1.6
Sum of 14–18	97.7	67.9	96.8	44.9	97.3	79.3	76.4	94.2	49.6	45.5	93.1	61.5	52.5	74.5	36.8	39.8	92.0	50.6	34.6
Sum of 19–26	2.3	32.1	3.2	55.1	2.7	20.7	23.6	5.8	50.4	54.5	6.9	38.5	47.5	25.5	63.2	60.2	18.0	49.4	66.4

Each fatty acid is expressed as a percentage of the total unsubstituted fatty acids in each sphingolipid. Small amounts of minor fatty acids including 12:0, 13:0, 13:1, 14:1, 15:0, 15:1, 16:1, 19:0, 19:1, 21:0, 21:1, and 27:1 were detected and quantified but are not included in the table.
GM, gray matter; WM, white matter; M, myelin.

55 yr old man. One major gross difference between the brain of the baby and that of the adult was a thin ribbon of white matter in the former and a thick mass of white matter in the latter. One major ultrastructural difference is that myelin sheaths in the baby are not as thick as in the adult since there are fewer windings of the unit membrane around each axon. Thus, infants and adults differ not only in their total mass of cerebral white matter but also in the myelin content of their white matter. The major difference, then, between myelin in the baby and that in the adult is in its quantity rather than in its quality. Since myelin is "chemically mature" at an early age it is tempting to predict that a specific chemical composition must be reached before myelin can be formed; one in which saturated glycerophosphatides and long-chain sphingolipids predominate. Studies of myelin at even earlier ages are in progress to test this prediction further.

We wish to thank V. Shamburger, S.R.M. Connerton, and J. Plesset for their valuable assistance in the laboratory. We are indebted to Dr. Robert Stein, University of California at Los Angeles, for standards of known aldehydes from 9 to 16 carbons and to Drs. H. H. Hofstetter, N. Sen, and R. T. Holman of the Hormel Institute for supplying us with a copy of their article prior to publication in which carbon numbers (equivalent chain lengths) of a variety of polyunsaturated fatty acids of known structure are given. This study was supported in part by National Institutes of Health Grants NB-04136, HE-08429, and 62-56-H-7197; Los Angeles County Hospital Attending

TABLE 5 HYDROXY FATTY ACID COMPOSITION OF SPHINGOLIPIDS

Fatty Acid	Cerebroside												Cerebroside Sulfate											
	10-month old			6-yr old			9-yr old			55-yr old			10-month old			6-yr old			9-yr old			55-yr old		
	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M
14h:0	32.0	2.7	1.1	13.5	0.8	0.8	14.0	1.8	1.4	0.5	0.6	0.8	30.0	15.7	0.2	12.0	2.6	2.1	17.3	1.0	0.7	1.6	2.4	2.1
16h:0	20.0	3.0	0.8	40.0	1.0	1.0	5.0	1.0	0.2	0.2	1.0	0.2	15.5	5.0	3.1	17.0	1.0	1.6	16.9	9.0	8.0	9.6	6.3	6.3
18h:1	4.1	0.6	tr.	1.9	0.6	tr.	tr.	0.3	1.0	0.3	0.3	tr.	5.6	2.7	1.1	1.9	0.3	tr.	tr.	0.2	0.2	1.6	0.5	0.3
18h:0	5.6	1.5	1.3	2.0	3.8	5.2	2.0	0.6	2.3	1.7	0.6	0.6	7.1	4.2	2.4	3.2	0.9	2.2	1.9	0.8	1.0	3.8	2.2	3.4
20h:0	1.8	0.4	4.5	1.0	0.5	0.6	1.0	0.3	1.1	0.6	0.8	1.2	2.0	0.9	3.6	4.1	1.5	0.9	4.0	1.6	1.5	3.3	1.6	1.0
22h:0	4.5	10.0	9.5	1.8	7.0	9.8	4.8	4.0	11.8	8.5	11.0	12.0	8.0	10.3	1.1	16.6	8.4	7.8	13.0	8.7	17.1	16.3	13.2	13.3
23h:0	2.8	6.6	9.2	2.0	12.3	10.1	10.1	14.9	14.4	15.3	13.0	12.8	4.0	4.8	7.4	9.3	13.0	10.2	9.2	10.3	9.5	15.0	11.4	10.0
24h:1	5.8	14.5	12.3	11.3	18.7	20.2	22.2	20.5	24.6	26.4	24.0	24.0	6.2	12.1	19.5	12.0	17.7	11.8	12.6	13.8	15.6	16.0	8.4	9.6
24h:0	16.9	43.0	49.2	18.2	38.0	34.2	23.3	39.6	28.9	27.3	34.0	32.3	16.0	29.7	42.7	17.5	38.3	49.1	13.5	38.0	32.8	21.7	46.2	40.0
25h:1	1.7	2.2	1.9	1.7	4.3	6.6	4.0	3.6	4.8	6.8	3.0	3.2	tr.	1.4	2.8	1.7	3.0	3.6	3.7	5.2	3.5	1.6	5.4	5.0
25h:0	tr.	3.1	4.4	1.0	3.7	2.9	4.1	6.2	2.4	5.1	4.0	4.5	0.5	1.9	3.6	tr.	5.3	3.5	2.6	5.8	4.2	1.1	tr.	3.5
26h:1	tr.	6.6	4.6	2.1	5.8	5.3	4.1	5.1	2.5	6.0	4.1	7.0	tr.	1.6	8.0	tr.	4.0	2.4	tr.	4.2	4.2	1.4	tr.	2.3
26h:0	tr.	4.3	1.2	tr.	2.4	2.6	1.8	1.6	tr.	tr.	2.7	2.1	tr.	tr.	3.2	tr.	1.9	tr.	tr.	0.8	0.9	tr.	tr.	1.7
Sum of 14–18h	66.5	9.1	3.2	57.4	8.5	7.0	23.0	4.2	7.7	4.0	4.2	1.9	63.3	29.6	7.1	38.8	6.0	8.9	41.4	11.6	10.7	18.6	13.8	13.6
Sum of 19–26h	33.5	90.9	96.8	42.6	91.5	93.0	77.0	95.8	93.3	96.0	95.8	98.1	36.7	70.4	92.9	61.2	94.0	90.1	58.6	88.4	89.3	81.4	86.2	86.4

Each fatty acid is expressed as a percentage of the total hydroxy fatty acids of each sphingolipid. Other fatty acids including 12h:0, 13h:0, 13h:1, 14h:1, 15h:0, 15h:1, 17h:0, 19h:0, and 21h:0 were detected and quantified but are not included in the table.
GM, gray matter; WM, white matter; M, myelin.

Cerebroside (*continued*)									Cerebroside Sulfate											
6-yr old			9-yr old			55-yr old			10-month old			6-yr old			9-yr old			55-yr old		
GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M	GM	WM	M
3.0	0.5	0.9	4.7	0.8	1.2	1.3	0.5	0.4	6.0	0.7	0.9	2.5	0.6	0.5	3.0	0.5	0.1	0.5	0.4	0.3
33.0	7.5	8.2	30.4	8.3	8.3	9.7	2.1	2.0	50.0	6.4	4.0	40.0	3.4	4.4	32.0	4.0	4.1	2.4	9.3	8.5
20.0	10.5	2.3	21.3	3.3	5.3	3.7	0.7	3.2	16.6	5.1	2.0	15.5	1.4	0.8	9.6	1.5	1.3	1.0	1.6	1.3
35.6	17.4	10.1	34.3	7.9	12.9	11.0	7.6	7.8	16.4	7.7	10.0	11.6	4.3	2.9	13.1	5.1	5.4	2.6	6.1	3.9
0.1	1.2	0.4	tr.	0.4	0.6	0.8	1.0	1.1	0.6	6.9	1.7	0.6	0.7	0.2	0.5	0.5	0.3	1.1	1.6	1.3
tr.	0.2	tr.	tr.	0.4	0.3	0.2	0.3	0.2	tr.	1.5	2.3	tr.	0.6	0.2	0.5	0.3	0.3	0.2	0.2	tr.
0.5	1.3	1.7	tr.	1.8	1.8	1.3	3.1	1.4	0.8	6.0	5.7	1.6	2.3	1.0	1.3	2.0	2.2	1.6	1.4	1.4
tr.	0.6	tr.	tr.	0.8	1.0	0.9	1.1	0.3	0.5	0.5	0.3	1.2	0.9	0.3	tr.	0.6	0.4	0.7	0.5	tr.
0.2	3.1	2.0	tr.	3.1	3.1	2.7	4.2	2.9	0.6	1.4	1.0	1.2	4.0	2.1	1.2	3.3	2.0	3.2	2.3	1.2
4.2	29.7	38.3	4.4	40.0	35.5	37.8	44.0	38.8	3.0	28.3	31.0	10.2	30.3	43.7	17.0	38.7	42.3	37.6	36.2	42.7
2.0	12.5	13.8	2.1	11.3	12.2	10.2	15.1	14.2	3.0	21.3	24.0	5.4	19.1	20.9	10.3	16.1	17.9	17.7	15.6	14.7
tr.	4.7	3.6	0.4	7.0	6.4	8.8	4.3	12.9	tr.	2.1	2.6	3.0	7.7	7.1	5.2	9.0	7.4	13.6	11.2	7.3
tr.	2.6	7.1	tr.	3.5	2.9	2.5	5.0	7.9	tr.	1.4	1.8	2.2	7.2	4.8	1.3	5.0	4.0	5.8	5.5	6.1
tr.	6.8	7.2	tr.	7.4	7.1	6.8	8.0	5.4	tr.	8.2	8.5	2.1	12.7	9.5	2.8	9.8	7.0	10.9	6.0	8.3
tr.	1.2	0.9	tr.	1.0	0.7	1.6	1.3	0.9	tr.	2.2	1.7	tr.	3.5	1.3	tr.	1.8	1.5	0.1	1.2	2.0
93.01	36.	24.0	93.1	22.7	28.4	25.7	11.3	14.0	91.5	21.2	19.4	72.5	11.0	8.9	60.1	12.9	13.7	7.5	18.3	15.0
7.0	63.9	76.0	6.9	77.7	71.6	74.3	88.7	86.0	8.5	78.8	80.6	27.5	89.0	91.1	39.9	87.1	86.3	92.5	81.7	85.0

Staff Project #1605, and a contribution from the North American Aviation Donate Once Club.

Manuscript received April 1, 1965; accepted June 25, 1965.

REFERENCES

1. Radin, N. S., and Y. Akahori. *J. Lipid Res.* **2:** 335, 1961.
2. Gatt, S., and E. R. Berman. *J. Neurochem.* **10:** 73, 1963.
3. O'Brien, J. S., D. L. Fillerup, and J. F. Mead. *J. Lipid Res.* **5:** 109, 1964.
4. O'Brien, J. S., D. L. Fillerup, and J. F. Mead. *J. Lipid Res.* **5:** 329, 1964.
5. O'Brien, J. S., and G. Rouser. *J. Lipid Res.* **5:** 339, 1964.
6. Ställberg-Stenhagen, S., and L. Svennerholm. *J. Lipid Res.* **6:** 146, 1965.
7. Johnson, P. V., and F. A. Kummerow. *Proc. Soc. Exptl. Biol. Med.* **104:** 201, 1960.
8. Downing, D. T. *Australian J. Chem.* **14:** 150, 1961.
9. Baker, R. W. R. *Biochem. J.* **79:** 642, 1961.
10. Carroll, K. K. *J. Lipid Res.* **3:** 263, 1962.
11. O'Brien, J. S., and E. L. Sampson. *J. Lipid Res.* **6:** 537, 1965.
12. Farquhar, J. W. *J. Lipid Res.* **3:** 21, 1962.
13. O'Brien, J. S., and G. Rouser. *Anal. Biochem.* **7:** 288, 1964.
14. Woodford, F. P., and C. M. van Gent. *J. Lipid Res.* **1:** 188, 1960.
15. Mohrhauer, H., and R. T. Holman. *J. Lipid Res.* **4:** 151, 1963.
16. Mohrhauer, H., and R. T. Holman. *J. Neurochem.* **10:** 523, 1963.
17. Leopold, F. *Z. Physiol. Chem.* **285:** 182, 1950.
18. Debuch, H. *Z. Physiol. Chem.* **327:** 65, 1962.
19. Kishimoto, Y., and N. S. Radin. *J. Lipid Res.* **1:** 79, 1959.
20. Maturana, H. R., *J. Biophys. Biochem. Cytol.* **7:** 107, 1960.
21. Peters, A. J., *J. Biophys. Biochem. Cytol.* **8:** 431, 1960.
22. Bunge, M. B., R. P. Bunge, and G. D. Pappas. *J. Cell Biol.* **12:** 448, 1962.
23. Witting, L. A., C. C. Harvey, B. Century, and M. K. Horwitt. *J. Lipid Res.* **2:** 412, 1961.
24. O'Brien, J. S. *Science* **147:** 1099, 1965.