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Composition and Synthesis of Gangliosides in Bovine Testis, Sperm and Seminal Plasma

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ABSTRACT

Gangliosides were identified as constituents of bovine testis, sperm and seminal plasma. Based on sialic acid content, the average concentrations of gangliosides in bovine testis, sperm and seminal plasma were 1.6, 2.25 and 0.30 nmoles/mg protein. Seven chromatographically distinguishable gangliosides were found. The structures of five testicular gangliosides were shown to be N-acetylneuraminylgalactosylglucosyl ceramide (GM_3)[1]; N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosyglucosyl ceramide (GM_2); $(N\text{-acetylneuraminyl})_2$-galactosylglucosyl ceramide (GD_3); galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide (GM_1); and N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide (GD_{1a}). The structure of the major ganglioside constituent of bovine sperm was shown to be identical to GM_3. Saturated fatty acids of even carbon numbers from C_{14} to C_{24} were found in gangliosides. Appreciable amounts of n-uncosanoic (C_{21}) and n-tricosanoic (C_{23}) acids were also present. Testis tissue contained the galactosyl- and sialyltransferases for the synthesis of N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide (GD_{1a}) starting from glucosyl ceramide.

INTRODUCTION

Gangliosides, glycosphingolipids which contain sialic acid, were first identified in brain tissue by Klenk (1935). Gangliosides have been shown to be synthesized in a stepwise manner by transfer of carbohydrates from sugar nucleotides to glycolipid acceptors (Basu et al., 1965, 1973; Kaufman et al., 1966, 1968; Roseman, 1970). Gangliosides have also been identified in a large number of extraneural tissues (Ledeen et al., 1968; Puro et al., 1969, 1970; Svennerholm, 1965) and in many cultured cell lines (Yogeeswaran et al., 1970; Renkonen et al., 1970). Recently, gangliosides have been identified as constituents of sea urchin *(Echinocardium cordatum)* gonads (Kochetkov et al., 1976), porcine testis (Suzuki et al., 1975) and rat testis (Keenan et al., 1972).

The composition and synthesis of gangliosides in bovine testis, sperm and seminal plasma was examined. These studies were undertaken to determine if the ganglioside composition of bovine testis tissue and sperm were similar. The enzymatic capacity for ganglioside synthesis was shown to exist in particulate preparations, from testicular tissue.

MATERIALS

Glucosyl- and lactosylceramides were isolated from bovine milk fat globule membranes (Kayser and Patton, 1970). These compounds have the structures glucosyl (1 → 1) N-acylsphingosine and β-galactosyl (1 → 4)-glucosyl (1 → 1) N-acylsphingosine (Fujino et al., 1970). Ceramide and the gangliosides N-acetylneuraminylgalactosylglucosyl ceramide (GM_3), N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide (GM_2), galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide (GM_1) and $(N\text{-acetylneuraminyl})_2$ galactosylglucosyl ceramide (GD_3) were obtained as in previous studies (Keenan, 1974). The gangliosides N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide (GD_{1a}) and galactosyl-N-acetylgalactosaminyl-$(N\text{-acetylneuraminyl})_2$-galactosylglucosyl ceramide (GD_{1b}) were isolated from bovine brain using methods developed by Ledeen et al. (1973). N-acetylneuraminic acid and neuraminidase from *Clostridium welchii* were from Sigma Chemical Co., St. Louis, MO. Gas chroma-

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[1] Abbreviations: GM_3, N-acetylneuraminylgalactosylglucosyl ceramide; GD_3, $(N\text{-acetylneuraminyl})_2$-galactosylglucosyl ceramide; GM_2, N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide; GM_1, galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide; GD_{1a}, N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosyl ceramide; GD_{1b}, galactosyl-N-acetylgalactosaminyl-$(N\text{-acetylneuraminyl})_2$-galactosylglucosyl ceramide. These ganglioside abbreviations are those of Svennerholm (1963).

tographic column packings and methyl esters were from Supelco, Bellefonte, PA. Unlabeled CMP-N-acetylneuraminic acid was prepared according to Kean (1970). Unlabeled UDP-galactose (UDPGal), UDP-glucose (UDPGlc) and free carbohydrates were from Sigma. UDP-[^{14}C]-galactose (285 Ci/mole) was from New England Nuclear while CMP-N-[4,5,6,7,8,9-^{14}C]-acetylneuramic acid (214 Ci/mole) was from Amersham/Searle.

METHODS

Extraction Procedure

Bovine testes were obtained from Beutler Meat Co., Lafayette, IN. Bovine sperm and seminal plasma samples were obtained from the Purdue University Dairy Farm, W. Lafayette, IN and from American Breeders Service, De Forest, WI. Sperm were isolated from pooled ejaculates by centrifugation and were washed several times by resuspension in phosphate-buffered saline. Small samples of tissue (0.5 to 1.0 g) sperm or seminal plasma were homogenized in a Polytron Homogenizer (Kinematica, Lucerne) and the homogenate subjected to protein analysis by the method of Lowry et al. (1951). The remaining tissue (2–3 kg for testis, 4–5 g for sperm and 15–20 ml for seminal plasma) was homogenized in a Waring blender in methanol. Lipids were extracted by stirring the homogenates with 10 volumes of chloroform-methanol (2:1 v/v) for two days. The homogenate was filtered through cheesecloth and the resulting particulate again homogenized in 10 volumes of chloroform-methanol (1:1 v/v) overnight with stirring. The combined extracts were evaporated to near dryness.

Purification of Testis Gangliosides

The residue remaining after removal of solvent from lipid extracts was redissolved in chloroform-methanol (2:1 v/v) and gangliosides were recovered by partitioning according to the method of Folch et al. (1957). The upper phases were dialyzed, evaporated to dryness and ester lipids were removed by saponification in 0.5N methanolic NaOH at 40°C for 2–3 h. The solution was concentrated to one half its volume and dialyzed for two days against several changes of distilled water at 4°C. The dialysate was evaporated to dryness and the residue dissolved in chloroform-methanol (1:1, v/v). Ganglioside content was then determined by sialic acid assay (Warren, 1959). Crude gangliosides obtained in this manner were purified to homogeneity by thin layer chromatography.

Purification of Sperm and Seminal Plasma Ganglioside

The dried residue from the extraction procedure was dissolved in methanol-chloroform-H_2O (60:30:8, v/v/v). This solution was placed on a column of DEAE-Sephadex and the ganglioside fraction was eluted according to Ledeen et al. (1973). The ganglioside fraction was evaporated to dryness and the residue was solubilized in 0.1N methanolic NaOH. This solution was incubated for 2–3 h at 40°C to saponify esterified fatty acids. The solution was concentrated to one half of its volume, placed in a dialysis bag with 0.5M EDTA (tetrasodium salt) and dialyzed in the cold againat distilled water for two days. The content of the bag was then evaporated to dryness.

The resulting residue was dissolved in chloroform-methanol (4:1, v/v) and the solution applied to a small column of Unisil (Clarkson Chemical Co., Williamsport, PA) packed in chloroform. The ganglioside fraction was eluted by the procedure of Ledeen et al. (1973). The ganglioside fraction was evaporated to dryness, and then dissolved in a small volume of chloroform-methanol (1:1, v/v). Crude gangliosides obtained in this manner were purified to homogeneity by thin layer chromatography.

Thin-Layer Chromatography

Plates coated with 250 μm layer of silica gel G were used. For ganglioside separation, plates were developed in chloroform-methanol-28 percent ammonia-water (60:35:7:3, by volume) or n-propanol-28 percent ammonia (7:3, v/v). Gangliosides were visualized with resorcinol spray (Svennerholm, 1957). For isolation of individual gangliosides, chromatoplates were placed in a tank containing iodine crystals. Bands of silica gel containing gangliosides were scraped into sintered glass funnels and gangliosides were eluted with chloroform-methanol-water (1:1:0.2, by volume).

Analytical Methods

Sialic acid was determined by the method of Warren (1959). Sphingosine was determined according to Lauter and Trams (1962) using the hydrolysis procedure of Sweeley (1963). Total hexose was determined with anthrone reagent with an equimolar mixture of glucose and galactose as the standard (Spiro, 1966). N-Acetylhexosamines were determined by the modified Elson-Morgan procedure (Levvy and McAllan, 1959). Glucose, galactose and N-acetylgalactosamine were measured separately as the alditol acetates by gas-liquid chromatography (Vance and Sweeley, 1967). For this determination a 0.3 × 180 cm glass column packed with 3 percent SP 2340 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) was used. The column oven was temperature-programmed from 150 to 270°C at 3°C/min, with a nitrogen carrier gas flow rate of 25 ml/min, in an F & M Model 402 gas chromatograph with a flame ionization detector. Fatty acid methyl esters were prepared by hydrolysis with 14 percent BF_3 in methanol for 10 min at 100°C (Morrison and Smith, 1964) and were separated on a 0.3 × 180 cm glass column packed with 10 percent SP 2340 on 100/120 mesh Chromosorb W with temperature programming from 150 to 250°C at 4°C/min. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

To determine if ceramide was linked to glucose, in bovine testis gangliosides, a partial acid hydrolysis was performed on samples (Rauvala, 1976). This was followed by gas-liquid chromatography.

Mild Acid Hydrolysis

Neutral glycolipids were generated, as an aid to identification, from gangliosides by treatment with 0.001 M aqueous H_2SO_4 for 1 h in a boiling water bath (Frohwein and Gatt, 1969). Neutral glycolipids

were recovered by solvent partition and were examined by thin-layer chromatography in the chloroform-containing solvent system. Visualization was by spraying the chromatoplates with 50 percent aqueous H_2SO_4.

Enzymatic Hydrolysis

To release sialic acid, reaction mixtures contained 0.1 μmole ganglioside, 500 μg sodium taurocholate, 100 μmoles 0.05 M acetate buffer, pH 5.2 and 0.04 units of neuraminidase in a final volume of 0.2 ml. Incubation was for 18 h at 37°C. Reactions were stopped with 3 ml chloroform-methanol (1:1, by volume), the mixture was washed with water and the products were examined by thin-layer chromatography as for mild acid hydrolysates.

Glycosyltransferase Assays

Bovine testis tissue was homogenized in three volumes of 0.32 M sucrose-14mM 2-mercaptoethanol and filtered through cheesecloth. Homogenates were centrifuged at 1,000 X g for 10 min to remove debris. The resulting supernatant was centrifuged at 176,000 X g for 1 h and the pellet, designated total particulate fraction, was resuspended in sucrose-2-mercaptoethanol and used as the enzyme source.

For incorporation of galactose into gangliosides and neutral glycolipids, complete reaction mixtures contained (in μmoles) in a final volume of 0.1 ml: Glycolipid acceptor, 0.05; Triton X-114, 100μg; cacodylate-HCl, pH 7.3, 15; $MnCl_2$, 2.5; UDPGal, 0.05 (12.5 X 10^6 cpm/μmole); and 0.6 mg enzyme protein. For sialyl transferase assays, complete reaction mixtures contained (in μmoles) in a final volume of 0.1 ml: Glycolipid acceptor, 0.05; Tween 80–Triton CF-54 (1:2, w/w), 0.6 mg; cacodylate-HCl, pH 6.35, 15; $MgCl_2$, 1.0; CMP-N-acetylneuraminic acid, 0.05 (1.8 X 10^6 cpm/μmole); and 0.6 mg enzyme protein. All incubations were for 2 h at 37°C, after which time reactions were stopped by the addition of 0.1 ml of methanol. Reaction mixtures were applied onto Whatman 3 MM paper strips which were developed (descending) overnight with 1 percent sodium tetraborate (Chien et al., 1973). The origins were cut from the strips, placed in vials with 15 ml of chloroform-methanol-H_2O (1:1:0.2, by volume) and incubated, with shaking, for 1 h at 37°C. The extracts were evaporated, residues were solubilized in 1.0 ml of hyamine hydroxide and counted by liquid scintillation techniques. Specific activity values were calculated from cpm of radioactive sugar transferred to lipids.

RESULTS

Ganglioside Content

Bovine testicular tissue contained an average of about 99 nmoles of ganglioside sialic acid per g wet tissue weight (Table 1). Ganglioside sialic acid amounted to 1.6 nmoles per mg of protein. Bovine sperm contained 2.25 nmoles of ganglioside sialic acid per mg of protein while seminal plasma contained only 0.3 nmoles of ganglioside sialic acid per mg protein (Table 1). These values were obtained for ganglioside fractions isolated by the method of Ledeen et al. (1973). While not readily amenable to large-scale preparative isolation, this method yields nearly quantitative recovery of gangliosides, including the lower ganglioside homologs.

Distribution of Gangliosides

At least seven different gangliosides were observed on thin-layer chromatograms obtained from bovine testis tissue, sperm, and seminal plasma (Figs. 1–3). Visual comparison of thin-layer patterns obtained from a number of testis, sperm and seminal plasma samples suggested a variation in distribution of individual components among samples. In all samples of testis tissue and seminal plasma examined, Bands 1, 4 and 5 were the major constituents while for one sample of sperm (From Purdue University Dairy Farms) examined the major constituent was Band 3 (Fig. 2) and all other sperm samples (American Breeders Service) contained Band 1 as the major constituent.

Carbohydrate Composition

The thiobarbituric acid assay (Warren, 1959) demonstrated the presence of sialic acid in all fractions. Glucose, galactose and galactosamine were the only sugars observed on gas chromatography. Glucosamine was not detected in any of the ganglioside fractions examined.

TABLE 1. Ganglioside content of bovine testis, sperm and seminal plasma.

Source	Ganglioside sialic acid
Bovine testis (5)	99.16 ± 19.25 nmoles/g wet weight
Bovine testis (5)	1.6 ± 0.25 nmoles/mg protein
Bovine sperm (2)	2.25 nmoles/mg protein
Bovine seminal plasma (2)	0.30 nmoles/mg protein

Data are expressed as mean ± S.D. Numbers in parentheses are the number of samples analyzed.

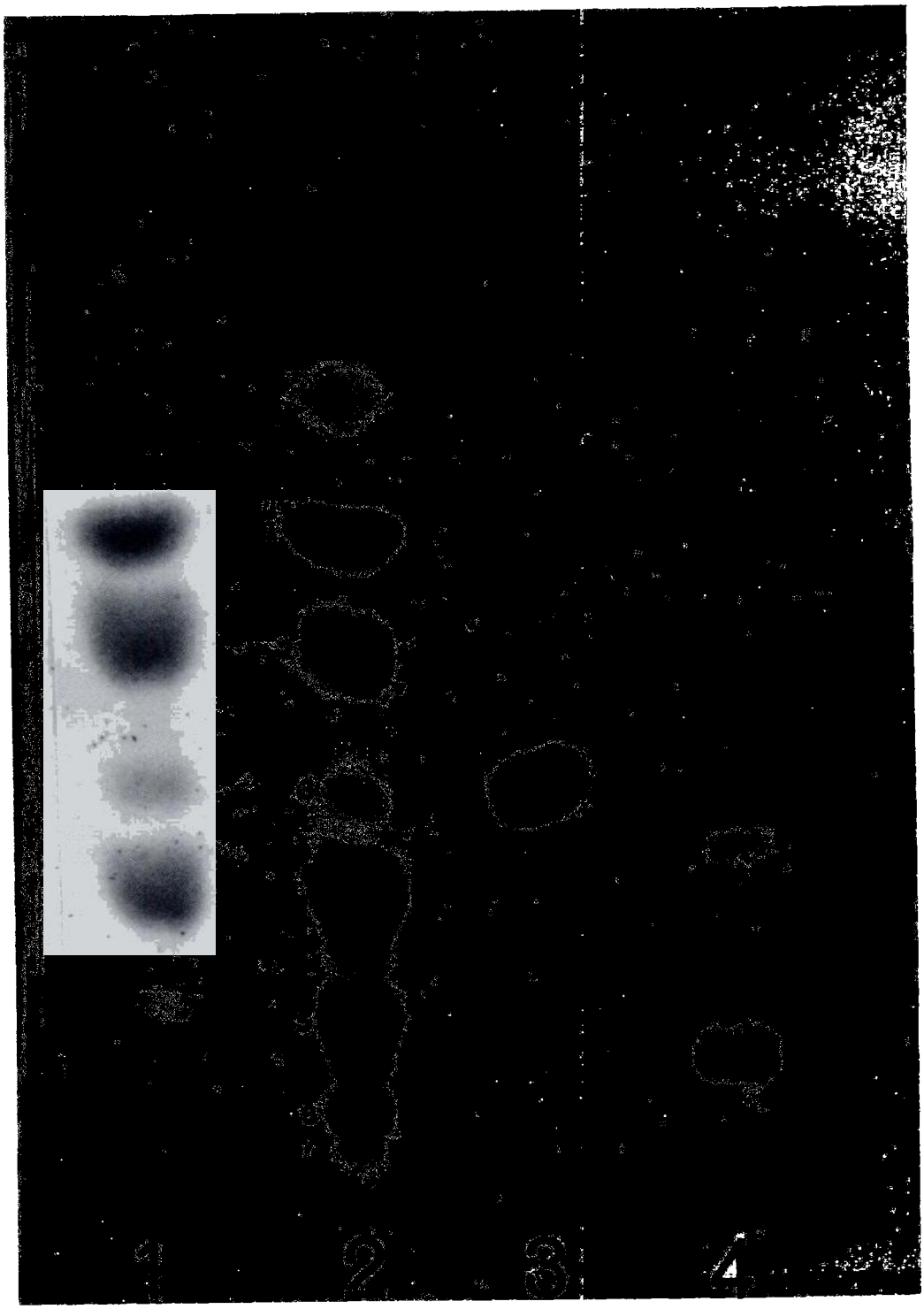

FIG. 1. Thin-layer chromatogram of ganglioside fractions from bovine testis tissue. The silica gel G plate was developed in *n*-propanol-28 percent ammonia (7:3, v/v) and sprayed with resorcinol reagent. (1) Reference GM_3, GM_2, GD_3 and GM_1 in order of decreasing chromatographic mobility; (2) bovine testis gangliosides, 80 nmoles of sialic acid; (3) GD_3; and (4) GD_{1a} (major spot).

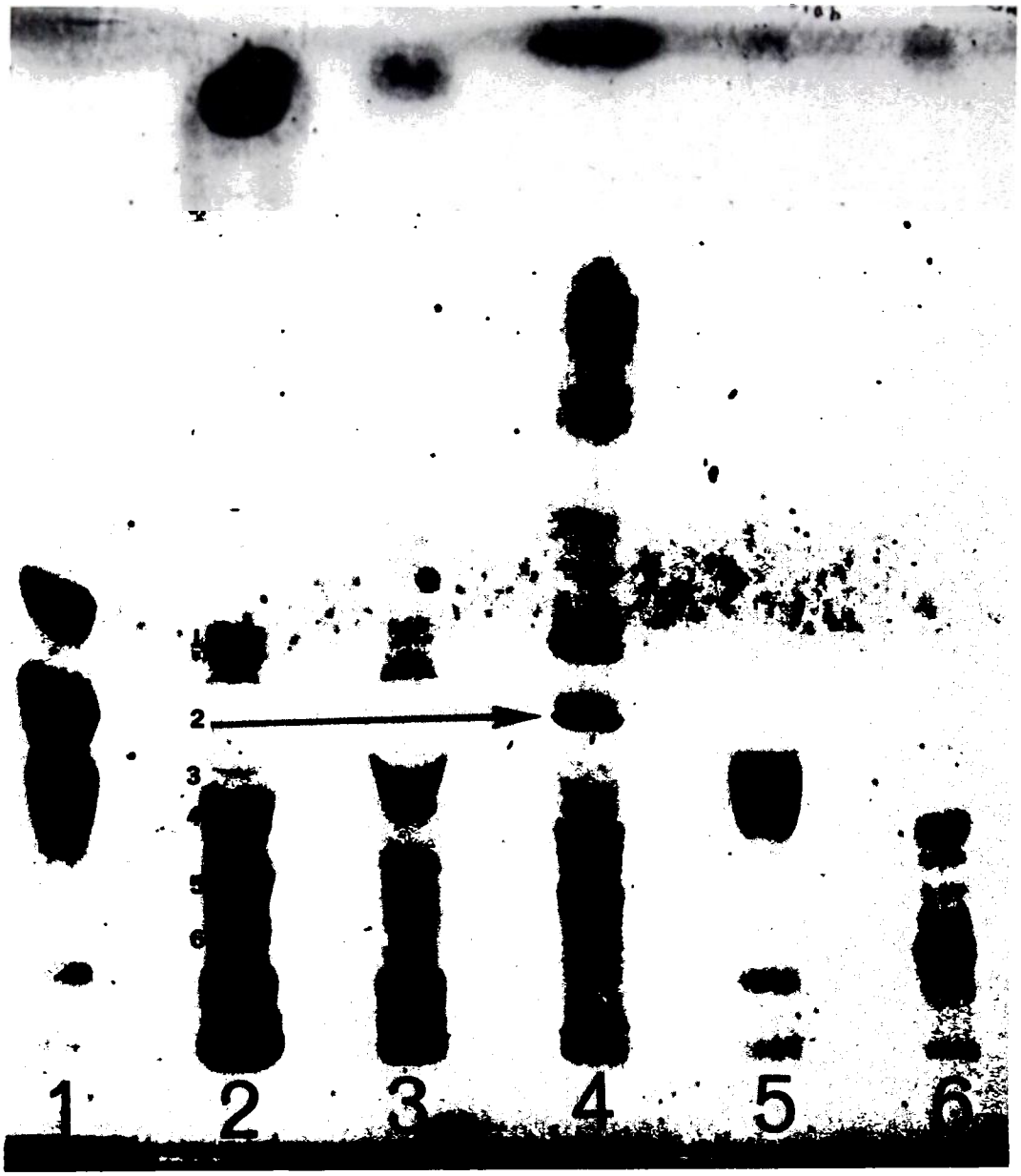

FIG. 2. Thin-layer chromatogram of ganglioside fractions from bovine testis tissue (4), seminal plasma (2), and sperm (3). The silica gel G plate was developed in chloroform-methanol-28 percent ammonia-water (60:35:7:3, by volume) and sprayed with resorcinol reagent. (1) Reference GM_3, GM_2 and GM_1 in order of decreasing chromatographic mobility; (5) GD_3; and (6) GM_1, GD_{1a}, and GD_{1b}, in order of decreasing chromatographic mobility).

Structural Characterization

Fractions enriched in the five less polar gangliosides were obtained either by Folch extraction or column chromatography. These gangliosides were designated TG I, TG II, TG III, TG IV, TG V (TG is for testis ganglioside) and SGI (SG is for sperm ganglioside) in order of decreasing chromatographic mobility. These gangliosides were purified to homogeneity by preparative thin-layer chromatography and subjected to structural characterization.

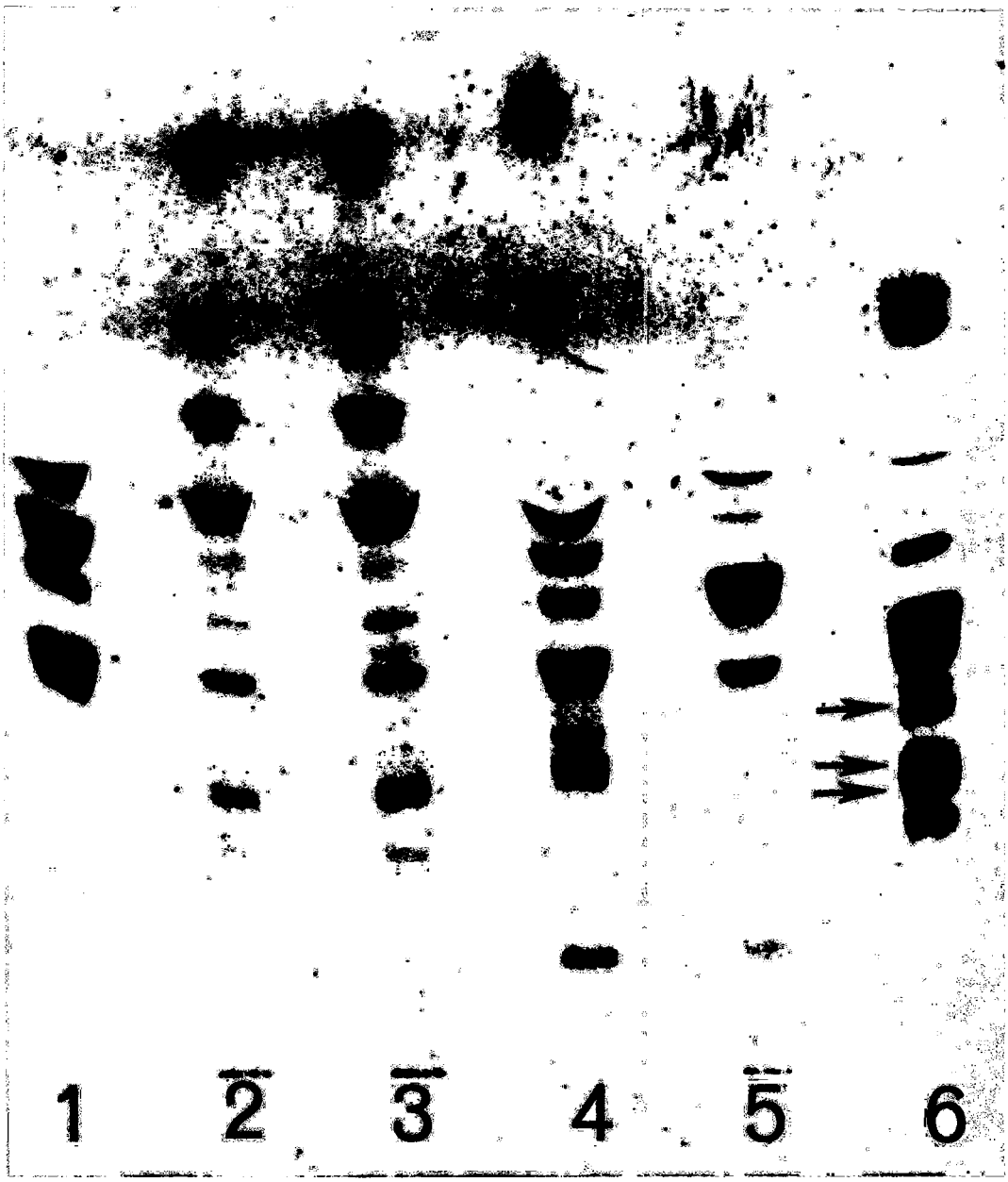

FIG. 3. Thin-layer chromatogram of ganglioside fractions from bovine testis tissue and sperm. The silica gel G plate was developed in *n*-propanol-28 percent ammonia-water (70:30:5, by volume) and sprayed with resorcinol reagent. (1) Reference GM_3, GM_2 and GM_1 in order of decreasing chromatographic mobility; (2) sperm gangliosides, 30 nmoles of sialic acid; (3) sperm gangliosides, 60 nmoles of sialic acid; (4) testis gangliosides, 60 nmoles of sialic acid; (5) GD_3; and (6) GM_1 (major constituent), GD_{1a} (single arrow) and GD_{1b} (double arrow).

Ganglioside TG I had a thin-layer mobility identical to that of GM_3 (Fig. 2). The molar ratios of spingosine to sialic acid to galactose to glucose were 1.00:0.91:0.99:0.94 (Table 2). Mild acid hydrolysis of TG I yielded one major glycolipid which migrated with lactosyl ceramide, and neuraminidase treatment of TG I yielded a neutral glycolipid with the same migratory properties as lactosyl ceramide. These are the expected products from GM_3.

TABLE 2. Molar ratios of ganglioside constituents.

Component	Molar ratios					
	TG I	TG II	TG III	TG IV	TG V	SG I
Sphingosine	1.00	1.00	1.00	1.00	1.00	1.00
Sialic acid	0.91	0.91	1.91	0.93	2.19	1.03
Galactose	0.99	0.97	1.10	1.80	1.92	1.03
Glucose	0.94	0.83	0.91	0.98	0.93	0.96
N-acetylgalactosamine	0.0	0.82	0.0	0.96	0.90	0.0

Individual gangliosides were isolated from bovine testis or sperm and purified to chromatographic homogeneity.

Partial acid hydrolysis, followed by removal of free carbohydrates and complete hydrolysis of the remaining glycolipid, showed glucose to be the last sugar removed from ceramide. Thus, the structure sialic acid-Gal-Glc-ceramide (GM_3) can be suggested for TG I.

Ganglioside TG II had a thin-layer mobility identical to that of GM_2 (Fig. 2). The molar ratios of sphingosine to sialic acid to galactose to glucose to galactosamine were 1.00:0.91:0.97:0.83:0.82 (Table 2). Mild acid hydrolysis of TG II yielded two major neutral glycolipids, one which migrated with lactosyl ceramide and another which chromatographed as a trihexosyl ceramide (the predicted product from GM_2). Neuraminidase treatment yielded unchanged TG II; GM_2 is not attacked by neuraminidase. Stepwise acid hydrolysis followed by gas-liquid chromatography showed that glucose was linked to ceramide. Thus, the structure GalNAc-(sialic acid)-Gal-Glc-ceramide (GM_2) can be assigned to ganglioside TG II.

Ganglioside TG III had a mobility identical to that of GD_3 (Fig. 2). The molar ratios of sphingosine to sialic acid to galactose to glucose were 1.00:1.91:1.10:0.91 (Table 2). Mild acid hydrolysis to TG III yielded one major glycolipid which migrated with lactosyl ceramide. Neuraminidase treatment of TG III resulted in an 80 percent conversion to a neutral glycolipid which migrated with lactosyl ceramide. The remaining product has an R_f identical with that of GM_3. Partial acid hydrolysis, followed by isolation of free carbohydrates and complete hydrolysis of the remaining glycolipid, showed glucose to be the last sugar removed from ceramide. Together these results suggest the structure of TG III to be sialic acid-sialic acid-Gal-Glc-ceramide (GD_3).

The thin-layer mobility of ganglioside TG IV was identical to that of GM_1 (Fig. 2). The molar ratios of sphingosine to sialic acid to galactose to glucose to galactosamine were 1.00:0.93:1.80:0.98:0.96 (Table 2). Mild acid hydrolysis to TG IV yielded two major neutral glycolipids, one with migratory properties identical to lactosyl ceramide and the other which chromatographed as a tetrahexosyl ceramide (the expected product from GM_1). Neuraminidase treatment yielded unchanged TG IV. Stepwise acid hydrolysis followed by gas-liquid chromatography showed that glucose was linked to ceramide. Thus, the structure Gal-GalNAc-(sialic acid)-Gal-Glc-ceramide (GM_1) was suggested for ganglioside TG IV.

Ganglioside TG V had a thin-layer mobility identical to that of GD_{1a} (Fig. 2). The molar ratios of sphingosine to sialic acid to galactose to glucose to galactosamine were 1.00:2.19:1.92:0.93:0.90 (Table 2). Mild acid hydrolysis of TG V yielded two major neutral glycolipids, one which migrated with lactosyl ceramide and the other which chromatographed as a tetrahexosyl ceramide (The expected product of GD_{1a}). Neuraminidase treatment resulted in about 90 percent conversion to a compound which migrated with ganglioside GM_1. The remaining compound was unchanged TG V. Partial acid hydrolysis, followed by isolation of free carbohydrates and complete hydrolysis of the remaining glycolipid, demonstrated that glucose was linked to ceramide. These results suggest the structure of TG V to be sialic acid-Gal-GalNAc-(sialic acid)-Gal-Glc-ceramide (GD_{1a}).

Ganglioside SG I had a thin-layer mobility identical with GM_3. The molar ratios of sphingosine to sialic acid to galactose to glucose were 1.00:1.03:1.03:0.96 (Table 2). Mild acid hydrolysis of SG I yielded one major glycolipid

TABLE 3. Fatty acid composition of individual gangliosides isolated from bovine testis and sperm.

Acid	TG I	TG II	TG III	TG IV	TG V	SG I
14:0	3.2	2.2	1.5	2.8	2.8	0.7
16:0	28.7	3.3	7.8	4.6	20.6	32.9
16:1	9.3	6.5	8.8	9.1	3.7	...
18:0	28.7	12.7	15.0	15.7	8.3	17.3
18:1	9.3	...	...	...	7.3	18.7
20:0	5.3	25.8	20.8	21.7	15.1	13.3
21:0	...	18.1	18.7	18.6	6.9	9.9
22:0	5.3	11.1	11.2	11.4	16.5	6.1
23:0	4.8	12.3	10.4	10.1	13.8	0.4
24:0	5.3	8.1	5.8	6.2	5.0	0.5

Data are given as weight percent of total fatty acid methyl esters. Fatty acids are abbreviated as number of carbons:number of double bonds. Results are averages of duplicate analyses.

which migrated with lactosyl ceramide. Neuraminidase treatment yielded lactosyl ceramide. Stepwise acid hydrolysis followed by gas-liquid chromatography showed that glucose was linked to ceramide. Thus, the structure sialic acid-Gal-Glc-ceramide (GM_3) was suggested for ganglioside SG I.

Fatty Acid Composition

The individual gangliosides from bovine testis and sperm were characterized by a high content of long chain (20–24 carbon atoms) fatty acids as well as large amounts of 16:0 and 18:0 (Table 3). All even carbon fatty acids from 14.0 and 24.0 were contained in ganglioside fractions while the only odd carbon acids found in any quantity were 21:0 and 23:0. Only small amounts of unsaturated fatty acids were observed; these being 16:1 and 18:1. Variation in fatty acid patterns was evident from sample to sample. Of note is the lack of detectable amounts of 18:1 in TG II, TG III and TG IV. With few exceptions, some degree of homology was evident in comparing individual gangliosides from testis (Table 3).

In vitro Biosynthesis

Sialytransferase active with glycolipid acceptors were present in bovine testis total particulate fractions (Table 4). Lactosyl ceramide was the most active acceptor followed in order by GM_1 and GM_3. Glucosyl ceramide, trihexosyl ceramide, GM_2, GD_{1a} and GD_{1b} were inactive or showed only low acceptor activity. Detergents were required for activity of the sialyltransferase with lactosyl ceramide as glycolipid acceptor (Table 4). There was no stimulation of activity with cardiolipid or phosphatidyl glycerol. This was contrary to results obtained by Keenan et al. (1974) with rat liver Golgi apparatus and by Keenan (1974) with bovine mammary total particulate fractions.

Under optimum conditions, only low specific activities in UDPGal:GM_2 galactosyltransferase were observed in testis particulate fractions (Table 5). Boiling the particulate fraction abolished galactose incorporation, thus suggesting enzymatic activity. Detergents were necessary for maximal activity and GM_3, GM_1 and GD_3 were inactive as acceptors under these assay conditions (Table 5). As expected, glucosyl, lactosyl and trihexosyl ceramides were active acceptors for galactose. These biosynthetic data demonstrated that the sialyl- and galactosyltransferases necessary for synthesis of the gangliosides isolated and identified from testis and sperm were present in bovine testis tissue.

DISCUSSION

These results show that gangliosides are present in bovine testis, sperm and seminal plasma. The percentages of total sialic acid which were lipid-bound in testis, sperm and seminal plasma were 8.6, 6.9 and 0.6 percent, respectively. These values are comparable to those lipid-bound sialic acid levels found in other extraneural tissues (Ledeen et al., 1968; Puro et al., 1969; Puro, 1970; Svennerholm, 1965 and Yogeeswaran et al., 1970).

At least 7 chromatographically distinguishable gangliosides occur in bovine testis, sperm and seminal plasma. Five of these gangliosides are homologous with gangliosides GM_3, GM_2,

TABLE 4. Characterization of CMP-N-acetylneuraminic acid:glycolipid sialytransferase activities in bovine testis particulate fractions.

Reaction mixture	Acceptor	^{14}C incorporated (pmoles/ mg protein/hr)
Complete	Lactosyl ceramide	2086
Complete	None	205
Plus cardiolipin (100 μg)	Lactosyl ceramide	1247
Plus phosphatidyl glycerol (100 μg)	Lactosyl ceramide	1782
Minus detergents	Lactosyl ceramide	40
Heat inactivated enzyme (2 min, 100°C)	Lactosyl ceramide	15
Complete	Glucosyl ceramide	490
Complete	Trihexosyl ceramide	195
Complete	GM_3	785
Complete	GM_2	125
Complete	GM_1	1251
Complete	GD_3	400
Complete	GD_{1a}	242
Complete	GD_{1b}	249

Complete reaction mixtures contained (in μmoles unless otherwise stated) in final volumes of 0.1 ml: cacodylate-HCl, pH 6.35, 15; $MgCl_2$, 1; CMP-N-acetylneuraminic acid (1.8×10^6 cpm/μmole), 0.05; glycolipid acceptor, 0.05; Tween 80 – Triton CF-54 (1:2, w/w), 0.6 mg; and 0.6 mg particulate protein. Incubations were for 2 h at 37°C. Assay methods are described in the Methods. Results are representative values obtained from several separate assays.

GM_1, GD_3 and GD_{1a} in composition and probable sequence of carbohydrates. Gangliosides GM_3, GM_1 and GD_{1a} were most abundant in bovine testicular tissue. These gangliosides from bovine testis contained all even carbon saturated fatty acids from 14:0 to 24:0 as well as substantial amounts of 21:0 and 23:0. This fatty acid pattern is characteristic of that found in gangliosides from other tissue (Keenan, 1973) as well as a ganglioside isolated

TABLE 5. Characterization of UDP-galactose:glycolipid galactosyl transferase activity in bovine testis particulate fractions.

Reaction mixture	Acceptor	^{14}C incorporated (pmoles/ mg protein/hr)
Complete	GM_2	67
Complete	None	41
Plus cardiolipin (100 μg)	GM_2	78
Plus phosphatidyl glycerol (100 μg)	GM_2	62
Minus detergents	GM_2	30
Minus active, plus heat inactivated enzyme (2 min, 100°C)	GM_2	29
Complete	Glucosyl ceramide	190
Complete	Lactosyl ceramide	135
Complete	Trihexosyl ceramide	121
Complete	GM_3	38
Complete	GM_1	23
Complete	GD_3	6

Complete reaction mixtures contained (in μmoles unless otherwise stated) in final volumes of 0.1 ml: cacodylate-HCl, pH 7.3, 15; $MnCl_2$, 2.5; UDPGal (12.5×10^6 cpm/μmole), 0.05; glycolipid acceptor, 0.05; Triton X-114, 100 μg; and 0.6 mg particulate protein. Incubations were for 2 h at 37°C. Assay methods are described in the text. Results are representative values obtained from several separate assays.

from boar testis (Suzuki et al., 1975).

Due to limited availability of adequate numbers of sperm, the structure of only one sperm ganglioside was established (SG I; equivalent to GM_3). However, in view of their coincident chromatographic mobility with standards and/or their occurrence in testis, it can be suggested that GM_2, GM_1, GD_3, GD_{1a} and GD_{1b} occur in sperm. The striking difference in ganglioside patterns observed with sperm samples from an individual bull in the Purdue herd and the composite sample from American Breeders Service cannot be explained. Unfortunately, the animal from the Purdue herd was sold before follow-up samples could be collected.

The sialytransferases and galactosyltransferases involved in the synthesis of neutral glycolipids and gangliosides were present in testis tissue. The sialytransferases for the conversion of lactosyl ceramide to GM_3 and for the conversion of GM_1 to GD_{1a} had high activity. The sialytransferase for the conversion of GM_3 to GD_3 showed modest activity *in vitro*. The low levels of GD_3 observed (Fig. 1) could be due to preferential conversion of GM_3 to GM_2 which in turn is converted to GM_1.

Of note is the fact that cardiolipid or phosphatidyl glycerol did not stimulate the activity of CMP-N-acetylneuraminic acid:glycolipid sialytransferase. Stimulation of this transferase activity by cardiolipid and phosphatidyl glycerol had been observed in rat liver Golgi apparatus (Keenan et al., 1973) and in bovine mammary gland particulate fractions (Keenan, 1974).

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