Structure and composition of sulfatides isolated from livers of patients with metachromatic leukodystrophy: galactosyl sulfatide and lactosyl sulfatide

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Abstract The livers of four patients with metachromatic leukodystrophy contained galactosyl sulfatide and lactosyl sulfatide, whereas these substances were undetectable in normal human liver. On the basis of methanolysis and permethylation studies, both sulfatides were shown to be substituted with sulfate at the C-3 position of the galactose moiety. Examination of the fatty acid compositions of these sulfatides showed that $C_{22:0}$ and higher 2-hydroxy and nonhydroxy fatty acids predominated in both. Both sulfatides contained the same long-chain bases, predominantly sphingosine, dihydrosphingosine, and phytosphingosine. Using as criteria the proportion of lactosyl sulfatide to galactosyl sulfatide, and the fatty acid and long-chain base compositions, the liver sulfatides from subjects with metachromatic leukodystrophy closely resemble those in the kidney and differ from those in brain and peripheral nerve.

Supplementary key words fatty acids · sphingosines · hexose · gas–liquid chromatography

Metachromatic leukodystrophy (MLD) is a genetically determined disorder associated with accumulation of sulfatides due to a diminished activity of cerebroside sulfatase (1, 2). The sulfatide excess is most striking and damaging in the nervous system, where it results in destruction of myelin. However, sulfatides also accumulate in a variety of other organs, particularly the kidney, gallbladder, and liver. Detailed studies of MLD sulfatides in brain, peripheral nerve, kidney, and urine have shown that they have the same composition and structure as those from normal individuals (3–10). An interesting by-product of these studies is the observation that sulfatide composition is to some extent organ specific. Thus, both in MLD and in the normal, sulfatides extracted from the kidney differ from those in brain in respect to fatty acid composition (7) and in respect to the proportion of various sphingosine bases (10).

These differences between brain and kidney sulfatides have proven of interest in respect to the pathophysiology of MLD. When this disease was first described, it was postulated that it represented a primary disorder of the nervous system (11). It was proposed that the destruction of myelin led to the release of sulfatides into the bloodstream and that these lipids then accumulated in excretory organs, such as the kidney and the gallbladder. However, the above-mentioned differences between the composition of brain and kidney sulfatides, as well as in vivo radioisotope studies (12), argued against this hypothesis. It is now considered likely that the sulfatides accumulated in MLD kidney are synthesized within that organ (7). This concept is supported by the facts that the normal human kidney contains higher levels of sulfatides than other visceral organs (2) and that the degradative enzyme cerebroside sulfatase is deficient in MLD kidney (1).

To our knowledge, no studies have been made of the structure of human liver sulfatides, either MLD or normal. In fact, in our experience the sulfatide levels of normal human liver are so low that they cannot be demonstrated by usual techniques (13). The proposition that there is sulfatide accumulation in MLD liver depends, to a considerable extent, upon histochemical studies that demonstrate abnormal quantities of metachromatic lipids. However, such histochemical reactions could also result from an excess of other acidic lipids, such as steroid sulfates, which are known to accumulate in one form of MLD (14). We report here studies of the sulfatide levels, compositions, and structures in the postmortem livers of four patients with MLD.

MATERIALS AND METHODS

Materials

Specimens of human liver, obtained at autopsy from the Massachusetts General Hospital, were stored first in dry

Abbreviations: galactosyl sulfatide, galactosyl-3-sulfate ceramide, ($SO_3H \rightarrow 3$)Gal-ceramide; lactosyl sulfatide, galactosyl-3-sulfate-glucosyl ceramide, ($SO_3H \rightarrow 3$)Gal(1 → 4)Glc-ceramide; MLD, metachromatic leukodystrophy; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; NFA, nonhydroxy fatty acid; HFA, hydroxy fatty acid.

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ice and later at −60°C: patient J. S., late infantile MLD, no. 34897, age 2 yr, tissue stored 0.5 yr; R. E., late infantile, no. 25021, age 3.5 yr, tissue stored 9.5 yr; L. B., juvenile, no. X-556, age 18 yr, tissue stored 4 yr; D. C., late infantile, Kennedy Memorial Hospital, age 3 yr, tissue stored 5 yr.

Quantitative determination of liver sulfatide levels

These determinations were performed as described by Moser et al. (13).

Preparation of lipid extract

The following methods were used for large-scale isolation of sulfatides for structure determination (infrared and permethylation studies). For smaller-scale fatty acid and long-chain base composition studies the methods were suitably modified (reduced column sizes, elution volumes, etc.). The frozen sample of liver (patient R. E., 45 g) was thawed, homogenized in a Waring blender with 2 vol of water, and lyophilized. The dry tissue was extracted with 20 vol of chloroform–methanol 2:1 (v/v) at room temperature. The extract was filtered, and the residue on the filter paper was reextracted with one-fifth the original volume of chloroform–methanol 2:1 (v/v) and refiltered. The combined filtrates were evaporated to dryness in vacuo at 40°C. About 3 g of total lipids was obtained. The dry weight of the liver residue was about 12 g.

The total lipids were subjected to mild alkaline hydrolysis to remove glycerolipids by the method of Rapport and Lerner (15), modified as follows. Methanolysis was performed in a mixture of 1 N methanolic sodium hydroxide and chloroform 1:2 (v/v) at 37°C for 1 hr. After acidification to pH 4 with 1 N aqueous HCl, the lipids were extracted from the hydrolyzate by adding 4 vol of chloroform–methanol 2:1 (v/v), shaking vigorously for 2 min, and centrifuging. After discarding the upper phase, the lower phase was dialyzed against distilled water at 4°C for 20 hr to remove traces of inorganic contaminants. The solvents were evaporated and the dried lipids were subjected to column chromatography.

Fractionation by column chromatography

The lyophilized material (400 mg) was dissolved in a minimal amount of chloroform–methanol 2:1 (v/v) and then applied to a column (2.5 × 60 cm, equilibrated with chloroform) packed with 50 g of activated Florisil (60–100 mesh, preheated at 100°C for 6 hr; Floridin Co., Pittsburgh, Pa.). The column was eluted successively with (*a*) 300 ml of chloroform, (*b*) 500 ml of chloroform–methanol 9:1 (v/v), (*c*) 500 ml of chloroform–methanol 4:1 (v/v), (*d*) 1000 ml of chloroform–methanol 7:3 (v/v), (*e*) 1000 ml of chloroform–methanol 3:2 (v/v), and (*f*) 500 ml of chloroform–methanol 1:1 (v/v). The effluents were collected in 10-ml fractions.

This method is modified from Hori, Itasaka, and Kamimura (16); the modification results in sulfatides being eluted in solvents *d* and *e*, together with slight amounts of trihexosyl ceramide and globoside. The presence of sulfatides in the effluent from these solvents was monitored by TLC of aliquots of the fractions on silica gel G in the solvent system described below (chloroform–methanol–water 24:7:1 [v/v/v]), in which galactosyl sulfatide has greater mobility than lactosyl sulfatide.

Isolation of sulfatides by preparative thin-layer chromatography

The fractions containing sulfatides were dried in vacuo and subjected to preparative TLC on glass plates (20 × 20 cm) coated with silica gel G (thickness 0.25 mm, Analtech, Inc., Newark, Del.). Chloroform–methanol–water 24:7:1 (v/v/v) was used as the solvent system. The lipids were made visible by exposure to iodine vapor. The gel containing each sulfatide was scraped from the plates and packed into separate columns (1 × 10 cm), and sulfatides were recovered from the silica gel by elution with 150 ml of chloroform–methanol–water 100:50:7.5 (v/v/v). The eluate was evaporated to dryness in vacuo. Preparative TLC was repeated on the residue, using *n*-propanol–water–15 M NH_4OH 6:2:1 (v/v/v) as solvent system, for separation of the sulfatides from any residual glycolipid contaminants (modified from Ref. 13). Standard trihexosyl ceramide (galactosyl-galactosyl-glucosyl ceramide) and globoside were gifts from Dr. R. Brady. Sulfatides were recovered from the silica gel plates as previously described.

Detection of glycolipids was occasionally provided by use of Molisch's reagent, consisting of 2% α-naphthol (w/v) in methanol–water 1:1 (v/v) and overspraying with 95% H_2SO_4.

Infrared analyses

Infrared spectra of samples (1 mg each) were taken from KBr disks with a Beckman infrared spectrophotometer, model IR-33.

Fatty acid analyses

The samples (1–2 mg) were methanolyzed in sealed tubes by heating at 80°C for 18 hr in 1 ml of 1 N methanolic HCl. The methyl esters of fatty acids were extracted three times with 1 ml of *n*-hexane. After evaporation, the residue was transferred to a 3 mm × 5 cm column containing Unisil (100–200 mesh, Clarkson Chemical Co., Williamsport, Pa.) equilibrated with the first solvent, and the hydroxy fatty acid (HFA) methyl esters were separated from the nonhydroxy fatty acid (NFA) methyl esters employing the method of Kishimoto and Hoshi (17). Elution was performed with (*a*) hexane–benzene 8:2 (v/v), (*b*) hexane–benzene 6:4 (v/v), and (*c*) benzene; the fraction eluted with solvent *b* contained only NFA methyl es-

ters and that with solvent *c* only HFA methyl esters. The HFA esters were trimethylsilylated with hexamethyldisilazane and trimethylchlorosilane in pyridine (17) before GLC. The methyl esters were then applied to a Hewlett-Packard 7624A flame ionization gas chromatograph, using 25% DEGS coated on 80–100 mesh Chromosorb W (high performance AW-DMCS, Applied Science Laboratories, Inc., State College, Pa.) in a 3.5 mm × 2 m column at 180°C with helium as the carrier gas. For qualitative analysis of fatty acid composition, NFA methyl ester mixtures KD and KF (Applied Science) and an HFA mixture obtained from bovine cerebroside (18) (Supelco, Inc., Bellefonte, Pa.) were used. For quantitative analysis, $C_{19:0}$ and $C_{19h:0}$ (gifts from Dr. Y. Kishimoto) were employed as internal standards.

Analyses of hexose components

After removal of the fatty acid methyl esters from the methanolysis mixture, the acidic methanol solution was treated with enough AG I-X8 resin (OH^- form, Bio-Rad Lab., Richmond, Calif.) to neutralize the solution. Methanol was removed from this solution under a stream of nitrogen, and the trimethylsilyl derivatives of methylglycosides were prepared from the dry residue as described above for HFA methyl esters. The reaction mixture was injected onto a column containing 3% OV-1 coated on 80–100 mesh Chromosorb W (high performance AW-DMCS, Applied Science) at 160°C. Hexose components were identified by reference to hexose standards subjected to the same procedure.

Long-chain base analyses

Sulfatides (1–2 mg) were methanolyzed in sealed tubes according to the method of Gaver and Sweeley (19). After extraction of fatty acid methyl esters with equal volumes of *n*-hexane, the hydrolyzate was adjusted to pH 11.0 with 1 N NaOH, the long-chain bases were extracted by adding 4 vol of diethyl ether, and the dried residue was oxidized with lead tetraacetate as described by Karlsson and Mårtensson (20). The aldehydes obtained were extracted with *n*-hexane and analyzed by GLC at 150°C using the same type of DEGS column as was used for analysis of fatty acid composition. Calibration of the column was achieved using aldehydes from the following sources: $C_{16:1}$ from sphingosine and $C_{16:0}$ from dihydrosphingosine (Supelco); $C_{15:0}$ from phytosphingosine (yeast sphingolipid, a gift from Dr. M. Hoshi); $C_{16:0}$ (K & K Laboratories, Inc., Plainview, N.Y.); and $C_{16:1}$ and $C_{18:1}$ from mixed human brain ganglioside (gift from Dr. R. McCluer).

Permethylation

Sulfatides (about 5 mg) were subjected to permethylation with methyl iodide in the presence of methylsulphinyl carbanions, as described by Hakomori (21), followed by methanolysis as described above for fatty acids. The methylated methyl hexosides were identified by GLC using 3% ECNSS-M coated on 100–120 mesh Gas-Chrom Q (Applied Science) at 140°C, employing as standards methylated hexoses derived from hematosides of beef adrenal gland (gift from Dr. R. McCluer).

Desulfation of sulfatide and methylation of the resulting neutral glycolipids

Sulfatides (5 mg) were dissolved in 2 ml of 0.05 N methanolic HCl and then hydrolyzed for 4 hr at room temperature (22). After drying in vacuo, resulting neutral glycolipid was permethylated as described above. The methylated methyl hexosides obtained after subsequent hydrolysis in methanolic HCl as described previously were identified by GLC on Gas-Chrom Q coated with 3% ECNSS-M, as above. The authentic standards methyl α- and β-2,3,4,6-tetra-*O*-methylgalactoside and methyl α- and β-2,3,6-tri-*O*-methylglucoside were obtained from galactosyl ceramide (Supelco) and from authentic lactosyl ceramide (gift from Dr. R. Brady).

RESULTS

Identification and structure of sulfatides

As the sulfatides were difficult to separate completely from alkali-stable glycolipids on the Florisil column, further purification was achieved by preparative TLC. The sulfatides isolated by this technique were purified by crystallization from hot methanol. The resulting products, galactosyl sulfatide (15 mg) and lactosyl sulfatide (10 mg), each gave two closely overlapping spots in both TLC systems (see Materials and Methods), due to the presence of both hydroxy and nonhydroxy fatty acids in each sulfatide. Galactosyl sulfatide had a hexose content of 20.2% (theoretical value 20.6%, based on an assumed composition of $C_{18:1}$ long-chain base and $C_{23:0}$ acid) and lactosyl sulfatide had a 33.5% hexose content (theoretical value 34.7%). Both reacted on thin-layer plates with Molisch's reagent and were distinguishable by TLC from other glycolipids; also, the desulfated galactosyl sulfatide and lactosyl sulfatide had R_F values corresponding to galactosyl ceramide and lactosyl ceramide, respectively.

The infrared spectra of the two sulfatides showed typical amide peaks at 1650 and 1550 cm^{-1} and showed also the characteristic absorption for sulfate at 1250 cm^{-1}. The absorption at 3450 and 1060 cm^{-1} (hydroxyl) was stronger in the case of lactosyl sulfatide, presumably because of greater hexose content (Fig. 1).

The hexose component in each sulfatide was determined by GLC of trimethylsilyl derivatives of methyl hexosides. The results, shown in Fig. 2, indicate that galactosyl sulfatide contained only galactose as the hexose component,

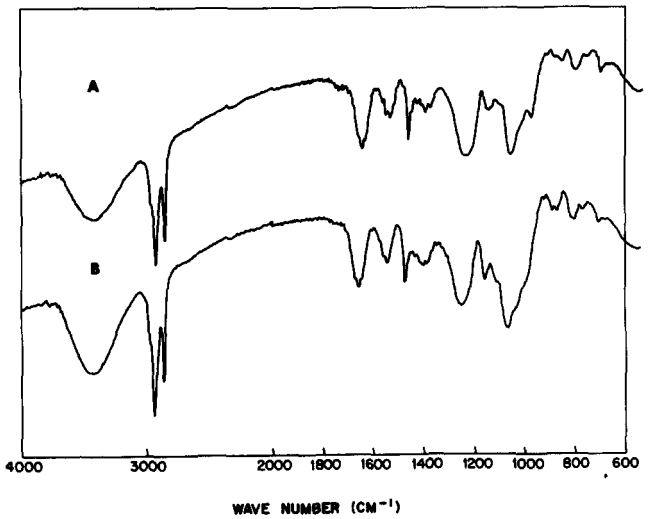

Fig. 1. Infrared spectra of liver sulfatides. See text for details. *A*, galactosyl sulfatide; *B*, lactosyl sulfatide.

while lactosyl sulfatide contained glucose and galactose in a 1:1 molar ratio.

The permethylated sulfatides were methanolyzed, and the methylated sugars were determined by GLC. As shown in Fig. 3*A*, two peaks were obtained from galactosyl sulfatide. Peak 1 corresponded to methyl β-2,4,6-tri-*O*-methylgalactoside and peak 2 corresponded to methyl α-2,4,6-tri-*O*-methylgalactoside (23). Lactosyl sulfatide, as shown in Fig. 3*B*, gave peak 1 corresponding to methyl β-2,3,6-tri-*O*-methylglucoside, peak 2 corresponding to methyl β-2,4,6-tri-*O*-methylgalactoside, and peak 3 corresponding to a mixture of the α anomers of these derivatives.

Fig. 4 indicates that after methylation and methanolysis of desulfated lactosyl sulfatide, peak 1 corresponding to methyl α,β-2,3,4,6-tetra-*O*-methylgalactoside, peak 2 corresponding to methyl β-2,3,6-tri-*O*-methylglucoside, and peak 3 corresponding to the α anomer of the latter glycoside were obtained.

Thus, the data obtained from examination of the methylated, methanolyzed derivatives of the sulfated and desulfated galactosyl and lactosyl sulfatides require (*a*) that galactosyl sulfatide contain galactose linked to ceramide at C-1 and sulfated at C-3; (*b*) that lactosyl sulfatide contain equimolar amounts of glucose and galactose; (*c*) that this glucose be substituted at C-1 and C-4 and galactose at C-1 and C-3; and (*d*) that the galactose be sulfated at the C-3

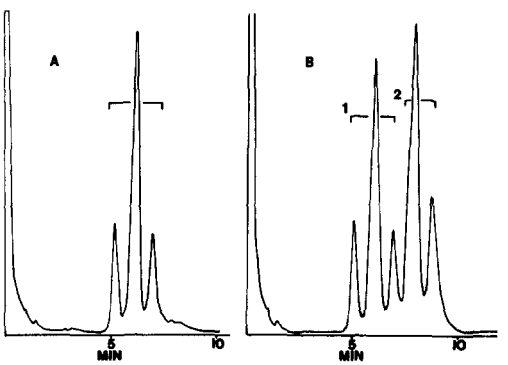

Fig. 2. GLC of the trimethylsilyl derivatives of methyl hexosides obtained from methanolysis of liver sulfatides. See text for details. *A*, galactose from galactosyl sulfatide; *B*, galactose (*1*) and glucose (*2*) from lactosyl sulfatide.

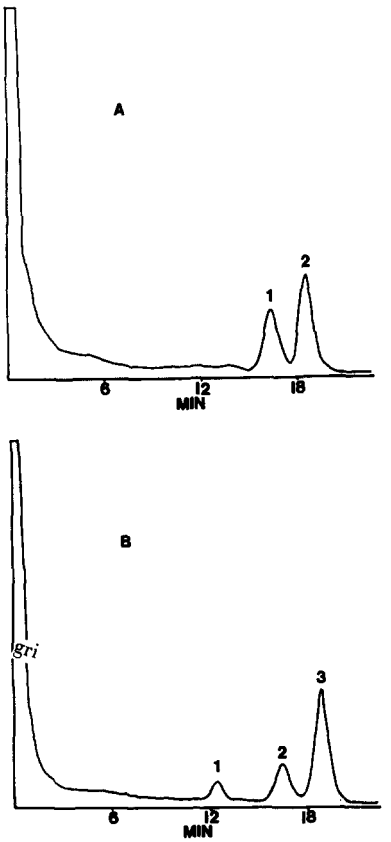

Fig. 3. GLC of methylated hexosides obtained from permethylation and methanolysis of liver sulfatides. See text for details. *A:* (*1*) methyl β-2,4,6-tri-*O*-methylgalactoside and (*2*) methyl α-2,4,6-tri-*O*-methylgalactoside from galactosyl sulfatide. *B:* (*1*) methyl β-2,3,6-tri-*O*-methylglucoside, (*2*) methyl β-2,4,6-tri-*O*-methylgalactoside, and (*3*) α anomers of (*1*) and (*2*) from lactosyl sulfatide.

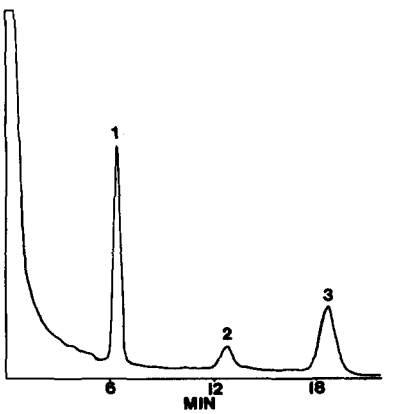

Fig. 4. GLC of methylated hexosides obtained after permethylation and methanolysis of desulfated liver lactosyl sulfatide. See text for details. *1*, methyl α- and β-2,3,4,6-tetra-*O*-methylgalactoside; *2*, methyl β-2,3,6-tri-*O*-methylglucoside; *3*, methyl α-2,3,6-tri-*O*-methylglucoside.

TABLE 1. Sulfatides in MLD liver[a]

	J.S.	D.C.	R.E.	L.B.	Controls (2)
Galactosyl sulfatide	300.1	324.3	256.6	238.4	ND[b] (<20)
Lactosyl sulfatide	180.4	276.5	239.2	136.2	ND (<20)

[a] Expressed as μg/g dry wt.
[b] Not detectable.

position. These findings for lactosyl sulfatide are most readily explained by assigning the same structure as that determined for kidney lactosyl sulfatide, ($SO_3H \rightarrow$ 3)Gal(1 → 4)Glc-ceramide (24).

Confirmation of the structure suggested here for galactosyl sulfatide was provided by the finding that the methylated hexose derived from this sulfatide had a mobility identical with that of 2,4,6-tri-*O*-methylgalactose standard on silica gel G using acetone–water–15 M NH_4OH 250:3:1.5 (v/v/v) as solvent. (This experiment was kindly performed by Drs. Pierre and Anne Stoffyn according to Ref. 24.) Thus, the structure is the same as that found for brain (25) and kidney (7) galactosyl sulfatide, ($SO_3H \rightarrow$ 3)Gal-ceramide.

Sulfatide levels and fatty acid compositions

Table 1 shows the data obtained after quantitative determination of the levels of galactosyl and lactosyl sulfatides in the MLD livers studied as well as in two control cases (from Table 2 of Ref. 13, where these data were summarized in preliminary form). The fatty acid compositions of these sulfatides are presented in Tables 2 and 3. Each sulfatide contained both NFA and HFA; the longer-chain fatty acids ($C_{22:0}$ and above) constituted more than 70% of the total fatty acids. The two sulfatides had similar distributions of NFA, but galactosyl sulfatide contained more $C_{23h:0}$ and $C_{24h:0}$ than did lactosyl sulfatide. Both sulfatides contained more HFA than NFA, as shown in Table 4.

TABLE 2. Nonhydroxy fatty acid compositions of liver sulfatides[a]

Fatty Acid[b]	Galactosyl Sulfatide			Lactosyl Sulfatide		
	J.S.	D.C.	R.E.	J.S.	D.C.	R.E.
$C_{16:0}$	6.6	2.2	4.1	2.0	1.1	1.2
$C_{16:1}$	3.4			3.1		
$C_{18:0}$	14.4	6.9	7.8	6.5	4.5	6.5
$C_{18:1}$	1.6	1.7	4.3	1.2	0.9	2.8
$C_{20:0}$	4.3	4.1	4.9	7.8	7.3	8.7
$C_{21:0}$	0.3	0.4	1.0	0.9	1.0	0.9
$C_{21:1}$	0.1					
$C_{22:0}$	16.0	26.5	24.8	30.0	33.4	35.0
$C_{23:0}$	8.9	17.7	16.5	11.8	13.7	12.4
$C_{23:1}$				0.4	0.5	
$C_{24:0}$	17.6	30.6	24.4	23.7	27.4	22.1
$C_{24:1}$	26.6	10.7	12.3	12.6	10.0	10.6

[a] Expressed as percentage of total NFA in each sulfatide.
[b] Number of carbon atoms: number of double bonds.

TABLE 3. Hydroxy fatty acid compositions of liver sulfatides[a]

Fatty Acid[b]	Galactosyl Sulfatide			Lactosyl Sulfatide		
	J.S.	D.C.	R.E.	J.S.	D.C.	R.E.
$C_{16h:0}$	6.3	3.5	3.4	4.5	4.5	10.1
$C_{16h:1}$	1.0			0.4		
$C_{18h:0}$	1.5	0.9	1.3	4.5	5.2	3.4
$C_{18h:0}$				0.2	0.3	
$C_{20h:0}$	0.8	1.4	0.6	7.0	7.5	1.5
$C_{20h:1}$	1.6		0.9			4.0
$C_{21h:0}$		0.4	0.4		1.2	1.1
$C_{22h:0}$	20.0	21.0	21.5	22.9	27.2	20.1
$C_{22h:1}$				1.0		
$C_{23h:0}$	20.9	28.8	28.8	15.6	15.7	18.2
$C_{23h:1}$	0.6			0.8	0.8	
$C_{24h:0}$	30.3	38.3	33.7	25.4	25.4	24.1
$C_{24h:1}$	15.6	5.7	9.3	16.6	12.2	17.5

[a] Expressed as percentage of total HFA in each sulfatide.
[b] Number of carbon atoms: number of double bonds.

Long-chain base compositions

The long-chain base compositions of both sulfatides are presented in Table 5. Each sulfatide consisted mainly of sphingosine, with lesser amounts of dihydrosphingosine and phytosphingosine. The presence of phytosphingosine in MLD liver sulfatide was confirmed by preparation of its dinitrophenyl derivative and comparison on TLC with the dinitrophenyl derivative of authentic phytosphingosine (20). Phytosphingosine was present in higher concentration in galactosyl than in lactosyl sulfatide.

DISCUSSION

The studies presented here show that MLD liver contains an excess of galactosyl and lactosyl sulfatides and that the structures of these compounds correspond to those already determined for galactosyl sulfatide in kidney (7) and brain (4, 6, 7, 25) and for lactosyl sulfatide in kidney (22, 26). The absolute level of sulfatide in MLD liver is only about one-third that in normal kidney and about one-thirtieth that in MLD kidney (7); although normal kidney contains an appreciable amount of sulfatide (7, 26), using current methodology we have not been able to demonstrate sulfatide in normal liver (13). Compared with normal, the relative excess of sulfatide in MLD liver may thus be equal to or greater than that in brain or kidney.

TABLE 4. Percentage distributions of nonhydroxy and hydroxy fatty acids of sulfatides isolated from MLD liver[a]

	Galactosyl Sulfatide			Lactosyl Sulfatide		
	J.S.	D.C.	R.E.	J.S.	D.C.	R.E.
NFA	47.0	39.6	35.9	35.9	34.6	33.5
HFA	53.0	60.4	64.1	64.1	65.4	66.5

[a] Expressed as percentage of total sulfatide.

TABLE 5. Long-chain base compositions of MLD liver sulfatides[a]

Aldehyde	Probable Parent Base[b]	Galactosyl Sulfatide			Lactosyl Sulfatide		
		J.S.	D.C.	L.B.	J.S.	D.C.	L.B.
$C_{14:0}$	C_{16}-SH_2	1.2	1.4	5.2	0.4	tr	12.0
$C_{14:1}$	C_{16}-S	2.1	3.8	2.0	2.7	0.9	4.0
$C_{15:0}$	C_{18}-P	4.8	4.7	10.0	2.4	0.8	9.3
$C_{15:1}$	C_{17}-S	3.1	2.9	2.5	1.8	2.7	2.4
$C_{16:0}$	C_{18}-SH_2	6.2	4.5	19.2	4.1	3.2	12.6
$C_{16:1}$	C_{18}-S	69.4	69.9	47.9	74.7	72.9	50.4
$C_{17:0}$	C_{20}-P			tr	0.2		tr
$C_{17:1}$	C_{19}-S	tr	tr	tr	0.3	tr	
$C_{18:0}$	C_{20}-SH_2	2.1	1.5	tr	tr	tr	tr
$C_{18:1}$	C_{20}-S	2.3	2.8	3.4	3.3	1.3	1.2
Unidentified	Unknown	8.8	8.5	9.8	10.1	18.2	8.1

[a] Expressed as percentage of total long-chain base in each sulfatide.
[b] S, sphingosine; SH_2, dihydrosphingosine; P, phytosphingosine.

MLD liver contains similar amounts of galactosyl and lactosyl sulfatides. Lactosyl sulfatide was first demonstrated in normal kidney and amounts to between one-third and one-half the amount of galactosyl sulfatide (26); to our knowledge lactosyl sulfatide has not been reported to be present in brain or peripheral nerve. In MLD kidney, lactosyl sulfatide has been reported to be about one-fourth the level of galactosyl sulfatide (5); in MLD liver, the ratio of lactosyl to galactosyl sulfatide is higher than in any site reported so far.

Compositional studies have shown consistent differences between brain and kidney sulfatides. In the first place, the long-chain bases of normal urinary and kidney sulfatides contain up to 10% phytosphingosine (10), whereas this hydroxy long-chain base is said to be practically absent from animal brain (20) and is present in only trace amounts in human brain.[2] The phytosphingosine in liver and kidney probably is of dietary origin, since Assmann and Stoffel (27) have shown that phytosphingosine administered orally or intravenously is incorporated to a significant extent into the sphingolipids of liver and kidney, but hardly at all into those of brain. Secondly, both normal and MLD kidney sulfatides contain much greater amounts of $C_{22:0}$ and, in general, somewhat smaller proportions of $C_{24:0}$ and $C_{24:1}$ and longer-chain fatty acids than do the normal and MLD brain and peripheral nerve sulfatides (5, 7–10, 26–30). Longer-chain HFA normally occur in high concentration in animal brain, and to a much smaller extent in kidney and skin, but are absent from liver (31). It is of interest then that MLD liver galactosyl and lactosyl sulfatides contain a high proportion of HFA, equivalent to that in MLD kidney (5, 7) and higher than that in normal kidney (5) or in MLD brain (7), though not as high as in peripheral nerve (8).

Thus, using three criteria, the proportion of lactosyl to galactosyl sulfatide, the percentage of phytosphingosine base, and the fatty acid composition, the sulfatides in MLD liver resemble those in normal or MLD kidney and differ from those in normal or MLD brain or peripheral nerves.

These structural properties of sulfatides make it highly unlikely that the sulfatides in MLD liver were transported to that organ from the brain. It is established that sulfatide accumulation in MLD is due to a genetically determined severe diminution of cerebroside sulfatase in a wide variety of tissues (32–34). The degradative defect in MLD liver in a sense may "freeze" the sulfatides synthesized in this organ and thus facilitate the demonstration of a compound that normally is present in very low concentration. It should be noted that sulfatide synthesis has been demonstrated in nonneural tissues such as rat and mouse kidney (35, 36); however, this point has not been specifically examined in human liver. We have already referred to the fact that long-chain HFA have not been found in normal mammalian liver. The observation that MLD liver sulfatides contain a high proportion of long-chain HFA suggests either that the liver is nevertheless capable of synthesizing these compounds or that they were transported to it from other sites and then incorporated into sulfatides by analogy with what has been proposed (27) in the case of phytosphingosine.

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