Galactose Metabolism by the Mouse with Galactose-1-Phosphate Uridyltransferase Deficiency

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ABSTRACT

The ability of mice deficient in galactose-1-phosphate uridyltransferase (GALT) to metabolize galactose was determined in animals weaned to a mouse chow diet for a 4-wk period. When given [^{14}C]galactose intraperitoneally, these animals slowly oxidized the sugar, excreting only 5.5% of the dose as $^{14}\text{CO}_2$ in 4 h, whereas normal animals excreted 39.9%. These results mimic those seen in human galactosemic patients given isotopic galactose. When given 10 μmol of [^{13}C]galactose, normal animals excrete small amounts of labeled galactose and galactonate but no galactitol in urine whereas GALT-deficient mice excrete significant amounts of all of these as labeled compounds in urine. When challenged with galactose, only about 20% of the dose is excreted in urine, and even on the chow diet, significant amounts of galactose, galactonate, and galactitol are excreted in urine. These compounds are also found to be present in liver, kidney, and brain, except that galactonate is not found in brain. Galactose-1-phosphate accumulates in red blood cells to levels found in humans exposed to large amounts of galactose, and galactose-1-phosphate is found in increased amounts in liver, kidney, and brain of GALT-deficient animals. There was no difference in the hepatic concentration of uridine diphosphate galactose and uridine diphosphate glucose between normal and GALT-deficient mice. The explanation for the presence of galactose and its

conversion products in tissues and urine of affected mice appears to be related to the presence of approximately 1.75% of galactose-containing carbohydrates in the chow, which becomes bioavailable to mice. Despite the presence of galactose and its metabolites in tissues and urine and impaired ability to oxidize the sugar, the GALT-deficient animals are indistinguishable from normal animals and do not exhibit the phenotype of humans with GALT-deficiency galactosemia. (*Pediatr Res* 48: 211–217, 2000)

Abbreviations

GALT, galactose-1-phosphate uridyltransferase
Gal-1-P, galactose-1-phosphate
N/N, normal mice
G/N, heterozygous normal and GALT deficient
G/G, homozygous for a deficiency of GALT
UDPgal, uridine diphosphate galactose
UDPglu, uridine diphosphate glucose
BSTFA, *N,O*-bis-(trimethylsilyl)trifluoroacetamide
TMCS, trimethylchlorosilane
CHOP, Children's Hospital of Philadelphia
GC-MS, gas chromatography–mass spectroscopy

After more than 60 y of experience with the diagnosis and dietary therapy of galactosemia caused by GALT deficiency, the pathophysiology of the disorder has remained unclear (1). Enigmatic has been the origin of both the rapid development of liver, kidney, eye, and central nervous toxicity occurring in newborns exposed to large amounts of galactose in milk and the long-term diet-independent complications of cognitive im-

pairment, speech and learning disorders, ataxia, and primary ovarian failure in females. To enhance the study of the pathogenesis of GALT deficiency and further our understanding of the cause of the galactosemic phenotype, we made mice deficient in GALT activity using gene-targeting techniques (2).

We have previously reported that, in mice in which exons 6–8 of the mouse GALT gene were removed, there was a complete absence of hepatic GALT activity in 7-d-old suckling animals. The plasma of these animals contained high levels of circulating galactose, and their red blood cells and liver tissue accumulated high levels of Gal-1-P. Despite the absence of liver GALT and the presence of Gal-1-P in these tissues, the

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homozygous GALT-deficient suckling animals appeared normal and were indistinguishable from normal littermates.

The present studies were performed to further characterize the effect GALT deficiency on the disposition of galactose. We have assessed the ability of the GALT knockout mice to metabolize the sugar. After weaning the animals to a normal mouse chow diet for 4 wk, we quantitated their ability to oxidize [^{14}C]galactose to $^{14}\text{CO}_2$, measured urinary metabolites after administering both unlabeled galactose and 1- ^{13}C galactose, and quantitated the presence in various tissues of galactose, Gal-1-P, galactitol, and galactonate. The results of these studies compared with those in normal mice form the basis of this report.

METHODS

Animals. Fifty-four animals were included in the study: 20 were normal CF-1 mice (N/N), 29 were CF-1 mice homozygous for a deficiency of GALT (G/G), and five were heterozygous for (G/N) as shown by Southern blotting of DNA from tail snips at 21 d of age. Normal mice bred in the CHOP animal facility were derived from animals purchased from Charles River Company (Wilmington, MA, U.S.A.). The GALT-deficient mouse colony at CHOP was established from founders from Dr. Nancy Leslie's colony at the Children's Hospital Research Foundation in Cincinnati, OH, U.S.A. All mice were weaned at 21 d to mouse chow 5015 obtained from Purina Mills, Inc (Richmond, IN, U.S.A.) and maintained on this diet for 4 wk, at which time *in vivo* metabolic studies were performed or animals were killed to determine galactose metabolite concentrations in various tissues.

The 5015 mouse chow diet is a complete life cycle diet containing 17.5% protein, 11% fat, and 2.5% fiber, with the remainder consisting of complex carbohydrates. Three samples of this diet were analyzed with a GC-MS method by Dr. K. Gross of the U.S. Department of Agriculture, who found this diet to contain an average of 1.75% of acid-hydrolyzable galactose, which could be bioavailable in the rodent intestine by enzymatic digestion. The high galactose diet used contained 40% galactose, in which the free sugar replaced the complex carbohydrate portion of the 5015 mouse chow (Purina Mills, Inc). All animal studies were performed under protocols approved by the CHOP Institutional Animal Care and Use Committee.

Oxidation of [^{14}C]galactose. Mice consumed water and diet *ad libitum* until the time of study. Each animal was injected with 0.5 mL of saline intraperitoneally containing 0.5 μCi of 1- ^{14}C galactose, and expired CO_2 was continuously collected for up to 10 h. All galactose oxidation studies were performed in tandem with G/G and N/N animals.

The $^{14}\text{CO}_2$ collection was accomplished by placing the animal in a small chamber through which room air was pulled by vacuum, maintaining an air flow of 300 mL/min measured by a Manostat flow meter (Manostat, New York, NY, U.S.A.). The effluent stream from the chamber passed through a Kontes (Kontes Glass Co. Vineland, NJ, U.S.A.) midjet bubble collector containing 15 mL of benzethonium hydroxide solution to trap expired CO_2 . Total trapping of $^{14}\text{CO}_2$ was accomplished

under these conditions as shown by experiments in which $^{14}\text{CO}_2$ was liberated from [^{14}C]NaHCO₃ in the chamber. One hundred-microliter aliquots were taken every 15 min from the collection reservoir for counting of labeled carbon in the expired CO_2 . The collection reservoir was replaced every 30 min with a clean reservoir containing 15 mL of nonsaturated benzethonium hydroxide. The 100- μL aliquots were added to scintillation vials containing 10 mL of Ecolite (+) (ICN Pharmaceuticals Inc, Costa Mesa, CA, U.S.A.), and the $^{14}\text{CO}_2$ was counted in a Packard liquid scintillation counter, model 2000 (Packard Instrument Co., Downers Grove, IL, U.S.A.).

Urinary metabolites after galactose administration. Two types of studies were performed in which the metabolic fate of administered galactose was monitored by measurement of urinary galactose, galactonate, and galactitol. In the first type, 1.8 mg (10 μmol) of 1- ^{13}C galactose was injected intraperitoneally to animals either fed chow *ad libitum* or fasted for 12 h followed by 24 h without food while urine was collected. In the second, G/G mice were challenged with nonisotopic galactose loading, which was performed by intraperitoneal injection of 76 mg into *ad libitum* fed animals fasted subsequently during the 24-h urine collection or by placing the normal chow fed animals on a 40% galactose chow diet for 24 h while urine was collected. Urine was collected in specially designed cages for mice, which separated urine from feces and prevented any feed from contaminating the urine.

Tissue, red blood cells, and urine preparation for metabolite analysis. Animals were killed by cervical dislocations. Blood samples were obtained by cardiac puncture using a heparinized syringe, after which liver and kidneys were immediately harvested and pressed between metal tongs that had been cooled in liquid nitrogen. The head was severed into liquid nitrogen for later brain tissue preparation. All tissues were stored at -80°C . Frozen tissue samples were weighed and homogenized in a chilled Potter-Elvehjem glass homogenizer containing 5 mL of 3% perchloric acid chilled to 4°C . After homogenization with three strokes of a polytetrafluoroethylene pestle, the mixture was transferred to 15-mL centrifuge tubes and spun at $900 \times g$ for 10 min. The supernatant was transferred to a different 15-mL centrifuge tube, neutralized with KOH, and centrifuged again at $900 \times g$ for 10 min. This final supernatant was the tissue extract, which was either analyzed directly or frozen at -80°C for future analysis.

Red blood cells were prepared by centrifuging whole blood to obtain a packed red cell pellet, which, after washing three times with normal saline, was frozen at -80°C .

Urine was frozen at -80°C until needed for GC-MS analysis. After thawing, the urine was treated with urease and subsequently with ethanol to precipitate any protein.

GC-MS metabolite analysis. A tissue extract (200 μL) was transferred to a 10-mL glass tube, to which 25 nmol of heptadecanoic acid in methanol was added as an internal standard. The sample was dried under a stream of nitrogen. After adding 1 mL each of methanol and methylene chloride, the sample was redried under a stream of nitrogen. The residue was trimethylsilylated with 0.2 mL each of BSTFA with 1% TMCS and pyridine at 80°C for 1 h. Urine samples were prepared by the method previously reported (3).

An HP 6890/5973 GC-MS (Hewlett-Packard Co., San Fernando, CA, U.S.A.), analyzer equipped with electron ionization was used for analysis. Gas chromatographic separation was performed with temperature programming on a fused silica DB-5 capillary column (30 m $\times$ 0.25 mm, J&W Scientific). Temperature for injector and detector was 250°C and 270°C, respectively. Identification of galactose, galactitol, and galactonate was based on the comparison of retention time and mass spectra with standard compounds. Quantitation of these compounds was calculated by standard curves based on relative peak area of specific ion for each compound compared with the ion 327 of the internal standard. Ions used in quantitation for [^{12}C]galactose, [^{12}C]galactitol, and [^{12}C]galactonate were 191, 217, and 292, respectively; for [^{13}C]galactose and [^{13}C]galactonate, 192 and 293, respectively. Enrichment in the urine after [^{13}C]galactose administration was calculated by the ratio of 192 to 191 for [^{13}C]galactose and the ratio of 293 to 292 for [^{13}C]galactonate. Because ions from [^{13}C]galactitol after trimethylsilylation overlapped ions from [^{12}C]galactitol because of its symmetrical chemical structure, a standard curve for [^{13}C]galactitol enrichment was obtained from the ratios of 218 to 217 from a preparation of different concentrations of [^{13}C]galactitol and [^{12}C]galactitol. Urinary enrichment of [^{13}C]galactitol was calculated by this standard curve. Total concentration of galactitol in urine collected after the [^{13}C]galactose study was calculated according to the whole peak area on gas chromatography. Concentration of urinary [^{13}C]galactitol and [^{12}C]galactitol was calculated by the enrichment of [^{13}C]galactitol and the total concentration of galactitol.

Gal-1-P and nucleotide sugar analysis. Red blood cell gal-1-P was determined enzymatically in both erythrocyte hemolysates and tissue homogenates (4). Levels in some tissues were quantitated by phosphorus nuclear magnetic resonance spectrometry, which verified the enzymatic results (5). The concentration of UDPgal and UDPglu was measured by a published HPLC method (6).

Materials. [^{14}C]galactose, specific activity, 55 mCi/mmol, was purchased from American Radiolabeled Chemicals, Inc (St. Louis, MO, U.S.A.), and [^{13}C]galactose, 99% atom-enriched, was purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.A.).

Statistics. Averages are given as mean $\pm$ SEM. Comparison of data for normal and galactosemic mice was evaluated using the *t* test.

RESULTS

Oxidation of [^{14}C]galactose. The oxidation of [^{14}C]galactose to $^{14}\text{CO}_2$ during 4 h after i.p. injection is shown in Figure 1A for four N/N, four G/G GALT-deficient, and five G/N heterozygote mice. The normal mice readily oxidized the galactose to $^{14}\text{CO}_2$, reaching a peak rate in 25–30 min with a subsequent decline. In contrast, the G/G mice liberated $^{14}\text{CO}_2$ at a low but detectable rate without a brisk rise and with a markedly different pattern compared with the normal animals. Heterozygote animals oxidized the sugar at a slightly slower initial rate and exhibited a lower peak rate than the normal mice.

Figure 1B shows the cumulative excretion of radioactivity in the expired CO_2 , determined by integration of the area under the curves in Figure 1A. The counts represented by these areas divided by the administered dose of the radioactive sugar at each time point are plotted as percents of the dose *versus* time. In the 4-h period, normal animals oxidized 39.9% of the administered [^{14}C]galactose, whereas the G/G mice metabolized only 5.5% of the dose ($p < 0.001$). Heterozygote animals oxidized 37.5% of the dose in the same time period. Although the early rate of the G/N mice differed from that of the N/N animals, there was no difference in the cumulative conversion to $^{14}\text{CO}_2$ in 4 h. In additional studies in two G/G mice in which the excretion of $^{14}\text{CO}_2$ was followed for 10 h, the cumulative excretion slowly increased and approximated 7.5% of the injected sugar. Two G/G mice that were fasted 15 h before administration of the labeled sugar exhibited the same slow rate of $^{14}\text{CO}_2$ excretion as the other animals fed *ad libitum* until the time of study.

Urinary excretion of [^{13}C]galactose and metabolites. Because the technique for collecting expired air after [^{14}C]galactose administration did not permit the collection of urine and GC-MS is an excellent method for metabolite identification, quantitation, and ^{13}C isotopic analysis, the metabolic fate of galactose was examined by administration of [^{13}C]galactose. In these studies, the urinary excretion of galactose, galactonate, and galactitol was measured after the injection of 1.8 mg (10 μmol) of 99% atom-enriched [^{13}C]galactose in 50-g mice. The results are shown in Table 1. The use of GC-MS permits quantitation of both ^{12}C normal carbon-containing compounds and ^{13}C -labeled material, thus enabling assessment of the fate of the injected isotope on the background of ingested or endogenously produced galactose. In the studies with normal mice, the amount of urine collected in 24 h was augmented by placing two animals together in each collection apparatus. The data represent the average of two such studies. Parallel studies were performed with G/G animals.

The results in normal animals were similar whether the isotope was injected into nonfasted animals or into animals fasted 12 h before the urine collection. Only small amounts equaling about 1% of the dose of 10 μmol were excreted as galactose or galactonate. No labeled galactitol was detected ($<0.001 \mu\text{mol}/\text{mouse}$). Very small amounts of [^{12}C]galactose and [^{12}C]galactonate were found.

In contrast, G/G animals which were not fasted before injection of the isotope excreted much larger amounts of the [^{13}C]galactose injected (35.6%) than normals. This was also true for [^{13}C]galactonate. Isotopically labeled galactitol was found. The total excretion of isotopically labeled material represents about 47% of the 10 μmol given, most of which was as the injected sugar. These animals excreted in excess of 20 μmol of [^{12}C]galactose, [^{12}C]galactonate, and [^{12}C]galactitol, more than half being accounted for by galactitol, which was higher than galactonate by a factor of 3.

When G/G animals were fasted 12 h before injection of [^{13}C]galactose and then fasted during the urine collection, 23% of the ^{13}C dose was excreted, again more than half as galactose itself. Labeled galactonate and galactitol were found. The

Figure 1. *A*, The rate of oxidation of [1-¹⁴C]galactose by normal (●; $n = 4$), GALT-deficient (○; $n = 4$), and heterozygous (◆; $n = 5$) mice. Bars indicate SEM of the mean. Only the rate at 22.5 min for the heterozygous group differs from the normal mice ($p < 0.01$). *B*, The calculated cumulative excretion of ¹⁴CO₂ as a percentage of injected radiolabeled galactose. Bars indicate SEM of the mean. GALT-deficient animals significantly differ from the normal animals ($p < 0.001$).

excretion of naturally occurring ¹²C compounds was markedly lower in animals fasted 12 h before injection than in animals fed before injection. The galactose excretion was reduced almost 9-fold, and the galactonate and galactitol excretion, by more than 3-fold.

Galactose and metabolite excretion after galactose challenge. Table 2 shows the urinary excretion of galactose, galactonate, and galactitol after two types of challenge in GALT-deficient G/G animals. In the first, in which 76 mg of the sugar (1.5 g/kg) was injected intraperitoneally, 81.5 μ mol or 19.3%

of the 422 μ mol injected were found as these three compounds, with galactose representing 14% of the injected dose while galactonate was 3% and galactitol 2.2%. When fed a 40% galactose diet and consuming a calculated 2222 μ mol of galactose, each mouse excreted 10.6% and 11.1% (~244 μ mol) of the injected galactose as the three compounds, nearly all as galactose itself.

Erythrocyte and tissue galactose-1-phosphate. Because measurement of red blood cell gal-1-P is a prevalent method of assessing dietary status of treatment of human galactosemic

Table 1. Mouse urinary metabolites after $[1-^{13}\text{C}]$ galactose administration

Animals	Galactose ($\mu\text{mol}/24\text{ h}/\text{mouse}$)		Galactonate ($\mu\text{mol}/24\text{ h}/\text{mouse}$)		Galactitol ($\mu\text{mol}/24\text{ h}/\text{mouse}$)	
	^{12}C	^{13}C	^{12}C	^{13}C	^{12}C	^{13}C
No prior fasting*						
N/N ($n = 4$)‡	0.09	0.07	0.02	0.01	ND	ND
G/G ($n = 3$)§	7.27	3.56	3.27	0.65	10.54	0.48
Prior fasting†						
N/N ($n = 2$)‡	0.02	0.05	0.12	0.10	ND	ND
G/G ($n = 4$)‡	0.86	1.42	0.71	0.76	3.24	0.16

* Ten micromoles (1.8 mg) $[1-^{13}\text{C}]$ galactose was administered intraperitoneally after *ad libitum* chow feeding followed by 24 h of fasting when urine was collected. This protocol of not fasting before injection of the sugar parallels that of the oxidation studies of Figure 1.

† Animals were fasted 12 h before ingestion of $[1-^{13}\text{C}]$ galactose and subsequently fasted during the 24-h urine collection.

‡ Two animals were placed together in a metabolic cage for urine collection.

§ Data from urine collected on two animals paired and one animal alone.

ND, not detected, $<0.001\text{ nmol}/24\text{ h}$.

Table 2. Twenty-four-hour urinary metabolites of mice after a galactose challenge

Animal*	Condition	Galactose ($\mu\text{mol}/24\text{ h}$)	Galactonate ($\mu\text{mol}/24\text{ h}$)	Galactitol ($\mu\text{mol}/24\text{ h}$)
G/G	76 mg galactose i.p.†	59.4	13.0	9.1
G/G	40% galactose‡	206.9	10.3	19.8
G/G	40% galactose‡	172.4	21.6	33.0

* Each study was performed on a single animal fed chow *ad libitum* until experiment was started.

† A mouse fed chow for 4 wk was injected with 76 mg (422 μmol) of galactose i.p. (1.50 g/kg) and urine collected for 24 h while the animal had no food intake.

‡ Urine was collected from each animal fed mouse chow for 4 wk that consumed a 40% galactose diet for the 24 h of urine collection. The animals consumed 1 g of chow or 400 mg (2222 μmol) of galactose during the collection period.

subjects by lactose restriction, the mouse red blood cell level of this metabolite was measured during the chow diet ingestion. Of cells from 17 normal mice, nine had nondetectable levels ($<0.04\text{ mg/dL}$) whereas eight had levels ranging from 0.5 mg/dL to 1.3 mg/dL of packed red blood cells. The level in red blood cells of 14 G/G mice ranged from 22.4 to 46.0 mg/dL, with a mean ($\pm\text{SEM}$) of $29.3 \pm 2.2\text{ mg/dL}$.

Table 3 reveals the levels of gal-1-P in liver, kidney, and brain of N/N and G/G mice. The sugar phosphate concentration in normal tissue ranged from $0.02 \pm 0.00\text{ }\mu\text{mol/g}$ wet wt in brain to $0.11 \pm 0.02\text{ }\mu\text{mol/g}$ wet wt in liver. There was no statistical difference between the level in liver and kidney. The level in both differed from that in brain ($p < 0.001$). Table 3 also shows that G/G mice, while eating the chow diet, have significantly increased liver, kidney, and brain gal-1-P compared with N/N animals ($p < 0.001$). The highest gal-1-P concentration was found in liver ($1.4 \pm 0.16\text{ }\mu\text{mol/g}$ wet wt) and the lowest in brain, similar to that observed in the normal mice. The difference between liver and kidney or brain is highly significant, $p < 0.001$ for kidney and $p < 0.01$ for brain.

Liver, kidney, and brain galactose, galactitol, and galactonate. Normal mice ingesting chow have no detectable galactose, galactitol, or galactonate in liver, kidney, or brain (Table 3). G/G mice have measurable quantities of these substances in these tissues except for brain, in which no galactonate was detected. The lowest level of galactose ($0.12 \pm 0.01\text{ }\mu\text{mol/g}$) was found in brain and differed significantly from $0.43 \pm 0.03\text{ }\mu\text{mol/g}$ in kidney, which had the highest level, and from liver ($0.27 \pm 0.05\text{ }\mu\text{mol/g}$; $p < 0.02$). Liver and kidney levels also

Table 3. Galactose and its metabolites in mouse tissues*

Tissue	Galactose ($\mu\text{mol/g}$ wet wt)	Galactitol ($\mu\text{mol/g}$ wet wt)	Galactonate ($\mu\text{mol/g}$ wet wt)	Gal-1-P ($\mu\text{mol/g}$ wet wt)
Liver				
N/N	ND (13)	ND	ND	0.11 ± 0.02
G/G	0.27 ± 0.05 (10)§	0.09 ± 0.01 ¶¶	0.14 ± 0.03 ‡‡	1.4 ± 0.16 †‡
Kidney				
N/N	ND (13)	ND	ND	0.08 ± 0.01
G/G	0.43 ± 0.03 (5)§§	0.25 ± 0.01	0.07 ± 0.00	0.48 ± 0.06 †**
Brain				
N/N	ND (13)	ND	ND	0.02 ± 0.00
G/G	0.12 ± 0.01 (5)	0.16 ± 0.03 ¶¶	ND	0.22 ± 0.02 †

* Mice were weaned to a mouse chow diet at 3 wk of age and maintained on the chow for 4 wk when the tissues were obtained. The numbers in parentheses refer to the number of animals whose tissues were analyzed. Values are mean $\pm\text{SEM}$.

ND, not detected, $<0.01\text{ }\mu\text{mol/g}$.

Gal-1-P: † $p < 0.001$ G/G vs NN; ‡ $p < 0.001$ liver vs brain and kidney; ** $p < 0.01$ kidney vs brain; † $p < 0.001$ G/G vs NN; ‡ $p < 0.001$ liver vs brain and kidney; ** $p < 0.01$ kidney vs brain.

Galactose: § $p < 0.02$ liver vs brain and kidney; §§ $p < 0.001$ kidney vs brain.

Galactitol: ¶ $p < 0.05$ brain vs liver and kidney; ¶¶ $p < 0.001$ liver vs kidney.

Galactonate: ‡‡ $p < 0.001$ liver vs kidney.

were significantly different ($p < 0.02$), such that for tissue levels, kidney > liver > brain.

Kidney had the highest concentration of galactitol (0.25 $\mu\text{mol/g}$) and liver the lowest (0.09 $\mu\text{mol/g}$), statistically different at $p < 0.001$. Brain had an intermediate level, which differed from both liver and kidney ($p < 0.05$). Galactonate was found in liver and kidney, with the liver concentration being significantly 2-fold higher (0.14 *versus* 0.07 $\mu\text{mol/g}$; $p < 0.05$).

Liver UDPgal and UDPglu concentrations. The report of a possible deficiency in human galactosemics of hepatic UDPgal related to an inability of the galactosemic patient to form UDPgal as a result of absent GALT prompted comparison of the levels of UDPgal and UDPglu in liver tissue of N/N and G/G mice. Table 4 demonstrates the levels in liver of these animals. There was no significant difference between the N/N and G/G mice in levels of either nucleotide sugar. The average $\pm$ SEM for normal liver UDPgal was 1.0 ± 0.03 $\mu\text{mol/mg}$ protein and 1.0 ± 0.03 $\mu\text{mol/mg}$ protein for liver of G/G animals. UDPglu was 1.9 ± 0.14 $\mu\text{mol/mg}$ protein and 1.9 ± 0.06 $\mu\text{mol/mg}$ protein for normal and galactosemic liver, respectively. Similarly, there was no difference in the ratio of UDPglu to UDPgal.

DISCUSSION

In our initial report describing the creation of a mouse deficient in GALT, we described the biochemical phenotype of 7- to 9-d-old suckling animals, showing increased gal-1-P in red blood cells and liver and the presence of galactose and galactitol in plasma (2). GALT-deficient mice fed mouse chow were also shown to grow normally and females to achieve pregnancy with normal size litters. In the present studies, we have assessed the ability of young adult GALT-deficient mice weaned to mouse chow for 4 wk to metabolize galactose and determined the presence and extent of alternative metabolic pathways. This was done using radiolabeled and stable isotopically labeled galactose and relied on sensitive GC-MS techniques for the quantitation of galactose and its alternative pathway metabolites, galactitol and galactonate.

From the experiments measuring $^{14}\text{CO}_2$ in expired air for 4 h after i.p. injection of $[1-^{14}\text{C}]$ galactose, it is evident that there is marked impairment of galactose metabolism in GALT-deficient mice, 39.9% oxidized by N/N *versus* 5.5% by G/G

animals. The extent of metabolism and the slow rate of $^{14}\text{CO}_2$ liberation by G/G animals mimic almost exactly the observations in classic galactosemic humans given $[1-^{14}\text{C}]$ galactose or $[1-^{13}\text{C}]$ galactose (7, 8), in which there is a slow linear oxidation of galactose during a 5-h period. The labeled CO_2 excretion curve of normal mice is similar to normal humans except there is much less variability in the extent of oxidation in the inbred mice than in normal humans (8). Although the cumulative excretion of G/N mice does not differ from that of N/N mice, the measurement of the initial rate of oxidation may make the delineation of G/N animals from N/N a possibility.

The low level of galactose oxidation to $^{14}\text{CO}_2$ by G/G mice, which increased to 7.5% by 10 h, could not be caused by residual GALT activity inasmuch as coding for the active site of the enzyme was removed and the absence of liver GALT demonstrated (2). An alternative metabolic pathway must be postulated whereby labeled CO_2 is produced from galactose. In the galactosemic human, from data showing the preferential oxidation of 1-C-labeled galactose to 2-C-labeled sugar (9), the suggestion has been made that the conversion of galactose to galactonate, which may subsequently be decarboxylated to yield CO_2 and xylulose, is the explanation. Such a pathway has been demonstrated in rat liver (10). That the galactonate pathway is functional in G/G mice is evidenced by the presence of galactonate in urine when these animals are presented with a large galactose challenge or are given $[1-^{13}\text{C}]$ galactose. Galactonate has been shown to be present in the urine of normal humans given a galactose load and in urine of classic galactosemics on lactose-restricted diets (11). Its synthesis has been shown in human red blood cells (12).

The impairment of galactose metabolism in the G/G mice demonstrated by their low capability to oxidize the sugar to CO_2 was substantiated by their large urinary excretion of galactose, as well as increased amounts of galactitol and galactonate when they were given 1.5 g/kg (76 mg to a 50-g animal). This was also shown when these animals were placed on a 40% galactose diet for 24 h (Table 2). Of interest is the fact that only about 20% of the i.p. galactose challenge of 420 μmol and 10% of galactose intake of mice on the 40% galactose diet (2220 μmol) was excreted in the urine as galactose and its metabolites. Much of the galactose administered apparently was taken into tissues as gal-1-P, galactitol, and galactonate, as well as galactose itself.

Table 4. Nucleotide sugar concentrations in liver of normal and galactosemic mice

	Normal			G/G mice		
	UDP-Gal ($\mu\text{mol/mg/protein}$)	UDP-Glu ($\mu\text{mol/mg/protein}$)	Ratio (UDP-Glu/UDP-Gal)	UDP-Gal ($\mu\text{mol/mg/protein}$)	UDP-Glu ($\mu\text{mol/mg/protein}$)	Ratio (UDP-Glu/UDP-Gal)
	1.0	1.9	1.9	1.0	2.3	2.2
	0.9	1.9	2.0	1.1	1.7	1.6
	1.1	2.1	1.9	0.9	1.6	1.7
	1.0	2.0	2.0	1.1	2.4	2.1
	1.0	1.9	2.0	1.0	1.6	1.6
	0.9	1.6	1.9	1.0	2.1	2.2
Average	1.0	1.9	1.9	1.0*	1.9*	1.9*
SEM	0.03	0.06	0.02	0.03	0.14	0.12

* Not significantly different; N/N *vs* G/G.

The results of the studies quantitating [$1\text{-}^{13}\text{C}$]galactose disposition into urinary metabolites was, indeed, revealing, especially inasmuch as the analytical technique permitted the quantitation of both ^{12}C - and ^{13}C -labeled compounds. Several important findings were made. First, G/G animals fed chow excrete galactose, galactonate, and galactitol, the origin of the galactose being either from the diet or from endogenous production with metabolites formed as a result. Second, the injected [^{13}C]galactose was oxidized to galactonate, as well as reduced to galactitol with greater conversion to galactonate. Third, normal animals converted the labeled galactose to galactonate but no galactitol. All of these observations support the direct oxidation of galactose to galactonate by these animals.

The increased excretion of galactose and its metabolites in the urine of G/G mice fed normal chow was puzzling until the chow was analyzed for galactose content and found to contain 1.75% of galactose as complex carbohydrates. This galactose could be bioavailable to the rodent whose intestine possesses the ability to hydrolyze such carbohydrates and liberate free galactose. Thus, 17.5 mg of galactose is potentially available in a 50-g animal ingesting 1 g of chow in 24 h. This amounts to 350 mg/kg, which is a large quantity to an organism with impaired galactose metabolism.

It was no wonder then that in addition to the urinary excretion of galactose and metabolites, these substances were found in tissues of G/G mice fed chow (Table 3). Gal-1-P in red blood cells from the same mice ranged from 22 to 46 mg/dL of packed red blood cells compared with undetectable levels in cells from most normal N/N mice. Such levels would be considered to indicate a large exposure to galactose by human patients who on the best galactose-restricted diets have levels ranging from 3 to 5 mg/dL of packed red blood cells (13). The high levels of red blood cell gal-1-P seen in G/G mice on chow are only seen in newly diagnosed human infants before or during initiation of a galactose-free formula. Liver, kidney, and brain also showed accumulation of substantial amounts of gal-1-P, with the highest concentrations in the liver followed by kidney and brain. Indeed, the concentration of gal-1-P was the highest of the accruing metabolites in all three tissues. Galactitol was found in liver, kidney, and brain, although mice are known to have little aldose reductase activity in tissues in contrast to humans (14). Galactonate was not found in the brain of G/G mice but was present in liver and kidney.

The GALT-deficient mouse permitted a comparison of liver UDPgal and UDPglu with hepatic levels of normal animals. Ng *et al.* (15) had reported that UDPgal in red blood cells and liver from galactosemic humans was significantly lower than that of normal subjects. The use of more-appropriate methods (16) indicated that the erythrocytes, as well as white blood cells and cultured fibroblasts (17), did not have UDPgal levels different from the normal subjects. An analysis of liver UDPgal from galactosemic humans has not been performed by HPLC methods to substantiate the findings of Ng *et al.* (15). The data reported here indicate that the concentration of UDPgal in liver of mice with no GALT activity does not differ from that of normal animals.

The G/G mice, as reported earlier (2), showed no evidence of galactose toxicity in the suckling period and grew normally

during the 4 wk after weaning to mouse chow. They exhibited no cataracts and were indistinguishable from normal animals on chow diets. They showed no adverse effect of being placed on a high galactose diet for 24 h or injected with a galactose load. This was seen despite very high levels of gal-1-P in red blood cells and tissues. There are little data on tissue metabolite levels in galactosemic humans other than for red blood cells. In two human autopsy brain specimens from classic galactosemic patients, the levels of galactitol were 12 and 22 $\mu\text{mol/g}$ (18, 19), which are two orders of magnitude higher than 0.16 $\mu\text{mol/g}$ in the brain of G/G mice on chow diet. Mice are resistant to galactose-induced cataracts because of low levels of aldose reductase in their tissues (20). It may be that high levels of galactitol in combination with high levels of gal-1-P are required to reproduce the pathologic abnormalities detected in some human tissues. Despite an impaired ability to oxidize galactose and abnormal galactose and metabolite concentration in various organs, the G/G mice lack evidence of the human phenotype of galactosemia. It appears that an absence of GALT is necessary but in itself is not sufficient to cause disease. This presents another aspect of the enigma of galactosemia (21).

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