Changes in the fatty acid composition of cerebrosides and sulfatides of human nervous tissue with age

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ABSTRACT Sphingogalactolipids (galactocerebrosides and sulfatides) have been isolated in almost quantitative yields from normal human nervous tissue (mostly brain) at different ages and their fatty acid compositions have been determined by gas–liquid chromatography.

The ratio of hydroxy acids to normal acids increased slightly during myelination and then remained rather constant; in adults the ratio for cerebrosides was about 2, and for sulfatides, 0.6–0.8. In adult nervous tissue the two predominant fatty acids of cerebrosides and sulfatides were the C_{24} monounsaturated and 2-hydroxy saturated acids. The infant brain galactolipids had (compared with child and adult) a lower percentage of C_{22}–C_{26} fatty acids and a much lower percentage of monoenoic acids, both of normal and hydroxy acids. Low activities of fatty acid elongation and desaturation systems during myelination are inferred.

Fatty acid changes with age were the same for cerebrosides and sulfatides but occurred later in the sulfatides, which supports the hypothesis that the cerebrosides are precursors of the sulfatides. The adult pattern of fatty acid composition with regard to degree of unsaturation and total percentage of C_{22}–C_{26} acids was reached as early as at 2 yr of age, but the percentage of odd-numbered (C_{23} and C_{25}) fatty acids continued to increase up to the age of 10–15 yr.

The fatty acid composition of the galactolipids of peripheral nerves differed mainly in its lower percentages of C_{25} and C_{26} acids and higher percentages of C_{22} and C_{16} acids. This composition is thus intermediate between those of brain and of extraneural organs.

KEY WORDS cerebrosides · sulfatides · fatty acid composition · man · brain · cerebral cortex · white matter · age variation · spinal medulla · peripheral nerve

Since 1958 we have been engaged in a systematic study of the distribution and chemical characterization of the different sphingolipids in the human nervous system in normal and pathological conditions. Studies on the lipid analysis of human fetal and newborn infant brain (1), the chemistry of gangliosides (2), and the fatty acid composition of human brain sphingomyelins (3) have hitherto been reported. The present paper concerns complete fatty acid analyses of cerebrosides and sulfatides from the human nervous system. After work started on the present study, several reports were published on the fatty acid composition of these lipids in brain. Radin and Akahori (4) made a careful study of the fatty acids of human brain cerebrosides, and Bernhard and Lesch (5) determined the fatty acid composition of cerebrosides and sulfatides from regions of the brain of a 69 yr old man. Recently O'Brien and collaborators (6–9) reported on the fatty acid composition of cerebrosides and sulfatides from bovine and human brain, while Eng et al. (10) studied only the 2-hydroxy fatty acids in white matter of infant and adult brains.

In our study a larger quantity of material has been analyzed and the fatty acids of the different sphingolipids have been isolated from the same tissue sample in order that the possible metabolic relationships of the sphingolipids might be studied.

MATERIALS AND METHODS

Tissue Sources

With some exceptions, the tissue materials were the same as those described in our study of sphingomyelins (3). Because large changes were found to occur in the fatty

Abbreviations: h, hydroxy fatty acid; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; C, chloroform; M, methanol; W, water; HAc, acetic acid; DEAE-cellulose, diethylaminoethyl cellulose. Fatty acids are designated by chain length: number of double bonds.

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acid patterns of cerebrosides and sulfatides with age in early infancy, but only small individual differences in adults, three additional infants were included in the material; only four brains from cases older than 15 yr were analyzed. The source of specimens for isolation of glycosphingolipids is recorded in Table 1.

Chemicals

Only redistilled solvents were used (3). Impurities found in analytical reagent grade methanol were extracted with the fatty acids and gave unidentified peaks on GLC. The impurities disappeared upon redistillation.

Screened silicic acid (Baker, A.R.) was used (3) in the main part of the study. Because it was impossible to obtain more of the same brand, silicic acid A.R. from Mallinckrodt was used in the latter part of the study. It was found unnecessary to screen this adsorbent, which we purified by suspending it twice in redistilled methanol, each time decanting the methanol after 20 min. The silicic acid was reactivated at 120°C for 16 hr before use. Diethylaminoethyl (DEAE) cellulose (Whatman) was processed as described elsewhere (10). Silica Gel G (Merck) was used for analytical and preparative thin-layer chromatography (TLC).

Analytical Methods

Phosphorus was determined by a micro modification (11) of the method of Lowry, hexose with an orcinol method after hydrolysis with 3 N HCl in chloroform–ethanol for 3 hr (2), glucose with glucose oxidase (12), and sulfate by reductive hydrolysis to hydrogen sulfide (13). Analytical TLC was usually performed with chloroform–methanol–water (C–M–W) 65:25:4 as the solvent, but for the separation of gluco- and galactocerebrosides from each other and from contaminating free fatty acids, less polar mixtures were also applied, such as C–M–W 75:25:3, C–M–HAc 75:25:5, and C–M 9:1. The plates were sprayed with 50% sulfuric acid in water or anisaldehyde–sulfuric acid in glacial acetic acid (15).

Isolation of Cerebrosides and Sulfatides

The isolation of crude samples of cerebrosides and sulfatides has been reported previously (3,14). The glycolipids still contained large amounts of other lipid material, especially when isolated from infant brain and cerebral grey matter. All glycolipids were rechromatographed on silicic acid columns (1 g of silicic acid per 10 mg of glycolipid) prepared in chloroform. The samples were applied in chloroform and elution was started with 10 column volumes of C–HAc 20:1, which eluted free fatty acids and cholesterol (partly oxidized). The cerebrosides and sulfatides were then eluted with 10 column volumes each of C–M 9:1, C–M 4:1, and C–M 1:1. The effluent was collected in about 75 tubes, the volume in each tube depending on the size of the silicic acid column. The tubes containing pure galactocerebrosides and sulfatides, as determined by TLC analysis, were combined. The tubes contaminated with simple gangliosides (16) were combined and a final purification was achieved by preparative TLC (17).

TABLE 1 Source of Tissue Used for the Isolation of Cerebrosides and Sulfatides

Subject	Sex	Age and Cause of Death	Tissue Used
FB 104-6	2 M, 1F	Combined newborn, full-term, lived for 1–3 days; asphyxia	Cerebral hemispheres
FB 107	M	Newborn, full-term, lived for 1 hr; subdural hemorrhage	Frontal and parietal lobes, cortex and white matter
CB 110	M	10 Days; asphyxia	Cerebral hemispheres
CB 109	M	2 Months	Right cerebral hemisphere
CB 111	M	7 Months; hydrocephalus op. and pneumonia	Frontal lobe, white matter
CB 108	F	8 Months; accident	Temporal lobe, total
CB 102	F	17 Months; congenital heart disease	Frontal lobe, total and temporal lobe, white matter
CB 112	F	25 Months; fire accident	Frontal lobe, cortex and white matter
CB 103	M	4 Yr; road accident	Frontal lobe, total
CB 106	M	8 Yr; road accident	Frontal lobe, total
CB 107	F	15 Yr; congenital heart disease	Frontal lobe, total and temporal lobe, cortex and white matter
AB 102	M	16 Yr; road accident	Temporal lobe, cortex and white matter
AB 104	F	32 Yr; cancer ovarii	Frontal lobe, cortex and white matter
AB 105	F	33 Yr; acute nephritis	Parietal lobe, cortex, and white matter
AB 108	F	77 Yr; bronchopneumonia	Frontal lobe, cortex and white matter
AB 109	F	77 Yr; emphysema and bronchopneumonia	Spinal medulla and spinal nerve roots
AB 110	M	72 Yr; acute myocardial infarction	Femoral nerves
AB 111	M	72 Yr; cerebral hemorrhage	Femoral nerves

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Qualitative and Quantitative Analyses of Isolated Cerebrosides and Sulfatides

The isolated cerebrosides and sulfatides were tested for phosphorus, total hexose, and sulfate. Only glycolipids containing less than 0.01% P were used for GLC. The cerebrosides and sulfatides of white matter contained nearly theoretical amounts (95–102%) of galactose and fatty acids, but in newborn brains and cerebral gray matter, the hexose and fatty acid contents were 80–95% and 70–80% of the calculated values, respectively. IR spectroscopy showed that the latter cerebrosides were contaminated with adsorbents (18); they had a slightly yellow-brown discoloration. An almost white compound was obtained on rechromatography of these cerebrosides on Florisil, but the recovery of cerebroside hexose was only 80–95%. The cerebrosides retained could be removed from the Florisil columns with C–M–W 5:5:1. They differed on TLC from the ordinary cerebrosides in showing two very diffuse confluent spots with considerable tailing. The same behavior on TLC had been found earlier for serum glycolipids eluted by C–M–HAc from DEAE-cellulose columns (19). GLC of the fatty acids showed several large anomalous peaks which could not be identified as any of the common saturated and monoenoic acids. To determine whether the glycolipids were altered during isolation we extracted a newborn brain (FB 107) immediately after autopsy and performed all steps in a nitrogen atmosphere in a dark room, using solvents freshly deaerated with nitrogen. No markedly smaller amounts of altered cerebrosides could be detected and consequently it appears that altered forms of cerebrosides do occur naturally in the tissues, as was found by Rouser, Galli, Lieber, Blank, and Privett (20) for phosphatidyl ethanolamine of bovine heart.

All cerebrosides and sulfatides from infant brains or cerebral gray matter of child brains were tested for glucose by the glucose oxidase method, by paper chromatography in 1-butanol–pyridine–water 6:4:3, and by zone electrophoresis in borate buffer (17). These independent methods showed the glucose concentration to be less than 2% of the total hexose in all cerebrosides and sulfatides subjected to GLC.

Preparation of Fatty Acid Methyl Esters and Separation of Normal and Hydroxy Esters

The methanolysis and isolation of the methyl esters of fatty acids were performed as for sphingomyelins (3). The esters of normal and hydroxy acids were separated by two different methods. When large samples of cerebrosides and sulfatides were analyzed, about 50 mg was hydrolyzed. After gravimetric determination of the total amount of methyl esters, normal and hydroxy acids were separated on 10-g columns of silicic acid. The normal acids were eluted with 50 ml of chloroform, and the hydroxy acids with the subsequent 150 ml of the same solvent. The columns were further eluted with C–M 4:1 to test the completeness of the elution. The separation of the methyl esters was monitored by TLC (about 100 μg of esters applied to the plate). Chloroform or methylene chloride was used as solvent, and the esters were made visible by spraying with sulfuric acid. Contamination with less than 1% of other organic compounds could be detected. The amount of the esters was determined gravimetrically on a microanalytical balance. We also tried the colorimetric hydroxamate method (21) for the quantitative determination of the esters, but it proved to be less accurate and to require relatively large amounts.

The newborn and infant brains (FB 104–106, FB 107, CB 109, and cerebral cortex of CB 112) provided only 2–25 mg of glycolipids for GLC. To 2–5 mg of lipid, known amounts of methyl 19-methyl eicosanoate (methyl isoheneicosanoate) and methyl 2-hydroxy-19-methyl eicosanoate were added before methanolysis as internal standards. (These esters were chosen as standards because they did not coincide with any of the natural esters from brain glycolipids during GLC on Apiezon, Reoplex, or diethylene glycol adipate or succinate, see below.) The methyl esters were then isolated by preparative TLC on Silica Gel G, 0.25 mm thick, with methylene chloride as solvent. The ester bands were made visible by spraying with bromothymol blue solution, scraped off, and eluted from the adsorbent with C–M 9:1. The amounts of normal and hydroxy acids were calculated from the GLC analysis and the known concentration of the internal standards.

In a few experiments the content of normal and hydroxy acids in cerebrosides and sulfatides from cerebral gray matter was determined by both methods (Table 2). Similar values were found for normal acids, but the content of hydroxy acids was found to be 5–20% lower by the internal standard procedure when galactolipids from cerebral gray matter and infant brains were analyzed. Clearly visible amounts of column adsorbents and impurities remaining at the origin of the TLC (first method) contaminated the hydroxy acid fraction but not the normal acid fraction; these impurities did not influence the fatty acid analysis by GLC, but gave a falsely high weight for the hydroxy acids.

The internal standards only became available during the course of the study and consequently they were used with a limited number of samples (Table 2). A comparison of the results obtained with the gravimetric and the internal standard methods shows that the latter is preferable, especially during analysis of glycolipids from tissues in which the glycolipid concentration is low or the hydroxy acids represent only a small percentage of total fatty acids.

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TABLE 2 Percentage of Hydroxy Acids in Total Fatty Acids of Glycolipids from Human Brain and Nervous Tissue

Tissue Sample		Cerebrosides		Sulfatides	
		Gravimetric Detm.	Internal Standard	Gravimetric Detm.	Internal Standard
		%			
FB 104-6	T	50		50	
FB 107	CC		50		25
	W		55		17
CB 110	T	60	52	44	25
CB 109	T	60	56	34	20
CB 111	W	67			
CB 108	T	61		30	
CB 102	T	64		44	
	W	69		46	
CB 112	CC		65		35
	W		55		32
CB 103	T	63		42	
CB 106	T	62		49	
CB 107	T	67		44	
	CC				58
	W				33
AB 102	CC	80	63	62	
	W	64	60	45	37
AB 104	CC	73		51	
	W	65		43	
AB 105	CC	71		60	
	W	65		38	
AB 108	CC	73	65	55	54
	W	60	60	44	44
AB 109					
Spinal medulla		55		44	43
Spinal roots		49		33	23
AB 110 + 111					
Peripheral nerve		60		36	

FB, fetal; CB, child; AB, adult; T, total (gray + white); CC, cerebral cortex; W, white matter. All samples were of brain except AB 109–111.

Gas–Liquid Chromatography

GLC of the methyl esters was performed on 10% Reoplex and diethylene glycol adipate polyester columns, before and after hydrogenation, on Perkin-Elmer gas chromatographs 154 or 800 with flame ionization detectors. The hydroxy acids were analyzed as their trimethylsilyl derivatives (22). The mixture of normal fatty acids employed as a reference standard previously (3) was also used in this study. The reference standard of hydroxy acids was composed of the following 2-hydroxy acids: 18h:0, 18h:1, 22h:0 and 24h:1.

Only columns that gave quantitative recoveries of the reference standard mixtures were used.

The peaks were identified from log retention diagrams (3) and by isolation of the esters and subsequent mass spectrometry (3). Quantification was by weighing of the peak area. All results are means of duplicates of unhydrogenated and hydrogenated samples, expressed in weight percentages of total fatty acid methyl esters.

RESULTS

Recovery of Cerebrosides and Sulfatides, and Their Purity

It has previously been shown (14) that crude extracts of sulfatides were obtained in quantitative yield with the present isolation procedure. The recovery of cerebrosides from cerebral white matter was quantitative but some cerebrosides from infant brain and cerebral gray matter were retained on the DEAE-cellulose and Florisil columns. Sulfatides from the latter two sources were also lost during the final purification on Florisil. The retained glycolipids, eluted from Florisil with C–M–W 5:5:1, had markedly altered lipophilic portions. The total recovery of cerebrosides and sulfatides from cerebral white matter, calculated from determinations of lipid hexose, was 98.0% (95–102%) and from cerebral cortex and infant brains, not more than 80–90%.

After these purifications the cerebroside fraction was still contaminated with ceramide dihexosides, which represented 1–2% of total cerebrosides in cerebral white matter and up to 5% in the infant brain. The ceramide dihexosides were isolated by TLC from samples FB 104–106, FB 107, CB 103, and CB 112. They all contained glucose and galactose in equimolar proportions. Their fatty acid composition was very similar to that of the monohexosides (galactocerebrosides) in CB 103 and in the white matter of FB 107 and CB 112, but in FB 104–106 and the cerebral cortex of FB 107 and CB 112 the ceramide dihexoside fraction contained more C_{18} than the ceramide monohexoside fraction. Because of these results the fatty acid analyses of cerebrosides from child and adult total brain and from cerebral white matter were performed on samples which still contained the ceramide dihexosides. From the other two sources, infant brains and cerebral cortex, the ceramide dihexosides were removed by preparative TLC on silica gel (17) prior to fatty acid analysis of the cerebroside samples. TLC on borate plates showed that the cerebroside samples used for the fatty acid analyses contained no glucocerebrosides.

The sulfatide fractions were all contaminated with various amounts of monosialogangliosides, mainly ceramide monosialyl lactosides (G_{M3}). This contamination was negligible in the sulfatide fractions from cerebral white matter and from total brains of older children and adults, but it could constitute 0.2–0.3 of the sulfatide samples from infant brains and cerebral cortex. It was not detected at first because, when large samples of sulfatides were applied to the analytical TLC plates, the major fraction of the gangliosides moved together with the sulfatides. The two glycolipids were completely separated by repeated chromatography on Silica Gel G or Florisil columns loaded with 2–5 mg of glycolipid per

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g of adsorbent. Because this contamination was not detected until some time after the beginning of the study, no new material could be obtained from the cerebral cortex of AB 104. All the other samples from infant brain and cerebral cortex were freed from gangliosides before analysis. The contamination of sulfatides with gangliosides is negligible in normal cerebral white matter and the samples from this source were not further purified before fatty acid analysis.

In other sources—kidney (13), liver, and blood plasma (L. Svennerholm, unpublished results)—the presence of ceramide dihexoside sulfate has been indicated. We have been unable to detect this sulfatide in the present material but minute amounts were isolated from brain tissue of children with disorders associated with an increased level of ceramide dihexosides (L. Svennerholm, unpublished results).

Hydroxy Acid Content

Only 2-hydroxy acids were found in the brain glycolipids. Their concentration in cerebrosides and sulfatides is given in Table 2. The cerebrosides contained a higher proportion of hydroxy acids (50–70%) than the sulfatides (20–60%). The difference was, in general, more pronounced in cerebral white matter than in cerebral cortex. The concentration of hydroxy acids in cerebrosides and sulfatides increased during the myelination period and then remained fairly constant during adult life. It should be noted, however, that the figures obtained by gravimetric analyses are uncertain, particularly for the hydroxy acid content in sulfatides.

Normal Fatty Acids in Brain Cerebrosides and Sulfatides

There is a wide variety of fatty acids in brain cerebrosides and sulfatides, with chain lengths mainly from 16 to 26 carbons. In addition, we found small amounts of C_{14} and C_{15} (in general less than 0.5%), and also isolated and identified C_{27} and C_{28} by countercurrent distribution of the fatty acids from 10 g of cerebrosides. Representative samples of the fatty acids from cerebrosides and sulfatides were analyzed by GLC at higher temperatures and for longer times, but no fatty acid with elution time longer than that of 28:1 could be detected.

Cerebrosides (*Table 3*). Stearic acid comprised nearly 25% of the normal fatty acids in samples from the new-

TABLE 3 Composition of Normal Fatty Acids in Brain Cerebrosides

Samples		Individual Fatty Acids													Totals	
		16:0	18:0	18:1	20:0	22:0	22:1	23:0	24:0	24:1	25:0	25:1	26:0	26:1	22–26	Monoenes
FB 104-6 (newborn)	T	2.3	23.8	0.7	3.4	10.4	0.5	1.9	29.5	15.1	1.6	0.8	3.4	5.5	68.9	22.8
FB 107	CC	4.7	18.3	2.1	2.7	6.5	1.0	2.2	18.2	28.5	2.1	3.1	1.8	5.8	69.8	41.5
(newborn)	W	2.2	17.1	0.9	2.7	8.3	1.2	1.9	26.0	24.7	1.9	1.3	3.0	7.5	76.2	37.2
CB 110 (10 days)	T	2.1	19.4	0.7	2.6	8.1	0.6	2.0	31.1	16.9	2.1	1.3	4.1	7.9	74.4	27.8
CB 109 (2 months)	T	0.7	12.8	0.4	2.6	8.2	1.2	1.8	26.8	26.4	2.0	1.8	3.1	11.2	83.0	41.6
CB 111 (7 months)	W	1.2	11.1	1.6	1.8	5.5	1.4	2.2	17.4	34.4	2.3	4.0	2.1	13.3	83.4	56.0
CB 108 (8 months)	T	0.4	8.5	0.4	1.8	5.6	1.3	2.4	20.0	35.0	2.3	3.6	2.3	15.1	88.3	65.2
CB 102	T	0.6	8.0	tr.	1.5	6.0	0.5	3.9	24.7	30.7	4.5	5.0	2.9	10.1	89.3	47.3
(17 months)	W	0.4	7.8	tr.	1.7	6.7	0.3	5.8	28.5	22.3	6.2	4.7	3.7	10.3	88.9	38.0
CB 112	CC	0.4	5.7	0.2	0.9	3.4	0.7	3.2	18.4	40.0	4.8	6.5	2.2	12.0	92.0	60.2
(25 months)	W	0.3	5.1	0.1	0.8	3.3	0.7	3.5	20.1	40.2	4.6	6.3	1.9	12.0	93.4	60.1
CB 103 (4 yr)	T	0.3	5.5	tr.	0.7	2.7	0.6	3.7	13.8	42.7	4.4	9.2	1.6	11.0	92.7	64.6
CB 106 (8 yr)	T	0.3	7.4	tr.	0.7	2.5	0.8	4.1	13.7	43.8	3.5	10.7	0.7	9.6	90.7	66.4
CB 107 (15 yr)	T	1.6	10.2	tr.	1.9	5.6	0.3	9.1	28.5	18.6	8.7	4.9	2.0	4.0	82.3	28.4
AB 102	CC	0.5	8.5	tr.	1.1	2.7	0.6	4.3	15.0	36.6	4.9	11.5	1.2	10.9	88.8	60.7
(16 yr)	W	0.6	6.6	tr.	1.2	3.2	0.6	5.1	16.7	33.4	6.5	11.4	1.5	11.2	90.7	57.7
AB 104	CC	0.5	6.3	tr.	1.1	2.7	0.6	4.3	14.4	38.1	5.2	11.9	2.1	11.0	91.3	62.6
(32 yr)	W	0.4	8.9	tr.	1.0	2.6	0.6	4.4	15.1	38.7	5.8	10.0	2.1	8.6	88.8	58.8
AB 105	CC	1.7	10.0	1.1	1.2	2.8	0.4	3.8	12.8	40.4	3.2	11.7	0.7	8.3	85.1	63.0
(33 yr)	W	0.9	9.5	0.7	0.9	2.6	0.4	4.2	13.6	43.8	4.0	9.9	1.0	6.7	87.1	62.5
AB 108	CC	1.3	10.1	0.5	1.6	3.6	0.5	4.8	15.0	34.3	5.7	10.7	1.3	8.9	85.6	55.7
(77 yr)	W	0.5	6.5	0.5	0.8	2.4	0.6	3.9	12.5	44.5	4.4	12.0	0.9	8.9	91.2	67.7

The following were detected in small amounts (in general less than 1%): 16:1, 17:0, 19:0, 20:1, 21:0, and 23:1. For abbreviations see Table 2. Values are weight percentage of methyl esters.

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born brains, but its concentration rapidly dropped with age to a level between 5 and 10%. The fatty acids C_{22}–C_{26} increased from about 70% at birth to 90–95%, their highest concentration, at the age of about 2 yr, after which there was a tendency to a very slight decrease with age. The most obvious change was a significant increase in unsaturation, monoenes increasing from 25–40% at birth to 60% or more from 2 yr and on. C_{22}, which constitutes a large proportion of the fatty acids in visceral glycolipids (22, 23) occurred to the extent of about 10% at birth but then rapidly decreased to a final level of 2–3%. All these changes seemed to be completed during the first 2 yr of life. The odd-numbered acids C_{23} and C_{25} reached their final level somewhat later, apparently at about 10–15 yr of age.

Gray and white matter from one newborn and one child brain (25 months of age) and from four adult brains were separated. There was no clear-cut difference between the fatty acid patterns of cerebrosides from cerebral cortex and white matter either in child or adult brains, but there were somewhat more of the shortest fatty acids in the cerebral cortex than in the white matter of the newborn.

There were two exceptions to the described pattern of development, CB 102 and CB 107. In these two brains, the concentration of unsaturated fatty acids was lower than expected for the age. The two children had both died from congenital heart failure. As it is known that a failing circulation will lead first to a serious hypoxemia of the temporal lobe, the cerebrosides and sulfatides were isolated from this lobe as well. The content of unsaturated cerebroside fatty acids was even smaller in this brain region.

Sulfatides (*Table 4*). The normal acids of sulfatides underwent changes similar to those in the cerebroside fatty acids during development. However, in the sulfatides of newborn brain, the concentration of C_{16} and C_{18} was nearly 50% larger than in the cerebrosides and the diminution with increasing age was also more pronounced. This resulted in a slightly higher final concentration of the C_{22}–C_{26} fatty acids in sulfatides (about 95–97%), and in a somewhat higher content of monounsaturation. As in the case of cerebrosides, CB 102 showed a diminished unsaturation compared to others of the same age; fatty acids of the total tissue of CB 107 did not differ significantly from those of AB 102 at the same

TABLE 4 Composition of Normal Fatty Acids in Brain Sulfatides

Samples		Individual Fatty Acids													Totals	
		16:0	18:0	18:1	20:0	22:0	22:1	23:0	24:0	24:1	25:0	25:1	26:0	26:1	22–26	Monoenes
FB 104-6 (newborn)	T	12.6	33.9	1.6	4.1	7.4	0.5	1.2	17.5	13.0	0.9	0.5	2.0	3.3	46.4	19.1
FB 107	CC	7.2	24.4	1.4	2.8	4.2	0.9	1.6	11.7	31.2	2.0	3.2	1.2	6.2	62.8	43.8
(newborn)	W	9.8	29.0	5.9	2.7	5.8	1.3	1.0	14.7	20.5	1.1	0.9	1.6	4.5	51.6	33.6
CB 110 (10 days)	T	3.3	24.1	0.4	3.2	6.8	1.1	1.4	22.4	22.8	1.5	1.2	3.1	7.6	68.3	33.6
CB 109 (2 months)	T	1.7	20.2	0.4	3.6	7.7	1.7	1.1	21.3	28.0	1.2	1.1	2.3	8.3	73.2	40.1
CB 108 (8 months)	T	0.9	7.9	0.4	2.1	5.7	1.8	1.6	17.8	39.4	1.6	2.6	2.3	13.9	87.4	58.9
CB 102 (17 months)	W	1.0	5.4	0.5	1.3	6.6	0.8	3.2	31.2	24.0	4.7	3.3	5.7	10.4	90.7	40.0
CB 112	CC	0.9	4.0	2.0	1.0	3.7	0.7	2.4	20.1	38.8	3.8	5.0	2.6	13.4	91.1	60.8
(25 months)	W	0.3	2.3	0.2	0.6	3.3	0.6	2.5	20.9	42.4	3.6	5.6	2.3	14.2	96.2	63.8
CB 103 (4 yr)	T	0.3	2.6	0.2	0.5	2.3	0.5	3.2	17.3	43.5	3.5	10.1	2.2	12.6	96.1	67.8
CB 106 (8 yr)	T	0.5	1.3	0.3	0.3	1.7	0.4	2.9	13.3	45.8	4.5	12.5	1.2	13.1	96.3	73.0
CB 107	T	0.4	2.1	0.2	0.3	2.1	0.5	4.1	19.3	38.2	6.6	10.3	2.4	11.3	95.7	61.5
(15 yr)	CC	2.8	5.8	1.0	1.2	3.3	0.3	5.5	21.7	28.4	8.5	9.4	2.4	8.0	88.2	47.8
	W	0.9	2.5	0.6	0.4	2.1	0.4	4.2	19.2	39.6	5.8	10.8	1.3	10.3	94.7	62.7
AB 102	CC	0.6	3.0	0.5	0.3	1.5	0.3	3.1	16.0	43.4	6.0	10.6	2.2	11.6	95.5	67.9
(16 yr)	W	1.0	3.1	0.8	0.3	1.7	0.3	3.1	16.9	45.2	4.8	10.8	1.0	10.1	94.8	67.8
AB 104 (32 yr)	W	0.4	3.0	0.4	0.4	2.0	0.4	4.0	18.6	38.7	7.0	9.5	2.3	11.9	95.3	62.0
AB 105	CC	1.3	4.8	0.5	0.8	2.2	0.3	4.0	13.5	40.0	5.7	13.4	1.7	10.3	91.8	65.2
(33 yr)	W	1.3	5.3	1.3	0.5	1.5	0.4	2.6	8.8	52.2	3.3	11.4	0.9	8.7	90.7	75.1
AB 108	CC	tr.	2.3	1.0	0.5	1.7	0.6	3.1	11.7	41.0	5.6	14.9	1.9	14.6	96.0	73.0
(77 yr)	W	0.3	1.7	0.1	0.3	1.4	0.4	3.6	14.0	44.1	5.4	13.6	1.6	12.2	97.2	71 3

The following were detected in small amounts (in general less than 1%): 16:1, 17:0, 19:0, 20:1, 21:0, and 23:1. For abbreviations see Table 2. Values are weight percentages of methyl ester.

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age, but those of the cerebral cortex also had a diminished unsaturation in CB 107.

2-Hydroxy Acids in Brain Cerebrosides and Sulfatides

The hydroxy acids differed from the normal acids in that they displayed less unsaturation and contained fewer medium-chain (C_{16}–C_{18}) acids. However, the same trends are clearly seen with development.

Cerebrosides (*Table 5*). 18h:0 was 4% in newborn brain and dropped to less than 1% in adult brain. The degree of unsaturation increased from about 10% at birth to more than 30% at 2 yr of age. 22h:0 showed the same decrease with age as 22:0. The increase of odd-numbered hydroxy acids was still more pronounced than the increase of odd-numbered normal acids. The decreased unsaturation of CB 102 and CB 107 was also evident for the hydroxy acids.

Sulfatides (*Table 6*). Sulfatides showed the same changes on a more pronounced scale: 18h:0 decreased from 15% to less than 1%, and 22h:0 from 20% to 5%. The increase in unsaturation was also evident. The monoenes increased from 13% to about 30%. The odd-numbered acids decreased slightly during the first 2 months but then increased rapidly, the maximum level being reached at the age of about 10 yr.

Fatty Acids of Spinal Medulla and Peripheral Nerve

Only one sample each of spinal medulla, spinal roots, and peripheral nerve was analyzed (Tables 7 and 8). Compared to brain, the cerebrosides and sulfatides of spinal medulla contained a smaller number of fatty acids (which consisted mainly of C_{24} and 18:0), a larger concentration of 18:0, and a smaller concentration of C_{25}–C_{26}. The fatty acids were slightly more unsaturated than in the brains of the same age. Except for these rather small differences, the glycolipid fatty acid patterns were similar in brain and spinal medulla. Spinal nerve roots and peripheral nerve showed large differences from the brain pattern, with much less unsaturation in normal and hydroxy acids. The concentrations of C_{22} and C_{23} were larger and those of C_{25} and C_{26} much smaller, both for normal and for hydroxy acids. The differences in the normal acid pattern showed the same tendency already observed for sphingomyelins (3) and confirm the assumption that the sphingolipid fatty acids of peripheral

TABLE 5 COMPOSITION OF 2-HYDROXY ACIDS IN BRAIN CEREBROSIDES

Samples		Individual Fatty Acids										Totals	
		18h:0	20h:0	22h:0	23h:0	24h:0	24h:1	25h:0	25h:1	26h:0	26h:1	22–26	Monoenes
FB 104-6 (newborn)	T	4.3	1.5	18.2	5.8	52.3	7.4	2.4	0.5	3.5	3.6	94.2	12.0
FB 107	CC	5.3	1.9	14.6	8.6	39.6	14.8	2.9	2.5	2.1	3.8	93.3	24.6
(newborn)	W	3.3	1.3	17.5	5.9	49.4	11.8	2.4	0.8	1.8	3.3	95.4	17.4
CB110 (10 days)	T	3.1	1.2	16.1	6.3	53.2	8.0	2.8	0.8	4.1	3.9	95.6	13.1
CB 109 (2 months)	T	2.1	1.1	16.4	6.2	47.8	12.7	2.6	1.0	3.6	5.8	96.7	20.1
CB 111 (7 months)	W	1.1	0.7	10.5	8.4	41.3	19.8	3.1	2.6	2.4	8.9	98.1	32.4
CB 108 (8 months)	T	1.0	0.5	12.5	8.9	41.3	18.9	3.6	1.9	2.6	8.0	98.4	29.6
CB 102	T	0.9	0.5	9.9	11.7	43.2	13.7	5.9	2.7	3.5	6.0	98.5	23.6
(17 months)	W	1.1	0.6	12.3	13.1	49.4	11.5	4.6	0.8	2.5	3.0	98.0	16.1
CB 112	CC	0.8	0.4	8.2	11.5	41.8	17.9	6.1	2.8	2.4	7.1	98.8	28.8
(25 months)	W	0.7	0.3	8.6	11.5	43.0	18.0	5.5	2.6	2.3	6.3	98.9	28.0
CB 103 (4 yr)	T	0.6	0.3	7.0	14.1	42.5	18.7	5.2	3.2	1.5	5.9	99.0	28.7
CB 106 (8 yr)	T	0.2	0.4	7.9	14.7	33.8	25.4	5.1	4.3	1.4	5.5	99.2	36.3
CB 107 (15 yr)	T	0.2	0.5	11.6	21.9	44.2	9.4	6.2	2.0	1.1	1.5	99.0	14.0
AB 102	CC	0.1	0.4	8.5	15.9	36.9	21.2	6.7	3.7	1.3	3.7	99.3	30.0
(16 yr)	W	0.1	0.4	7.8	16.3	41.5	17.1	6.9	4.0	1.1	3.5	99.2	25.6
AB 104	CC	0.3	0.4	7.5	15.4	29.4	23.3	7.1	6.1	2.6	6.8	99.2	37.2
(32 yr)	W	0.7	0.3	6.8	15.4	36.4	16.8	7.2	4.5	2.6	4.7	98.9	27.4
AB 105	CC	0.5	0.4	8.2	17.1	32.4	22.7	6.0	5.4	1.4	5.1	99.0	33.9
(33 yr)	W	1.0	0.2	7.2	17.7	37.9	19.4	6.8	4.0	1.1	4.1	98.9	28.1
AB 108	CC	0.5	0.4	8.3	19.5	40.7	10.7	9.7	3.8	2.0	3.8	98.9	18.9
(77 yr)	W	0.6	0.2	6.5	17.4	40.0	17.9	6.5	4.3	1.1	4.8	99.1	27.7

The following were detected in small amounts (in general less than 1%): <18, 19h:0, 21h:0, 22h:1, 23h:1 (3.5% in FB 107 CC). For abbreviations see Table 2. Values are weight percentages of the 2-hydroxy acid methyl acids.

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TABLE 6 Composition of 2-Hydroxy Acids in Brain Sulfatides

Samples		Individual Fatty Acids										Totals	
		18h:0	20h:0	22h:0	23h:0	24h:0	24h:1	25h:0	25h:1	26h:0	26h:1	22–26	Monoenes
FB 104-6 (newborn)	T	14.7	5.4	19.5	7.2	33.0	9.5	2.6	0.6	3.8	1.5	78.9	12.8
FB 107	CC	13.9	4.4	13.2	7.8	22.0	22.0	3.6	3.3	2.7	4.9	81.1	32.0
(newborn)	W	12.4	3.5	16.6	5.0	36.3	15.7	3.4	tr	2.3	2.1	82.5	19.1
CB 110 (10 days)	T	11.0	3.5	16.2	5.4	37.2	14.0	2.1	0.9	3.8	3.0	83.8	19.1
CB 109 (2 months)	T	8.9	3.1	16.6	4.7	38.8	14.6	1.9	0.8	3.3	4.6	86.6	21.3
CB 108 (8 months)	T	3.5	1.3	11.7	5.9	36.9	22.8	2.1	1.3	4.0	8.3	94.8	34.2
CB 102 (17 months)	W	0.9	1.0	9.7	8.0	47.2	18.0	3.4	1.2	3.3	5.4	97.1	25.5
CB 112	CC	1.1	0.7	7.3	8.1	37.9	23.0	5.4	2.9	3.0	8.9	98.1	36.4
(25 months)	W	0.6	0.4	7.6	8.7	40.9	21.5	5.1	2.7	2.9	8.0	98.8	33.6
CB 103 (4 yr)	T	0.4	0.3	5.9	11.7	43.4	18.4	7.1	2.6	1.8	7.3	99.2	29.2
CB 106 (8 yr)	T	0.3	0.3	4.6	12.5	37.5	19.4	7.9	5.6	2.0	9.0	99.3	34.8
CB 107 (15 yr)	T	0.5	0.3	5.3	15.2	39.6	21.5	6.7	3.7	1.0	5.2	98.8	31.2
AB 102	CC	1.7	0.6	7.6	15.0	40.3	17.3	6.9	3.1	1.4	4.1	97.2	26.0
(16 yr)	W	0.3	0.2	4.7	13.4	40.4	20.2	10.8	3.7	2.9	3.6	99.5	27.5
AB 104 (32 yr)	W	0.7	0.5	3.9	12.7	47.1	22.0	6.4	1.9	1.7	1.8	98.0	26.3
AB 105	CC	0.4	0.4	6.1	16.2	37.1	16.0	9.9	5.0	2.5	5.7	99.0	27.1
(33 yr)	W	0.6	0.2	4.9	12.0	26.9	33.9	5.8	6.4	1.9	5.9	99.1	47.6
AB 108	CC	0.3	0.3	4.8	13.5	34.8	19.5	9.5	6.4	2.4	7.7	99.1	34.2
(77 yr)	W	0.2	0.3	4.2	14.3	42.3	15.3	8.6	5.5	1.9	6.5	99.3	28.2

The following were detected in small amounts (in general less than 1%): <18, 19h:0, 21h:0, 22h:1, 23h:1. For abbreviations see Table 2. Values are weight percentages of the 2-hydroxy acid methyl esters.

nerve have a composition between that of brain and that of extraneural organs.

DISCUSSION

Purity of Cerebrosides and Sulfatides

The present study has clearly demonstrated the difficulties of preparing pure samples of cerebrosides and sulfatides from cerebral cortex of child and adult brain and from whole infant brain. With the present elution scheme, the galactocerebrosides were eluted together with glucocerebrosides and ceramide dihexosides in silicic acid chromatography. Almost complete separation of the glycolipids could be achieved with gradient elution or frequent changes of solvent systems with an increasing polarity, but tubes containing overlapping fractions nevertheless appeared. It seems advisable to use only the pure portions of a certain glycolipid for fatty acid analyses. However, the total fraction has to be examined if accurate figures are to be obtained, because the elution of the glycolipids from the adsorbent columns depends not only on the carbohydrate moiety, but also on the chain length, degree of unsaturation, and substitution of fatty acids and sphingosines. When the goal is quantitative recovery of galactocerebrosides and sulfatides, the risk of contamination with glucolipids (glucocerebrosides, ceramide dihexosides) increases. Such an admixture of the galactolipids with glucolipids has to be avoided because the two groups have different fatty acid patterns in brain tissue.

It is still more important to use well-defined compounds when pathological material is analyzed because a deviation from the normal fatty acid pattern may be accorded some pathogenetic significance. The risk of contamination of cerebrosides and sulfatides is much larger in most pathological materials studied (24) because the concentrations of glucocerebrosides, ceramide dihexosides, and monosialogangliosides are often several times larger than in normal material.

Selection of Normal Brain Material

One of the major difficulties in a study such as this is to collect pure, normal material. Brain specimens obtained from subjects who have had no previous symptoms or signs of neurological or mental disease and who have died suddenly in an accident can in general be regarded as adequate normal material without any neuropathological examination. Such material can be obtained from children and adults but very seldom from infants, and yet it

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TABLE 7 Composition of Normal Acids in Cerebrosides and Sulfatides from Nervous Tissue Other than Brain

	Individual Fatty Acids												Totals	
	16:0	18:0	18:1	20:0	22:0	24:0	24:0	24:1	25:0	25:1	26:0	26:1	22–26	Monoenes
Cerebrosides														
Spinal medulla (AB 109)	0.3	16.3	tr	1.2	2.1	2.4	8.9	53.9	1.8	5.9	0.7	4.8	81.9	66.0
Nerve roots (AB 109)	0.4	7.3	0.2	3.3	10.5	9.3	33.5	27.0	4.0	1.3	0.9	1.7	88.2	30.2
Femoral nerve (AB 110-1)	0.7	5.2	0.5	2.8	10.6	10.3	37.0	22.8	4.9	1.4	1.5	1.6	90.1	26.3
Sulfatides														
Spinal medulla (AB 109)	1.4	10.4	1.5	0.9	2.0	2.5	10.7	53.2	2.0	6.2	1.1	5.6	84.7	68.3
Nerve roots (AB 109)	1.2	5.0	5.3	2.1	8.0	7.4	30.8	29.5	3.8	1.0	1.0	1.8	84.4	40.0
Femoral nerve (AB 110-1)	1.6	5.6	5.0	2.0	8.4	8.4	32.9	23.9	5.1	0.9	1.8	2.2	83.6	34.5

The following were detected in small amounts (in general less than 1%): 17:0, 19:0, 20:1, 22:1, 23:1. Values are weight percentages of methyl esters.

TABLE 8 Composition of 2-Hydroxy Acids in Cerebrosides and Sulfatides from Nervous Tissue Other than Brain

	Individual Fatty Acids										Totals	
	18h:0	20h:0	22h:0	23h:0	24h:0	24h:1	25h:0	25h:1	26h:0	26h:1	22–26	Monoenes
Cerebrosides												
Spinal medulla (AB 109)	2.7	0.5	8.0	12.3	38.5	26.5	3.5	2.9	1.0	3.2	96.7	33.4
Nerve roots (AB 109)	0.5	0.7	18.1	19.5	51.1	5.6	2.4	0.4	0.9	0.5	98.5	6.5
Femoral nerve (AB 110-1)	0.3	0.6	16.5	20.7	51.8	4.2	3.0	0.8	1.1	0.8	98.9	5.8
Sulfatides												
Spinal medulla (AB 109)	1.1	0.3	6.4	11.0	38.6	28.4	4.7	3.6	2.0	3.8	98.5	35.8
Nerve roots (AB 109)	0.4	0.8	16.5	18.2	49.1	9.3	2.6	0.5	1.8	0.7	98.6	10.5
Femoral nerve (AB 110-1)	0.2	0.6	14.2	20.5	53.6	5.1	3.5	0.3	1.3	0.6	98.9	6.0

The following were detected in small amounts (in general less than 1%): 16h:0, 16h:1, 17h:0, 18h:1, 19h:0, 21h:0, 22h:1, and 23h:1. Values are weight percentages of methyl esters.

is during the infant period that large changes occur in lipid amounts and composition of brain. In the present study we obtained only one such infant specimen and were obliged to use brain material from infants who died from other disorders. The specimens of newborn brain were from infants who had died from asphyxia, but without the brain showing any signs of macroscopic changes. Obviously a newborn infant who dies soon after delivery may well have suffered from an inherited disorder or some chronic prenatal injury, or been exposed to a long-standing hypoxemia during parturition. All these events will lead to disturbances which can seriously affect the composition of the brain lipids. Therefore, FB 107 is to be regarded as the most "normal" in our newborn material even though the infant died from a rupture of the transverse sinus after a precipitate delivery. Except for the hemorrhage, histological brain examination showed a normal picture.

In other studies of human brain lipids, the "normal material" is often presented as deriving from "persons who died from other than neurological disorders." This is hardly acceptable, since careful selection and description of the "normal material" is of great importance for the validity and evaluation of the results.

Anatomical Localization of Cerebrosides and Sulfatides

Our study of the fatty acid composition of the sphingomyelins demonstrated distinctly different fatty acid patterns in cerebral gray and white matter (3). The cerebrosides and sulfatides from the two sources, on the

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other hand, showed very similar fatty acid patterns. There were no differences either in fatty acid composition between various regions of the cerebral hemispheres. Since the concentration of galactolipids is negligible in brain before myelination (2), it is likely that the galactolipids are confined to the myelin sheath in cerebral gray matter too. Our results do not agree with those of O'Brien and Sampson (9), who reported distinctly different fatty acid patterns for cerebrosides and sulfatides from cerebral gray and white matter and from the myelin of three children. It is difficult to explain this marked discrepancy. The very large concentrations of 16:0 and 18:1 and the low percentage of hydroxy acids reported by O'Brien and Sampson in cerebrosides and sulfatides of cerebral gray matter suggests to us that their samples were heavily contaminated with other compounds. However, their figures for the fatty acid composition in the brain of a 55 yr old man are very similar to ours, as are the data on cerebroside fatty acids of adults reported by Suomi and Agranoff (25). Menkes, Philippart, and Concone (26) recently reported figures for the fatty acid composition of cerebrosides and sulfatides in infant and adult brains which are similar to those of O'Brien and Sampson (9): in the infant brains, 16:0, 18:0, and 18:1 represented the major normal acids. We consider it likely that their preparations of cerebrosides and sulfatides from this age group consisted largely of the glycolipids which we removed as contaminants. An admixture of gangliosides and other ceramide oligosaccharides in the cerebroside and sulfatide preparations from gray matter is a plausible explanation for their figures for 18:0 being much higher than ours.

Changes in the Fatty Acid Pattern with Age

The relatively low concentration of long-chain fatty acids in the galactolipids in infant brains may indicate a relative lack of the chain elongation enzyme system at birth. At the same time, the concentration of monoenes is also low in the galactolipids, which suggests a low activity of the desaturation system (27) for the fatty acids. The initiation of myelination is followed by a rapid rise in both the absolute amount and the relative concentration of very long-chain acids (C_{24}–C_{26}) and of monoenes, and this suggests a rapid increase in the activity of the two enzyme systems. It seems that the increase in unsaturation of fatty acids with myelination is not confined to galactolipids, because Kishimoto, Davies, and Radin (28) found a striking increase of polyunsaturated fatty acids of glycerophospholipids in rat brain during myelination. The adult fatty acid pattern of galactolipids is reached as early as 2 yr, at least in terms of the degree of unsaturation and the concentration of even-numbered, very long-chain acids. The increase of the odd-numbered acids C_{23} and C_{25} continues for much longer, which is consistent with the finding of Hajra and Radin (29) that the turnover of these acids is very slow in rat brain cerebrosides.

Apart from the two studies of the galactolipid fatty acids of human brain(9,26) discussed earlier in this paper, a study of the influence of aging on the cerebroside fatty acid pattern of *rat* brain has been carried out by Kishimoto and Radin (30). There are some similarities between their results and ours: the odd-numbered fatty acids showed the greatest increase with age, and C_{22} underwent a relative decrease. There are also several dissimilarities: in human brain the concentration of unsaturated fatty acids increased rapidly and reached a considerably higher level than in rat brain. C_{20} and C_{22} were rather small fractions in human galactolipids and rapidly declined during the first 2 yr of development. The relatively large content of medium-chain acids (C_{16}–C_{18}) and the low degree of unsaturation at the onset of myelination either did not occur in rat brain or was missed because the rats were too old in the youngest age group (about 22 days).

Metabolic Relationship between Cerebrosides and Sulfatides

The present study has shown that cerebrosides and sulfatides have very similar fatty acid patterns in adult human brains. The only consistent differences are a lower percentage of hydroxy acids and a lower level of C_{18} fatty acids in sulfatides.

This similarity may indicate either that cerebrosides and sulfatides have a common fatty acid pool or that the one galactolipid is the immediate precursor of the other. In vitro studies (31, 32) have shown that the sulfatides are formed from the cerebrosides by the transfer of sulfate from 3′-adenosine 5′-phosphosulfate. Another fact which supports the hypothesis that the sulfatides are formed mainly from the cerebrosides is that, while the changes with age in the fatty acid patterns of the two galactolipids run parallel, there is a distinct lag in the fatty acid changes of the sulfatides.

Changes in Fatty Acid Pattern in Heart Failure

The lower degree of unsaturation of the galactolipid fatty acids in the two brains from patients who died from congenital heart failure calls for some comment. It might be an effect of diminished oxygen tension in the brain, since oxygen is necessary for the desaturation reaction (33). This idea is supported by the finding of a fairly normal degree of unsaturation in the sulfatide sample from the white matter of the older child brain, since the turnover of myelin sulfatides is very slow once myelination is complete (34), and it may be assumed that the sulfatides of white matter were mainly formed before the congestive failure became serious. On the other hand, sulfatides

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continue to turn over in cerebral gray matter and this is reflected by a decreased unsaturation of the fatty acids.

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