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Localization of Some Glycolipid Glycosylating Enzymes in the Golgi Apparatus of Rat Kidney

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Cell fractions from rat kidney were isolated and studied for their ability to synthesize several possible intermediates in the biosynthesis of sulfatides and gangliosides. The enzymes studied include UDP-Gal:ceramide galactosyltransferase, UDP-Gal:glucosylceramide galactosyltransferase, UDP-Gal:galactosylceramide galactosyltransferase, and CMP-NAN:lactosylceramide sialyltransferase activities. The initial glycosylation of ceramide was found to be present in all of the kidney cell fractions studied. The remaining glycosylating enzymes were largely localized in the Golgi apparatus of kidney. Thus, in addition to modifying glycoproteins for secretion, the Golgi apparatus in kidney is involved in the modification of a number of glycolipids which are destined to form cell membrane components.

Key words: Golgi, glycolipid biosynthesis, glycosyltransferases, kidney cell fractions

Golgi apparatus has been isolated from liver (1–3) and shown to be the main locus in the hepatocyte for the addition of galactose and sialic acid to secreted glycoproteins (4–6). These sugars are added stepwise to the nonreducing end of the carbohydrate chains of the glucoprotein by the action of specific galactosyl or sialytransferases which use nucleotide sugars as glycosyl donors (7). Analogous reactions have been shown to be involved in the biosynthesis of glycosphingolipids (Fig. 1) (7).

The biosynthesis of glycosphingolipids other than gangliosides has been reviewed by Morell and Braun (8). Ganglioside biosynthesis has recently been reviewed by Fishman and Brady (9). The reactions catalyzed by enzyme 1 (Fig. 1) have been shown to be present in kidney (10) and brain (11) microsomes. The synthesis of lactosylceramide (enzyme 4) has been described in spleen (12), kidney (13), and brain (14). A galactosyltransferase which adds a second galactose to galactosylceramide has also been shown to be present in rat kidney (15). The sialyltransferase which forms sialyllactosylceramide (GM_3) from lacto-

Abbreviations: CER – ceramide; Gal – galactose; Glu – glucose; UDP-Gal – uridine diphosphogalactose; UDP-Glu – uridine diphosphoglucose; PAPS – 3′-phosphoadenosine-5′-phosphosulfate; CMP-NAM – cytidine monophosphoryl-N-acetylneuraminic acid; GM_3 – sialyllactosylceramide, GD_3 – disialolactosylceramide.

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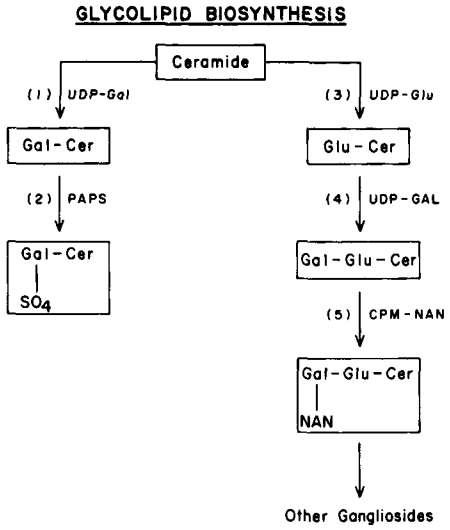

Fig. 1. Two possible pathways in the biosynthesis of sulfatide and gangliosides.

sylceramide (enzyme 5) was first described in embryonic chicken brain (16). GM_3 is a precursor of more complex gangliosides in this system (16). All of the transferase activities necessary to synthesize gangliosides from ceramide have also been shown to be present in mammary gland (17). The subcellular localization to these transferases in kidney, spleen, and mammary gland are not known. In brain, a number of glycolipid glycosyltransferases have been shown to be present in synaptosome fractions (18). Keenan et al. reported enzyme 5 to be highly enriched in Golgi apparatus isolated from rat liver, although activity was also found in isolated endoplasmic reticulum fractions (19).

In order to study the possible role of the Golgi apparatus in the biosynthesis of glycosphingolipids, we developed procedures for the isolation of Golgi apparatus from rat kidney (20), a tissue rich in glycolipids. By comparing the isolated Golgi fraction with isolated plasma membranes, endoplasmic reticulum, mitochondria, and nuclei, we showed that cerebroside sulfotransferase (reaction 2, Fig. 1) was localized mainly in Golgi membranes in kidney (21). This finding brought to light a new function for the Golgi apparatus, that is, that Golgi modifies not only mucopolysaccharides and glycoproteins, but glycolipids as well. Glycolipids are not normally secreted but are destined for use in cell membranes.

In this paper we report further studies aimed at localizing some of the other galactosyl- and sialyltransferases involved in the biosynthesis of sulfatide and gangliosides in kidney. These include UDP-Gal:ceramide galactosyltransferase (enzyme 1, Fig. 1), UDP-Gal: glucosylceramide galactosyltransferase (enzyme 4) and CMP-NAN:lactosylceramide sialyltransferase (enzyme 5).

METHODS

Isolation of Cell Fractions

Male Holtzman rats, 300–350 g, fed ad libitum were used throughout. The isolation of cell fractions from rat kidneys (9) and livers (2, 22) have been described previously.

Purity of the fractions was estimated using marker enzymes as detailed previously (2, 21, 22).

Enzyme Assays

UDP-Gal was obtained from Calbiochem, (Los Angeles, California) UDP-[^{14}C]-Gal uniformly labelled in the galactose moiety was obtained from New England Nuclear Corporation (Boston, Massachusetts). It was diluted with carrier to a specific activity of about 1 mCi per mmole before use. CMP-[^{14}C]-NAN(sialic 4-^{14}C), about 1 mCi/mmole, was purchased from New England Nuclear Corporation and used without additional carrier. Hydroxy fatty acid (HFA) ceramides, a natural mixture from bovine brain, and glucosyl and galactosyl ceramides were purchased from Applied Science Laboratories Inc. (State College, Pennsylvania). N-Lignoceroyl-DL-dihydrolactocerebroside was purchased from Miles Laboratories. Inc. (Elkhart, Indiana). Human serum transferrin was obtained from Sigma Chemical Company (St. Louis, Missouri). It was desialylated by treatment with 0.05 N sulfuric acid for 1 h at 80°C followed by dialysis as described by Spiro and Spiro (23). Authentic sialyllactosylceramide (GM_3) was the kind gift of Dr. Charles Sweeley, Department of Biochemistry, Michigan State University, East Lansing, Michigan. Cardiolipin was purchased from Sylvania Chemical Company, Orange, New Jersey.

UDP-Gal:ceramide galactosyltransferase (enzyme 1) was assayed as described by E. Costantino-Ceccarine and P. Morell (10). The assay mixture contained in order of addition, 100 μg protein (lyophylized); HFA ceramides (added in benzene and evaporated) 25 μg; Tris-HCl, pH 7.8, 75 μmoles; EDTA, 0.15 μmoles; dithiothreitol, 0.15 μmoles; and UDP-[^{14}C]-Gal 0.25 μmoles; in a total volume of 130 μl.

UDP-Gal:cerebroside galactosyltransferase (enzyme 4) assays contained, in order of addition, sodium cacodylate, pH 6.6, 40 μmoles; $MnCl_2$, 0.2 μmoles; cutscum, 750 μg; protein, 50 μg; glycosyl or galactosyl ceramide, 50 μg suspended first at 10 mg/ml by homogenization in 1% (wt/wt) cutscum in water; UDP-[^{14}C]-Gal, 0.10 μmoles; in a total volume of 100 μl.

CMP-NAN:lactosylceramide sialyltransferase (enzyme 5) was assayed in a mixture containing, in order of addition, imidazole·HCl, pH 6.5, 80 μmoles; a mixture of Triton CF-54:Tween 80 (2:1, wt/wt) 200 μg; protein, 20 μg; N-lignoceroyl dihydrolactocerebroside, 50 μg, suspended first at 10 mg/ml by homogenization in 1% (w/v) Triton CF-57:Tween 80 (2:1, wt/wt) in water; and CMP-[^{14}C]-NAN, 0.10 μg; in a total volume of 100 μl. For cell fractions with low levels of activity, up to 100 μg protein was used, but the ratio of detergent/protein was kept constant.

All incubations were for 1 h at 37°C. Reaction was stopped by addition of 3.0 ml chloroform:methanol (2:1, vol/vol). Radioactive cerebrosides were recovered and counted as described previously for assay of sulfatide formation (21). For measurement of GM_3 formation, the chloroform:methanol extracts were washed once after addition of 0.6 ml 2% (wt/vol) $CaCl_2$ plus 0.3 ml 1 N formic acid to form 2 phases (24). The lower phase was then washed 3 more times with an "upper phase" made by mixing the appropriate volumes of chloroform, methanol, water, $CaCl_2$, and formic acid. The final lower phase was dried and counted as described previously (21).

UDP-Gal:N-acetylglucosamine galactosyltransferase of the fractions was assayed essentially as described previously (22) except that the final concentration of N-acetylglucosamine used was 2 mM since higher concentrations are slightly inhibitory. CMP-NAN:glycoprotein sialyltransferase was assayed by a modification of the method of Schachter et al. (4). The assay mixture contained, in order of addition, sodium 2-(N-morpholino)

TABLE I. Specific Activities* of Glycoprotein Galactosyltransferase and Sialyltransferase Enzymes in Subcellular Fractions of Rat Liver and Kidney

	Galactosyltransferase		Sialyltransferase	
Fraction	Liver	Kidney	Liver	Kidney
Homogenate	6	7	7	2
Nuclei	2	3	2	1
Rough microsomes	12	4	8	2
Smooth microsomes	41	38	33	9
Golgi	764	650	550	135
Mitochondria	0	2	0	1
Plasma membranes	5	1	23	3
Supernatant	0	0	0	0

*Specific activities expressed as nmoles galactose of N-acetylneuraminic acid transferred per hour per mg protein at 37°C.

ethanesulfonic acid (Mes) pH 5.7, 5 μmoles; protein, 20–100 μg; Triton X-100, 300 μg; desialylated human serum transferrin, 750 μg; CMP-[^{14}C]-NAN, 20 nmoles; in a total volume of 55 μl. After incubation at 37°C for 1 h, 0.02 ml of 0.3 M EDTA was added and the assay mixture placed on ice. Aliquots were spotted on Whatman No. 3 filter paper disks, dried, and protein-bound radioactivity determined as described by Mans and Novelli (25).

RESULTS

Table I summarizes the distribution in the liver and kidney subcellular fractions of galactosyltransferase and sialyltransferase activities involved in glycoprotein biosynthesis. Both activities appear to be concentrated mainly in the Golgi apparatus fraction in liver and in kidney. The low levels of activity found in other cell fractions probably reflect contamination of these fractions with Golgi membranes (21, 22).

A number of glycolipid galactosyltransferases are involved in the synthesis of glycolipids. We have measured 2 of these activities in our fractions. They are enzymes 1 and 4 (Fig. 1). The results are summarized in Table II. The activities are present in kidney but are very low in liver. Both are membrane bound. Enzyme 1 differs from enzyme 4 and from glycoprotein galactosyltransferases in that it does not appear to be localized in Golgi membranes but is distributed in all the membranous fractions studied. In contrast to the enzyme from rat brain (26, 27), we found that enzyme 1 from kidney was not stimulated by added lecithin, in the presence or absence of Triton X-100. Enzyme 4 which converts glucosylceramide to lactosylceramide, a precursor of gangliosides, appears to be a Golgi enzyme. In the course of studying the specificity of this reaction, we found that galactosylceramide was a better acceptor for galactose than glucosylceramide in this assay system (Table II). The distribution of this activity in kidney cell fractions was similar to that of enzyme 4. It is not clear whether these activities are due to the same or to different enzymes. The activity with galactosylceramide as substrate is stimulated greatly by the addition of manganese (Fig. 2). Magnesium will not substitute for manganese in this reaction. Addition of detergents also stimulates the activity up to a point (Fig. 3), after which some detergents become inhibitory.

TABLE II. Specific Activity* of Some Glycolipid Galactosyltransferases in Rat Kidney and Liver Subcellular Fractions

Fraction	Ceramide[a] (1)↓UDP-Gal Gal-Cer		Gal-Cer ↓ UDP-Gal $(Gal)_2Cer$		Glu-Cer (4) ↓ UDP-Gal Gal-Glu-Cer
	Kidney	Liver	Kidney	Liver	Kidney
Homogenate	0.110	0.015	0.2	0.03	0.1
Rough microsomes	0.048	0.01	0.2	0.03	0.2
Smooth microsomes	0.087	0.03	0.2	0.13	0.4
Golgi	0.113	0.03	18.0	0.14	8.0
Mitochondria	0.030	0.01	0.1	0.01	0.1
Plasma membranes	0.057	0.01	0.3	0.09	0.2
Supernatant	0.001	–	0.0	–	0.0

*Expressed as nmoles galactose transferred per hour per mg protein at 37°C.
[a]Ceramides containing hydroxy fatty acids only were used as substrate (10).

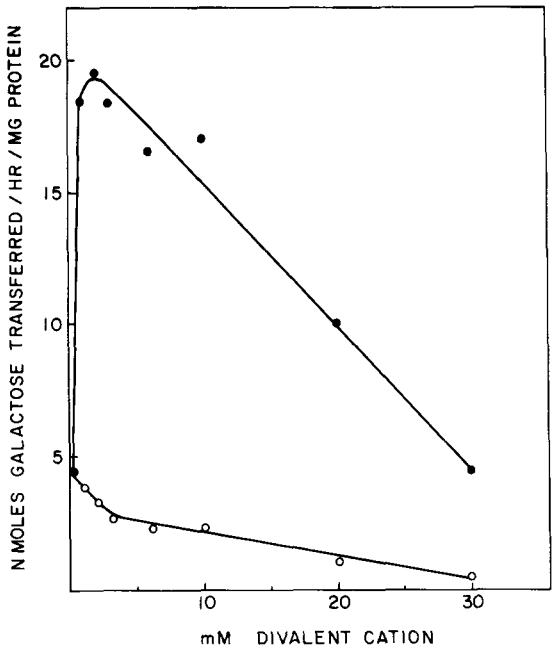

Fig. 2. Effect of varying concentrations of divalent cations in the form of their chloride salts on UDP-Gal:galactosylceramide galactosyltransferase activity of rat kidney Golgi apparatus. •—•) Mn^{2+}; ○—○) Mg^{2+}. Other constituents added as described under "Methods". The amount of protein used was 30 μg.

We next investigated the ability of kidney Golgi fractions to synthesize GM_3 (enzyme 5). It was reported by Keenan et al. (19) that added cardiolipin stimulates the formation of GM_3 by rat liver Golgi. We therefore first studied the effect of added cardiolipin on enzyme 5 activity of rat kidney Golgi. The results are summarized in Fig. 4. Addition of cardiolipin was slightly stimulatory at pH above 6.5, while at pH 6.4 cardiolipin

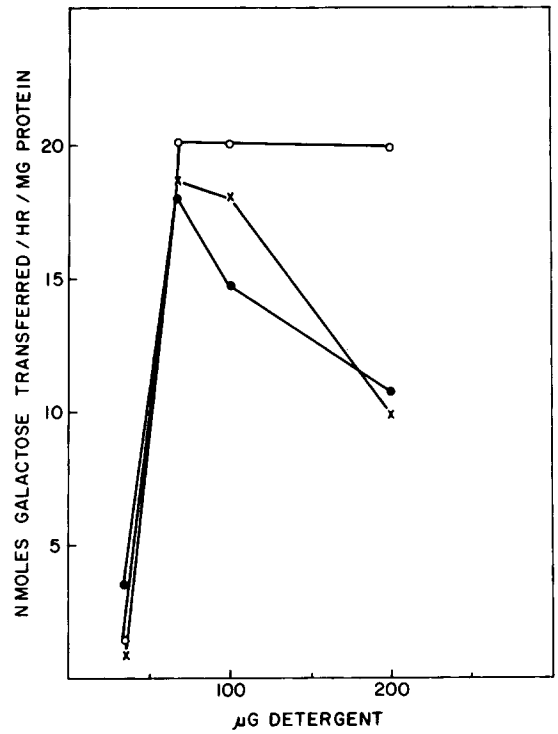

Fig. 3. Effect of varying detergent concentration and type on UDP-Gal:galactosylceramide galactosyltransferase activity of rat kidney Golgi apparatus. The final volume of the assay mixture was 100 μl and contained 20 μg protein. Other constituents added as described under "Methods." o—o) Triton CF-54:Tween 80, 2:1 wt/wt; x—x) Triton X-100; •—•) cutscum.

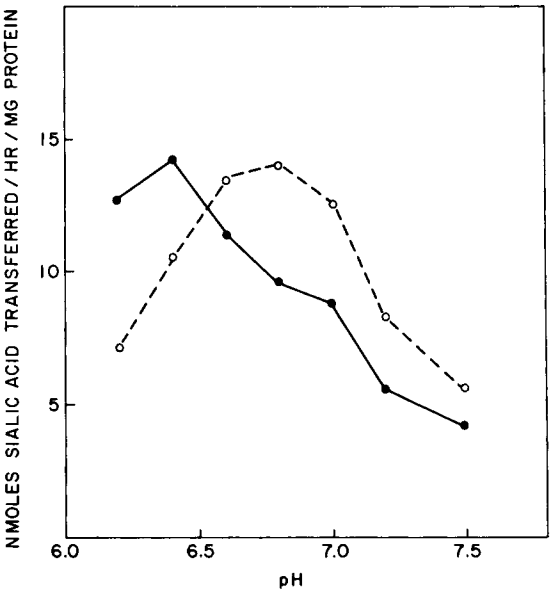

Fig. 4. Effect of varying pH of the assay mixture on CMP-NAN:lactosylceramide sialyltransferase activity of rat kidney Golgi apparatus. 40 μg protein and 10 mM Mg^{2+} were used throughout. Other constituents as described under "Methods." •—•) Without added cardiolipin; o—o) 7.3 μg cardiolipin were added to each assay before addition of the enzyme.

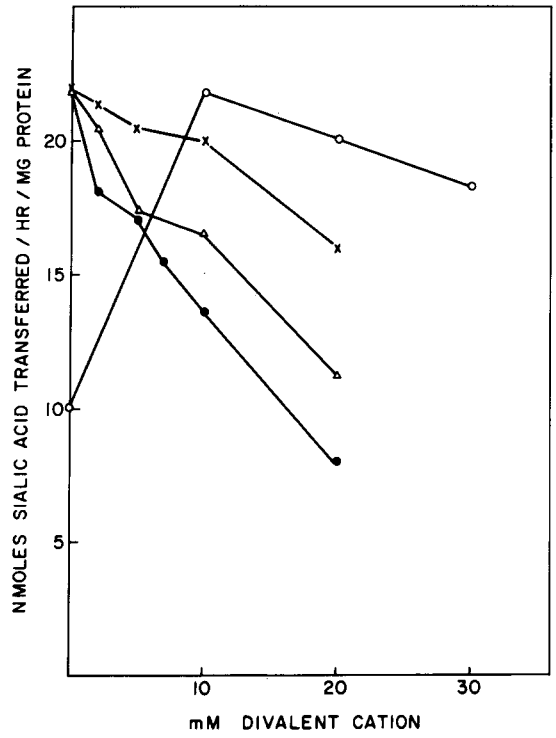

Fig. 5. Effect of divalent cations in the form of their chloride salts on CMP-NAN:lactosylceramide sialyltransferase activity of rat kidney Golgi apparatus. 40 μg protein were used throughout. x—x) Mg^{2+}; •—•) Ca^{2+}; ○—○) Mg^{2+} plus 7 μg cardiolipin, pH 6.6. All but the latter were run at pH 6.4.

was inhibitory. At pH 6.6 in the presence of cardiolipin, 10 mM magnesium was necessary for maximum activity (Fig. 5). At pH 6.4 in the absence of added cardiolipin, however, added magnesium was not necessary and was slightly inhibitory at high concentrations.

Addition of cardiolipin to the assay was also reported to change the nature of the products formed when rat liver Golgi was used as a source of enzyme (19). In the absence of cardiolipin, the main product was found by these authors to be GM_3, but when cardiolipin was added, the products were a mixture of GM_3 and disialolactosylceramide (GD_3). We investigated this possibility by incubating either rat liver or rat kidney Golgi in the presence and absence of cardiolipin at optimum pH and magnesium concentrations in each case. After removal of nonlipid contaminants, the products were chromatographed on thin-layer plates. As shown in Fig. 6, one main radioactive product was obtained using either Golgi preparation in the presence or absence of added cardiolipin. This product comigrated with a standard preparation of GM_3. A small second peak was detected using some preparations of liver Golgi. The concentration of this product was not enhanced by added cardiolipin, and it did not migrate as GD_3, that is, about midway between the origin and GM_3 (28).

The distribution of enzyme 5 activity was determined in purified cell fractions from liver and kidney in the absence of added cardiolipin. The results are summarized in Table III. The highest specific activity of the enzyme was found in the Golgi fractions from both tissues. The low levels of activity found in the other cell fractions are most likely due to contamination of these fractions with Golgi apparatus.

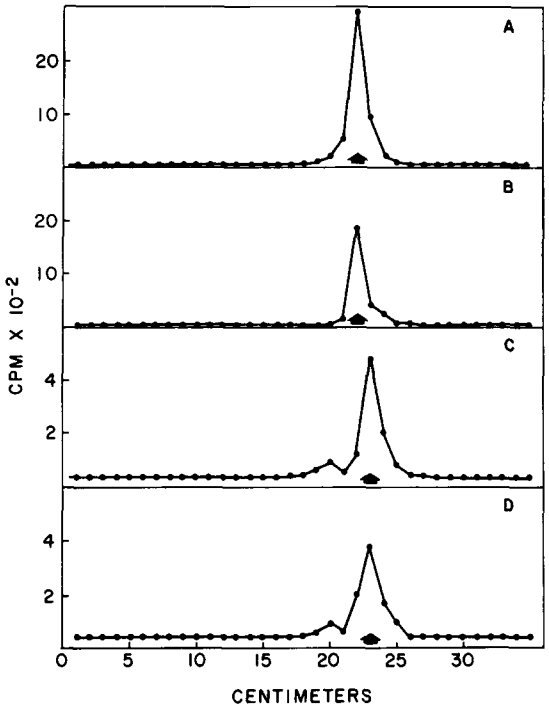

Fig. 6. Thin-layer chromatography of products of CMP-NAN: lactosylceramide sialyltransferase activity of rat kidney or liver Golgi apparatus. About 30 μg protein were used in each assay. Other constituents were as described under "Methods." After washing to remove nonlipid components, the chloroform:methanol, 2:1 (v/v) extracts were spotted on silica Gel G plates and developed 2 times with chloroform:methanol:2.5 N NaOH (65:45:9) (39). The arrows indicate the migration of standard GM_3. A) Rat kidney Golgi. B) Rat kidney Golgi plus 10 mM Mg^{2+} and 7 μg cardiolipin. C) Rat liver Golgi. D) Rat liver Golgi plus 10 mM Mg^{2+} and 7 μg cardiolipin.

TABLE III. Specific Activity* of Glycolipid Sialyltransferase in Rat Kidney and Liver Subcellular Fractions

	Gal-Glu-Cer (5) ↓ CMP-NAM NAN-Gal-Glu-Cer	
Fraction	Kidney	Liver
Homogenate	0.5	0.07
Rough microsomes	0.9	0.07
Smooth microsomes	2.4	0.70
Golgi	61.4	12.5
Mitochondria	1.2	0.0
Plasma membranes	1.2	0.2
Nuclei	0.1	0.06
Supernatant	0.0	0.0

*Specific activity expressed as nmoles N-acetylneuramic acid transferred per hour per mg protein at 37°C.

DISCUSSION

In addition to modifying glycoproteins for secretion, the Golgi apparatus appears to be involved in the modification of glycolipids as well. The initial glycosylation of ceramide to form galactosylceramide, however, does not appear to be exclusively a Golgi enzyme but is present in all of the membrane fractions from kidney. This is in sharp contrast to the sulfotransferase which converts galactosylceramide to sulfatide which we have shown previously is mainly localized in the Golgi apparatus in kidney (21). UDP-Gal:ceramide galactosyltransferase activity has also been demonstrated at a relatively high level in myelin from brain of young rats (27), indicating that in neuronal tissue that is actively forming myelin, plasma membranes can carry out this function.

Golgi apparatus from rat kidney is the main locus of galactosyltransferases which convert glucosylceramide to lactosylceramide or galactosylceramide to digalactosylceramide. These enzymes do not appear to be present in liver Golgi, and are therefore distinct from protein galactosyltransferase. UDP-Gal:ceramide galactosyltransferase also is very low in liver. This probably explains why liver, as a tissue, is low in all glycolipids.

Our studies illustrate further that Golgi apparatus from different tissues can vary considerably in its function. The most consistent function we have found in Golgi is UDP-Gal:N-acetylglucosamine galactosyltransferase, the enzyme involved in transferring the penultimate galactose to secreted glycoproteins (29). This enzyme has been shown to be present in Golgi from liver (1, 2, 4), kidney (21), pancreas (1), lung (30), thyroid (31), mammary gland (32), and testes (33). The enzyme also appears in a soluble form in milk (32), and serum (34). A slower-moving isozyme not found in normal serum has been shown to be present in sera of patients with carcinomas (35).

The sialotransferase which forms sialyllactosylceramide from lactosylceramide is present in both kidney and liver Golgi, although the activity is much higher in kidney than in liver. In both liver and kidney, this activity appears to be concentrated mainly in the Golgi apparatus and is not present to any appreciable extent in other cell fractions. In our assays using purified Golgi preparations from either liver or kidney, we could not detect the synthesis of disialylactosylceramide in the presence or absence of added cardiolipin. This is in contrast to the results of Keenan et al. (19). Since we used a synthetic, saturated lactosylceramide as substrate while Keenan et al. used lactosylceramide isolated from milk fat globule membranes, it is possible the enzyme transferring the second sialic acid would not work with our substrate. Another possibility which we have not explored is that the extraction procedure we have used to isolate the products of the reaction discriminates between the mono- and disialyllactosylceramides and that we have only recovered the monosialylceramide.

In the absence of added cardiolipin, we found no stimulation of enzyme 5 by added Mg^{2+}. It has been shown recently by Kemp and Stoolmiller, however, that treatment with EDTA inhibits enzyme 5 activity in neuroblastoma cells and that the activity is restored by addition of 1 mM Mg^{2+} or 2 mM Mn^{2+} (36). Our enzyme preparations were not treated with EDTA and thus may contain enough bound Mg^{2+} to satisfy the requirements of this enzyme.

In contrast to neuronal tissues, GM_3 and GD_3 are the major gangliosides of kidney (37). GM_3 appears to be the precursor of the more complex gangliosides of brain (16). GM_3, the product of enzyme 5 action (Fig. 1), is the major ganglioside present in newborn rat kidney cells in tissue culture. Changes in levels of this ganglioside during viral trans-

formation have been shown to be related to changes in the level of enzyme 5 activity (37) in the transformed cells. The induction of enzyme 5 in HeLa cells by treatment with butyrate also markedly alters the morphology of these cells and inhibits their growth (38).

The biological role of gangliosides is as yet poorly understood. Evidence that these lipids, as plasma membrane components, are involved in determining cell morphology, and may act also as cell surface receptors for viruses and hormones is accumulating (9). It would appear that in kidney, the Golgi apparatus plays a major role in the biosynthesis of these components.

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NOTE ADDED IN PROOF

When GD_3 (the kind gift of Dr. Robert Ledeen, Albert Einstein College of Medicine, Yeshiva University, Bronx, N.Y.) was added to the assay mixture for enzyme 5, it was not recovered in the chloroform layer after washing to remove non-lipid contaminants. Chromatography of the original reaction mixture as described in Fig. 6, however, resulted in separation of CMP-NAN, GD_3, and GM_3. When this was done for assays containing rat liver Golgi with or without added Mg^{2+} and cardiolipin, two radioactive peaks were observed which comigrated with authentic GM_3 and GD_3. In the presence of added Mg^{2+} and cardiolipin, the amount of GD_3 increased while the amount of GM_3 remained the same. This confirms the observations of Keenan et al. (19).

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