Characterization and quantitative determination of gangliosides and neutral glycosphingolipids in human liver

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Abstract The neutral and acidic glycolipids from the liver of an 11-year-old male were quantitatively isolated and characterized. The total concentration of gangliosides was also determined in samples from five other human livers. Lipid-bound sialic acid varied between 190–248 nmol/g with a mean value of 212 nmol/g. The major ganglioside was G_{M3}, which represented 91.6% of the sialic acid. Besides G_{M3} and G_{M1}, a wide variety of other minor monosialogangliosides were isolated. Gangliosides of the gangliotetraose series with up to four sialic acids were demonstrated in human liver for the first time. The composition of the ceramide portion of the gangliotetraose gangliosides was considerably different from that of the "visceral" gangliosides, G_{M3}, G_{D3}, and L_{M1} (sialosyl-lactoneotetraosylceramide), which suggests that these two groups of gangliosides are biosynthesized in two different pools. The concentration of the neutral glycolipids was approximately the same as that of the gangliosides. Lactosylceramide was the largest fraction, closely followed by galactosylceramide, glucosylceramide, and globotriaosylceramide. The ceramide composition of the neutral glycolipids resembled that of the "visceral" gangliosides, suggesting that they are metabolically related. 2-Hydroxy fatty acids were found in glucosyl-, galactosyl-, and lactosylceramides as well as in ganglioside G_{M3}.—**Nilsson, O., and L. Svennerholm.** Characterization and quantitative determination of gangliosides and neutral glycosphingolipids in human liver. *J. Lipid. Res.* 1982. **23:** 327–334.

Supplementary key words glucosylceramide • galactosylceramide • lactosylceramide • globotriaosylceramide • globotetraosylceramide • normal fatty acids • 2-hydroxy fatty acids • sphingosine bases

Our knowledge about the concentration and distribution of glycosphingolipids in human extraneural tissue is scanty (1, 2). The glycolipids in renal, splenic, and intestinal tissue have been the subject of a few studies (1, 2), but the only comprehensive study of the liver glycolipids is the one published by Kwiterovich, Sloan, and Fredrickson in 1970 (3). This is remarkable since the liver is readily accessible to biopsy, which is useful in the diagnosis of inherited disorders of the glycolipid metabolism (4), and in assessment of the effect of enzymic replacement therapy (5, 6), and of the toxicity of certain drugs. Differentiation as well as malignant transformation of cells is accompanied by changes in the composition of the glycolipids in the cell membranes (7). In the light of the above observations, more accurate knowledge of the concentration of the glycosphingolipids in liver is necessary. During the last decade the methods for extracting and separating glycolipids have been improved (8, 9), and very sensitive chromatographic methods capable of measuring the concentrations of glycosphingolipids in even small biopsy specimens have been developed (10, 11). It was therefore decided to make a careful reinvestigation of the neutral and acidic glycolipids in liver tissue from young normal humans. The results could be used as reference values in the study of biopsy material from patients with metabolic disorders, and might at the same time shed some light on the possible function of the glycolipids as receptors, recognition substances, or adhesion molecules.

EXPERIMENTAL

Chemicals

All organic solvents and chemicals used were of analytical quality. Light petroleum (boiling range 42–45°C), used for GLC analyses of fatty acids and sphingosine bases, was redistilled and tested for purity as previously described (12). TLC plates, Kiselgel 60, 20×20

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; C-M-W, chloroform–methanol–water; TMS, trimethylsilyl. Ganglioside abbreviations follow the nomenclature system of Svennerholm (Svennerholm, L. 1977. *Eur. J. Biochem.* **79:** 11–21). G_{M3}, II^3NeuAc-LacCer; G_{D3}, $II^3(NeuAc)_2$-LacCer; L_{M1}, IV^3NeuAc-nLcOse$_4$Cer; G_{M2}, II^3NeuAc-GgOse$_3$Cer; G_{M1}, II^3NeuAc-GgOse$_4$Cer; G_{D1a}, IV^3NeuAc,II^3-NeuAc-GgOse$_4$Cer; G_{D1b},$II^3(NeuAc)_2$-GgOse$_4$Cer; G_{T1a}, $IV^3(NeuAc)_2$,II^3NeuAc-GgOse$_4$Cer; G_{T1b}, IV^3NeuAc,$II^3(NeuAc)_2$-GgOse$_4$Cer; G_{Q1b}, $IV^3(NeuAc)_2II^3(NeuAc)_2$-GgOse$_4$. Sphingosine nomenclature follows the system of Karlsson (Karlsson, K-A. 1970. *Lipids.* **5:** 878–891). t18:0, tri-OH-sphinganine; d18:0, sphinganine; d18:1, 4-sphingenine; d18:2, 4,cis14-sphingadienine; t20:0, tri-OH-eicosasphinganine; d20:0, eicosasphinganine; d20:1, 4-eicosasphingenine.

cm; HPTLC plates, Kiselgel 60, 20×20 cm, and Kiselgel G70–230, and 230–400 mesh, were obtained from Merck AG, Darmstadt, Federal Republic of Germany. Florisil, 60–100 mesh, was obtained from Floridin Co., Talahassee, FL. The anion exchange resin Spherosil-DEAE-Dextran was a gift from Institute Mérieux, Lyon, France. Sephadex G-25, fine, was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. D-Glucose, D-galactose, D-mannose, D-glucosamine hydrochloride, and D-galactosamine hydrochloride were all obtained from Pfahnstiehl Laboratories, Waukegan, IL. Vibrio cholerae sialidase was purchased from Behringwerke AG, Marburgh-Lahn, Federal Republic of Germany. β-Galactosidase and β-*N*-acetylhexosaminidase isolated from jack beans were gifts from Drs. S. C. and Y-T. Li, Tulane University, LA. All gangliosides and glycolipids used as references were prepared in this laboratory.

Tissue material

The livers were obtained from six subjects, who had died in accidents. The liver from an 11-year-old boy was used for the large scale isolation of glycosphingolipids. Total gangliosides were determined in liver samples (10–20 g) from the five other subjects, aged 5, 8, 15, 23, and 48 years. Surrounding connective tissue and large blood vessels were removed before homogenization.

Isolation of the glycosphingolipids

Extraction from the tissue. The liver (620 g) was homogenized in a scissor homogenizer with 3 volumes of water. A total lipid extract was obtained from the homogenate by addition of chloroform and methanol to give 10 volumes (w/v) of chloroform–methanol–water (C–M–W) 4:8:3 (v/v/v) (8). After centrifugation the tissue residue was reextracted with 5 volumes of C–M–W 4:8:3. The combined lipid extracts were filtered through Celite to remove insoluble tissue material.

Crude separation of the glycosphingolipids. **Fig. 1** shows a flow scheme of the procedures used for the isolation of glycosphingolipids. The total lipid extract was evaporated to dryness (with addition of isobutanol to prevent foaming) and dissolved overnight in 200 ml of C–M–W 60:30:4.5. Undissolved material was removed by centrifugation and the supernatant was evaporated to dryness and dissolved in chloroform. A crude separation of the glycosphingolipids was obtained by column chromatography on 200 g of silicic acid, 70–230 mesh. The following elution scheme was used: *1*) 10 vol (per g adsorbent) of chloroform; *2*) 10 vol of C–M 9:1; *3*) 15 vol of C–M 4:1; and *4*) 5 vol of C–M–W 30:60:20. Fraction 1 did not contain glycolipid material and was not analyzed further. Fractions 2 and 3 were purified further by column chromatography on 50 g of silicic acid, 230–400 mesh. The same elution scheme was used, but the C–M 9:1 and 4:1 eluents, respectively, were collected in 15-ml fractions and the elution was monitored by TLC. Fractions migrating as mono-, di-, and triglycosylceramides were pooled, and contaminating phospholipids were removed by peracetylation and chromatography on Florisil (13).

Fraction 4, from the silica column, contained all the gangliosides and higher neutral glycosphingolipids. The glycosphingolipids were separated into neutral and acidic lipids with the aid of the anion exchange resin Spherosil-DEAE-Dextran. The gangliosides were eluted with a discontinuous gradient of potassium acetate in methanol; this elutes the gangliosides according to the number of sialic acid moieties (9). The neutral fraction was purified by peracetylation and chromatography on Florisil (13). Contaminating phospholipids in the ganglioside fractions were hydrolyzed with 0.5 M KOH in methanol–water 1:1 for 12 hr at room temperature. The samples (10–20 g) from the five other livers were analyzed in the same way, except that the gangliosides were eluted directly with 0.5 M potassium acetate from the anion exchange column, and only total ganglioside NeuAc was determined.

Final separation and quantification of the glycosphingolipids. The final separation of glycosphingolipids into fractions with a uniform carbohydrate moiety was performed by preparative TLC. Monohexosyl ceramides were separated into glucosyl- and galactosylceramide on borate-impregnated TLC plates developed in C–M–2.5 M ammonia in water 40:10:1 (14). The other neutral glycolipids and sulfatide, eluted together with diglycosylceramides, were separated with C–M–W 65:25:4 as developing solvent. The amount of the individual neutral glycolipids was measured by determination of their hexose content by the orcinol method (15), and quantitative densitometry of the glycolipids separated on TLC plates together with 2.5, 5.0, and 7.5 nmol of each glycolipid. The plates were visualized with cupric acetate (16), and then scanned with a Zeiss KM3 TLC scanner at 450 nm and integration with a Varian 100 CDS computer.

The gangliosides were quantified with the resorcinol method (17). The composition of the mono-, di-, and polysialoganglioside fractions was determined by densitometric analyses at 620 nm of TLC plates visualized with resorcinol reagent (8). The solvent systems used were C–M–0.25% KCl (aqueous) 60:35:8, C–M–2.5 M ammonia 60:40:9, and propanol–0.25% KCl 3:1. The analyses were performed on both standard TLC and HPTLC plates.

The composition of the monosialoganglioside fraction was determined after the major part of ganglioside G_{M3} had been isolated by column chromatography with C–M–W 65:25:4 as eluent. The other gangliosides were isolated by preparative TLC and rechromatography in

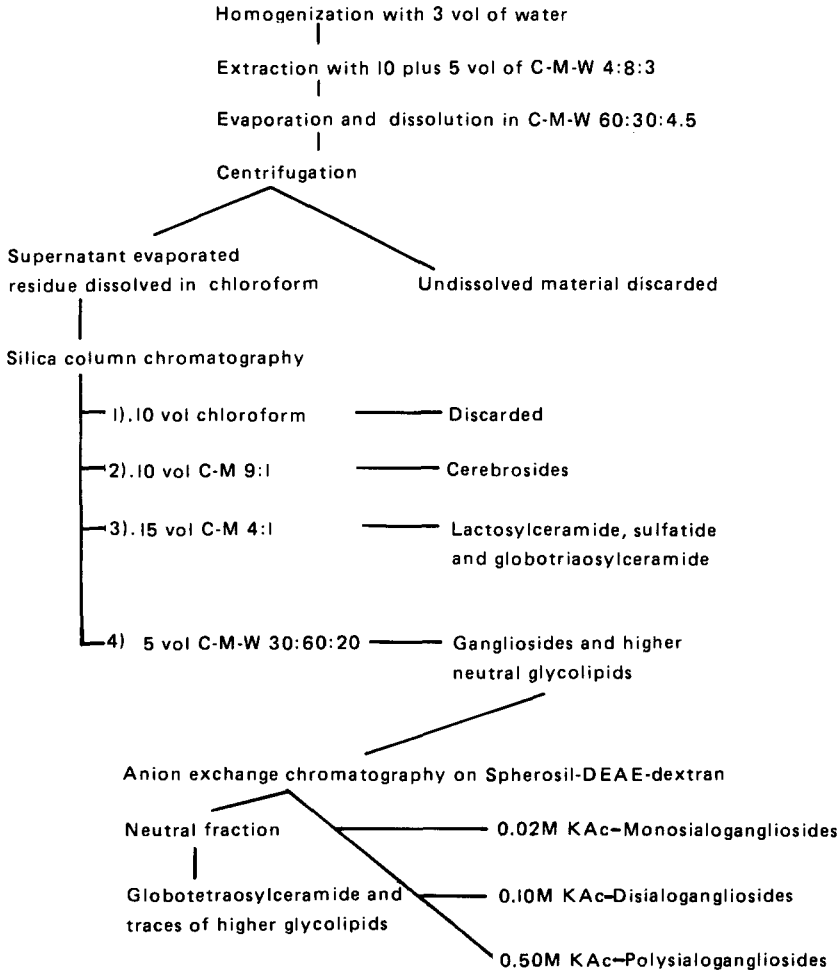

Fig. 1. Isolation procedure for the quantitative isolation of glycosphingolipids from the liver of an 11-year-old male. The different fractions were purified further as described in the text.

different solvents until homogeneity of the neutral carbohydrate moiety was achieved.

Determination of carbohydrate components

The carbohydrate components of the isolated glycolipids were quantitatively determined as their alditol acetates by GLC (12). The fatty acids and sphingosine bases were determined after hydrolysis in 1.0 M HCl in methanol–water 82:18 (18). The 2-hydroxy and normal fatty acids were separated as their methyl esters on TLC plates developed in methylene chloride. The 2-hydroxy fatty acids were then analyzed as their TMS derivatives. The proportion between normal and 2-hydroxy fatty acids was determined by adding 20 nmol of the methyl ester of C21:0 to the separated acids as internal standard. All GLC analyses were performed on a Perkin-Elmer F22 Gas Chromatograph connected to a HP 3352 Lab Data system. The alditol acetates were separated on a 3% ECNSS-M column, sphingosine bases on a 15% DEGS column, normal fatty acids on 3% OV-1, and 15% DEGS, and 2-hydroxy fatty acids on a 3% OV-1 column.

Enzymic hydrolysis

Gangliosides were also analyzed by sialidase hydrolysis in 100 μl 0.01 M Tris-maleate buffer pH 6.2 for 16 hr at 37°C. The products of the hydrolysis were analyzed on HPTLC plates developed in C–M–W 60:32:7, and visualized with orcinol or resorcinol spray. Neutral glycolipids were analyzed by sequential degradation with β-galactosidase and β-*N*-acetylhexosaminidase as described previously (19).

Analysis of the sialic acids

Aliquots from the ganglioside fractions, corresponding to about 300 nmol NeuAc, were pooled and hydrolyzed with 1 M formic acid for 30 min in a boiling water bath. Released sialic acid was isolated with anion exchange chromatography on Dowex 2×8, 200–400 mesh, and analyzed by TLC.

RESULTS

The total gangliosides were determined in six normal livers, and ranged from 190 to 248 nmol NeuAc/g, with a mean value of 212 nmol/g. The concentration of gangliosides in the large scale isolation was 214 nmol/g. The monosialoganglioside fraction, obtained after anion exchange chromatography, constituted 93.5% of the total ganglioside NeuAc; the disialoganglioside fraction comprised 5.1%, and the polysialoganglioside fraction, 1.4%. G_{M3} was the major ganglioside and constituted more than 90% of the total gangliosides. In addition to G_{M3}, nine other gangliosides were isolated from the monosialoganglioside fraction, but only three of them occurred in an amount that permitted definitive identification. They were shown to be G_{M2}, L_{M1} (sialosyl-lactoneotetraosylceramide), and G_{M1}. **Fig. 2** shows TLC of the gangliosides isolated from the monosialoganglioside fraction. The major disialoganglioside was G_{D3}, which constituted 2.6% of the total ganglioside NeuAc. The occurrence of gangliosides G_{D1a} and G_{D1b} was also established. As in the monosialoganglioside fraction, a large number of other gangliosides were also detected in the disialoganglioside fraction by TLC, but, owing to their low concentration, no attempt was made to isolate and purify them to homogeneity. The polysialoganglioside fraction contained three gangliosides, as judged by TLC. They were identified as G_{T1a}, G_{T1b}, and G_{Q1b}; the major ganglioside in this fraction was G_{T1b}. **Table 1** gives the concentration and distribution of the gangliosides isolated. The identification of the isolated gangliosides was based on TLC migration, comparison with known standards before and after sialidase treatment, and determination of their carbohydrate moieties (see Table 3 below). *N*-Acetylneuraminic acid was the only detectable sialic acid; the eventual existence of *O*-acetylated neuraminic acid could not be determined inasmuch as alkaline hydrolysis was used during the isolation procedure.

Table 2 gives the concentrations of the major neutral glycolipids and the sulfatides. The monohexoside fraction was separated into glucosyl- and galactosylceramides on borate-impregnated TLC plates. The substances migrated on standard TLC plates like authentic standards of glucosyl- and galactosylceramide, respectively. On borate-impregnated TLC-plates the glucosylceramide migrated in three bands, with the two faster ones like authentic glucosylceramide. The third band was isolated and proved to be glucosylceramide with 2-hydroxy fatty acids. Analyses of the carbohydrate components as alditol acetates revealed the presence of glucose and galactose,

TABLE 1. Ganglioside composition in the liver of an 11-year-old male

Ganglioside	nmole NeuAc/g	% NeuAc
Monosialogangliosides	200	93.5
G_{M3}	196	91.6
G_{M2}	0.5	0.2
L_{M1}	1.1	0.5
G_{M1}	1.4	0.7
Unidentified[a]	0.9	0.4
Disialogangliosides	11.0	5.1
G_{D3}	5.6	2.6
G_{D1a}	2.5	1.2
G_{D1b}	1.6	0.7
Unidentified[b]	1.3	0.6
Polysialogangliosides	3.0	1.4
G_{T1a}	0.1	0.05
G_{T1b}	2.4	1.1
G_{Q1b}	0.5	0.2
	Total 214.0	

[a] Fig. 2 shows a thin-layer chromatogram of six of the gangliosides in this fraction.

[b] This fraction contained at least five different gangliosides. Owing to the low concentration, no attempts to isolate them into purity were made.

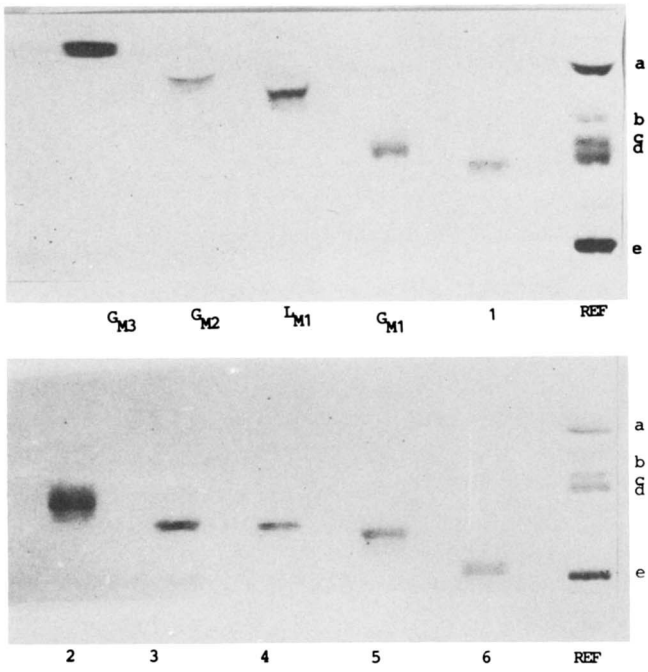

Fig. 2. Monosialogangliosides isolated from human liver. The gangliosides were isolated from the monosialoganglioside fraction obtained after anion exchange chromatography on Spherosil-DEAE-dextran. The gangliosides were then isolated as described in the text (Experimental). Ganglioside 1 (upper plate) and gangliosides 2–6 (lower plate) could not be definitively identified owing to the low amount obtained. Chromatographic conditions: HPTLC plates, Merck, Kiselgel 60, 10 × 20 cm; solvent: C–M–2.5 M ammonia 60:40:9; 40-min development. The reference gangliosides were from an adult human brain, with the addition of G_{M2} isolated from a Tay-Sachs brain; a) G_{M2}, b) G_{D3}, c) G_{M1}, d) G_{D1a}, e) G_{D1b} and G_{T1b}. The gangliosides were visualized with resorcinol reagent.

TABLE 2. Major glycosphingolipids isolated from the liver of an 11-year-old male

Glycosphingolipid	nmol/g
Glucosylceramide	34
Galactosylceramide	50
Lactosylceramide	54
Globotriaosylceramide	25
Globotetraosylceramide	14
Oligohexosylceramides	<1
Total amount neutral	~178
Sulfatide	1

respectively. The identification of the other asialo glycolipids was based on TLC migration, determination of the carbohydrate components (**Table 3**), and sequential enzymic degradation.

Ceramide composition. **Table 4** gives the fatty acid distribution and the proportions of normal (unsubstituted) and 2-hydroxy fatty acids of the isolated glycosphingolipids. The composition of the sphingosine bases is given in **Table 5**. Glucosylceramide, galactosylceramide, lactosylceramide, and ganglioside G_{M3} were all shown to contain both normal and 2-hydroxy fatty acids. There was a striking difference in the ceramide composition between the "visceral" gangliosides (G_{M3}, G_{D3}, and L_{M1}) and the gangliosides belonging to the gangliotetraose series. All the gangliotetraose gangliosides had a large proportion of stearic acid (>50%), and small proportions of lignoceric acid (24:0) and nervonic acid (24:1). The "visceral" gangliosides had only a small proportion of stearic acid (<10%), while C-24 fatty acids constituted nearly 50% of the fatty acids. The fatty acid distribution of the neutral glycosphingolipids resembled that of the "visceral" gangliosides. They were characterized by a large proportion of behenic acid (22:0), large proportions of C-24 fatty acids, but a very small proportion of stearic acid. Moreover the "visceral" gangliosides and the neutral glycolipids had a similar composition of the long chain bases. The major sphingosine base was d18:1, while the gangliotetraose gangliosides contained both d18:1 and d20:1 as major sphingosine bases.

DISCUSSION

The isolation procedure used in this study was chosen to achieve as complete an extraction and recovery of the glycosphingolipids as possible. The water-rich solvent mixture gave a total lipid extract from which the major portion of non-lipid contaminants could be easily removed, without any measurable loss of glycolipids, by evaporation and resolubilization in chloroform–methanol–water 60:30:4.5. For further purification of lipid extracts, solvent partition is generally used since it gives an upper phase with the gangliosides and non-lipid contaminants, and a lower phase with the bulk of the other lipids. This step was omitted because the least polar gangliosides, which are the major gangliosides in human liver, would be partitioned in both the upper and lower phase. Anion exchange chromatography without previous purification of the lipid extract is unsuitable for the large scale isolation of glycosphingolipids from sources where they are a minor part of the lipids. This is because very large columns and solvent volumes are necessary to prevent loss of monosialogangliosides. Seyfried, Ando, and Yu (20) used a column of Sephadex A-25, with ca. 1000-ml bed volume and a 9-liter total volume of the lipid extract applied to the column for the isolation of ganglioside G_{M3} from 550 g of human liver.

TABLE 3. Sugar composition of glycosphingolipids isolated from the liver of an 11-year-old male

Glycosphingolipid	Glc	Gal	GlcNAc	GalNAc	NeuAc[a]
	mol/mol glycosphingolipid				
Glucosylceramide	1.00				
Galactosylceramide	0.09	1.00			
Lactosylceramide	1.00	0.97			
Globotriaosylceramide	1.00	1.98			
Globotetraosylceramide	1.00	2.09		0.96	
Sulfatide	0.11	1.00			
G_{M3}	1.00	0.89			0.94
G_{D3}	1.00	0.97			2.10
L_{M1}	1.00	1.94	0.91		1.07
G_{M2}	1.00	0.95		0.87	1.04
G_{M1}	1.00	2.08		0.90	0.96
G_{D1a}	1.00	2.10		0.84	2.12
G_{D1b}	1.00	2.07		0.91	1.94
G_{T1b}	1.00	1.83		0.87	2.79

[a] The NeuAc was determined with the resorcinol method.

TABLE 4. Fatty acid composition of glycosphingolipids isolated from the liver of an 11-year-old male

Glycosphingolipid		%	16:0	18:0	20:0	22:0	22:1	23:0	23:1	24:0	24:1	25:0	25:1	26
								% of total fatty acids						
Neutral glycosphingolipids:														
Glucosylceramide	N	80	8	2	2	25	3	12	1	35	10	1	1	
	H	20			2	17	1	17	6	46	11	1	1	
Galactosylceramide	N	40	4	11	2	6	3	5	1	14	39	3	11	3
	H	60			1	9	1	18	4	33	22	7	6	3
Lactosylceramide	N	90	13	3	3	21	2	8	1	26	22	1	1	
	H	10			2	13	1	15	15	33	18	2	1	
Globotriaosylceramide	N	100	8	3	3	19	3	9	1	33	19	1	1	
Globotetraosylceramide	N	100	5	3	4	19	3	7	1	31	25	1	1	
Gangliosides:														
G_{M3}	N	70	7	9	3	21	3	10	1	30	16	1	1	
	H	30			1	20	2	17	6	37	15	3	1	
G_{D3}	N	100	10	8	4	18	3	10		32	13	1	1	
L_{M1}	N	100	13	8	3	19	3	7	1	30	14	2	1	
G_{M2}	N	100	9	52	8	12	3	3		9	2	1		
G_{M1}	N	100	6	50	8	10	2	4		13	3	1		
G_{D1a}	N	100	5	46	9	13	4	6		16	1	1		
G_{D1b}	N	100	4	61	13	12	3	2		5	1			
G_{T1b}	N	100	7	65	13	8	2	1		4	1			

N, normal fatty acids; H, 2-hydroxy fatty acids.

DEAE-Sephadex does not give a complete recovery of the most polar gangliosides (8) and was therefore not suitable for our purpose, i.e., to secure complete recovery of all the liver glycolipids, including the most polar ones. The use of silica column chromatography is practical in the isolation and separation of glycosphingolipids from organs rich in simple lipids, as these can be easily removed without losses of the glycosphingolipids. The separation of the lipid extract into four fractions, the first with the simple lipids, which could be directly discarded, the second and the third with the major portion of the simple neutral glycolipids, and the fourth with all the gangliosides and higher neutral glycolipids, proved best for quantitative isolation and further separation of the glycolipids. The fourth fraction was applied directly to anion exchange chromatography on Spherosil-DEAE-

TABLE 5. Sphingosine composition of glycosphingolipids isolated from the liver of an 11-year-old male

Glycosphingolipid	t18:0	d18:0	d18:1	d18:2	t20:0	d20:1
			% of total sphingosine bases			
Neutral glycosphingolipids:						
Glucosylceramide	3	4	87	3	2	1
Galactosylceramide	1	3	92	3	1	2
Lactosylceramide	2	4	86	4	2	3
Globotriaosylceramide	2	4	86	5	2	3
Globotetraosylceramide	2	5	86	4	1	3
Gangliosides:						
G_{M3}	4	5	83	3	2	4
G_{D3}	3	4	85	3	2	5
L_{M1}	3	3	89	3	1	2
G_{M1}	2	4	70	2	1	21
G_{D1a}	2	4	65	2	2	27
G_{D1b}	2	4	51	1	1	43
G_{T1b}	1	2	56	2	1	38

Dextran, and the gangliosides were eluted according to the number of sialic acids.

The values of the concentration of lipid-bound sialic acid, 214 nmol of *N*-acetylneuraminic acid per g fresh weight (in the large scale isolation) and 190, 196, 209, 215, and 248 nmol/g in the five other liver samples analysed, are essentially the same as that reported by Seyfried et al. (20), viz 213 nmol/g. Although the number of liver samples assayed was small, the ganglioside value for liver seemed to be fairly constant. Kwiterovich et al. (3) reported a wider range for six samples of human liver, 58–192 nmol/g, and a lower mean value, 133 nmol/g, but their experimental procedure included a partition step, for which reason the low value and wide range could be explained by losses of gangliosides in the lower phase during the partition.

With our isolation and separation methods we were able to isolate gangliosides with up to four sialic acids. These gangliosides were of the gangliotetraose series and their existence in human liver has never been demonstrated before. A large number of minor monosialogangliosides were also isolated and, though they were all in very low concentrations, they might nevertheless play an important role on the cell membrane as receptors for various substances, or as recognition structures for adjacent cells, which are some of the assumed purposes of gangliosides (21).

The gangliosides of the liver tissue might be derived from liver cells, reticulohistiocytic cells, connective tissue, or nerve tissue. The present study does not warrant any conclusions about the possible localization of the gangliosides to certain structures. The ceramide composition of the individual gangliosides, however, suggests that they are synthesized in at least two different sites. The gangliosides belonging to the gangliotetraose series had a ceramide portion that showed certain similarities with that of brain gangliosides (18). In the liver gangliosides of the gangliotetraose series, as well as in gangliosides isolated from brain tissue, stearic acid was the major fatty acid, and both sphingenine and eicosasphingenine were the major sphingosine bases. However, the liver gangliosides contained more behenic acid (22:0) and C-24 fatty acids than the corresponding gangliosides of the brain. Three other gangliosides, G_{M3}, G_{D3}, and L_{M1} (which occur in much higher concentrations in extraneural organs, particularly in mesenchymal tissues, than in brain tissue) had a ceramide composition that differed remarkably from that of the gangliosides of the gangliotetraose series. In these three "visceral" gangliosides the proportion of C-24 fatty acids was much larger, and that of stearic acid smaller, than in the "brain" gangliosides, and they also contained C-18 sphingosines almost exclusively. Ganglioside G_{M3} contained 2-hydroxy fatty acids also. The presence of 2-hydroxy fatty acids in gangliosides was first reported by Rauvala (22) in human kidney gangliosides. Seyfried et al. (20) confirmed this finding in G_{M3} isolated from human liver. The value found by them was as high as 43%, which is higher than that found by us, namely ca. 30%. The neutral glycolipids closely resembled the three "visceral" gangliosides in ceramide composition, which suggests that they are synthesized from the same ceramide pool.

The neutral glycolipid fraction was approximately as large as the ganglioside fraction. The existence of galactosylceramides in human liver was clearly demonstrated for the first time. Coles, Hay, and Gray (23) were the first to report sex specific differences in the glycolipid composition and strain specific differences have also been reported (24). Specific differences in glyco-conjugate composition in the human blood group system have been known for some time, but it has not been known that the ABO blood group system will also influence the pattern of the simple monoglycosylceramides. Nilsson and Svennerholm[1] recently observed a wide variation of the ratio of glucosyl- to galactosylceramides in red blood cells with ABO blood group. It is possible that previous studies of the neutral liver glycolipids have, by chance, been performed on material from subjects belonging to a blood group in which the proportion of galactosylceramide is very low, and thus was not reported earlier. Although large efforts were made to remove all large blood vessels and nerves from the liver tissue samples, it cannot be excluded that a portion of the galactosylceramide was derived from nerve tissue. It is also interesting to note that it proved possible to isolate glucosylceramides with 2-hydroxy fatty acids. Hammarström (25) demonstrated the occurrence of glucosylceramide with 2-hydroxy fatty acids in the brain of young mice, but to our knowledge the existence of glucosylceramide with 2-hydroxy fatty acids in human tissues has not been demonstrated previously.

This study has shown a much higher complexity of the glycolipids, especially the gangliosides, in human liver than previously known. For the understanding of their biological function, further studies about their structures and cellular localization should be carried out.

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