Gangliosides of human, bovine, and rabbit plasma

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Abstract Gangliosides were isolated from human, bovine, and rabbit plasma and were quantified by gas–liquid chromatography. Purification was achieved by sequential use of partitioning in solvents, DEAE-Sephadex chromatography, base treatment, and silicic acid chromatography. Human and bovine plasma yielded slightly more than 1 μmole of lipid-bound sialic acid/100 ml; for rabbit plasma the value was 0.28 μmole/100 ml. The total bovine plasma ganglioside fraction contained equal amounts of *N*-acetylneuraminic and *N*-glycolylneuraminic acids, rabbit plasma gangliosides had about 1% of the latter, and the human plasma sample contained only the former. Thin-layer chromatography revealed important differences among the plasmas from the three species, but all possessed hematosides and hexosamine-containing gangliosides. The approximate ratios of these two categories, based on sialic acid content, were (hematosides: hexosamine-type): human, 2:1; rabbit, 3:2; and bovine, 2:3. The fatty acid compositions of both categories were characteristic of extraneural gangliosides and included six major acids: palmitic, stearic, behenic, tricosanoic, lignoceric, and nervonic. The major long-chain base in each sample was sphingosine, while only a trace of the C_{20} isomer was detected.

Supplementary key words hematosides · sialic acid · fatty acids · sphingosine bases · DEAE-Sephadex

Gangliosides were first recognized as constituents of blood by their discovery in erythrocytes by Yamakawa and Suzuki in 1951 (1). A recent report by Marcus and Cass (2) indicated the presence of lipid-bound sialic acid in human plasma, and this was subsequently confirmed by Tao and Sweeley (3), who isolated hematoside[1] from the same source. The present study extends the survey to bovine, rabbit, and human plasma and demonstrates the presence of both hematosides and hexosamine-containing gangliosides in all three species. Some portions of this work have been presented in preliminary communications (4, 5).

MATERIALS AND METHODS

Plasma preparation and extraction

Fresh bovine blood was obtained from a local abattoir; rabbit blood was from normal mature laboratory animals. Both samples were treated with ACD anticoagulant and centrifuged at 2500 *g* for 30 min at 10°C. The supernatant fluid was recentrifuged at 8000 *g* for 30 min to obtain the final cell-free plasma. Human plasma from an adult male, treated with ACD, was obtained from the blood bank and was centrifuged at 8000 *g* for 30 min.

Between 20 and 100 ml of plasma was placed in a large round-bottomed flask and 10 vol of C–M 1:1 was added. The mixture was stirred vigorously at room temperature for 1 hr and filtered through a sintered glass funnel of medium porosity. The insoluble portion was reextracted with 5 vol of C–M 1:2 and filtered, and the filtrates were combined. Further treatment in the same manner with 0.5 vol of isotonic saline plus 5 vol of C–M 1:2 did not extract additional ganglioside.

Purification

(a) *Solvent partitioning.* The above combined filtrate was evaporated to dryness and redissolved with C–M 1:1 in 30-ml centrifuge tubes using vortex mixing and mild sonication (a small amount of insoluble material was later solubilized on addition of water.). Each tube was adjusted to a composition of C–M 2:1, the final

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; NANA, *N*-acetylneuraminic acid; NGNA, *N*-glycolylneuraminic acid; EDTA, ethylenediaminetetraacetate; C–M, chloroform–methanol; ACD, acid–citrate–dextrose.

[1] The term "hematoside" is used here to designate the subcategory of ganglioside which lacks hexosamine. The principal form is lactosylceramide with sialic acid bonded to the C-3 hydroxyl of galactose. The second category is designated as "hexosamine-containing gangliosides" or "slow-migrating species."

volume being 15–20 ml/10 ml of original plasma. This was partitioned with 0.2 vol of water according to Folch, Lees, and Sloane Stanley (6), and the lower phase was repartitioned three times with "pure solvents upper phase" containing no salt. The combined upper phases were evaporated to near dryness, dissolved in 15–25 ml of water, and 1–2 ml of 0.5 M EDTA (tetrasodium salt) was added. The mixture was dialyzed for 2 days in the cold against distilled water, and the bag contents were then lyophilized to dryness.

(b) *DEAE-Sephadex chromatography*. The above residue was dissolved in 50 ml of methanol–chloroform–water 60:30:8 (solvent A) and applied to a column of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, Piscataway, N.J.). The DEAE-Sephadex was prepared by washing 2.2 g of the resin several times with methanol–chloroform–0.8 M sodium acetate 60:30:8 (solvent B) followed by equilibration in the same solvent overnight. This treatment converted the resin from the chloride to the acetate form and conditioned it for use with organic solvents. The resin was then washed several times with solvent A; the slurry was poured into a column 14 mm in diameter and washed with 100–200 ml of the same solvent. After slow application of the sample, the column was eluted with 150 ml of solvent A to remove all noncharged lipid contaminants. Gangliosides together with a small amount of acidic lipid contaminants were then eluted with 180 ml of solvent B, and the solvent was removed by evaporation.

(c) *Base treatment*. The above dried residue from solvent B, containing sodium acetate, was treated with 10–20 ml of 0.2 N NaOH in CH_3OH and incubated at 37°C for 1 hr with frequent agitation. The sample was evaporated to one-fourth volume, taken up in an excess of water, and dialyzed for 2 days in the cold against distilled water. The bag contents were lyophilized to dryness.

(d) *Unisil chromatography*. 1 g of Unisil, 200–325 mesh silicic acid (Clarkson Chemical Co., Williamsport, Pa.), was packed as a chloroform slurry in a column 12 mm in diameter and washed with 50 ml of chloroform. Unisil was used without prior activation. The above dry sample was dissolved in 6 ml of C–M 1:1 with the aid of sonication and slight warming; 14 ml of chloroform was added, bringing the solvent ratio to 85:15, and the mixture was applied to the Unisil. Elution with 200 ml of C–M 85:15 removed substances such as sulfatides and free fatty acids which may have been carried through to this stage. The ganglioside fraction was then eluted in relatively pure form with 150 ml of C–M 2:3; the same solvent was used to rinse the original flask employed for lyophilization and the rinsings were applied to the Unisil column. Distilled solvents were used in the final stages of purification.

Sialic acid analysis

Quantification of sialic acid was carried out by the GLC procedure described previously (7). Ganglioside samples containing 0.5–5.0 μg of sialic acid were dissolved in 2 ml of 0.05 N HCl in CH_3OH and heated at 80°C for 1 hr. After extraction with hexane to remove small quantities of fatty acid esters, the methanolic solution containing methyl ester methyl ketosides of sialic acid was evaporated to dryness and the products were converted to trimethylsilyl ether derivatives. Quantification by GLC was based on the major β peak only, using an empirical calibration curve.

Since the yield of β-ketoside from methanolysis differed to a small but measurable extent for hematosides compared with hexosamine-containing gangliosides (brain ganglioside mixture), the calculation for sialic acid in each type of plasma was based on a weighted average of the slopes for these two standard curves. The calculated results were virtually the same when we employed a simple average slope computed from the standard curves for hematosides and hexosamine-containing gangliosides (7). This gave rise to the following equation:

$$\text{Sialic acid } (\mu g) = (1.67)(R)(IS)$$

where R is the ratio of the peak area of sialic acid β-methyl ketoside methyl ester to that of internal standard (phenyl *N*-acetyl-α-D-glucosaminide) as measured on the gas–liquid chromatogram, IS represents the quantity of added internal standard (μg), and 1.67 is the composite constant.

GLC analyses were carried out with a Hewlett-Packard F & M model 402 instrument equipped with flame ionization detector. Helium carrier gas was employed at a flow rate of 70 ml/min and a pressure of approximately 40 psi. Column packings, obtained from Supelco, Inc. (Bellefonte, Pa.), were of two types: OV-1, 3% on Chromosorb W HP (100–120 mesh), and OV-225, 3% on Supelcoport (100–120 mesh). Both columns were glass, U-shaped, 6 ft × 4 mm, and were operated at 205°C. Peak areas were measured with an Infotronics CRS-101 electronic integrator.

Neuraminidase reaction and quantification of hematosides

Ganglioside samples containing 0.1–0.3 μmole of sialic acid were dissolved in 1 ml of 0.1 M acetate buffer (pH 5) containing 0.1% $CaCl_2 \cdot 2H_2O$. A small volume of neuraminidase from *Clostridium perfringens* (type VI, Sigma Chemical Co., St. Louis, Mo.) containing 0.1 unit of activity was added. The solution was covered with 0.2 ml of toluene and incubated at 37°C for 2–3 days. During this period four more additions of 0.1 enzyme unit were made at intervals. The toluene was removed with a stream of nitrogen and the reaction mixture was treated

with 20 vol of C–M 2:1. The resulting precipitate was removed by filtration, and the filtrate was evaporated to dryness. The lipid residue was suspended in a small volume of dilute EDTA and dialyzed for 2 days in the cold against distilled water. After lyophilization the dry residue was suspended in a small volume of chloroform and applied to a column containing 1 g of Unisil packed with chloroform. Elution with 50 ml of chloroform was followed by elution with 150 ml of C–M 85:15, the latter solvent removing lactosylceramide (the neuraminidase product of hematoside). Monosialoganglioside, the neuraminidase product of the slower-migrating gangliosides, was eluted with C–M 2:3. GLC quantification of lactosylceramide was carried out according to the procedure of Vance and Sweeley (8); monosialoganglioside was assayed by the above GLC procedure.

Fatty acid and long-chain base analyses

The fatty acid compositions of hematosides and hexosamine-type gangliosides were determined separately by utilizing the two fractions resulting from neuraminidase treatment: lactosylceramide and monosialoganglioside. Samples containing 0.2–0.4 μmole of glycolipid were dissolved in 2 ml of 0.75 N HCl in CH_3OH and heated at 80°C for 20 hr. Fatty acid methyl esters were extracted with hexane and processed as described previously (9); a TLC purification step preceding GLC was essential to remove contaminants. GLC analysis was performed with two types of columns: OV-1 (nonpolar) and EGSS-X (polar). Standards of fatty acid methyl esters, obtained from Applied Science Laboratories (State College, Pa.) were used for identification. Quantification was carried out with the OV-1 column after establishing identity with both columns. Peak areas were measured with an Infotronics CRS-101 electronic integrator, and data were reported as weight percentages (no correction for possible differences in detector response).

Long-chain bases were analyzed according to the procedure described previously (9). To first liberate the bases, samples containing 0.2–0.4 μmole of ganglioside (or lactosylceramide) were heated in 2 ml of methanol–water–concentrated HCl 82:9.4:8.6 at 70°C for 22 hr, according to the procedure of Gaver and Sweeley (10). The isolated bases were oxidized with periodate, as described by Sweeley and Moscatelli (11), and the resulting aldehydes were analyzed by GLC on both OV-1 and OV-225 columns (vide supra). Parallel samples were hydrogenated prior to methanolysis, and the resulting saturated bases were processed similarly. Standard aldehydes were prepared from hydrogenated and normal brain ganglioside standards.

Thin-layer chromatography

Gangliosides were chromatographed on precoated plates of silica gel G, 250 μm thick, obtained from Analtech, Inc. (Newark, Del.). The plates were freshly activated by heating at 110°C for 40 min; they were then cooled in a desiccator. Two ascending runs in succession were carried out with chloroform–methanol–2.5 N NH_4OH 60:40:9 as solvent, the plate being thoroughly dried in a vacuum desiccator between runs. Resorcinol–HCl reagent (12) was employed as spray to detect gangliosides. Lactosylceramide (product of neuraminidase) was identified by TLC with chloroform–methanol–2.5 N NH_4OH 65:35:8 as solvent. Fatty acid methyl esters were purified on plates precoated with 250 μm of silica gel HR (Analtech), using *n*-hexane–ethyl ether 7:3 as solvent. The latter separated normal from hydroxy fatty acid esters, which were run as standards and detected with water spray. In these runs the plate was first washed by allowing C–M 2:1 to migrate the length of the plate before activation. The zones corresponding to normal and hydroxy fatty acid esters were each eluted with C–M 85:15.

RESULTS

Quantification

After purification, lipid-bound sialic acid was quantified by GLC. This procedure allows simultaneous measurement of NANA and NGNA, provided methanolysis is carried out under mild conditions to avoid cleavage of sialic acid *N*-acyl bonds (7). Even these mild conditions, however, cause a small amount of *N*-acyl cleavage, and each sialic acid thus gives rise to three GLC peaks (Fig. 1). Only the largest of these (methyl β-ketoside methyl ester) was used for quantification. This was done in an empirical manner, employing standards of brain ganglioside mixture and hematosides; since these two categories give somewhat different yields, an average (or weighted average) of the slopes for the two standard curves was used for maximum accuracy. The method has proved highly sensitive and reliable for ganglioside preparations from diverse sources, provided the gangliosides have been purified to a reasonable degree.

The ganglioside fraction of bovine plasma contained approximately equal amounts of NANA and NGNA,

TABLE 1. Ganglioside content of plasma

	Sialic Acid	NGNA
	μmoles/100 ml	%
Human (4)	1.13 ± 0.17	0
Bovine (3)	1.31 ± 0.13	50.9 ± 2.8
Rabbit (6)	0.28 ± 0.03	1.1 ± 0.3

Data are averages of three or more samples ± SD. Plasma volumes were corrected for the volume of ACD. Numbers in parentheses represent the number of independent analyses.

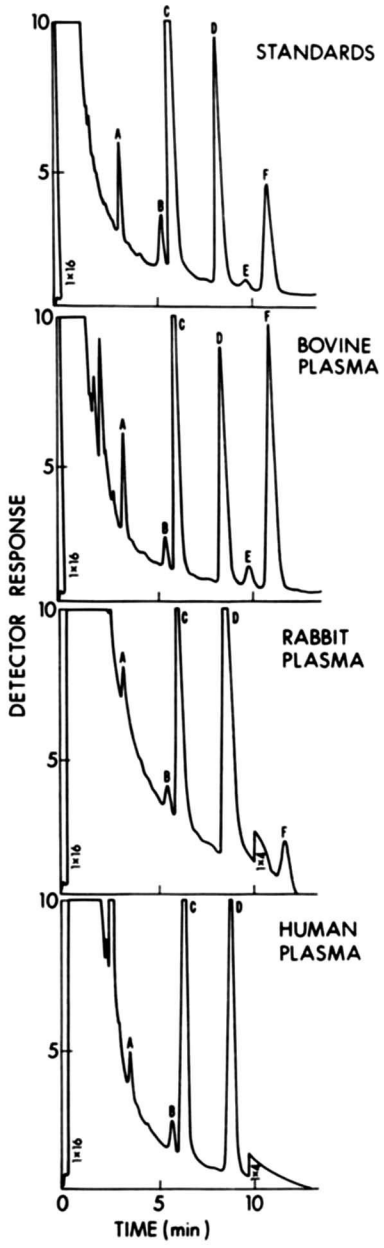

FIG. 1. Gas–liquid chromatogram of sialic acids on OV-1. Samples were heated for 1 hr at 80°C in 0.05 N methanolic HCl, and the products were converted to TMS derivatives. The 6-ft column was operated isothermally at 205°C. Peak identification: *A*, methoxyneuraminic acid methyl ester (deacylated product); *B*, α-methyl ketoside methyl ester of NANA; *C*, β-methyl ketoside methyl ester of NANA; *D*, phenyl *N*-acetyl-α-D-glucosaminide (internal standard); *E*, α-methyl ketoside methyl ester of NGNA; *F*, β-methyl ketoside methyl ester of NGNA. Peak *F* was discernible in all samples except human plasma gangliosides.

while that from rabbit plasma had only a small proportion of the latter (Table 1 and Fig. 1). The identification of these two sialic acids was confirmed by GLC with the more polar OV-225 column (7). The human plasma sample contained NANA but no trace of NGNA.

Ganglioside yields for the three species are summarized in Table 1. Slightly more than 1 μmole of lipid-bound sialic acid/100 ml was isolated from human and bovine plasma, while rabbit plasma yielded approximately one-fourth this quantity. These species differences were substantiated for several samples from a number of individuals. The values in Table 1, representing yields, are probably close to the true plasma levels, since recent experiments[2] with tissue gangliosides subjected to the same purification procedure indicated recoveries of about 93%. The ratio of hematoside to hexosamine-containing ganglioside in plasma, computed on the basis of sialic acid, also differed for the three species: human, 2:1; rabbit, 3:2; and bovine, 2:3.

Thin-layer chromatography

TLC patterns of total plasma ganglioside samples are depicted in Fig. 2. Significant differences were evident between species, although all three samples contained a mixture of hematosides and slower-migrating gangliosides. The presence of hexosamine in the latter was demonstrated by the GLC technique (13), both *N*-acetylgalactosamine and *N*-acetylglucosamine being detected for all three species. These two sugars were present in a ratio of 16:10, respectively, in the human and bovine plasma samples. Glucose and galactose were the other sugars identified, besides sialic acid.

The results of neuraminidase treatment are shown in Fig. 3. Hematosides were removed through conversion to lactosylceramide (not visible), and the latter was

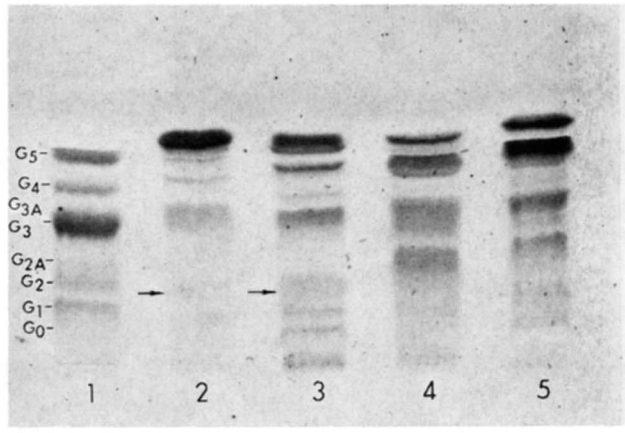

FIG. 2. Thin-layer chromatogram of plasma gangliosides. Channel *2*, human; *3*, rabbit; *4*, bovine. Channel *1* is bovine brain ganglioside standard plus Tay-Sachs ganglioside (G_5). Channel *5* is ganglioside mixture from bovine adrenal medulla; the top two bands are NANA-hematoside and NGNA-hematoside, respectively. Silica gel G, 250 μm thick, precoated (Analtech); two ascending runs in chloroform–methanol–2.5 N NH_4OH 60:40:9; resorcinol spray. All bands were purple except those indicated with arrows, which were yellow-brown. The symbols are based on the system of Korey and Gonatas (18). For correlation with other systems, see Ref. 19.

[2] Yu, R. K., and R. W. Ledeen. Unpublished observations.

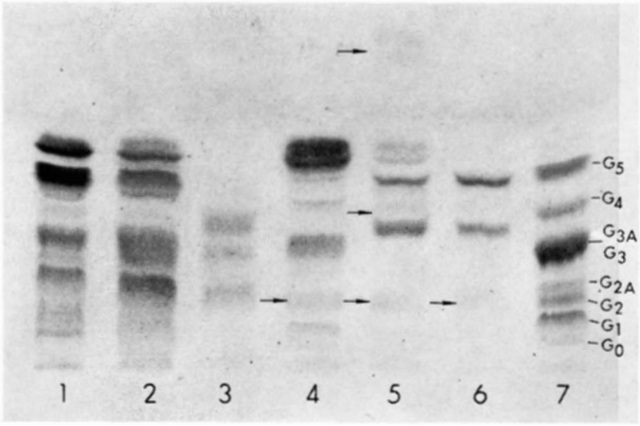

FIG. 3. Thin-layer chromatogram of plasma gangliosides before and after neuraminidase treatment. Channel *2*, bovine before treatment; *3*, bovine after; *4*, human before; *5*, human after; *6*, rabbit after. Channel *1* is ganglioside mixture from bovine adrenal medulla and *7* is bovine brain ganglioside standard plus Tay-Sachs ganglioside (G_5). Conditions same as Fig. 2. All bands were purple except those indicated with arrows, which were yellow-brown. The symbols are based on the system of Korey and Gonatas (18). For correlation with other systems, see Ref. 19.

identified in each of the three samples by a separate TLC system. The presence of fast-moving sialic acid-positive bands after exhaustive neuraminidase reaction indicated the possible presence of Tay-Sachs ganglioside (G_5 = G_{M2}) or an unknown species. This applied particularly to the human and rabbit samples. The slow-migrating gangliosides lost only part of their sialic acid during exhaustive neuraminidase treatment, giving products which migrated in the general vicinity of the major brain monosialoganglioside. The bovine sample had additional slower-migrating bands.

Analysis of apolar constituents

After neuraminidase treatment, lactosylceramide was separated from the remaining enzyme-resistant (presumably monosialo-) gangliosides by Unisil chromatography. Analysis of these two fractions gave the lipophilic compositions for hematosides and hexosamine-containing gangliosides, respectively. The fatty acid patterns (Table 2) of the two groups differ to some extent, even though derived from the same plasma, but all samples displayed the characteristic extraneural pattern with significant amounts of 16:0, 18:0, 22:0, 24:0, and 24:1. No hydroxy fatty acids were found in any of the fractions.

Long-chain base composition of human plasma gangliosides is summarized in Table 3. Since this type of analysis seems especially prone to artifact formation (9), the aldehyde products were identified by comparison with standards on both OV-1 and OV-225 columns as well as by semilog plots. Additional confirmation was obtained with the saturated aldehydes generated from hydrogenated gangliosides, both columns again being employed. Sphingosine was by far the most abundant base in both hematosides and hexosamine-containing gangliosides, while minor amounts of the C_{16} and C_{17} homologs were also detected. No eicosasphingenine was found in the hematoside fraction, but a very small amount of this C_{20} sphingosine was detected in the hexosamine-containing gangliosides.

TABLE 2. Fatty acid composition of plasma gangliosides

	Human		Bovine		Rabbit
	Hematoside	Hexosamine-containing	Hematoside	Hexosamine-containing	Hexosamine-containing
	%		%		%
14:0[a]	4.8		4.1		
15:0	1.5	1.1	1.6	0.2	1.7
16:0	25.0	26.8	40.7	25.5	22.6
16:1	0.8	trace	1.8	1.2	0.8
17:0	1.0	0.1	0.6	0.9	0.2
18:0	18.4	28.3	19.0	22.8	20.6
18:1	0.7	2.5	1.9	3.7	4.2
19:0	trace	0.2	trace	0.1	0.6
20:0	6.1	4.1	1.1	2.8	8.8
21:0	1.4		0.7	0.4	
22:0	16.4	13.1	8.7	14.5	16.1
22:1		trace		trace	0.9
23:0	9.1	3.6	6.1	9.8	6.4
24:0	12.2	9.5	9.8	12.6	7.1
24:1	2.7	10.8	3.9	5.6	10.4

Fatty acids were analyzed as methyl esters on an OV-1 column; identifications were confirmed with an EGSS-X column. Data are presented as percentages based on peak areas. Hematosides from rabbit plasma were not analyzed.

[a] Number of carbon atoms: number of double bonds.

TABLE 3. Long-chain base composition of human plasma gangliosides

Parent Base	Aldehyde	Hematoside	Hexosamine-containing Gangliosides
		%	%
d16:1[a]	14:1	7.1	5.7
d17:1	15:1	2.5	2.5
d17:0	15:0	5.3	2.8
d18:1	16:1	82.1	88.0
d18:0	16:0	3.1	0.8
d20:1	18:1		0.2

Long-chain bases were analyzed as aldehydes following periodate oxidation. Data are presented as percentages based on peak areas. Quantification was performed with an OV-225 column; identifications were confirmed with OV-1.

[a] Number of hydroxyl groups–number of carbon atoms: number of double bonds.

DISCUSSION

In the early stages of this work, different extraction procedures were compared for relative efficiency. The procedure described under Methods, involving direct

extraction of whole plasma with C–M, was finally adopted as the method of choice because of simplicity and favorable yields. Satisfactory results were also obtained with an alternate procedure wherein the plasma was first dialyzed (with added EDTA) and the lyophilized residue was then extracted with chloroform–methanol–water 10:10:2. In this case, however, re-extraction of the protein residue with chloroform–methanol–0.12 M NaCl 10:10:2 was necessary to remove the last 10–15% of lipid-bound sialic acid and thus bring the yield to the same level as that obtained with direct C–M extraction. EDTA was added to samples before dialysis to ensure removal of divalent cations which could have a deleterious effect on subsequent chromatography. A procedure involving direct extraction of the freeze-dried residue of plasma gave reduced yields in some cases.

Purification of the ganglioside fraction proved difficult because of the very small amount of this material relative to total lipid (ca. 0.7% for human plasma) and because plasma contains highly polar gangliosides as well as the less polar hematosides. A suitable procedure must give high yields of both types and also ensure removal of the small quantity of glycoprotein which invariably co-dissolves with lipid in the original C–M extraction. Satisfactory results were obtained with the sequence outlined: solvent partitioning, DEAE-Sephadex chromatography, base treatment, and Unisil chromatography.

Solvent partitioning, as normally performed with salt in the aqueous phase, is not satisfactory for tissues and fluids which contain substantial hematoside because of the latter's tendency to remain in the lower phase. However, repeated partitioning without salt, as employed here, gives virtually quantitative recovery of hematoside in the combined upper phase. On the other hand, a disadvantage of this type of exhaustive partitioning is that it results in considerable lipid contamination in the upper phase. Subsequent steps were designed to eliminate these impurities. Removal of excess salts by dialysis was necessary before applying the sample to DEAE-Sephadex. The latter was used as an ion exchange resin and was found to be a highly effective means of removing the large bulk of lipid contaminants which are dipolar ions or uncharged molecules and hence pass through in neutral solvent. Gangliosides (and other acidic lipids) bind to the resin when the latter is in the acetate form and they may be efficiently eluted with C–M containing aqueous acetate. However, a rather large excess of resin exchange equivalents was found necessary to bind all the applied ganglioside. Although DEAE-Sephadex is generally used with aqueous solvents, the mixed organic–aqueous solvent used here presented no difficulties provided preconditioning was carried out as described. In many respects the use of DEAE-Sephadex was found to be more effective and convenient for this particular purpose than the more traditional DEAE-cellulose.

Acidic lipid contaminants which partitioned into the upper phase were eluted together with gangliosides, and most of these were destroyed by the subsequent base treatment. The final chromatography on Unisil removed sulfatides and free fatty acids by elution with C–M 85:15, and gangliosides were then eluted in relatively pure form with C–M 2:3. The use of these two columns in sequence was found to be effective in removing protein contaminants as well. A similar purification procedure employing DEAE-Sephadex and Unisil has been utilized for gangliosides of a variety of tissues.[2]

Since hematoside comprises approximately two-thirds of the total human plasma ganglioside, the yield of this constituent in the present study is about twice the value (0.33 μmole/100 ml) reported by Tao and Sweeley (3). These workers used preparative TLC to isolate hematoside, and the difficulty in eluting gangliosides quantitatively from silica gel G (13) may account for all or part of this discrepancy. Ganglioside types other than hematoside were not reported by Tao and Sweeley (3). It is interesting to note that the concentration of lipid-bound sialic acid in human plasma is close to that reported for glucosylceramide and somewhat higher than that for the other glycosylceramides (8).

The present study demonstrates the existence of both hematosides and hexosamine-containing gangliosides in the plasma of the three species examined. The hexosamine-containing gangliosides resemble their brain counterparts in retaining a portion of neuraminidase-resistant sialic acid following exhaustive reaction with the enzyme. However, individual ganglioside structures have not yet been determined, and the presence of glucosamine indicates that some structures must have different oligosaccharide moieties than the characteristic species of brain. Glucosamine-containing gangliosides have been found in bovine spleen and kidney (14), human spleen (15), and human platelets;[3] these were present together with larger quantities of galactosamine-containing gangliosides and hematosides.

The lipophilic constituents showed the general characteristics of most extraneural gangliosides, i.e., the presence of several major fatty acids and predominance of the C_{18} long-chain base. The fatty acid pattern of the human hematoside sample showed general agreement with values reported by Tao and Sweeley (3). The long-chain base distribution resembled that previously found in adrenal medulla gangliosides (9) but differed from that reported in neutral glycosylceramides of bovine serum (16); the latter included significant amounts of

[3] Marcus, A. Personal communication.

C_{16}, C_{17}, C_{19}, and C_{20} homologs besides sphingosine and dihydrosphingosine.

Neither the origin nor the function of plasma gangliosides is known at present. The presence of only a fraction of a percent of eicosasphingenine in the hexosamine-containing gangliosides would seem to eliminate brain as a significant source. A recent in vivo study with porcine blood (17) demonstrated that certain of the plasma glycosphingolipids in this species are derived from the erythrocyte membrane, but similar determinations have not yet been carried out for the gangliosides. It will be of interest to know whether these substances can be absorbed from plasma into the erythrocyte membrane in the same manner that has been recently demonstrated for the Lewis blood group antigens (2). Studies are also in progress to determine whether gangliosides from plasma are able to cross the barriers into brain and/or cerebrospinal fluid.

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