Synthesis of Galactosylceramide and Glucosylceramide by Mouse Kidney Preparations*

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SUMMARY

A particulate fraction was prepared from kidneys, lyophilized, and coated with ceramide. After incubation with UDP-[14C]Gal and appropriate cofactors, the major radioactive component in the lipid fraction was galactosylceramide (Gal-Cer). If UDP-Glc was substituted for UDP-Gal, the product was glucosylceramide (Glc-Cer). Specific activity for Gal-Cer synthesis was present at comparable levels in kidney preparations from males or females at early ages. However, between 20 and 30 days of age there was a 5-fold increase in activity in preparations from male kidney, followed by a slight decline. In contrast, Gal-Cer synthesis in kidney preparations from female mice decreased slowly from 10 to 70 days of age. Despite the low levels of Gal-Cer in kidney, enzymatic activity for its synthesis was high, 0.8 nmole per mg of protein per hour in 30-day-old males. This was 40% of the specific activity for Gal-Cer synthesis in brains of the same animals.

Glc-Cer synthesis by kidney followed a developmental pattern markedly different from that for Gal-Cer formation. There was an increase in activity throughout the time period studied (10 to 64 days of age), but activity was most rapid between 30 and 40 days of age. Activity in preparations of female kidney followed a similar pattern but was lower than that of preparations from males at 10 days of age and increased less rapidly. In kidneys of mature male animals, the specific activity for Glc-Cer synthesis was twice that of the females.

Galactosylceramide (cerebroside) and glucosylceramide (gluco cerebroside) are related chemically but are markedly different with respect to their biochemical properties. Galactosylceramide was originally isolated and characterized as a component of brain (2) and has been further localized as a major component of the myelin sheath (see Reference 3 for a review). Recently the terminal enzymatic step in the biosynthetic pathway for this compound, ceramide:UDP-galactose galactosyltransferase, has been demonstrated in cell-free preparations (4-6).

Free glucosylceramide is found in very low concentrations in brain tissue (7); its major metabolic significance is as an intermediate in the metabolism of gangliosides (8). The biosynthetic pathway for glucosylceramide synthesis is analogous to that for galactosylceramide synthesis and involves a ceramide:UDP-glucose glucosyltransferase (9). Developmental studies of the enzymatic activities for monoglycosylceramide synthesis using brain preparations have been carried out and the results interpreted in view of the presumed structural and physiological roles of these compounds in brain (6, 10-12). The activities for synthesis of galactosylceramide and glucosylceramide vary markedly during development, and there is considerable interest in how these enzyme activities are controlled.

To gain a better understanding of monoglycosylceramide synthesis in brain, we wanted to characterize the relevant enzymatic activities in another organ. Although outside of the nervous system the concentration of simple glycosphingolipids is quite low (13, 14), these compounds have been studied in kidney (15, 16). In particular the glycolipid composition of mouse kidney has been analyzed, and marked qualitative and quantitative differences between male and female mice have been demonstrated (17). In a preliminary study, Coles and Gray (18) demonstrated that levels of activity of some kidney glycosyltransferases involved in sphingolipid synthesis were sex-dependent. We have partially characterized the enzymatic activities for galactosylceramide and glucosylceramide synthesis in kidney and carried out a developmental study.

EXPERIMENTAL PROCEDURE

Materials

Ceramides containing hydroxy fatty acids were prepared as described previously (4). Ceramides containing nonhydroxy fatty acids were purchased from Applied Science Laboratories. UDP-[14C]Gal and UDP-[14C]Glc were obtained from New England Nuclear and diluted in the desired specific activity with nonradioactive nucleotide sugars obtained from Sigma. The mouse colony (a Swiss Webster line obtained from the stock of Flora O'Grady, 2336 Gunther Avenue, New York, N.Y. 10461) was maintained in our facilities and litters reduced to eight animals at birth.
**Assay Procedures**

**Tissue Preparation**

The assay procedures for glycolipid biosynthesis were similar to those previously described for brain tissue (4, 5), with the important modification (11) of the use of lyophilized tissue as enzyme source. Mice at the ages indicated were killed by decapitation, and brains and kidneys were rapidly removed and put on ice. Adrenal glands were removed from the kidneys.

The brains were homogenized in a motor-driven Potter-Elvehjem homogenizer in 4 volumes (wt/vol) of water and lyophilized. Kidneys, because of their content of connective tissue, required harsher disruptive procedures. They were passed through a tissue press and homogenized in 4 volumes (wt/vol) of 0.25 M sucrose as above, and further processed in a Ten Broeck tissue grinder. The homogenate was centrifuged at 2700 × g for 10 min, the supernatant collected, and pellet washed by rehomogenization in 3 volumes of sucrose and centrifugation as before. The supernatants were combined and centrifuged for 45 min at 105,000 × g, and the pellet was suspended in water and lyophilized. All steps were carried out at 4°C.

With both brain and kidney material assays were performed on the morning following overnight lyophilization. Specific incubation mixtures were prepared as follows:

**Galactosylceramide Synthesis by Kidney Preparations**—The lyophilized kidney particulate preparation was homogenized in benzene (1 mg dry weight of tissue per ml) in a Dounce homogenizer with a loose-fitting pestle. Aliquots of this suspension, 20 or 40 μl (equivalent to about 12 or 24 μg of protein), were pipetted into screw-capped tubes, 25 μg of HFA-ceramide (25 μl of a solution at 1 mg per ml in benzene) and the mixture evaporated under a stream of nitrogen. Aliquots were also removed for protein determination (19). The incubation mixture, final volume of 130 μl, contained also 75 μmoles of Tris-HCl, pH 7.8, 0.15 μmole of EDTA, 0.15 μmole of dithiothreitol, and 0.3 μmole of MgCl2. UDP-[14C]Gal (12.5 nmoles at a specific activity of 20 μCi per μmole) was added last.

**Galactosylceramide Synthesis by Brain Preparations**—This assay was carried out as described above, except that input levels of lyophilized whole brain homogenate, homogenized in benzene as above, were 250 or 500 μl (about 150 or 300 μg of protein). The amount of substrate HFA-ceramide was raised to 100 μg, and the incubation mixture was buffered with Tris-HCl at pH 7.8.

**Glucosylceramide Synthesis by Kidney Preparations**—In this assay 50 μg of lecithin (added as 5 μl of a solution at 10 mg per ml in ethanol) were evaporated onto the test tube and then 250 or 500 μl of lyophilized whole brain homogenate were suspended at 1 mg per ml in benzene (150 or 300 μg of protein) and 100 μg of NFA-ceramide were dried down together under nitrogen.

**Workup of Incubation Mixtures**

Incubation tubes, prepared as above, were sonicated in an ice-filled sonicating cleaning bath for 20 s to disperse aggregates and were then incubated at 37°C with vigorous shaking for 120 min. The reactions were stopped by addition of 0.5 ml of chloroform-methanol (2:1, v/v) to each tube. Protein was removed by filtration with Celite through a sintered glass funnel. The reaction tube and the funnel were rinsed out with 0.5 ml of chloroform-methanol (2:1, v/v) and 0.6 ml of 1 M KCl was added. The two phases were separated by centrifugation, and the lower phase was washed once with 1.4 ml of 1.0 M KCl-methanol (1:1, v/v), once with 1.4 ml of water-methanol (1:1, v/v), and once with 1.4 ml of the upper layer formed from chloroform-methanol-0.06 M KCl (8:4:3, v/v/v). The lower phase was then transferred to glass scintillation vials and the tubes rinsed twice with 1 ml of chloroform-methanol (2:1, v/v). After evaporation to dryness under nitrogen, the lipid residue was suspended in 10 μl of scintillation fluid (15.4 g of Permablend 111 (Packard), 800 ml of Triton X-100 (Rohm and Hass), and 2.3 liters of toluene). Radioactivity was quantitated in a Packard Tri-Carb liquid scintillation counter.

**Calculation of Data**

All incubations were carried out in duplicate and at the two protein levels indicated to insure that the assay was linear with enzyme concentration. Values reported for the developmental studies at each time point are the average of specific activities calculated from these four values. Standard deviations were less than 10% in each case.

**Characterisation of Radioactive Products**

In some experiments, the radioactive products were characterized by separation by thin layer chromatography on 0.5-mm plates of Silica Gel G (Brinkmann). The plates were developed in chloroform-methanol-water (14:25:28, v/v/v) twice and dried for 20 min at 50°C between runs (4). Thin layer chromatography on borate-impregnated plates was used to differentiate glucosylceramide from galactosylceramide (4, 20). The radioactive products were located by autoradiography on Kodak "no screen" x-ray film and shown to coincide with authentic standards (4, 5). The radioactive spots were collected, and radioactivity was determined as above.

**RESULTS**

**Growth of Kidney during Development**

The wet weight and protein content of individual kidneys were determined at various ages (Fig. 1). Starting at about 20 days of age, the kidneys of male mice show a preferential increase in weight over growth rate of kidney slows down. This difference in growth rate of kidney slows down. This difference in weight is not merely due to water accumulation, since the protein accumulation of kidneys of male and female mice is also markedly different (Fig. 1).

**Synthesis of Monoglycosylceramides by Kidney Preparations**

**Assay Procedure**—Early experiments to demonstrate cell-free synthesis of galactosylceramide in kidney were done by incu-
motor-driven homogenizer and a tissue grinder. Protein content of an aliquot was determined by the procedure of Lowry et al. (19) for insoluble residues, which involves treatment of the tissue with 1 M NaOH at 37°.

Bating aliquots of whole homogenate with a suspension of ceramide coated onto Celite along with the other components of the incubation mixture. However, in confirmation of the results of Brenkert and Radin with the brain system (11) we observed that by coating the substrate ceramide directly onto the tissue much higher enzymatic activity could be obtained. To utilize this method quantitatively, it was necessary to obtain a homogenous suspension of the tissue in benzene so that aliquots could be accurately distributed to incubation tubes. This also insured that the tissue particles would be small enough so that the substrate ceramide could most effectively contact the tissue. We retained that the tissue particles would be small enough so that the substrate ceramide could most effectively contact the tissue. We therefore, rather than store enzyme preparations as pellets, stored enzyme preparations as whole homogenate in 0.25 M sucrose to be removed by low speed centrifugation after the initial homogenization had to be carried out in 0.25 M sucrose which interfered with the assay (presumably by not allowing the ceramide to be well coated onto the tissue).

Possible cofactor requirements were studied in experiments shown in Table I. Note that the magnesium ions actually somehow inhibit the formation of glucosylceramide. We retained MgCl₂ in the incubation mixture for this work since the development of this work was already under way. The kidney preparation showed a marked specificity for the galactosylation of HFA-ceramide as compared to NFA-ceramide. Synthesis of NFA-galactosylceramide with NFA-ceramide as acceptor was only about 12% that of HFA-galactosylceramide with HFA-ceramide. Synthesis of NFA-galactosylceramide, gave a product co-migrating with authentic standard upon chromatography in the absence (Fig. 2) or presence of borate. HFA-ceramide was equally effective as a substrate in terms of [14C]glucose incorporation. Although no standard of glucosylceramide with its presumed (9) physiological precursor, NFA-ceramide, gave a product co-migrating with authentic standard upon chromatography in the absence (Fig. 2) or presence of borate. HFA-ceramide was equally effective as a substrate in terms of [14C]glucose incorporated. Although no standard of glucosylceramide was available, the decreased mobility of the radioactive product when HFA-ceramide was the acceptor strongly suggests that the product was HFA-glucosylceramide.

**Table I**

| Conditions                      | UDP-Gal | UDP-Gluc |
|--------------------------------|---------|----------|
| EDTA                           | 38      | 75       |
| MgCl₂                          | 140     | 33       |
| + 0.9 amole of MgCl₂           | 77      | 84       |
| - MgCl₂, - EDTA                | 71      | 72       |
| - Dithiothreitol               | 96      | 62       |
| + 0.3 amole of ATP             | 99      |          |
| - MgCl₂, - EDTA                | 46      |          |
| - Dithiothreitol, -MgCl₂, - EDTA | 39  |          |
| ATP                            | 6       | 80       |
| HFA-ceramide                   | 12      |          |
| HFA-ceramide + 25 µg of NFA-ceramide | 11   |          |
| Lecithin                       | 70      |          |
| NFA-ceramide + 50 µg of HFA-ceramide | 102 |          |

**Conditions**

The assay conditions for glucosylceramide biosynthesis by kidney were similar to those for galactosylceramide synthesis except for the inclusion of ATP. Cofactor requirements are shown in Table I. The absence of lecithin caused a decrease of 30% in activity for glucosylceramide synthesis. We also noted that the lecithin, dissolved in ethanol, had to be added to the tube first and the solvent evaporated. The presence of even this low concentration of ethanol while the benzene was being evaporated from the tissue inhibited enzyme activity by about 50%. Presumably the slight increase in solvent polarity was sufficient to denature some enzyme. Synthesis of glucosylceramide with its presumed (9) physiological precursor, NFA-ceramide, gave a product co-migrating with authentic standard upon chromatography in the absence (Fig. 2) or presence of borate. HFA-ceramide was equally effective as a substrate in terms of [14C]glucose incorporated. Although no standard of glucosylceramides containing HFA acids was available, the decreased mobility of the radioactive product when HFA-ceramide was the acceptor strongly suggests that the product was HFA-glucosylceramide.

**Stability of Enzyme to Storage**—As indicated in Table II, there was considerable loss of enzymatic activity during the first few weeks of storage of lyophilized kidney particulate preparations at -60°C. Therefore, rather than store enzyme preparations as they were prepared and then assay them all together, each preparation was assayed immediately after lyophilization.
Effect of pH—A broad pH curve, with optimal activity between pH 7.4 and 8.2, was characteristic of both of the glycosyltransferases of kidney.

Time Course of Reaction—Formation of galactosylceramide by kidney preparations was linear with time up to 3 hours, while the glucosyltransferase activity was linear for 4 hours. Our experiments were routinely incubated for 2 hours as described above.

Acceptor Concentration—The activity for galactosylceramide synthesis by kidney preparations from 45-day-old male mice remained constant when the amount of HFA-ceramide was varied between 10 and 300 μg per tube. When 22-day-old animals were studied, activity started to fall off slightly below 20 μg or above 75 μg of HFA-ceramide per tube.

Sugar Nucleotide Concentration—Our data for glucosyl- or galactosylceramide as a function of nucleotide sugar concentration are presented in Fig. 3. A level of 0.006 mM (12.5 nmoles/130 μl of incubation mixture), slightly below saturating levels for three of the assays, was utilized because of financial considerations.

Protein Concentration—It was critical for the developmental studies that the enzyme concentration used be in the region of linearity with respect to product formation. Therefore, enzyme concentration curves were run with preparations at several different ages. As can be seen in Fig. 4, the results for galactosylceramide synthesis differed depending upon the age and sex of the mice—material from younger males or from females of any age saturating the system at very low levels of protein and then, under our assay conditions, becoming slightly inhibitory with increasing protein concentrations. In contrast, particulate preparations from male kidney did not saturate the system until higher concentrations were reached, and inhibition was not observed until much higher levels of protein, about 0.25 mg, were used. The observed inhibition of galactosylceramide synthesis at higher levels of input protein might be due to increasing the level of an enzyme activity which competes for available UDP-Gal (e.g., a hydrolase which attacks the nucleotide sugar).

Another possible explanation is the presence of an enzyme inhibitor, presumably present at higher levels in kidney preparations from females. This last possibility is unlikely since addition of preparations from female kidney did not depress enzyme activity in incubation mixtures containing particulate preparations from male kidney. In all cases, the levels of kidney particulate protein used for the developmental study, about 12 and 24 μg, were in the linear region.

The results for glucosylceramide synthesis (Fig. 5) show much less variation with regard to age and sex of the animal, although

**Table II**

**Stability of Enzymatic Activity for Monoglycosylceramide Synthesis in Kidney Preparations**

| Monoglycosylceramide formed in assay mixtures | Days of storage after lyophilization |
|---------------------------------------------|-------------------------------------|
|                                             | 16  | 20  | 40  | 80  |
|                                             | %   |     |     |     |
| Glucosylceramide                            | 96  | 63  | 59  | 59  |
| Galactosylceramide                          | 86  | 58  | 43  | 42  |

Fig. 2. Radioautograph of enzymatically synthesized galactosylceramide and glucosylceramide. Incubation conditions were as described under "Experimental Procedure" using particulate preparations from kidneys of 45-day-old mice as enzyme source. The nucleotide sugar, ceramide substrate, and sex of the animal from which the kidney homogenate was prepared were, respectively: Lane 1, UDP-Gal, HFA-ceramide, female; Lane 2, UDP-Gal, HFA-ceramide, male; Lane 3, UDP-Gal, NFA-ceramide, male; Lane 6, UDP-Glc, NFA-ceramide, female; Lane 7, UDP-Glc, NFA-ceramide, male; Lane 8, UDP-Glc, HFA-ceramide, male. Triplicate incubation mixtures were pooled before lipid extraction and chromatography (see "Experimental Procedure") except for Lane 8 where only two incubations were pooled. The darkening of the photographic plate in Lane 1 (at the area of Spot d) and of Lane 3 (at Spot e) was too light for reproduction due to the low levels of radioactivity incorporated (see Table I). Lane 4 contained a standard of beef spinal cord lipids; the same standard was included in Lanes 1 to 3. Lane 5 contained a standard of glucosylceramide, as did Lanes 6 to 8. The stippled lines represent spots made visible by iodine vapors. Spots c and d of Lanes 1 to 4 contain, respectively, the galactosylceramides having very long chain NFA and very long chain HFA (4, 5). The area directly below Spot c and the area directly below Spot d (stippled in Lane 4 but omitted from Lanes 1 to 3 for purposes of clarity) contain, respectively, the galactosylceramide having shorter chain NFA and shorter chain HFA (4, 5). Spots c and d of Lanes 5 to 8 presumably (21) contain the glucosylceramides containing very long chain NFA and shorter chain NFA.

Fig. 3. Monoglycosylceramide synthesis as a function of nucleotide concentration. Assays were conducted as described under "Experimental Procedure" using kidney preparations from 45-day-old mice as the enzyme source, except that the lyophilized enzyme source (particulate preparation from kidneys of 35-day-old male mice) was stored at -60°C as indicated. Values are averages of triplicate determinations.
FIG. 4. Synthesis of galactosylceramide by kidney preparations as a function of protein concentration. Particulate fractions obtained from four male or four female mice at 22 or 45 days of age were assayed as described under "Experimental Procedure" with HFA-ceramide as acceptor and UDP-[3H]Gal as donor, except that the input of kidney particulate fraction was varied as indicated. Results are the average of triplicate determinations with a mean deviation of less than 10%.

FIG. 5. Synthesis of glucosylceramide by kidney preparations as a function of protein concentration. The legend of Fig. 2 applies except that NFA-ceramide was the acceptor and UDP-[3H]Glc as the donor.

with older animals there is still a marked difference in activity between kidney from male and female mice. Assays for the developmental study were done at protein levels of 30 and 60 µg, which was in the linear region in all cases.

Developmental Study—Galactosyltransferase is present at comparable levels in kidney preparations from male or female mice at young ages. However between 20 and 25 days of age there is a 4-fold increase in activity in preparations from male kidney. As can be seen in Fig. 6, the level of galactosyltransferase activity peaks at about 30 days of age and declines slowly. In contrast, activity from kidney preparations of female mice declines slowly from 10 to 70 days of age. The net result is a 5-fold difference in enzymatic activity for galactosyltransferase after 30 days of age.

Enzymatic activity for glucosyltransferase (Fig. 7) increased with development for the time period studied, but most rapidly between 30 and 40 days of age. Enzymatic activity of preparations from female kidney followed the same general pattern but was lower than that of male kidney at early age points and increased less rapidly. By 64 days of age, activity in female kidney was less than half of the activity in male kidney.

Synthesis of Monoglycosylceramide by Brain Preparations

As a control for the work with kidney, monoglycosylceramide synthesis was assayed with brain preparations of different ages. The results (Fig. 8) are almost identical with those reported for rat brain by Brenkert and Radin (11). We have extended that study by showing that there is no difference in capacity of brain homogenates of male and female mice to synthesize monoglyco-
syleceramides. Note that endogenous levels of NFA-ceramide are high in brain, as evidenced by the significant synthesis of glucosylceramide in the absence of acceptor.

**DISCUSSION**

The ceramide:UDP galactosyltransferase of kidney differs in several respects from the brain enzyme. Our preliminary data on cofactor requirements (Table I) indicate that the kidney enzyme is inhibited by some endogenous substance which is chelated by EDTA. Magnesium or manganese ions inhibit at low concentrations, possibly by competing with EDTA complexing of this substance. Activity is not inhibited by ATP. These results are different from the divalent ion requirement and ATP inhibition observed for the brain enzyme (11). There is also a marked difference in the level of tissue protein required to saturate the system. Kidney tissue saturates the system at 25 to 50 µg of protein, while synthesis of galactosylceramide by brain tissue assayed under the same conditions is linear with respect to protein concentration to at least 500 µg. The marked substrate specificity for fatty acids containing hydroxy fatty acid in the N-acyl position as compared to utilization of nonhydroxy fatty acid containing ceramides was the same in kidney as in brain. By optimizing conditions with respect to galactosylceramide synthesis in kidney preparations we demonstrated incorporation of galactose into galactosylceramide at levels three orders of magnitude more efficiently than previously reported (23). The magnitude difference in the amount of product accumulated in the two organs (10-12, 22-24) that in brains of mice and rats this galactosyltransferase activity peaks between 15 and 20 days of age, when the rate of myelin accumulation is highest (12).

In kidney of male mice, enzymatic activity for galactosylceramide synthesis (Fig. 6) follows a pattern which is almost the reciprocal of the brain pattern, activity in kidney rising rapidly from 20 to 30 days of age, while activity in brain is declining. The activity in kidney presumably does not relate to the rate of product accumulation, for it remains elevated in the mature animal and does not diminish in activity as does the brain enzyme. In the adult animal potential activity for galactosylceramide synthesis is at almost the same levels in brain and kidney (Figs. 6 and 8). However, there are almost three orders of magnitude difference in the amount of product accumulated in the two organs. Galactosylceramide accounts for several percent of the dry weight of mature mouse brain (25), whereas total monoglycosylceramide accounts for a few hundredths of a percent of total dry weight of kidney (17). Presumably this high synthetic capacity for galactosylceramide synthesis in kidney is related to its rapid turnover of galactosylceramide. It is likely that such a rapid turnover is related to the role of galactosylceramide as a precursor of sulfatide (26) which turns over rapidly in kidney (27). This contrasts to the relative metabolic stability of brain galactosylceramide (28, 29).

The developmental curve for galactosylceramide synthesis in kidney of female mice is markedly different from the results obtained with males. Although capacity for galactosylceramide synthesis is lower, the ratio of enzymatic activity to galactosylceramide accumulated is still almost two orders of magnitude greater than that in brain.

The developmental pattern for glucosylceramide synthesis in kidney (markedly different from that in brain) showed a marked increase in enzymatic activity during development. This increase was both later in time and more gradual than the corresponding increase for galactosylceramide synthesis and less marked in the female than in the male. Synthetic capacity for glucosylceramide synthesis (Figs. 7 and 8) as related to the amount of substrate accumulated (as diglycosyl- or triglycosylceramide in the case of kidney (17) or as ganglioside in brain (25)) is several orders of magnitude greater in kidney than in brain.

Markedly higher kidney weights of males relative to females have been observed in many mammals (31, 32). It is also well known that injections of testosterone have a marked effect on renal tissue; the induced renal hypertrophy is localized in tubules, and the parietal layer of Bowman's capsule becomes thicker and acquires a brush border (31-34). The specific activity of a number of enzymes is elevated in kidney of male mice, relative to female mice, and these differences are obliterated by testosterone.
treatment of the female (35). Such changes attributable to testosterone are of the order of 50% on a specific activity basis. Also relevant is the observation of Y.-N Lin and N.S. Radin, who have carried out developmental studies of galactosylceramide galactosidase in rat kidney. 3 Although this activity decreases during development in both sexes, it declines more rapidly in the female. The difference in activity is 31% at 50 days of age.

The sex-related difference in glucosylceramide synthesis might fall into the category of a relatively nonspecific response due to a generalized anabolic action of testosterone. It would seem, though, that the 5-fold preferential synthesis of galactosylceramide by male, relative to female, kidney represents some highly specific response to male hormones. Indeed, Gray (36) who have carried out developmental studies of galactosylceramide by male, relative to female, kidney represents some generalized anabolic action of testosterone. It would seem, therefore, that galactosylceramide or sulfatide is implicated in certain active transport functions (38, 39). It is for speculation it should be mentioned that sulfolipids have been difference in galactosylceramide synthesis. As a departure point raises galactosylceramide synthetase activity severalfold to the levels found in male kidney and causes changes in glycolipid composition (37).

There are several possible explanations for this sex-related difference in galactosylceramide synthesis. At a departure point for speculation it should be mentioned that sulfolipids have been implicated in certain active transport functions (38, 39). It is possible, therefore, that galactosylceramide or sulfatide is engaged in some sort of transport function of quantitatively much greater significance in male, as compared to female, kidney.

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