Human urinary sulfatides in patients with sulfatidosis (metachromatic leukodystrophy)

MICHEL PHILIPPART, LOUIS SARLIEVE,* COLETTE MEURANT, and LASZLO MECHLER

Mental Retardation Research Center, The Neuropsychiatric Institute, Los Angeles, California 90024; and Departments of Pediatrics, Neurology, and Psychiatry, University of California, Los Angeles, California 90024

ABSTRACT The excretion of sulfatides in human urine was studied. 24-hr urine collections were filtered. Urinary glycolipids were extracted from the filter paper and fractionated on diethylaminoethyl cellulose and silicic acid columns, and by thin-layer chromatography. Fatty acids and long-chain bases were analyzed by gas–liquid chromatography of the corresponding esters and aldehydes. Glycosyl ceramide concentration was determined by gas–liquid chromatography of the trimethylsilyl ethers of the methyl glycosides.

Normal females were found to excrete larger amounts of dihexosyl ceramides than males. Sulfatides were detected in all urine specimens. In sulfatidosis, a hereditary sulfatide storage disorder known as metachromatic leukodystrophy, a large increase in sulfatide was readily apparent on a thin-layer chromatogram of the crude lipid extract. On comparing samples from normal individuals and patients with sulfatidosis, urinary sulfatide composition was remarkably similar to that previously reported in the kidney, including differences in fatty acid pattern. The determination of urinary sulfatides was a valuable confirmation of the deficiency in arylsulfatase A activity characteristic of sulfatidosis.

SUPPLEMENTARY KEY WORDS arylsulfatase A · neutral glycosyl ceramides · sphingosine · fatty acid · sexual differences · thin-layer chromatography · gas–liquid chromatography

Austin and his colleagues (1, 2) have provided evidence for a specific deficiency of ASA in the urine, brain, and organs of patients with sulfatidosis. The increased output of urinary sulfatides has been the basis for the development of two clinical tests: the search for metachromatic granules in the urinary sediment (3) and the fluff method, based on the precipitation of sulfatides at the interphase of a lipid extract equilibrated with water (4). Few precise determinations of urinary sulfatides have been reported (5) and the clinical use of such an approach has been debated (6). While the compositions of brain (7–10) and kidney (11–13) sulfatides had been established in normal individuals and in patients with sulfatidosis, we did not possess such data for urinary sulfatides. A possible correlation between sulfatide excretion and the stage of the disease (4) was not confirmed (6). The increased excretion of another glycolipid, dihexosyl sulfatide, is characteristic of sulfatidosis (5, 6, 14). Normal levels of urinary sulfatides are low; precise qualitative and quantitative analysis requires an elaborate fractionation of the many components found in the crude lipid extract (5). The present study was undertaken in order to establish the concentration and composition of urinary sulfatides in normal individuals and in patients with sulfatidosis.

MATERIALS AND METHODS

16 patients from 13 unrelated families were studied. Most patients were first seen in the early stage of the disease, which was generally suspected on finding metachromatic material in the urinary sediment (3). Nonbiochemical methods used to confirm the diagnosis included peripheral nerve conduction time (15). Three children (PN, DLo, and MaB), each of whom had an affected sibling, were still apparently healthy when first

Abbreviations: ASA, arylsulfatase A; C–M, chloroform–methanol; C–M–W, chloroform–methanol–water; DEAE, diethylaminoethyl; $C_{m:n}$, m number of carbon atoms:n number of double bonds; h, α-hydroxy fatty acid; LCB, long-chain bases; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

* Research Fellow of the INSERM, Paris, France. Present address: Centre de Neurochimie, Strasbourg, France.

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examined. In three patients (RLa, HV, and GB) the disease had been manifest for 2 yr or more.

Urine Collection

Routine urinalysis was first performed in order to exclude bacteriuria or pyuria. 24-hr samples of urine were collected as completely as possible and kept at 4°C during collection. The pooled specimens were filtered on Whatman No. 1 filter paper, previously extracted with C–M 2:1. The collection flask was carefully rinsed with distilled water to resuspend any sediment sticking to the walls. By comparing lipid extraction from the dry residue obtained by lyophilization of the urine before or after filtration, it was formerly shown that the bulk of urinary glycolipids (90% or more) is retained on the filter paper (16). This filtration procedure thus provided a convenient and efficient method to collect urinary glycolipids. For enzyme determinations (17) filtrated urine was dialyzed overnight against tap water, or the proteins were precipitated with $(NH_4)_2SO_4$ (18).

Arylsulfatase A Determination

Enzyme activity, using *p*-nitrocatechol sulfate as substrate (Sigma Chemical Co., St. Louis, Mo.) (18), and total protein (19) were measured in the dialyzed urine and the $(NH_4)_2SO_4$ precipitate. In five families with a case of sulfatidosis, parents and siblings were also examined. Controls were run on children hospitalized with neurological disorders and on healthy individuals aged 2–15 yr.

Extraction of Urinary Lipids

All solvents were of reagent grade and were redistilled prior to use. After drying at room temperature for a few hours, the lipid-impregnated filter papers were extracted with C–M 2:1 as previously described (16). Aliquots of the crude lipid extract were examined by TLC, using plates of Silica Gel G or HR (Merck), 250 μ thick, developed in C–M–W 50:21:3 or C–M–ammonium hydroxide 60:35:8. Pure cerebrosides and sulfatides prepared from human brain white matter (20) were used as comparison standards.

Glycolipids appeared as bluish (glucosyl ceramide) or purple spots on a white background after lightly spraying with 0.2% anthrone in concentrated sulfuric acid and heating at 120°C for 10 min. The coloration was stable for several hours if a glass cover plate was applied on top of the chromatogram to protect it from moisture; if exposed to moisture, the spots turned green and faded. Deliberate exposure of the chromatogram to water vapor caused glycolipids containing nonhydroxy fatty acids to quickly turn green, while glycolipids containing hydroxy acids remained purple. The crude lipid extract was fractionated into neutral and acidic glycolipids in DEAE cellulose (21). The neutral glycolipids were quantitated by combined TLC and GLC (22). The acidic lipids were dried, redissolved in 20 ml of C–M 2:1, and washed with 4 ml of water to extract ammonium acetate remaining from the DEAE eluate; small amounts of sulfatides soluble in the aqueous upper phase were recovered by extraction with 200 ml of chloroform. The crude acidic lipids were fractionated on 10 g of silicic acid (325 mesh, Sigma Chemical Co.) packed in a 12 mm I.D. column. Contaminating unidentified components, which were free from carbohydrate on hydrolysis, were first eluted with 100 ml of chloroform and 200 ml of C–M 95:5; monohexosyl and dihexosyl sulfatides were then obtained by elution with C–M 4:1. Cerebrosides, which could arise from desulfation of sulfatides, would appear in the C–M 95:5 eluate but were never observed in that fraction.

Glycolipids obtained under these conditions were fairly pure, as judged by the hexose content and by the absence of phosphorus (20). However, after hydrolysis some of the early control samples were found to contain large amounts of C_{16} and C_{18} fatty acids, which is unusual for glycosphingolipids. Mild saponification (23) revealed an impurity that remained in the sample. Thereafter, mild saponification was introduced as an obligatory purification step. Finally, monohexosyl and dihexosyl sulfatides were separated by preparative TLC, using C–M–W 50:21:3 as a solvent; they were visualized by spraying the plates with 0.0024% rhodamine G in water. The appropriate fluorescent bands were collected and the glycolipid was eluted from the gel with C–M–W 8:4:1.

To evaluate preparative losses, quadruplicate aliquots of some original crude lipid extracts were separated using a two-dimensional TLC system (24), with added phosphatidylethanolamine and lecithin to help identify the sulfatide area. GLC (25) of the sulfatide hexose determined under these conditions agreed with the amount of purified sulfatides with the 10% experimental error; losses in the same range have been found in the determination of urinary neutral glycolipids (22).

Sulfatide Composition

Purified sulfatides were methanolyzed for 24 hr at 75°C. Fatty acid methyl esters were extracted with hexane. The hydroxy fatty acid esters were separated from the nonhydroxy esters on small Florisil (Floridin Co., Tallahassee, Fla.) columns (20, 26). Purity of the fatty acid fractions was checked by TLC and successive treatment of the plate with rhodamine 6G, iodine, and sulfuric acid carbonization (27). Hydroxy fatty acids were etherified (26) prior to GLC analysis. After extraction of the methyl esters, the methanolysis mixture was

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dried under nitrogen and dissolved in 0.5 ml of 5 N sodium hydroxide and 1.5 ml of water. The LCB was recovered by extraction with three 4-ml volumes of ether (28). An aliquot of LCB was examined by qualitative TLC of the ether extract (29). Remaining LCB was submitted to periodate oxidation; the resulting aldehydes were freed of contaminants on small silicic acid columns and immediately analyzed by GLC (30).

After extraction of LCB, the aqueous residue was neutralized with 1 N HCl and lyophilized. Methyl glycosides were extracted with hot pyridine while crushing the salt residue. Sorbitol was added as an internal standard and trimethylsilyl derivatives were prepared (25, 31).

GLC of fatty acids was performed on Gas-chrom P containing 15% diethylene glycol succinate (Applied Science Laboratories Inc., State College, Pa.). A Barber-Colman Model 5000 gas chromatograph (Barber-Colman Co., Rockford, Ill.) with 6 ft, U-shaped columns and flame ionization detector was used isothermally at 210°C for the nonhydroxy fatty acids and at 220°C for the hydroxy ethers. Peaks were identified as previously described (7).

Methyl glycosides were analyzed on Chromosorb W, containing 3% SE-30, isothermally at 160°C. Sorbitol gave an area greater by a factor of 1.25 than the same weight of galactose or glucose. Hexose recovery was monitored by comparing GLC values with anthrone and orcinol determinations on the pure glycolipids (7). Optimal conditions for methanolysis were established by analysis of mixtures of pure glucosyl ceramides with galactose and sorbitol, lactose with sorbitol, and pure brain sulfatides with glucose and sorbitol.

RESULTS

Arylsulfatase A Activity

All patients with sulfatidosis had a sharply reduced enzyme activity compared with the normal children ($P < 0.005$) (Table 1). Among the relatives, five mothers and three siblings had low activity. This decreased the mean of that group, which was too small to be subdivided. Wide variations were found in children hospitalized with neurological disorders, although only one patient with Guillain-Barré syndrome actually fell within the range of sulfatidosis. Since random samples tended to give much wider variation, only 24-hr collections were taken into account. Activity found in the protein precipitate agreed fairly well with values obtained on freshly dialyzed urine. There was no apparent correlation between the age of onset or the duration of the disease and the amount of residual enzymatic activity.

Semiquantitative Estimation of Sulfatide Excretion

TLC examination of the crude urinary lipid extract with a concurrent standard of pure sulfatides was very useful in establishing the diagnosis of sulfatidosis. Among our 16 patients, 14 presented a definitely abnormal sulfatide excretion. Two patients did not excrete a strikingly elevated amount of sulfatide. However, the detection of dihexosyl sulfatide, which was excreted simultaneously, allowed us to distinguish these from normal controls. There was no obvious correlation between the age of onset or the duration of the disease and the sulfatide output. However, one of the two patients who had no definite increase of sulfatide when first examined excreted much more 1 yr later; the other patient could not be reexamined. Three siblings with normal arylsulfatase activity had a questionable increase in urinary sulfatides, but no dihexosyl sulfatides. All parents had a normal lipid pattern.

Concentration and Composition of Urinary Sulfatides

While 24-hr collections from patients with sulfatidosis allowed isolation of quantities of sulfatides in the milligram range, 10–30 liters of pooled urine were needed to adequately study the controls. Total lipids were expressed as mg per 100 ml of urine, since excretion expressed in these units did not appear to be significantly influenced by the age of the donor (Table 2). Excessive variations due to large fluctuations in daily urinary output (and possible losses in some of our bed-ridden patients) were thus canceled. Only two patients had definitely increased total lipid concentration (MR, ML), this being due mostly to an increase in triglycerides as shown

TABLE 1 Arylsulfatase A Activity in Urine from Patients with Sulfatidosis

	Number of Males	Number of Females	Age Range	Mean ± SD	Range
			yr	*µg p-nitrocatechol/hr/mg protein*	
Sulfatidosis	8	8	1–17	2.0 ± 2.3	0–8.6
Relatives of patients	8	6	5–50	37.0 ± 23.0	14.7–89.3
Normal children	7	7	2–15	50.1 ± 21.3	25.4–80.8
Hospitalized children	5	8	2–15	64.1 ± 57.4	5.5–201.0

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TABLE 2 Urinary Lipids and Sulfatides from Patients with Sulfatidosis and Normal Individuals

Donor	Age	Total Lipid	Sulfatide
	yr	*mg/100 ml urine*	*nmoles/100 ml urine*
Sulfatidosis			
Males			
AN	2	6.2	674
AL	2	3.9	388
MR	3	16.6	408
HV*	12	9.4	589
GB†	14	2.9	223
GB, after 2-yr interval	16	4.1	251
Mean ± SD		7.2 ± 4.7	422 ± 164
Females			
ML	8	20.6	980
RL	11	5.9	412
CC	13	2.6	110
MB†	13	2.0	168
MB, after 2-yr interval	15	4.6	305
Mean ± SD		7.1 ± 6.9	395 ± 311
All patients			
Mean ± SD		7.2 ± 5.8	410 ± 242
Normal			
Males‡			
TD	5	3.5	22
EM	6	2.0	15
LL	32	4.3	13
Females			
CD	11	2.2	9
Heterozygote female*			
MV	50	6.0	44
Normal mean			
5 males, 5 females	1–11	3.2, 1.9	
	12–50	3.8, 1.4	
10 males, 10 females	1–50	4.0, 1.5	

* Mother and son.
† Siblings.
‡ Controls used for sulfatide isolation.

by TLC of the crude extract. Sulfatide excretion was markedly increased in patients with sulfatidosis, with a wider range of values found in females. In the two patients reexamined after a 2-yr interval (GB, MB), similar sulfatide levels were found in the boy, while his sister had a 1.8-fold increase.

Analytical values for purified sulfatides (Table 3) agreed well with the calculated composition. Hexose, as shown by GLC, was at least 95% galactose with occasional traces of glucose, assumed to represent residual dihexosyl sulfatide. Dihexosyl sulfatides were found in normal urine, although their low concentration prevented a thorough characterization of fatty acids and LCB. In the only patient in whom dihexosyl sulfatides were determined, there were 22 nmoles per 100 ml of urine.

Though nonhydroxy fatty acids predominate not only in normal urinary sulfatides but also in brain and kidney sulfatides, this fraction was low in most of our patients with sulfatidosis.

TABLE 3 Composition of Urinary Sulfatides

Donor	Hexose	Long-chain Bases	Fatty Acids	Hydroxy/Nonhydroxy Fatty Acids Ratio
	wt % of pure sulfatide			
Sulfatidosis				
AN	18.6	31.1	43.4	2.3
AL	17.9	35.3	43.4	1.5
MR	22.2	35.6	40.0	1.0
HV	20.4	33.9	39.0	1.2
GB	21.0	N.D.	39.2	0.33
GB, after 2-yr interval	20.8	35.5	40.8	1.3
ML	20.5	29.0	38.9	1.5
RL	20.7	33.0	45.7	1.4
CC	17.5	34.0	44.0	4.0
MB	18.9	N.D.	42.4	0.7
MB, after 2-yr interval	19.6	35.7	39.9	4.0
Heterozygote				
MV	19.0	N.D.	39.6	0.83
Normal				
TD	19.9	34.3	41.6	0.25
EM	20.0	35.4	43.2	0.19
LL	19.8	32.8	40.1	0.86
CD	19.5	29.2	40.8	0.32
Calculated values (mol wt 937)*	19.3	32.2	41.6	0.2–0.4† 0.7‡

N.D., not determined.
* Ammonium salt of sulfatide monohexoside.
† Human brain sulfatide (8).
‡ Human kidney (13, 32).

LCB distribution (Table 4) was similar in patients and in normal controls. Identification was based on the equivalent chain length of the aldehydes derived from periodate oxidation. Identity of unsaturated aldehydes was established by GLC after hydrogenation. Phytosphingosines accounted for about 10% of the bases.

The nonhydroxy fatty acid composition is shown in Table 5. Behenic ($C_{22:0}$) and lignoceric ($C_{24:0}$) acids were predominant. Tricosanoic acid ($C_{23:0}$) was consistently lower, and unsaturated acids, mostly nervonic acid ($C_{24:1}$), were markedly higher than in control children. Similar differences between patients and controls were found in α-hydroxy fatty acid distribution (Table 6). The major acids were $C_{22h:0}$ and $C_{24h:0}$; there was less $C_{23h:0}$ and more $C_{24h:1}$ in the patients than in the controls. $C_{25h:0}$, $C_{25h:1}$, and $C_{26h:0}$ were increased in most patient samples, but they were not detected in control children.

Concentration of Neutral Urinary Glycosyl Ceramides

Glucosyl ceramide was the major neutral glycolipid in human urine (Table 7). In normal females, dihexosyl ceramide was significantly ($P < 0.001$) more abundant than in males. Despite a wide scattering of values, the mean level and range of the different hexosyl ceramides were similar in patients and controls.

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TABLE 4 Long-chain Bases of Urinary Sulfatides

	3-*O*-Methyl-C_{18}-S	C_{18}-S	C_{18}-SH_2	C_{17}-P	C_{18}-P	Not Identi-fied
			% of total LCB			
Sulfatidosis						
AN	23.0	59.0	3.8	2.8	7.6	3.8
AL	18.6	53.6	8.9	1.8	8.7	8.4
MR	23.4	50.8	8.6	0.4	4.3	12.5
VB	21.2	52.8	3.4	3.8	11.9	6.9
GB	22.4	55.4	4.5	0.7	9.4	7.6
GB, after 2-yr interval	19.3	48.5	14.5	3.2	8.2	6.3
ML	22.6	52.0	5.2	1.5	9.6	9.1
RL	24.6	57.0	3.8	0	5.7	8.9
CC	21.0	52.3	4.7	1.4	12.2	8.4
MB	23.0	49.0	8.9	3.5	8.0	7.6
MB, after 2-yr interval	16.8	62.2	6.7	2.0	10.2	2.1
Normal						
TD	9.8	42.8	10.0	2.5	5.4	29.5
EM	5.2	62.3	3.6	3.0	7.0	18.9
LL	14.6	42.5	6.4	0	17.8	18.7

C, carbon chain length; S, sphingosine; SH_2, dihydrosphingosine; P, phytosphingosine.

DISCUSSION

Austin, McAfee, and Shearer (1) found consistently low ASA activity in the urine of patients with sulfatidosis. The low values we observed in a few controls were generally not confirmed on repeating the test. Diurnal variations in excretion or incomplete removal of inhibitors such as phosphate ions might account for these low control values (18). Many of the younger and apparently normal siblings of the patients tended to have no ASA activity in urine or had activity below the mean of other normal children. These findings raised the problem of deciding which of these children would ultimately develop sulfatidosis and which were actually heterozygous for the abnormal gene. It has been suggested (34) that a very low activity could be regarded as the first manifestation of sulfatidosis prior to the development of clinical symptoms. This is indeed what one would expect on a theoretical basis in an inborn error of the metabolism. All present endeavors to diagnose such conditions on fetal amniotic cells rely on that assumption. More work is required, however, in order to document the appearance and variation of enzymatic activity in normal and heterozygous fetuses. Our results in three families with sulfatidosis confirm the predicted value of a low or absent ASA activity at least 1 yr prior to the clinical onset. In view of the occurrence of low enzymatic activity in a few hospitalized patients and in siblings who probably are heterozygous, it appears suitable, besides repeating the test on several occasions as recommended by Austin et al. (1), to substantiate the diagnosis by measuring sulfatide concentration in tissues or urine. In the majority of cases, this is easily accomplished using a semiquantitative test on crude urinary lipids (16).

Depending on the developing solvent mixture for TLC, sulfatides will migrate with either dihexosyl or trihexosyl ceramides. Dihexosyl ceramide concentration may be elevated in a number of circumstances, such as in urine from a female donor, in a specimen containing

TABLE 5 Nonhydroxy Fatty Acid Composition of Urinary Sulfatides from Patients with Sulfatidosis and from Normal Controls

	Urine																Kidney		
	Sulfatidosis												Controls				Sulfatidosis	Controls	
	Males						Females						Male	Male	Female	Male		10 to 17‡	60 to 90§
Fatty Acid	AN	AL	MR	HV*	GB†		ML	RL	CC	MB†		MV*	TD	EM	CD	LL	2‡		
									% of nonhydroxy fatty acids										
16:0	Tr.	3.4	3.9	12.0	2.9	...	6.7	3.9	5.6	4.7	...	...	Tr.	0.9	1.9	2.8	6	7	7.2
16:1	1.3	...	Tr.	6.1	7.0	...	2.0	1.7	1.3	3.6	...	...	Tr.	1.3	Tr.	0.3	..	..	0.1
18:0	1.2	4.1	14.7	2.7	7.9	4.2	10.3	6.3	3.4	6.4	1.8	4.4	8.0	4.6	7.2	1.7	3	4	1.7
18:1	2.0	2.0	2.2	6.7	11.9	...	6.4	5.7	...	...	...	...	1.3	1.3	3.4	...	..	2	0.4
20:0	5.1	5.4	4.9	2.6	7.8	4.6	2.8	5.7	5.6	3.6	4.7	6.4	5.6	4.5	4.8	5.5	6	3	3.9
21:0	2.2	0.7	0.8	0.6	6.7	0.9	8.0	1.3	0.8	2.3	...	0.6	1.8	0.7	0.9	1.4	..	..	0.7
22:0	32.0	28.0	30.0	24.0	23.7	39.0	37.0	18.7	35.4	27.0	48.0	36.4	35.7	33.6	36.7	32.7	40	26	22.9
22:1	Tr.	1.3	Tr.	0.4	...	0.9	...	2.1	1.7	1.9	...	...	...	0.7	0.2	1.6	..	..	1.7
23:0	9.5	9.5	7.8	5.9	7.5	7.0	6.9	8.1	6.3	5.1	8.8	5.7	16.2	19.8	16.7	18.3	8	10	10.0
24:0	32.6	33.0	28.4	24.0	24.5	30.0	19.9	25.6	28.4	30.6	34.7	27.2	30.0	21.2	27.1	27.2	24	24	29.5
24:1	8.9	11.3	5.9	4.7	...	10.0	...	12.2	10.0	7.5	1.9	6.1	1.4	1.2	0.9	3.9	10	16	18.5
25:0	3.4	1.0	0.5	8.9	...	Tr.	...	3.0	0.4	2.7	...	2.0	...	...	...	2.9	..	2	0.8

* Mother and son; MV is an obligatory heterozygote.
† Siblings.
‡ From Mårtensson, Percy, and Svennerholm (32); patient with sulfatidosis was 2 yr old; controls, 10–17 yr.
§ From Mårtensson (33); 60–90 yr old.

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TABLE 6 Hydroxy Fatty Acid Composition of Urinary Sulfatides from Patients with Sulfatidosis and from Normal Controls

Fatty Acid	Urine																Kidney		
	Sulfatidosis												Controls				Sulfatidosis	Controls	
	Males						Females						Male	Male	Female	Male		10 to 17‡	60 to 90§
	AN	AL	MR	HV*	GB†		ML	RL	CC	MB†		MV*	TD	EM	CD	LL	2‡		
	% of total hydroxy fatty acids																		
16h:0	1.7	0.2	1.2	2.3	1.9	1.9	3.7	2.4	2.5	6.3	1.5	1.3	...	...	0.5	0.3	2	2	3.5
17h:0	...	0.1	...	7.7	9.8	...	1.8	...	...	Tr.	0.6	2.9	Tr.	3.0	Tr.	0.5	..	..	0.1
18h:0	1.7	1.4	0.9	1.6	3.4	1.8	1.7	1.6	2.1	1.5	1.8	3.5	Tr.	3.3	3.9	0.8	2	1	1.7
19h:0	...	0.3	...	1.5	1.5	2.3	2.2	0.5	0.5	1.8	...	1.4	...	0.6	...	0.9	..	..	0.1
20h:0	3.7	3.8	2.7	3.9	3.6	3.9	4.0	3.5	6.0	1.6	6.1	6.6	4.2	3.5	3.8	2.4	5	2	3.0
21h:0	11.5	2.3	7.2	...	8.3	4.1	5.3	4.7	2.9	...	3.7	1.3	9.0	7.5	5.9	6.1	..	..	0.9
22h:0	16.4	22.0	23.2	20.0	13.6	18.8	16.4	13.7	19.1	18.5	27.0	23.0	17.7	18.0	18.6	18.5	27	17	17.6
23h:0	8.4	12.5	13.6	15.2	12.0	14.2	9.0	12.9	9.5	21.4	12.0	12.5	21.6	21.0	22.3	21.3	9	17	17.1
24h:0	25.8	40.0	38.4	27.8	25.0	30.3	22.2	34.0	30.8	25.2	24.7	31.5	38.4	39.0	36.2	40.8	33	26	27.4
24h:1	10.6	15.7	7.3	11.7	12.1	10.8	12.4	14.4	21.2	6.6	10.0	7.0	4.1	4.1	6.3	3.8	17	25	23.0
25h:0	10.5	1.1	5.3	...	3.6	4.2	3.0	2.2	2.2	...	2.2	1.0	...	...	...	2.1	..	2	1.2
25h:1	7.5	0.6	...	2.8	1.9	...	3.8	...	1.6	...	...	1.0	...	...	...	...	..	2	1.5
26h:0	...	...	...	2.0	Tr.	...	1.0	...	...	7.6	...	...	...	...	...	...	..	2	0.3
Saturated	80.8	83.7	92.5	85.2	86.0	82.5	74.7	77.5	77.2	83.9	90.0	87.0	90.9	95.9	93.7	94.2	83	72	73.9
Unsaturated	19.2	16.3	7.5	14.8	14.0	17.5	25.3	22.5	22.8	16.1	10.0	13.0	9.1	5.1	6.3	5.8	17	28	26.1

* Mother and son; MV is an obligatory heterozygote.
† Siblings.
‡ From Mårtenson, Percy, and Svennerholm (32); patient with sulfatidosis was 2 yr old; controls, 10–17 yr.
§ From Mårtensson (33); 60–90 yr old.

TABLE 7 Concentration of Neutral Urinary Glycosyl Ceramides in Patients with Sulfatidosis and in Normal Individuals

Donor	Number	Age	Galactosyl Ceramide	Glucosyl Ceramide	Dihexosyl Ceramide	Trihexosyl Ceramide	Tetrahexosyl Ceramide
		yr	*nmoles/100 ml urine ± SD*				
Normal							
Males	10	2–68	3.5 ± 3.6	32.7 ± 15.6	4.0 ± 0.7*	3.9 ± 3.7	3.7 ± 3.3
Females	10	1–68	2.9 ± 4.5	38.3 ± 23.4	19.9 ± 7.8*	2.1 ± 2.3	1.4 ± 1.6
Sulfatidosis							
Males	6	1–16	5.3 ± 8.7	17.9 ± 18.4	3.9 ± 2.6	1.0 ± 0.7	0.8 ± 0.4
Females	6	5–15	2.9 ± 2.9	28.2 ± 28.0	18.9 ± 19.8	1.6 ± 1.5	1.8 ± 1.2

* $P \leq 0.001$.

leukocytes (5), or in urine from patients with Hurler's syndrome (5) or Fabry's disease (16, 22). In males with this last condition, trihexosyl ceramides are equally increased. The use of different developing mixtures and of concurrent glycolipid standards is helpful in characterizing urinary glycolipids. Erythrocytes represent a good source of di-, tri-, and tetrahexosyl ceramides (22), and brain white matter contains large amounts of cerebrosides and sulfatides.

Sulfatide concentration in normal urinary sediment was reported by Wherrett (5) to range from 5–45% of the neutral glycolipids, while Desnick, Sweeley, and Krivit (22) did not detect any. The ion-exchange step used in the present study allowed the demonstration of a sulfatide fraction in all urine specimens. In normal males, sulfatide concentration tended to be higher than that of dihexosyl ceramides, while the reverse was observed in females. Sex differences in glycolipid distribution deserves further study; they were recently detected in mouse kidney, where they can be influenced by testosterone administration (35). Interestingly, the only obligatory heterozygote studied here (MV) excreted twice as much sulfatide as the highest control. Since all patients with sulfatidosis had a mean twentyfold increase in sulfatide levels, the usefulness of a semiquantitative estimation for routine purposes is apparent.

Urinary sulfatides had a pattern of LCB (28, 36) and of fatty acids which was almost identical with that of kidney sulfatides but different from brain sulfatides. The occurrence of 5–10% phytosphingosines in urinary sulfatides represents a particularly useful marker, since this type of base is found in the kidney but not in the

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brain. If a significant portion of urinary sulfatides originated in the brain, the proportion of phytosphingosine would be diluted accordingly. This was not observed and was further corroborated by differences in fatty acid distribution.

The high proportion of $C_{22:0}$ acids was similar in both patients and controls. The patients had less $C_{23:0}$ acids, less unsaturated acids, and a much higher proportion of hydroxy fatty acids than the controls. This pattern was quite similar to that reported by Mårtensson (11) in the kidney, confirming the idea of Desnick et al. that urinary lipids provide "an 'indirect biopsy' of the kidney" (22). Normal urinary sulfatides contained more $C_{23:0}$ acids and much less unsaturated acids than kidney sulfatides. Preparative losses involved in processing large quantities of urine might partly account for these differences. Another explanation might be that normal urine contains a sizable proportion of sulfatides derived from organs other than the kidney, or that a hypothetical selective excretion of sulfatides is impaired in sulfatidosis.

We have previously stressed the simplicity and the effectiveness of the filtering step used to collect urinary glycolipids (16). The amount of neutral glycolipids extracted from the filter (Table 7) compares favorably with the values obtained through a more elaborate technique (22), which in fact seems to yield lesser quantities of monohexosyl ceramides.

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