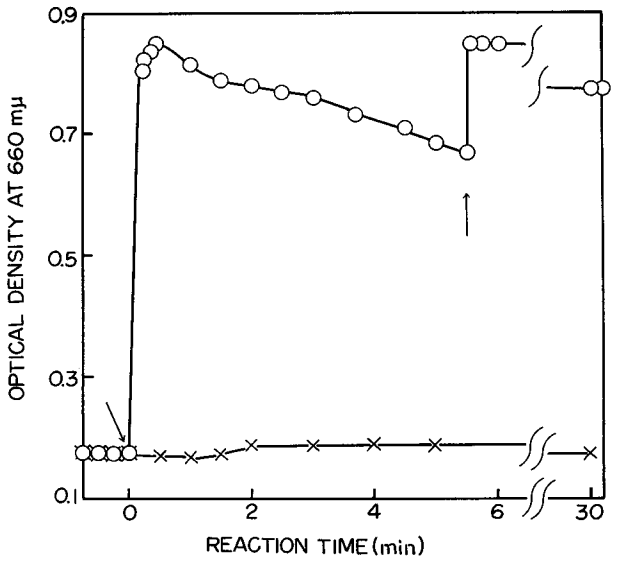

FIG. 2. Superprecipitation of actomyosin. NTP-myosin (1.5 moles of NTP per 4×10^5 g) and control myosin were prepared by the methods described in the text. Actomyosins were prepared by mixing myosin with F-actin at a weight ratio of 5:1.7. Superprecipitation of actomyosin was followed by measuring change in optical density at 660 mμ in a Cary model 14 spectrophotometer. The reaction mixture contained 0.5 mg of actomyosin per ml, 0.06 M KCl, 4 mM $MgCl_2$, and 0.1 mM ATP, pH 7.0 (5 mM Tris-maleate buffer), at 20°. ○, control actomyosin; ×, NTP-actomyosin. *First* and *second arrows* indicate addition of ATP and stirring of suspension, respectively.

would therefore be of importance in correlating the formation of the myosin-phosphate complex with muscle contraction. In the experiments shown in Fig. 2, superprecipitation of actomyosin reconstituted from myosin or NTP-myosin and F-actin at a weight ratio of 5:1.7 was measured in 0.06 M KCl-4 mM $MgCl_2$ at pH 7.0 and 20° after addition of 0.1 mM ATP, by following the change in optical density at 660 mμ (17). Binding of 1.5 moles of *p*-nitrothiophenol to 4×10^5 g of myosin completely prevented the superprecipitation of actomyosin. The same results were also obtained with NTP-myosins containing 0.8 and 1.3 moles of NTP per 4×10^5 g of myosin.

As discussed previously (1), myosin has 1 mole of active site per 4×10^5 to 5×10^5 g of protein. Furthermore, recent sedimentation equilibrium and osmotic pressure measurements[4] have shown that myosin has a molecular weight of 4.8×10^5. Taking all the results presented in this note into consideration, together with earlier findings (1–3), it now appears highly likely that *p*-nitrothiophenylation of 1 mole of phosphate-binding site per mole of myosin can completely prevent the actomyosin type of ATPase activity as well as the superprecipitation of actomyosin by ATP, although this modification does not affect the steady state activity of myosin-type ATPase and the F-actin-binding capacity of myosin. We therefore conclude that the formation of a reactive myosin-phosphate complex by ATP is an obligatory intermediate step in the actomyosin type of ATPase and is also a key reaction in superprecipitation of actomyosin by ATP.

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Incorporation of D-Galactose into Glycoproteins*

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Orosomucoid (1), fetuin (2), and other glycoproteins contain oligosaccharide side chains terminating in a trisaccharide unit as follows: sialic acid → galactose → *N*-acetylglucosamine → glycoprotein. Earlier studies (3, 4) on the biosynthesis of glycoproteins resulted in the isolation of enzymes from mammary gland and colostrum that transferred sialic acid from cytidine 5′-phosphate sialic acid to the galactose units in glycoproteins and related compounds. In the experiments outlined below, other glycosyltransferases, particularly galactosyltransferases, were studied.

Particulate preparations from rat tissues transferred galactose from uridine diphosphate galactose to *N*-acetylglucosamine and to orosomucoid pretreated with sialidase and β-galactosidase. Detailed studies of one galactosyltransferase system were conducted with a soluble preparation obtained from goat colostrum; this preparation also utilized UDP-*N*-acetylgalactosamine and UDP-glucose in place of UDP-galactose.

Other investigators (5, 6) have reported that milk and mammary gland contain a UDP-galactose:glucose galactosyltransferase; lactose was the product of the reaction. Since some

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TABLE I

*Galactosyltransferase activities in rat tissues**

Preparation	Endogenous	*N*-Acetylglucosamine		Glycoprotein I	
		Uncorrected	Corrected	Uncorrected	Corrected
Lung, particulate	5,000	16,000	11,000	5,500	500
Brain, particulate	17,800	26,000	8,200	18,500	700
Spleen, particulate	2,300	4,400	2,100	2,100	
Large intestine, particulate	10,800	24,800	14,000	8,000	
Mammary gland, supernatant	2,200	12,900	10,700	7,100	4,900
Mammary gland, particulate	12,000	179,000	167,000	130,000	118,000
Liver, nuclear	1,200	9,600	8,400	3,800	2,600
Liver, mitochondrial	1,000	4,400	3,400	2,100	1,100
Liver, microsomal	800	11,000	10,200†	4,200	3,400†

* Activities are given as the ^{14}C incorporated into products expressed as counts per min per mg of protein per hour of incubation; conditions were those given in Fig. 1, except that incubations were conducted for 1 hour, and the specific activity of the UDP-galactose-^{14}C was 940,000 cpm per μmole. Corrected values are the uncorrected minus endogenous values; the latter represent incorporation without added acceptors. The tissues were obtained from an exsanguinated female postpartum rat, 250 g, and they were homogenized in a Potter-Elvehjem homogenizer with 2 volumes of 0.1 M cacodylate-HCl buffer, pH 6.8. After centrifuging at 121 × *g* for 15 min, the supernatant fluid was centrifuged for 30 min at 39,000 × *g*, yielding a supernatant and particulate fraction; the latter was washed twice with buffer. The liver fractions were prepared according to Hogeboom (10). The results are considered qualitative since product formation was not shown to be proportional to time of incubation and protein concentration. No activity was detected in the supernatant fractions from lung, brain, spleen, and intestine, and in both fractions from erythrocytes.

† These products were isolated from a larger incubation in a different experiment. The ^{14}C-disaccharide was homogeneous on paper chromatography and corresponded to *N*-acetyllactosamine in rate of migration and color reaction. Both the glycoprotein and disaccharide gave ^{14}C-galactose on treatment with β-glactosidase (72 and 70% yields, respectively).

mammary gland preparations also utilized *N*-acetylglucosamine as an acceptor, the presence of another galactosyltransferase was suggested (6); here, the product was partially characterized as *N*-acetyllactosamine.[1] Goat colostrum Fraction A, described below, was inactive with glucose, but active with *N*-acetylglucosamine and glycoprotein; the results of fractionation studies suggested that the purified preparation contained more than one galactosyltransferase.

One substrate, Glycoprotein I, was prepared by pretreating orosomucoid (α_1-acid glycoprotein (7))[2] with purified sialidase, and β-galactosidase (8). Concentrations of Glycoprotein I are expressed as the theoretical sites available to accept galactose, *i.e.* the quantity of galactose removed from orosomucoid by β-galactosidase. Labeled sugar nucleotides were prepared as previously described (9).

The results of a survey of rat tissues for galactosyltransferase activity are shown in Table I. Many of the particulate preparations were active; in addition, they contained considerable quantities of unidentified endogenous acceptors. A liver microsomal preparation yielded products from *N*-acetylglucosamine and Glycoprotein I that appeared to be the same as, or similar to, those formed by colostrum Fraction A described below.

This soluble, partially purified galactosyltransferase system was prepared from frozen goat colostrum which was thawed, centrifuged at 18,000 × *g* for 30 min, adjusted to 0.07 M with $ZnSO_4$, and fractionated with solid ammonium sulfate at 4°. After pressure dialysis of the 40 to 75% ammonium sulfate fraction, it was further purified on Sephadex G-100, as shown in Fig. 1, giving Fraction A. The elution pattern, observed in a number of experiments, suggested two or more galactosyltransferase peaks in *A*. In different experiments, the enzyme or enzymes were purified 60- to 220-fold over the crude supernatant fraction, and they were obtained in about 20% yield. Preparative column disk electrophoresis on polyacrylamide gel was occasionally employed, and also showed an unsymmetrical distribution of galactosyltransferase activity in contiguous sections of the gel.

Kinetic studies with Fraction A showed (*a*) that the optimum conditions for assay were those given in Fig. 1, and that product formation was proportional to time of incubation and protein concentration at all stages of purification; (*b*) that Mn^{++} exhibited maximum activity at 0.075 M, and could be partially (25%) replaced by Mg^{++} at the same concentration, while Ca^{++}, Cu^{++}, Zn^{++}, Co^{++}, Ni^{++}, Fe^{++}, and Cd^{++} showed either slight or no activity; (*c*) that the approximate K_m values were 2.5×10^{-4} M for UDP-galactose, 1.3×10^{-3} M for *N*-acetylglucosamine, and 1.5×10^{-3} M for Glycoprotein I.

To characterize the product formed from Glycoprotein I, 15 μmoles of UDP-galactose-^{14}C (specific activity, 330,000 cpm per μmole), 0.7 mg of Fraction A, and 13 μmoles (32.5 mg) of Glycoprotein I were incubated for 4 hours, and exhaustively dialyzed. The ^{14}C-product, Glycoprotein II, contained 11 μmoles of ^{14}C-galactose in the protein fraction as determined electrophoretically (see accompanying paper (11)), equivalent to 85% of the theo-

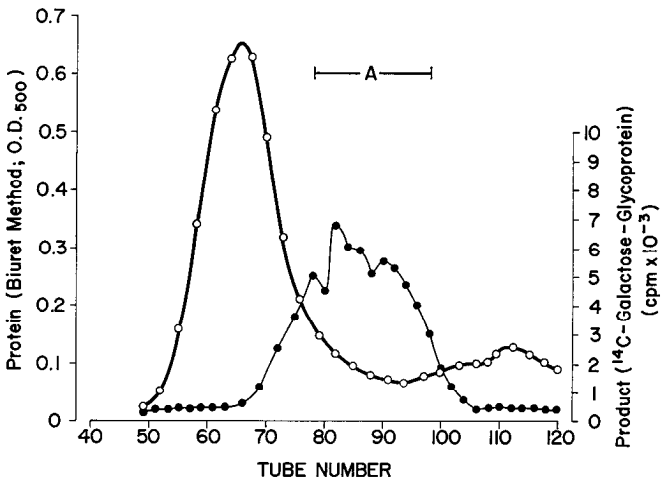

FIG. 1. Chromatography of ammonium sulfate fraction on Sephadex G-100. ○, protein determined by a modified biuret method; ●, galactosyltransferase activity. The column, 4 × 104 cm, was pretreated with 0.05 M KCl + 0.005 M 2-mercaptoethanol, and the protein fraction (85 mg) was applied and eluted with the same buffer. Enzyme incubation mixtures contained the following components (micromoles in 0.04 ml): Glycoprotein I, 0.5; $MnCl_2$, 3; 2-mercaptoethanol, 3; glycylglycine buffer, pH 7.7, 10; UDP-galactose-^{14}C, 0.15 (1-^{14}C-galactose, specific activity, 310,000 cpm per μmole); enzyme (*e.g.* 6 μg of combined Peak A). When *N*-acetylglucosamine was used as acceptor (2.5 μmoles), the buffer was cacodylate-HCl, pH 7.0. After 15 min at 37°, 5 μmoles of EDTA were added to stop the reaction; aliquots were transferred to Whatman No. 3MM paper saturated with 1% sodium tetraborate, and assayed by an electrophoretic method (11). Controls either contained EDTA or lacked enzyme. The elution profile with *N*-acetylglucosamine as acceptor paralleled that shown with Glycoprotein I except that it was slightly higher (about 10% at each point).

[1] *N*-Acetyllactosamine is 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine. All sugars were of the D configuration except L-fucose, and glycosides were pyranosides. Chemical analyses were performed by modifications of standard methods.

[2] Large quantities of purified orosomucoid were generously supplied by Dr. Karl Schmid, Boston University.

retical sites available in Glycoprotein I. Glycoprotein II was nonelectrodialyzable, precipitable with phosphotungstic acid and 80% ethanol, and gave ^{14}C-glycopeptides after digestion with Pronase. Purified β-galactosidase released 95% of the ^{14}C, identified as ^{14}C-galactose by paper chromatography and electrophoresis in 1% sodium tetraborate. Oxidation of 0.25 μmole of Glycoprotein II (based on ^{14}C content) with galactose oxidase (12) gave 0.17 μmole of aldehyde. With goat colostrum sialyltransferase (4), Glycoprotein II was at least as effective an acceptor as sialidase-treated orosomucoid. Finally, partial acid hydrolysis of Glycoprotein II, under conditions used for glycoproteins (2), gave a comparable yield, 7.4%, of a disaccharide (III) identified as ^{14}C-*N*-acetyllactosamine as described below.

To characterize the product formed from *N*-acetylglucosamine, the latter was incubated with UDP-galactose-^{14}C and Fraction A; the ^{14}C-product, Disaccharide IV, was obtained in 45% yield (10.8 μmoles) after purification by paper chromatography. Disaccharides III and IV were homogeneous in a paper chromatographic system that separated the three known position isomers of β-galactosyl-*N*-acetylglucosamine (2); they corresponded in migration rates, color reactions, and in cochromatography experiments with authentic *N*-acetyllactosamine. The following molar ratios were observed when Disaccharides III and IV were analyzed: ^{14}C, 1.00; galactose by the orcinol method, 1.19 for IV; hexosamine, 1.22 for III, and 1.04 for IV. Hydrolysis of Disaccharide IV with β-galactosidase gave ^{14}C-galactose identified by paper chromatography, electrophoresis, and with galactose dehydrogenase (13). Reduction of Disaccharide IV with $NaBH_4$, followed by acid hydrolysis, gave ^{14}C-galactose, but no ^{14}C-galactitol. Acid hydrolysis of Disaccharides III and IV gave glucosamine identified by paper electrophoresis (14), ion exchange chromatography (15), and ninhydrin degradation (16).

The galactosyltransferase activity of Fraction A was exhibited toward a number of acceptors, particularly toward those containing β-*N*-acetylglucosaminyl end groups.[3] Since different enzyme fractions showed different relative activities with Glycoprotein I and *N*-acetylglucosamine, we suggest that the two acceptors may be substrates for different galactosyltransferases. For example, the ratio of activity of *N*-acetylglucosamine to Glycoprotein I was 0.2 in the crude preparation, and 1.1 in Fraction A; similar changes were noted in fractions obtained from hydroxylapatite and disk electrophoresis columns. Also, crude preparations from rat tissues (Table I) showed markedly different activities with these substrates.

[3] Under the conditions described in Fig. 1, and where product formation was approximately proportional to time of incubation, the following glycoproteins showed the indicated relative activity as acceptors: Glycoprotein I, 100; fetuin pretreated with sialidase and β-galactosidase, 121; bovine submaxillary mucin, 31, pretreated with sialidase, 84; ovomucoid, 58; ovalbumin, 30; ovomucin, 10; porcine submaxillary mucin pretreated with sialidase, 7 (?). The following glycoproteins were inactive (less than 5% of Glycoprotein I): ribonuclease B, orosomucoid, fetuin, ovine and porcine submaxillary mucins, and the corresponding sialidase-treated materials (except porcine mucin). In a separate experiment, the following sugars were active: *N*-acetylglucosamine, 100; *N*-glycolylglucosamine, 12; methyl- and phenyl-β-*N*-acetylglucosaminides, 85 and 39, respectively; *N*,*N*′-diacetylchitobiose, 93; *N*,*N*′,*N*″-triacetylchitotriose, 99. Some inactive sugars (*i.e.* less than 10% as active as *N*-acetylglucosamine) included glucosamine; mannosamine, galactosamine, and their *N*-acetyl and *N*-glycolyl derivatives; glucose, galactose, mannose, fucose, and methyl α- and β-galactoside; methyl α-glycosides and α-1-P esters of *N*-acetylglucosamine and *N*-acetylgalactosamine; lactose and *N*-acetyllactosamine; *N*-acetyl- and *N*-glycolylneuraminic acid. The class of glycolipids described in the accompanying communication (11) were inactive with or without detergent (less than 2% compared with Glycoprotein I).

Fraction A was inactive with α-glycose 1-phosphates, UDP-*N*-acetylglucosamine, GDP-glucose, and GDP-mannose as glycose donors. With either Glycoprotein I or *N*-acetylglucosamine as acceptor, the following sugar nucleotides showed the indicated relative activities: UDP-galactose, 100; UDP-*N*-acetylgalactosamine, 1.3; UDP-glucose, 1.6. Whether one or more transferases are responsible for the activities with the different sugar nucleotides remains to be determined.

The *N*-acetylgalactosaminyltransferase activity was characterized by incubating a purified fraction with UDP-*N*-acetylgalactosamine-^{14}C and *N*-acetylglucosamine. The ^{14}C-product was obtained in 20% yield (4.5 μmoles) after purification by paper chromatography, and it was homogeneous when chromatographed in five solvent systems and subjected to paper electrophoresis at three pH values. Acid hydrolysis gave glucosamine and galactosamine (molar ratio, 1.0:0.97) that were separated by ion exchange chromatography, and further identified by paper chromatography, and electrophoresis, and ninhydrin degradation. Reduction of the disaccharide, followed by acid hydrolysis and *N*-acetylation gave *N*-acetylglucosaminitol and *N*-acetylgalactosamine as the only detectable products. The disaccharide is therefore *N*-acetylgalactosaminyl-*N*-acetylglucosamine; neither its anomeric configuration nor linkage is known.

The glucosyltransferase activity was partially characterized by incubating the ammonium sulfate fraction with UDP-glucose-^{14}C and *N*-acetylglucosamine. The ^{14}C-product was purified by paper chromatography, and obtained in a 47% yield (0.29 μmole). It appeared homogeneous and migrated like a disaccharide on paper chromatography; ^{14}C-glucose, but no ^{14}C galactose, was detected after acid hydrolysis.

In conclusion, glycosyltransferases, partially purified from goat colostrum, were active with UDP-galactose, UDP-*N*-acetylgalactosamine, and UDP-glucose when Glycoprotein I and *N*-acetylglucosamine were used as acceptors. The products of some of these reactions were characterized. The galactosyltransferase system replaced 85% of the galactose removed from orosomucoid by β-galactosidase; this reaction may be an important step in the biosynthesis of glycoproteins. Galactosyltransferases were also detected in preparations obtained from several rat tissues.

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Conversion of Tay-Sachs Ganglioside to Monosialoganglioside by Brain Uridine Diphosphate D-Galactose: Glycolipid Galactosyltransferase*

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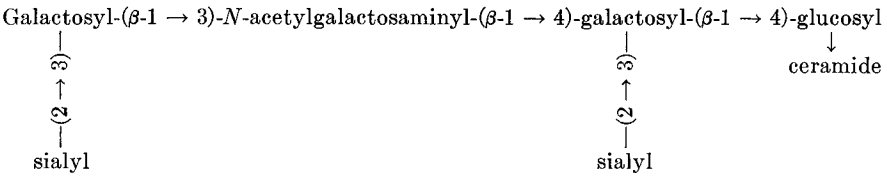
Brain gangliosides were first isolated by Klenk (1), and comprise a family of closely related glycosphingolipids. The major components of this family have recently been isolated by several groups of investigators (see review by Svennerholm (2)). Structural studies, particularly by Kuhn and Wiegandt (3), suggested that disialoganglioside is[1]



In earlier work on the biosynthesis of gangliosides (4), we employed a particulate preparation from embryonic chicken brain that catalyzed the synthesis of hematoside and monosialoganglioside when the system was incubated with the corresponding sialic acid-free glycolipids and CMP-sialic acid.[2] In the present studies, these findings have been extended; the particulate preparation contained a galactosyltransferase that catalyzed the following reaction.

Tay-Sachs ganglioside + UDP-galactose →

monosialoganglioside (+ UDP?)

Preliminary experiments also suggested that the preparation contained an *N*-acetylgalactosaminyltransferase that was active with hematoside; the postulated enzymatic product, Tay-Sachs ganglioside, has not yet been isolated.

The following substances were prepared by previously described methods: UDP-galactose and UDP-*N*-acetylgalactosamine labeled in the galactose and acetyl moieties, respectively (6); ceramide mono-, di-, tri-, and tetrasaccharides by partial acid hydrolysis of calf brain ganglioside followed by chromatography (7). Other substrates were either commercial preparations (*e.g.* the monosaccharides) or gifts.[3]

Galactosyltransferase activity was detected in homogenates of brain from embryonic chicken (7 to 20 days), pig fetus (82-mm fetus), and rat (9 days); maximum activity was observed with brains from 13- to 18-day-old embryonic chickens. A typical preparation was obtained from frozen 13-day embryonic chicken brain by homogenizing the thawed tissue with 4 volumes of a solution containing 0.25 M sucrose and 0.014 M 2-mercaptoethanol. The specific activity of the preparation was increased 4-fold when a particulate fraction, sedimenting between 3,000 and 39,000 × *g*, was collected; the activity was located in a light particulate layer overlaying a heavy residue. After washing the light layer twice with sucrose-mercaptoethanol solution, the particles were resuspended in the same solution to a final concentration of 8 mg of protein per ml; the activity was stable to storage for several months at −20°.

The optimum conditions for determining galactosyltransferase activity are given in Table I. As indicated in the table, EDTA completely inhibited the system, while Mn^{++} was required. This cofactor was employed when it was found to be an important component of the colostrum galactosyltransferase system (8). With the chicken brain particulate system, Co^{++} was about 20% as effective as Mn^{++} when tested at the same concentration, while the following ions were inactive: Mg^{++}, Zn^{++}, Ca^{++}, Ni^{++}, and Cu^{++}.

The results of specificity studies with the galactosyltransferase system are shown in Table II. Tay-Sachs ganglioside was the most active lipid acceptor; glycoproteins and *N*-acetylglucosa-

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[1] The nomenclature used in this report is as follows. Ceramide = *N*-acylsphingosine where the acyl group is primarily stearic acid; sphingosine = the C_{18} and C_{20} class of sphingosines and the corresponding dihydrosphingosines; all sugars are of the D configuration, and presumably pyranose ring structures; the location of glycosidic bonds is indicated by parentheses as shown in the abbreviated structure for disialoganglioside, where β refers to anomeric configuration of the sugar to the left of the parenthesis, while the arrow indicates where the anomeric carbon of the sugar at the left (C-1 except for sialic acid) is attached to the sugar at the right of the parenthesis; sialic acid = *N*-acetylneuraminic acid. By removal of 1 or more sugar residues from disialoganglioside, a series of ceramide oligosaccharides is obtained. All of the possible products have been isolated from natural sources or by partial acid hydrolysis of the ganglioside mixture or both. The following trivial names are used for these substances: ceramide mono-, di-, tri-, or tetrasaccharide corresponds to the disialoganglioside degradation products lacking sialic acid and where monosaccharide units are removed in a stepwise manner starting at the galactose end of the oligosaccharide chain (*e.g.* ceramide disaccharide = lactosylceramide); hematoside = sialyl-(2 → 3)-galactosyl-(β-1 → 4)-glucosyl → ceramide; Tay-Sachs ganglioside = disialoganglioside lacking the terminal galactose and sialic acid moieties; monosialoganglioside = disialoganglioside lacking 1 of the sialic acid residues; the term sialoganglioside is used in the literature (2) in preference to sialylganglioside.

[2] The two glycolipids appear to be substrates for different sialyltransferases. Embryonic chicken brain was used for these studies since this tissue rapidly accumulates glycosphingolipids during development (5).

[3] We are most grateful to the following individuals for their gifts of valuable compounds: Dr. Abraham Rosenberg for crude Tay-Sachs ganglioside (the material was purified by thin layer chromatography on silica gel G in a 1-propanol-water system (7:3)); Dr. Lars Svennerholm for standard monosialoganglioside and other glycolipids; Dr. Edward J. McGuire for the glycoproteins used in this study. Dr. Y. Kishimoto kindly performed the fatty acid analyses.