Generalized accumulation of neutral glycosphingolipids with G_{M2} ganglioside accumulation in the brain

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Abstract Analyses have been made of glycosphingolipids from visceral organs and brain of a patient with an unusual lipid storage disorder diagnosed initially as classical Tay-Sachs disease. Levels of the lipids from fresh-frozen sections of gray and white matter, kidney, spleen, liver, and heart from this patient were compared with those of normal juvenile controls, and the fatty acid composition of accumulated glycosphingolipids was compared with reference compounds. This patient was found to have abnormally high concentrations of a globoside in liver, kidney, and spleen, asialo G_{M2} ganglioside in brain and liver, and G_{M2} ganglioside in the brain. On the basis of these findings along with the clinical manifestations of Tay-Sachs disease with visceral involvement (hepatosplenomegaly) and demonstration of total deficiency of both A and B components of β-*N*-acetylhexosaminidase activity, this glycosphingolipidosis is the same as two previously reported cases of G_{M2} gangliosidosis with globoside accumulation and total β-*N*-acetylhexosaminidase deficiency.

Supplementary key words globoside · G_{M2} ganglioside · asialo G_{M2} · glycosphingolipid storage disease · total hexosaminidase deficiency

An interesting variation of Tay-Sachs disease was described by Sandhoff, Andreae, and Jatzkewitz in 1968 (1, 2). Biochemical analyses revealed abnormal amounts of normal kidney globoside, *N*-acetylgalactosaminyl-(1→3)-galactosyl-(1→4)-galactosyl-(1→4)-glucosyl ceramide, in visceral organs while G_{M2} ganglioside,[1] *N*-acetylgalactosaminyl-(1→4)-(*N*-acetylneuraminyl-[2→3])-galactosyl-(1→4)-glucosyl ceramide, accumulated in the brain along with the asialo form of this lipid.[2] The brain and other organs from this infant were completely devoid of β-*N*-acetylhexosaminidase activity (2, 3), and this enzymatic defect was postulated to be the cause of the unusual pattern of glycosphingolipid storage. A similar glycosphingolipidosis has been recently described by Suzuki et al. (4), and preliminary findings of similar cases were presented by O'Brien (5) and Young et al. (6).

In this report the chemical findings are presented of a child who died at age 18 months from a lipid storage disorder with clinical manifestations similar to those of classical infantile Tay-Sachs disease. However, hepatosplenomegaly was observed in addition to the usual neurological symptoms and the characteristic cherry-red spot in the retina, suggesting the possibility of visceral involvement (7). Neutral glycosphingolipids and gangliosides were isolated from cerebral gray and white matter, kidney, spleen, liver, and heart, and the levels of these lipids were compared with those of normal juvenile controls. The chemical structures of lipids found in abnormally high concentrations were partially elucidated from analyses of the carbohydrate constituents by gas–liquid chromatography, comparisons of thin-layer chromatographic behavior of the samples with those of refer-

Abbreviations: TMSi, trimethylsilyl.

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[1] Ganglioside nomenclature suggested by Svennerholm (32).

[2] The complete chemical structures of the globoside and ganglioside have not been determined, but our analytical data as well as those of Sandhoff et al. (1, 2) are consistent with the structures of normal kidney globoside and G_{M2} ganglioside.

ence standards, and mass spectrometry of the intact glycosphingolipids. The results of this study indicate that the patient had an unusual type of glycosphingolipidosis with visceral and CNS accumulation of a globoside and asialo G_{M2}, accompanied by accumulation of G_{M2} ganglioside in the brain. There was a total deficiency of both A and B components of β-*N*-acetylhexosaminidase activity.[3] The metabolic disorder is assumed to be the same as that described by Sandhoff et al. (1–3) and by Suzuki et al. (4).

MATERIALS AND METHODS

Portions of the patient's brain, kidney, heart, liver, and spleen, and comparable sections from normal juvenile organs (fresh-frozen at autopsy), were shipped in dry ice from Minneapolis to East Lansing for chemical analyses, except for the heart, which was analyzed at a later date in Minneapolis. Glycosphingolipids used as reference standards for thin-layer chromatography were obtained from washed human erythrocytes by the method of Vance and Sweeley (8). Heat-activated silicic acid (Unisil, 200–325 mesh, Clarkson Chemical Co., Williamsport, Pa.) was used in all silicic acid column chromatographic separations. Thin-layer chromatography was carried out on glass plates coated to a thickness of 250 μ with silica gel G (Brinkmann Instruments, Inc., Westbury, N.Y.) or with precoated thin-layer chromatography plates from Quantum Industries, Chicago, Ill. Gas–liquid chromatographic analyses were made with a Hewlett-Packard model F&M 402 gas chromatograph containing glass U-tube columns (6 ft × 1/8 inch I.D.) packed with 3% SE-30 or with 3% OV-1 methylsilicone liquid phases on 100–200 mesh Gas-Chrom P (Applied Science Laboratories Inc., State College, Pa.) or Supelcoport (Supelco, Inc., Bellefonte, Pa.). Hexamethyldisilazane, trimethylchlorosilane, and bis-trimethylsilyltrifluoroacetamide used for the preparation of trimethylsilyl (TMSi) derivatives were purchased from Applied Science Laboratories. Chemicals and solvents were analytical or reagent grade unless otherwise indicated.

Extraction and purification of lipids

Total lipids were extracted from sections of rinsed and blotted tissue with chloroform–methanol 2:1 by the method of Folch, Lees, and Sloane Stanley (9). Initially the tissue (5–15 g) was ground with a small volume of chloroform–methanol and some sand in a mortar; the homogenate was diluted with chloroform–methanol to a volume of 20 ml/g of tissue and filtered after 30 min. The residue was then extracted for 2 hr with refluxing chloroform–methanol 2:1 (20 ml per g). In most of the analyses a modification of the "Folch wash" was used to remove nonlipid contaminants. This procedure involved prolonged dialysis of a solution of total lipids in 30–40 ml of chloroform–methanol and changing the large volume of distilled water several times over a period of 2 days. Gangliosides were recovered from the upper aqueous layer in the dialysis tubing, and neutral glycosphingolipids were isolated from the lower chloroform layer. Ideally, this method minimizes the loss of low molecular weight gangliosides such as G_{M3} in the organic phase which often occurs in the conventional extraction procedure (10).

Neutral glycosphingolipids were purified by the procedure previously described in detail (8). A fraction (6–12 mg) of the crude mixture of sphingolipids obtained by silicic acid chromatography and mild alkali-catalyzed methanolysis was separated into individual species by preparative thin-layer chromatography in chloroform–methanol–water 100:42:6 with single development of the plate; preliminary identifications of the neutral glycosphingolipids were made by comparing their chromatographic behavior with that of reference compounds from human erythrocytes. Similarly, a portion (6–12 mg) of the crude ganglioside fraction was separated by preparative thin-layer chromatography in chloroform–methanol–2.5 N aqueous NH_3 60:40:9 with double development of the plate (11). Appropriate zones were scraped from the plates and gangliosides were recovered by eluting the silica gel with 40 ml of chloroform–methanol–water–pyridine 40:56:12:2. Individual fractions were identified by comparison of their thin-layer chromatographic properties with published values (11).

Gas–liquid chromatography of carbohydrate constituents and methyl esters of fatty acids

Purified glycosphingolipids were degraded in methanolic HCl to yield a mixture of methyl esters of the fatty acids (extracted from the methanolysate with hexane), methyl glycosides of the carbohydrate units, and sphingolipid bases. Galactosamine and the methyl ketal of methyl neuraminate were re-*N*-acetylated with acetic anhydride in methanol, and the volatile TMSi derivatives of the carbohydrates were prepared with a mixture of hexamethyldisilazane–trimethylchlorosilane–pyridine 2:1:5. Details of the procedures for methanolysis, acetylation, and isolation of the methyl ester and carbohydrate fractions have been reported by Dawson and Sweeley (12).

The composition of the oligosaccharide moieties of each of the isolated glycosphingolipids was determined

[3] We are grateful to Dr. K. Suzuki for permission to cite the results of his analyses of total hexosaminidase activity and the A and B components of this enzyme.

by gas–liquid chromatographic analysis of the TMSi derivatives with mannitol as an added internal standard. Molar galactose/glucose (gal/glc) ratios calculated from the gas chromatograms provided additional evidence for the identifications of neutral glycosphingolipids and gangliosides. These ratios also gave an approximate analysis of the composition of unresolved mixtures of galactosyl and glucosyl ceramides in ceramide monohexoside fractions and of lactosyl and digalactosyl ceramides in ceramide dihexoside fractions. Compounds that contained *N*-acetylgalactosamine were easily identified by the presence of appropriate peaks on gas chromatograms for the anomeric forms of the TMSi methyl glycoside of this sugar.

Although the yields of *N*-acetylneuraminic acid by the procedure used in this study were somewhat variable and consistently lower than theory, the observed *N*-acetylneuraminic acid/glucose molar ratios were used to estimate the number of sialic acid residues in isolated ganglioside fractions, assuming a yield of approximately 60% for sialic acid.

Methyl esters were determined by gas–liquid chromatography on 3% SE-30 or 3% OV-1 with linear temperature programming from 180 to 240°C at 2°/min. Areas of the peaks were calculated from the height of the peak at the summit and the width at half-height, and the composition was expressed as percentages of total area. The TMSi methyl glycosides from neutral glycosphingolipids were analyzed isothermally on 3% SE-30 at 170°C, whereas the mixture from gangliosides was separated with temperature programming on 3% SE-30 from 150 to 220°C at 2°/min. The carbohydrate composition was determined from ratios of yields of individual sugars, and the amount of glycosphingolipid was expressed as μmoles per gram wet weight, using TMSi mannitol as an internal standard to estimate the amount of TMSi methyl glucosides (or galactosides in the case of galactosyl ceramide) in the mixture (8, 12).

Mass spectrometry of glycosphingolipids

The TMSi derivatives of purified samples of glycosphingolipids from patient and control organs were prepared from 50–150 μg of the lipid and 200 μl of bis-trimethylsilyltrifluoroacetamide–pyridine 1:2 as previously described (13). An aliquot of the mixture was evaporated in a glass tube for analysis in the direct inlet of an LKB 9000 single-focusing mass spectrometer. Mass spectra were recorded at 3500 v and 70 ev electron energy with 60 μa electron current and an ion source temperature of 290°C. The TMSi derivatives of the glycosphingolipids were volatile at temperatures ranging from 125 to 200°C (direct inlet temperature), depending on the complexity of the oligosaccharide attached to ceramide.

RESULTS

Mass spectrometry of accumulated neutral glycosphingolipids

In Fig. 1, partial mass spectra of the TMSi derivatives of the accumulated ceramide tetrahexoside from the patient's kidney and the accumulated ceramide trihexoside from the patient's liver are compared with the spectrum of galactosylgalactosylglucosyl ceramide from control kidney. The mass spectra of globoside that accumulated in liver and spleen were the same as the spectrum of the kidney globoside. As previously found in mass spectral analyses of reference glycosphingolipids (13), the presence of an *N*-acetylhexosamine residue can be detected by a fragmentation ion at *m/e* 173, arising from C-2 and C-3 of the amino sugar derivative. This ion is prominent in the mass spectra of both of the stored neutral glycosphingolipids, but it is virtually absent from the mass spectrum of the reference ceramide trihexoside, which contains only glucose and galactose residues. The amino sugar can be assigned to the terminal position of the oligosaccharide chain if there are strong ions at *m/e* 420 and 330, but if these ions are absent and *m/e* 451 and 361 are present, the terminal residue is a neutral hexose (13). The mass spectra of both ceramide trihexoside and ceramide tetrahexoside from the patient have intense peaks at *m/e* 420 and 330 and the *N*-acetylgalactosamine residue can therefore be assigned unequivocally to the terminal position of the carbohydrate chain. The mass spectra of these lipids have other relationships of ion intensities, such as *m/e* 204, 243, and 271 compared with *m/e* 311, which support the assignments of asialo G_{M2} for the ceramide trihexoside and globoside for the ceramide tetrahexoside. A detailed account of how these relationships can be related to certain structural features has already been presented (13).

Composition of lipid fractions from visceral organs and brain

The concentrations of neutral glycosphingolipids in kidney, spleen, and liver from the patient and from a juvenile control are given in Table 1 along with a summary of previously published normal data. Lactosyl ceramide was the most abundant component in the normal juvenile liver, as found previously (14, 15), and the concentration of total neutral glycosphingolipids (0.22 μmole/g wet wt) was the same as that reported for adult liver (15). Glucosyl ceramide and lactosyl ceramide each accounted for about 35% of the 0.42 μmole/g wet wt of total neutral glycosphingolipids in normal juvenile spleen, whereas Svennerholm and Svennerholm (14) found relatively more lactosyl ceramide (50–60%) and less glucosyl ceramide (10–15%) in adult spleen. Globoside was a minor constituent in both liver and spleen.

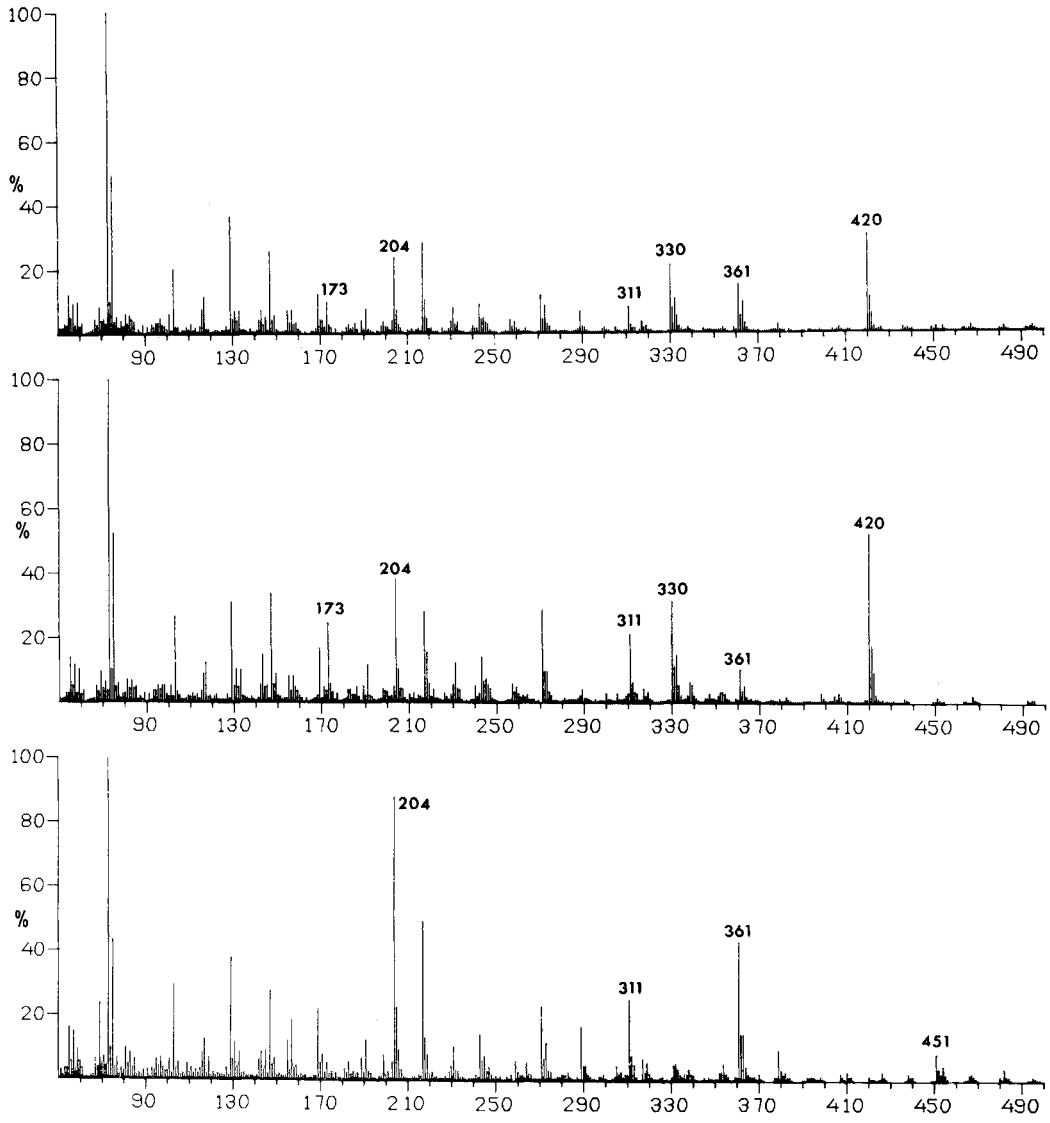

FIG. 1. Partial mass spectra of the TMSi derivatives of accumulated globoside from kidney (top), accumulated ceramide trihexoside (asialo G_{M2}) from liver (middle), and galactosylgalactosylglucosyl ceramide from normal kidney (bottom).

In normal kidney, however, globoside was found by Mårtensson (16) to be the major constituent (about 50% of total neutral glycosphingolipids) and was accompanied by a substantial concentration of galactosylgalactosylglucosyl ceramide. We found almost the same composition in the normal juvenile kidney, although the proportion of globoside was somewhat lower (Table 1). The ceramide monohexoside fraction of normal kidney was a mixture of glucosyl and galactosyl ceramides and the ceramide dihexoside fraction contained both lactosyl and digalactosyl ceramides, as previously reported by Mårtensson (16). Normal heart contained approximately equal proportions of the four neutral glycosphingolipids (total concentration 0.23 μmole/g wet weight).

In the patient, globoside was found in abnormally high concentrations in all of the visceral organs examined, but the storage of this glycosphingolipid was especially marked in liver and spleen (Table 1). Heart contained twice the level found in the control. The molar ratio of glucose/galactose/*N*-acetylgalactosamine was close to 1/2/1 in all of the samples, and the chromatographic behavior of the accumulated lipid was the same as that of globoside from control kidney and human erythrocytes.

Liver from the patient also contained abnormal amounts of a ceramide trihexoside with a 1/1/1 molar ratio of glucose/galactose/*N*-acetylgalactosamine. This finding agrees with the results of mass spectral analysis and supports the assignment of the accumulated ceramide trihexoside as asialo G_{M2}.

The ceramide monohexoside fraction from the patient's kidney contained about equal amounts of glucosyl

TABLE 1. Glycosphingolipids in visceral organs

Organ	Lipid	Patient[a]			Juvenile Control[a]			Normal[b]
		μmoles/g	gal/glc	galNAc	μmoles/g	gal/glc	galNAc	μmoles/g
Liver	Ceramide monohexosides[c]	0.12 ± 0.02	0.47	−	0.05 ± 0.01	0.06	−	0.05
	Lactosyl ceramide	0.09 ± 0.02	1.04	−	0.10 ± 0.02	1.11	−	0.08
	Ceramide trihexoside	1.11 ± 0.21	1.07	+	0.04 ± 0.01	1.95	−	0.04
	Globoside	0.30 ± 0.07	1.91	+	0.03 ± 0.01	1.92	+	0.03
Spleen	Glucosyl ceramide	0.30 ± 0.02	0	−	0.12 ± 0.07	0.08	−	
	Lactosyl ceramide	0.13 ± 0.01	0.93	−	0.16 ± 0.09	1.08	−	
	Galactosylgalactosylglucosyl ceramide	0.12 ± 0.02	1.41	−	0.06 ± 0.03	1.80	−	
	Globoside	2.04 ± 0.22	2.00	+	0.08 ± 0.02	1.77	+	
Kidney	Ceramide monohexosides[c]	0.12 ± 0.04	0.55	−	0.10 ± 0.02	0.52	−	0.11
	Ceramide dihexosides[d]	0.15 ± 0.02	8.41	−	0.14 ± 0.07	3.90	−	0.16
	Galactosylgalactosylglucosyl ceramide	0.13 ± 0.01	1.93	−	0.31 ± 0.17	2.11	−	0.32
	Globoside	0.67 ± 0.11	2.08	+	0.26 ± 0.12	1.98	+	0.48

[a] Reported levels are the averages of from two to six determinations with different tissue samples.
[b] Normal data for liver taken from Kwiterovich, Sloan, and Fredrickson (15); normal data for kidney taken from Mårtensson's values for dry weight of kidney (16).
[c] A mixture of glucosyl and galactosyl ceramides.
[d] A mixture of lactosyl and digalactosyl ceramides, and possibly some unresolved sulfatides (kidney).

and galactosyl ceramides, and there was no evidence for storage of either lipid. The liver from the patient had a slight increase in galactosyl ceramide, however, since the ceramide monohexoside fraction was increased along with a change in the molar galactose/glucose ratio to 0.47. The ceramide dihexoside fraction was recovered in normal amounts from the patient's kidney but may have contained a modest increase in the proportion of digalactosyl ceramide, based on the observed molar galactose/glucose ratios.

Ganglioside fractions from the visceral organs were examined by thin-layer chromatography. Although abnormal amounts of G_{M2} ganglioside did not appear to be present in tissue from the patient, there was not sufficient material for a detailed analysis of the gangliosides.

The composition of neutral glycosphingolipid fractions from cerebral gray and white matter is given in Table 2. Sulfatide and lactosyl ceramide cochromatographed in the thin-layer chromatography system used, but the proportion of lactosyl ceramide in the mixture could be calculated from galactose/glucose ratios. The levels of cerebroside and sulfatide were much lower than the control values in both areas of brain from the patient. Furthermore, the ceramide monohexoside fraction from both white and gray matter contained substantial quantities of glucosyl ceramide, as previously noted in this disorder and other gangliosidoses (4). There was an inexplicable difference in the chromatographic behavior of ceramide monohexoside fractions from the patient's brain, which could not be accounted for by α-hydroxy

TABLE 2. Glycosphingolipids in brain

Lipid	Patient			Control[a]		
	μmoles/g	gal/glc	galNAc	μmoles/g	gal/glc	galNAc
Gray Matter						
Ceramide monohexosides[b]				5.00	no glc	−
Band A	0.39	0.51	−			
Band B	0.39	18.0	−			
Sulfatide and lactosyl ceramide[c]	0.29 (25%)	5.09	−	1.66 (9%)	11.60	
Asialo G_{M2}	4.61	1.06	+	none		
Globoside	0.11	2.07	+	none		
White matter						
Ceramide monohexosides[b]				135.7	no glc	−
Band A	2.16	12.80	−			
Band B	4.00	no glc	−			
Sulfatide and lactosyl ceramide[c]	2.84 (6%)	16.10	−	57.10 (7%)	14.95	
Asialo G_{M2}	3.20	1.08	+	none		
Globoside	0.30	2.14	+	none		

[a] In normal juvenile gray and white matter, neutral glycospbingolipids corresponding to asialo G_{M2} and globoside could not be detected on thin-layer chromatography.
[b] In fractions from the patient, the ceramide monohexosides were separated by thin-layer chromatography into two well-resolved bands (A and B). In fractions from the control, these two bands were not resolved.
[c] Figures in parentheses represent proportion of lactosyl ceramide in the mixture, calculated from gal/glc ratio.

fatty acid content. Trihexosyl ceramide (tentative assignment as asialo G_{M2}) and tetrahexosyl ceramide (presumably globoside) were found in both gray and white matter of brain from the patient but were not observed in the lipids isolated from control brain. The level of the trihexosyl ceramide in the brain was much higher than that of the globoside, and on the basis of wet weight of tissue, represented the largest amount of stored neutral glycosphingolipid in any organ examined.

Analyses of ganglioside fractions are summarized in Table 3. The level of G_{M2} ganglioside in the patient's brain was about 10 times higher than normal in white matter and about 100 times higher than normal in gray matter, whereas all of the other gangliosides were within the normal range.

Gas chromatographic analysis of fatty acid methyl esters of isolated lipid fractions

The fatty acid composition of the stored visceral glycosphingolipids from the patient are compared with those of corresponding normal glycosphingolipids in Table 4. There was no remarkable difference in the composition of the globoside from kidney and spleen, and both fractions were quite similar to globoside from normal kidney and spleen except for the variation in proportions of lignoceric (24:0) and nervonic (24:1) acids.

The ceramide trihexoside from the patient's liver contained a high percentage (55%) of stearic acid, which distinguished it from the ceramide trihexoside (gal-gal-glc-ceramide) found in normal kidney and from globoside in normal liver. Other than the absence of 23:0, the

TABLE 3. Gangliosides in brain

	Patient					Control				
Ganglioside[a]	mg/g	μmoles/g	gal/glc	galNAc/glc	NANA/glc	mg/g	μmoles/g	gal/glc	galNAc/glc	NANA/glc
Gray matter										
G_{M3}		0.07	0.71		0.33		0.01	0.71		0.71
G_{M2}		5.43	1.10	0.77	0.64		0.04	1.14		0.80
G_{M1}		0.30	1.93	0.64	0.88		0.32	2.03	0.73	0.78
G_{D1a}		0.33	1.98	0.69	1.17		0.46	2.05	0.80	1.63
G_{D1b}		0.14	1.74	0.76	1.20		0.20	1.85	0.68	1.41
$(G_{T1})^b$		0.11	1.93	0.56	1.05					
Total	11.5					3.7				
White matter										
G_{M3}		0.02	0.72		0.50					
G_{M2}		0.92	1.03	0.70	0.60		0.01	1.12		0.71
G_{M1}		0.09	1.77	0.62	0.55		0.09	2.05	0.72	0.71
G_{D1a}		0.09	1.89	0.68	1.06		0.13	2.03	0.79	1.41
G_{D1b}		0.04	1.44	0.82	1.09		0.02	1.63	0.63	1.64
$(G_{T1})^b$		0.02	1.44	0.57	0.78		0.04	1.82	0.67	1.48
Total	2.5					0.8				

[a] Values (μmoles/g) are derived directly from gas–liquid chromatographic estimation of glucose after methanolysis of the lipid. Corrections have not been made for manipulative losses during isolation.

[b] The thin-layer chromatography fraction labeled G_{T1} gave poor ratios of carbohydrate units with the sample from the patient, and the fraction might therefore be a mixture.

TABLE 4. Fatty acid compositions of patient and normal visceral glycosphingolipids

	Globoside							Ceramide Trihexoside[a]	
	Kidney			Spleen		Liver		Liver	
Fatty Acid[b]	Patient	Normal Juvenile	Normal Adult[c]	Patient	Normal Juvenile	Our Patient	Suzuki (Ref. 4) Patient	Patient	Normal Juvenile
				% of total fatty acids					
16:0	5.8	7.1	5.5	5.3	6.8	7.0	8.4	6.2	12.6
18:0	3.0	3.4	3.2	4.6	2.7	9.4	14.7	55.0	6.1
18:1	0.5	1.1	0.3	1.0	1.0	2.8	1.3	4.7	2.2
20:0	6.4	6.2	6.8	3.1	4.1	3.7	6.0	7.2	5.2
22:0	23.4	22.7	20.6	13.3	21.4	11.4	26.8	5.8	25.7
22:1		0.9	1.9	1.7		3.7	1.3		
23:0	3.7	3.1	2.8	5.2	8.0	3.1	4.1	1.6	13.0
24:0	29.6	31.5	24.8	25.0	36.2	20.2	17.4	7.4	29.2
24:1	23.7	22.4	32.0	38.0	19.5	37.8	10.8	11.1	5.2

[a] Ceramide trihexoside from patient liver is asialo G_{M2} ganglioside, and from normal liver is galactosylgalactosylglucosyl ceramide.

[b] Fatty acid components are expressed as carbon chain length: number of double bonds.

[c] Values from Mårtensson's study of kidney glycosphingolipids (16).

TABLE 5. Fatty acid compositions of patient and Tay-Sachs cerebral glycosphingolipids

Fatty Acid[a]	Asialo G_{M2}			G_{M2} Ganglioside			Globoside
	Patient Gray Matter	Patient White Matter	Tay-Sachs[b]	Patient Gray Matter	Patient White Matter	Tay-Sachs[b]	Patient White Matter
				% of total fatty acids			
16:0	2.0	3.8	3.4	2.3	2.5	6.6	11.8
16:1				1.1	0.6		1.5
18:0	91.4	87.6	84.1	89.8	90.6	87.0	50.0
18:1	2.0	3.8	2.5	1.7	1.3	2.8	10.3
20:0	4.6	4.8	2.2	5.1	5.0	3.6	3.0
22:0			7.8				3.0
22:1							7.4
24:0							6.6
24:1							4.4

[a] Fatty acid components are expressed as carbon chain length:number of double bonds.
[b] Values from Taketomi and Kawamura (19).

fatty acid composition of this fraction was similar to that reported by Svennerholm for G_{M2} ganglioside and asialo G_{M2} that accumulated in liver in Tay-Sachs disease (17). The high percentage of 18:0 and the observation by mass spectrometry (Fig. 1, middle) that this lipid contained C_{18}-sphing-4-enine (*m/e* 311) and C_{20}-sphing-4-enine (*m/e* 339) in a ratio of about 4:1 suggest that at least a part of the lipid was of neural origin. The same conclusion was reached by Svennerholm (17) to account for the presence of C_{20}-sphing-4-enine (16% of total long-chain base) and high 18:0 in the accumulated liver G_{M2} ganglioside in Tay-Sachs disease.

The fatty acid composition of G_{M2} ganglioside, ceramide trihexoside, and ceramide tetrahexoside from the patient's brain are compared with lipids from Tay-Sachs brain in Table 5. With the exception of the ceramide tetrahexoside fraction, which contained substantial amounts of behenic (22:0), docosenoic (22:1), lignoceric, and nervonic acids, all of the lipids had mixtures of fatty acids consisting almost exclusively of palmitic (16:0), stearic, oleic (18:1), and arachidic (20:0) acids.

DISCUSSION

The biochemical findings in this patient are clearly different from those of classical Tay-Sachs disease. The key compound in differentiating this disorder from Tay-Sachs disease is globoside (I), the accumulation of which was most easily recognized in spleen. Globoside levels in plasma and urinary sediment were also increased (7). In brain, G_{M2} ganglioside (II) accumulation was about the same as that found in Tay-Sachs disease, but we were not able to demonstrate accumulation of this ganglioside in visceral organs. Some increase in the concentration of G_{M2} ganglioside in liver was observed by Suzuki et al. (4), however, and it is possible that our methods of analysis for ganglioside were not sufficiently sensitive for this study. Accumulation of the asialo derivative of G_{M2} ganglioside (III) in the liver and brain was much more pronounced than that found in Tay-Sachs disease (18), where it also accompanies storage of G_{M2} ganglioside (19–23). The results summarized in Fig. 2 parallel those reported first by Sandhoff et al. (1–3) and more recently by Suzuki et al. (4) for unusual biochemical findings in a G_{M2} storage disease with visceral accumulation of globoside.

GalNAc-Gal-Gal-Glc-Ceramide (I)

GalNAc-Gal-Glc-Ceramide (II)
|
NANA

GalNAc-Gal-Glc-Ceramide (III)

In both of these unusual cases with G_{M2} ganglioside and globoside accumulation, there was a total deficiency of both A and B components of β-*N*-acetylhexosaminidase activity, and the storage of the three glycosphingolipids with terminal *N*-acetylgalactosamine moieties was ascribed to this enzymatic defect (1–5). This finding provides another important biochemical difference from Tay-Sachs disease, where there is deficient activity of the A component and normal or increased amounts of the B component of β-*N*-acetylhexosaminidase (3, 24–27). Suzuki[3] has found that the A and B components of this enzyme were totally absent from samples of brain and liver from our patient, a finding which further strengthens the diagnosis of this disorder. We have concluded on the basis of the clinical manifestations and the biochemical evidence that the glycosphingolipidosis is the same as those previously described.

This genetic disorder has been referred to recently as Tay-Sachs disease variant I by Ohman, Ekelund, and Svennerholm (18). It may not be appropriate to refer to this disease as an unusual form of Tay-Sachs disease,

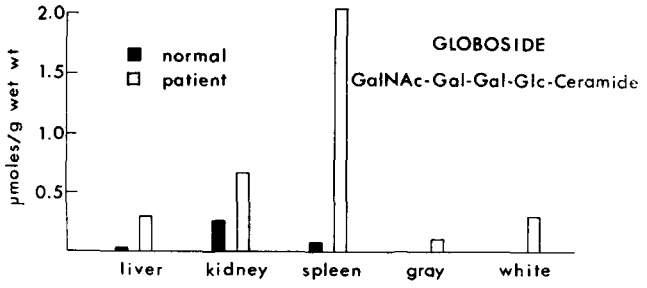

FIG. 2. Globoside concentration in patient and age-matched normal visceral and neural tissue. Ohman et al. (18) reported no increase of globoside in visceral organs of two patients with classical Tay-Sachs disease.

however, in view of the clinical (hepatosplenomegaly) and biochemical differences that have been observed. A general classification based on the nature of the stored lipid has been proposed for ganglioside storage diseases (28), but it is not completely descriptive when there is accumulation of several glycosphingolipids. An alternative system of nomenclature, proposed by Young et al. (6), specifies the nature of the enzymatic defect as well and is perhaps more appropriate. Thus, Tay-Sachs disease would be called G_{M2} gangliosidosis, type A_0B_H, indicating absence of component A and high amounts of component B of β-*N*-acetylhexosaminidase. It was suggested that the glycosphingolipidosis with total hexosaminidase deficiency be called G_{M2}-gangliosidosis, type A_0B_0. The latter designation fails to make an adequate distinction from Tay-Sachs disease in regard to the accumulation of globoside, however. We would like to propose that this disorder be called Sandhoff-Jatzkewitz disease.

The functional relationship of the A and B components of β-*N*-acetylhexosaminidase has not been clearly established. The accumulation of G_{M2} ganglioside can be related to the absence of the A component in Tay-Sachs disease, and the accumulation of globoside in Sandhoff-Jatzkewitz disease to the deficiency of the B component. The asialo derivative of G_{M2} is increased in both disorders, although the level is much greater in brain and liver when both A and B components are absent, suggesting that this glycosphingolipid can be hydrolyzed by both forms of β-*N*-acetylhexosaminidase. It has been pointed out by Sandhoff, however, that with purified preparations both components have similar substrate specificities (3).

Whether the actual genetic abnormalities in Tay-Sachs disease and Sandhoff-Jatzkewitz disease are related has been the subject of interesting speculation (29, 30). The A component of β-*N*-acetylhexosaminidase is a sialoglycoprotein with an isoelectric point at about pH 5 (3, 23, 29, 30). It can be converted by neuraminidase treatment to a more basic form that closely resembles and may be identical with the B component (29, 30), which has an isoelectric point at about pH 7.4 (3, 23, 29, 30). Whether the product from neuraminidase treatment of the A component has the same substrate specificity as the B component has not yet been demonstrated. Although the reverse reaction has not been examined experimentally, it has been suggested that the B component might be converted to the A component by addition of sialic acid residues (30, 31). The possibility of such a relationship between the two forms of β-*N*-acetylhexosaminidase suggests that the genetic defect in Tay-Sachs disease might not be in the synthesis of form A per se, but might involve the synthesis of the sialyl transferase necessary for addition of sialic acid to the B component, as shown in Fig. 3 (30, 31). An enzymatic block at this point might also explain the increased levels of B component (metabolic precursor) in Tay-Sachs disease (3, 24–27). In

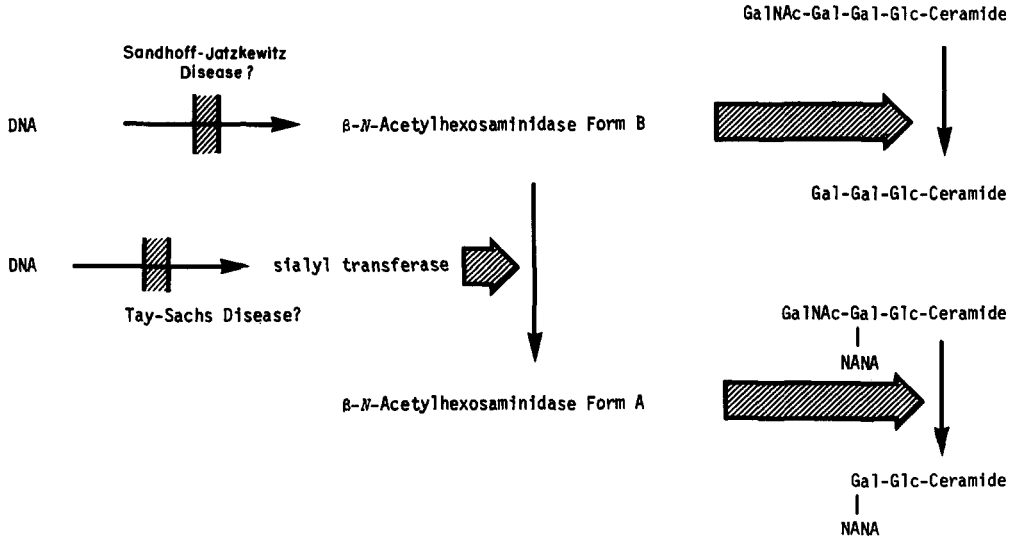

FIG. 3. Hypothetical difference in genetic defects in gangliosidoses with hexosaminidase deficiency.

Sandhoff-Jatzkewitz disease, the absence of both A and B components implies a genetic defect located in the synthesis of the B component, as shown in the hypothetical pathway (Fig. 3).

This work was supported by National Institutes of Health research grant AM 12434 and is published with the approval of the Director of the Michigan Agricultural Experiment Station as journal article no. 5450.

A portion of the work described in this paper was taken from the M.S. thesis submitted by PDS to Michigan State University.

Manuscript received 14 April 1971; accepted 1 September 1971.

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