Plasma Galactose and Galactitol Concentration in Patients With Galactose-1-Phosphate Uridyltransferase Deficiency Galactosemia: Determination by Gas Chromatography/Mass Spectrometry

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The plasma concentration of galactose and galactitol was measured in 27 patients with galactose-1-phosphate uridyltransferase (GALT) deficiency galactosemia on a lactose-restricted diet, 17 infants on lactose-free formula, and 21 infants and children on a normal diet, by a newly devised isotope dilution gas chromatograph/mass spectrometry (GC/MS) method. The method was linear in the range of 0.1 to 10 μmol/L for galactose and 1 to 20 μmol/L for galactitol with good reproducibility and a coefficient of variation less than 3%. The mean plasma galactose in 15 patients who were homozygous for the most common Q188R mutation of the GALT gene was 2.72 ± 0.70 μmol/L (mean ± SE) with a range of 0.58 to 3.98 in specimens obtained at regular clinic visits. In 12 patients with other GALT mutations, it was 2.45 ± 0.75 μmol/L. The mean value in nongalactosemic subjects on lactose-free formula was 0.52 ± 0.08 μmol/L, with a range of 0.12 to 1.25. The range in 21 normal subjects without diet restriction was 0.11 to 6.33 μmol/L, with a mean of 1.48 ± 0.32. The plasma galactitol level was 11.63 ± 0.46 and 10.85 ± 1.38 μmol/L in the 2 galactosemic groups. There was no relationship between plasma galactose and galactitol levels, with variable ratios of the two substances in the galactosemic patients. Galactitol was not detectable in the plasma of normal subjects. The red blood cell galactose-1-phosphate level was also measured in the galactosemic patients, and no relationship between plasma galactose and red blood cell galactose-1-phosphate was found. The galactose-1-phosphate concentration was 28 to 54 times higher than the ambient galactose. The low galactose concentration in the plasma of galactosemics on galactose-restricted diets in relation to the higher plasma galactitol and red blood cell galactose-1-phosphate is a metabolic enigma. The ability to measure plasma galactose accurately presents a new way of characterizing the galactosemic patient and the levels monitored over time may provide insight into the development of long-term complications associated with the disorder.

GALACTOSE-1-PHOSPHATE uridyltransferase (GALT) deficiency galactosemia is an enigmatic disorder characterized by a toxicity syndrome in the neonatal period and by later long-term complications despite the institution of a galactose-restricted diet.[1,2] As yet, there is no firm explanation for the defective speech and cognitive ability, neurologic syndromes, and ovarian failure observed in afflicted females.[3,4] Within the past decade, newer methods of characterizing galactosemic patients have become available, ie, genotyping for a mutation in the GALT gene[5] and measuring the ability of patients to oxidize ^{13}C-galactose.[6] Also, the determination of the metabolites, galactitol in urine and plasma[7,8] and galactonate in urine,[9] products of alternate metabolic pathways, has enhanced the ability to elucidate a biochemical phenotype. Indeed, measurement of the red blood cell galactose-1-phosphate level has long been used to follow the metabolic and dietary status of treated patients.[10]

What has been lacking in our knowledge of galactose metabolism in GALT-deficient patients is the level of galactose itself in plasma and how it may be related to the concentration of other circulating metabolites. We have devised an accurate and precise isotope-dilution gas chromatography/mass spectrometry (GC/MS) method for the quantitation of both plasma galactose and galactitol and determined their concentrations in 27 galactosemic patients of various genotypes. The relationship of the circulating galactose concentration to that of galactitol and galactose-1-phosphate measured in red blood cells isolated from the same blood sample was examined. Our results form the basis of this report.

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SUBJECTS AND METHODS

Materials

1-^{13}C-galactose (99 atom percent excess [APE]) and 2-^{13}C-galactitol (99 APE) were obtained from Omicron Biochemicals (South Bend, IN). ^{12}C-galactose and ^{12}C-galactitol were purchased from Pfanstiehl (Waukegan, IL). Acetic anhydride was from Supelco (Bellefonte, PA). All other reagents were from Sigma (St Louis, MO) and Fisher Scientific (Pittsburgh, PA). Dialyzed plasma was prepared from pooled human plasma collected from normal healthy subjects using a Slide-A-Lyzer dialysis cassette from Pierce (Rockford, IL). Each dialysis cassette was filled with 3 mL pooled plasma, and 5 cassettes were placed in a holding device in 1 L saline overnight at 4°C.

The method for quantitation of plasma galactose involved the formation of the aldononitrile pentoacetate derivative as first described by Pfaffenberger et al[11] and modified by Tserng and Kalhan,[12] who used it for the GC/MS analysis of ^{13}C-glucose in plasma. However, the analysis of plasma galactose by the GC/MS method is complicated by the fact that galactose exists in micromolar amounts in the presence of 4 mmol/L glucose, thus requiring adequate gas chromatographic separation of the two sugars or some means of removing the glucose. We first attempted to treat the plasma with glucose oxidase to convert the glucose to gluconate, thus removing the large amount of glucose interfering with the galactose chromatography. However, the glucose oxidase available commercially, while acting on glucose, contains trace activity of galactose oxidase and was found to act to some extent on galactose, making this approach undesirable. Using a fused silica

column (30 m × 0.25 mm, catalog no. 19091S-433; Hewlett-Packard, Palo Alto, CA) and appropriate temperature programing, we were able to clearly separate the 4-mmol/L glucose from 1-μmol/L galactose, enabling the quantitation of galactose. Galactitol, which is converted to the hexacetate, was easily separated from galactose with our gas chromatographic technique.

Method

The detailed method of the procedure is as follows. To 100 μL plasma, 0.25 nmol each of 1-^{13}C-galactose (99 APE) and 2-^{13}C-galactitol (99 APE) were added as internal standards. The plasma sample was deproteinized with 1 mL methanol. After centrifugation, the supernatant was evaporated to dryness under a stream of nitrogen. Hydroxylamine hydrochloride (2.1 mg) in 100 μL pyridine was added to the dried residue of the plasma extract and the mixture was heated at 90°C for 30 minutes. This was followed by the addition of acetic anhydride (75 μL) and heating at 90°C for an additional hour. After the sample cooled, water (1.5 mL) and methylene chloride (0.5 mL) were added, mixed, and centrifuged. The lower methylene chloride layer, which was transferred to a 1-mL vial using a Fisherband Gel-loading tip (Fisher Scientific), was dried under a stream of nitrogen and prepared for analysis by adding 100 μL ethylacetate to the dried extract. We then used a 1-μL aliquot of the derivatized preparation for GC/MS analysis.

Standard Samples

Samples were prepared by adding 0.01, 0.1, 0.25, 0.5, 1, 5, and 10 nmol galactose, 0.1, 0.25, 0.5, 1, 5, 10, and 20 nmol galactitol, and 0.25 nmol of each isotopic internal standard to 100 μL dialyzed plasma for quantitative calculation of galactose and galactitol. The standard samples were treated by the same procedure already described. A study of the precision of the method was made by a series of 5 injections of the same sample. The coefficient of variation for galactose and galactitol was 2.88% and 2.94%, respectively. Recovery was 98.70% ± 5.24% (mean ± SE) for galactose and 93.60% ± 1.69% for galactitol when 0.1, 0.25, 0.5, and 5 nmol were added to 100 μL plasma (1 to 50 μmol/L plasma).

GC/MS Analysis

A Hewlett-Packard 5890/5972 analyzer equipped with electron ionization was used for the analysis. Gas chromatographic separation was performed with temperature programing of the capillary column. The initial oven temperature was maintained at 125°C for 1 minute and increased to 190°C at a rate of 20°C/min, then to 215°C at a rate of 2.5°C/min, and finally to 300°C at a rate of 30°C/min. The oven was kept at the final temperature for 5 minutes. Data were collected by scan or the selected ion monitoring mode. The peaks of galactose and galactitol were first confirmed by mass spectra using the scan mode and later calculated from the data obtained using the selected ion monitoring mode. The ratio of *m/z* of ion 212 of galactose to *m/z* 213 of 1-^{13}C-galactose and the *m/z* 259 of galactitol to *m/z* 260 of 2-^{13}C-galactitol were used for galactose and galactitol quantitative analysis, respectively.

Figure 1A and B shows the total ion chromatograms of dialyzed normal plasma to which galactose and galactitol equivalent to 1 and 10 μmol/L and glucose equivalent to 4 mmol/L were added. The separation of galactose from the large amount of glucose was effectively performed. Figure 1C is a chromatographic analysis of the patient aged 4 years and 4 months with genotype S135L/H315H, and Fig 1D is for the patient aged 13 days with genotype S135L/Q188R in Table 1.

Figure 2 shows the isotope dilution standard curves indicating the linearity and ability to detect a lower limit of 0.1 μmol/L for galactose and 1 μmol/L for galactitol.

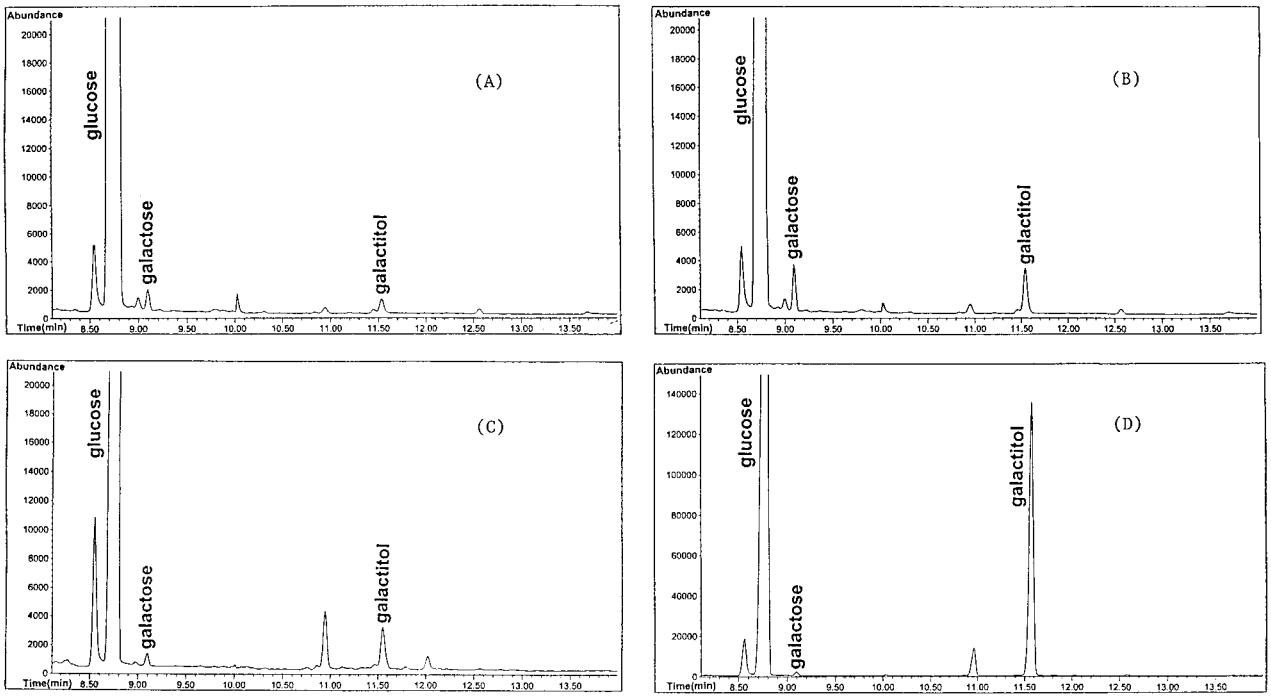

Fig 1. Plasma total ion chromatograms of standards and galactosemic patients. (A) 0.1 nmol galactose and galactitol and 400 nmol glucose in 100 μL dialyzed plasma equivalent to 1 μmol/L galactose and galactitol and 4 mmol/L glucose. (B) 1.0 nmol galactose and galactitol and 400 nmol glucose in 100 μL dialyzed plasma. (C) Plasma of patient aged 4 years and 4 months with genotype S135L/H315H. (D) Plasma of patient aged 13 days with genotype S135L/Q188R. Abundance scale for D is much larger than the others, making the galactose peak appear smaller than the others although the level is 5.22 μmol/L. See Table 1.

Table 1. Plasma Galactose and Galactitol and Red Blood Cell Galactose-1-Phosphate in Patients With Galactosemia

Genotype	Age	Galactose (μmol/L)	Galactitol (μmol/L)	Ratio of Galactitol to Galactose	Red Blood Cell Galactose-1-Phosphate (μmol/L)
Q188R/Q188R	2M	2.55	13.07	5.13	425
	2M	11.71	12.81	1.09	205
	9M	2.05	11.57	5.64	167
	9M	1.67	11.52	6.90	133
	5Y3M	0.58	12.38	21.34	72
	6Y9M	0.93	9.28	9.98	
	8Y	2.34	9.92	4.24	197
	9Y5M	3.57	10.67	2.99	137
	10Y	3.65	12.59	3.45	99
	10Y1M	1.09	9.70	8.89	
	10Y6M	1.57	9.91	6.31	190
	13Y	3.98	13.22	3.32	125
	13Y	2.15	11.27	5.24	91
	16Y	0.63	15.98	25.37	
	19Y6M	2.30	10.57	4.60	91
Mean ± SE		2.72 ± 0.70	11.63 ± 0.46	7.63 ± 1.76	161 ± 27.25
Other					
S135L/Q188R	13D	5.22‖	473.90‖	90.78‖	714‖
Q188R/unknown	1M	1.04‖	25.55‖	24.57‖	319‖
S135L/Q188R	1Y	3.12	11.95	3.83	61
Del/Del*	1Y8M	2.19	12.45	5.68	133
Q188R/T2359†	4Y	2.27	12.67	5.58	65
S135L/H315H	4Y4M	1.52	10.14	6.67	65
S135L/S135L	8Y9M	0.43	5.60	13.02	
Q188R/Y209C	9Y5M	0.92	9.27	10.07	
Q188R/A330V	10Y	6.49	18.70	2.88	61
S135L/Q188R	13Y4M	0.50	8.64	17.28	35
Q188R/A330V	14Y	6.74	15.21	2.26	65
S135L/S135L	16Y6M	0.36	3.82	10.61	
Mean ± SE		2.45 ± 0.75‡	10.85 ± 1.38‡	7.79 ± 1.53‡	69.29 ± 11.37§

Abbreviations: D, days; M, months; Y, years.
*Deletion.
†Stop codon.
‡No significant difference *v* Q188R/Q188R.
§$P = .02$ *v* Q188R/Q188R.
‖Not included in mean ± SE.

Subjects

Galactose and galactitol were quantified in plasma from 27 known galactosemic patients. Nonfasting plasma specimens were obtained on regular follow-up clinic visits after blood centrifugation to separate red blood cells for the routine red blood cell galactose-1-phosphate measurements. The plasma was frozen at −80°C until the analysis was performed. All of the patients were on galactose-restricted diets, albeit some only days after the diagnosis was made by the absence of red blood cell GALT activity. In older patients, the diet history was assessed and believed to be adequate, although no actual quantitation of dietary galactose was made. Red blood cell galactose-1-phosphate measurements were within the acceptable range of 2 to 5 mg/dL (76 to 190 μmol/L) or less in 23 of the patients above 2 months of age. Four patients aged 13 days to 2 months still had elevated levels even while on galactose-free formulas, which is not unusual for infants recently diagnosed and treated for a short time. Urine galactitol measurements were also made, with the levels indicating adequate dietary control in all but the young infants.

All of the patients had been genotyped previously and the GALT mutations were known.[8,9] Fifteen were homozygous for Q188R, the most common mutation, 8 were compounds of Q188R with another mutation, and 1 had an S135L mutation and an H315H where only a change in histidine coding was detected. The latter patient and the two S135L homozygotes were African-American. One patient had a homozygous deletion.

Seventeen infant subjects who did not have galactosemia were on galactose-free formulas, having been identified as possible galactosemics in a newborn screening program, but red blood cell GALT was found not to be absent. They were genotyped and 4 were found to be normal, 4 were Duarte homozygotes (N314D), 2 were Q188R/N314D, 5 were galactosemic heterozygotes, and 2 were N314D/normal. All had red blood cell GALT activity appropriately corresponding to their genotype. Twenty-one randomly obtained plasma specimens were available from infants and children aged 6 days to 11 years and 1 adult whose blood was sent to the Children's Hospital of Philadelphia metabolic laboratory for plasma amino acid analysis and found to be normal. No other parameters of galactose metabolism were measured, and it was assumed the subjects were on diets appropriate for their age. Red blood cell galactose-1-phosphate was determined by an enzymatic method.[13]

Statistical analysis was performed using Student's *t* test.

RESULTS

Plasma Galactose

The plasma galactose concentration in galactosemic patients on a galactose-restricted diet is shown in Table 1. Patients who were homozygous for the most common Q188R mutation, which is believed to be associated with the classic phenotype, had a mean plasma galactose level of 2.72 μmol/L, with a range

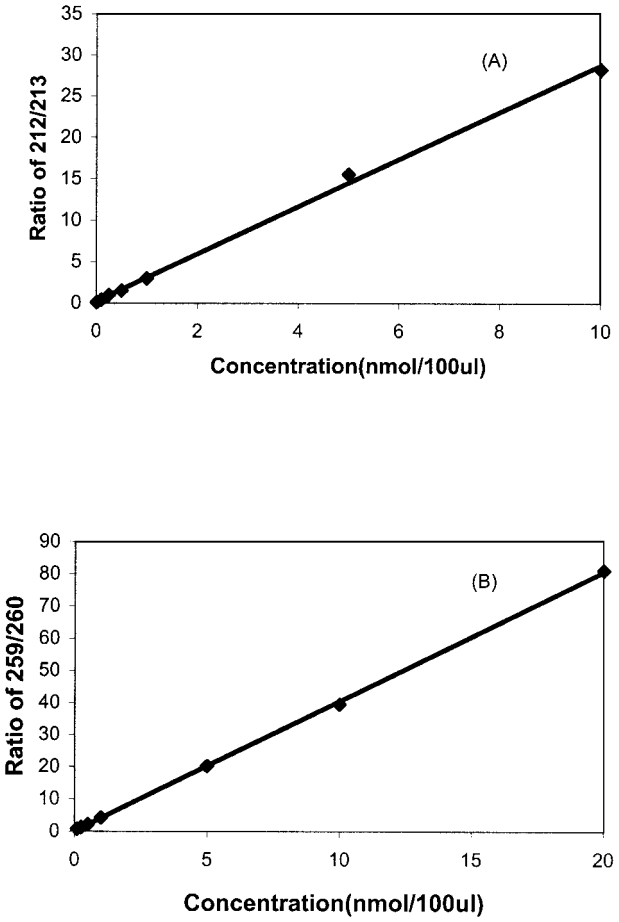

Fig 2. Isotope dilution standard curves. (A) Galactose. The ratio is the peak area of ion 212 of ^{12}C-galactose to the peak area of ion 213 of 1-^{13}C-galactose. (B) Galactitol. The ratio is the peak area of ion 259 of ^{12}C-galactitol to the peak area of ion 260 of 2-^{13}C-galactitol.

of 0.58 to 11.71. The latter value appears to be an outlier and may be due to a break in diet management. If that patient is omitted, the mean is 2.07 μmol/L. These values do not differ from the mean for patients with other genotypes (2.45 μmol/L), where the range in patients older than 2 months is 0.36 to 6.74 μmol/L. The lowest levels were found in S135L homozygote subjects who are African-American and known to be able to oxidize galactose in a near-normal fashion.[14,15] There was no relationship to the age of the patients in either group. In the Q188R/Q188R group, the 5-year-old had a level of 0.58 μmol/L, similar to that of the 16-year-old with a level of 0.63 μmol/L. The levels in all three 10-year-olds varied greatly. Surprisingly, there were a few patients in the homozygous Q188R group with values less than 1 μmol/L, similar to the low levels found in the patients who were homozygous for the S135L mutation.

Table 2 shows plasma galactose values in 14 patients who are galactosemic heterozygotes or who carry the Duarte N314D mutation on a galactose-free infant formula after being detected in a neonatal screening program as possibly galactosemic. The mean value (0.52 ± 0.08) differed significantly from values in the galactosemic groups.

Table 3 also shows plasma galactose values in normal subjects of various ages collected at random. Only 4 of 21 subjects had levels over 1 μmol/L. There was no relationship to age, and indeed, some values were in the range for patients on galactose-free diets (Table 2). The mean of 1.48 ± 0.32 μmol/L was 0.99 if the two highest values of 4 and 6.33 were omitted from the calculation.

Table 2. Plasma Galactose and Galactitol in Galactosemic Heterozygotes and Patients With the Duarte Genotype on a Galactose-Restricted Diet

Genotype	Age	Galactose (μmol/L)	Galactitol (μmol/L)
Normal/unknown*	10D	0.78	ND
N314D/N314D	11D	0.28	ND
N314D/N314D	12D	0.68	ND
Normal/unknown*	14D	0.82	ND
Q188R/N314D	15D	0.54	ND
N314D/normal	16D	0.54	ND
Q188R/normal	16D	0.37	ND
N314D/N314D	18D	0.41	ND
N314D/N314D	24D	0.21	ND
Q188R/N314D	2M	0.25	ND
Normal/unknown*	2M4D	0.22	ND
Normal/Q188R	2M25D	0.41	ND
N314D/normal	3M	1.25	ND
Mean ± SE		0.52 ± 0.08	

Abbreviations: ND, not detectable (<1 μmol/L); D, days; M, months.
*Normal/unknown heterozygote identified by a red blood cell GALT level of 50% of normal, isoelectric focusing pattern, and analysis of exon 6 and exon 10 of the GALT gene for Q188R and N314D mutation.

Table 3. Plasma Galactose and Galactitol in Normal Controls

Diet	Age	Galactose (μmol/L)	Galactitol (μmol/L)
Galactose-free	8D	0.52	ND
	10D	0.51	ND
	19D	0.12	ND
	21D	0.37	ND
Mean ± SE		0.38 ± 0.09	
Regular	6D	6.33	ND
	27D	0.43	ND
	45D	0.30	ND
	2M	1.37	ND
	6M	0.39	ND
	10M	0.85	ND
	1Y2M	0.41	ND
	1Y6M	0.15	ND
	1Y6M	0.82	ND
	2Y6M	4.00	ND
	2Y10M	0.14	ND
	3Y	0.24	ND
	3Y	0.73	ND
	3Y4M	0.38	ND
	4Y	0.11	ND
	5Y	0.80	ND
	5Y10M	0.93	ND
	7Y4M	0.72	ND
	9Y6M	0.60	ND
	11Y6M	0.20	ND
	26Y	1.85	ND
Mean ± SE		1.48 ± 0.32	

Abbreviations: ND, not detectable (<1 μmol/L); D, days; M, months; Y, years.

Plasma Galactitol

Plasma galactitol levels are shown in Table 1. The 15 Q188R homozygous patients had levels in a narrow range of 9.28 to 15.98 μmol/L, with mean of 11.63. Patients with other genotypes, omitting the patients aged 1 month and 13 days, had a greater range of 3.82 to 18.7 μmol/L but their mean did not differ versus Q188R/Q188R patients. There was no relationship of galactitol concentration with age in either group.

Galactitol was not detected in the plasma of patients with the Duarte mutation in combination with Q188R or in Q188R carriers (Table 2), nor was galactitol detected in the plasma of normal infants without a galactose-restricted diet.

Relationship of Plasma Galactose and Galactitol

Figure 3 is a plot of plasma galactose versus galactitol showing that, as a group, there is no significant relationship of the two substances. Within the Q188R homozygote group, for example, the patient aged 6 years and 9 months with a plasma galactose of 0.93 μmol/L has a corresponding galactitol level of 9.28 μmol/L, while the 8-year-old patient with a galactose of 2.34 μmol/L has essentially the same value (9.92 μmol/L). Again, the 2-month-old Q188R/Q188R patient with a galactose level of 11.71 μmol/L has a corresponding galactitol level of 12.81, while the 2 patients with the Q188R/A330V genotype with values above 6 μmol/L for galactose have a galactitol concentration of 15.21 and 18.70 μmol/L. The lack of correlation is shown in the ratio calculations in Table 1. In the Q188R/Q188R group, the ratio of galactitol to galactose is 1.09 to 25.37 with a mean of 7.63 ± 1.71, and in the other group, it is 2.88 to 90.78 with a mean of 7.79 if the 13-day-old and 1-month-old patients are excluded.

Relationship of Red Blood Cell Galactose-1-Phosphate to Plasma Galactose

Figure 4 shows no apparent relationship between plasma galactose and red blood cell galactose-1-phosphate, with both determinations made from the same blood specimen. Within the Q188R homozygous group, for example, the 10-year-old patient with a galactose level of 3.65 μmol/L in the plasma has 99 μmol/L red blood cell galactose-1-phosphate, while the individual aged 10 years and 6 months with a galactose level of 1.57 μmol/L has 190 μmol/L red blood cell galactose-1-phosphate. In the ''other'' group, 5 patients have red blood cell galactose-1-phosphate between 61 and 65 μmol/L, while the plasma galactose is between 1.52 and 6.74 μmol/L.

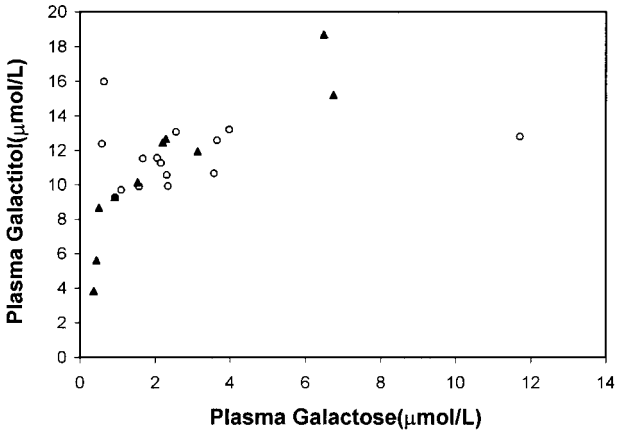

Fig 3. Relationship between plasma galactose and galactitol in galactosemic patients. ○, Q188R/Q188R homozygotes; (▲) other genotypes.

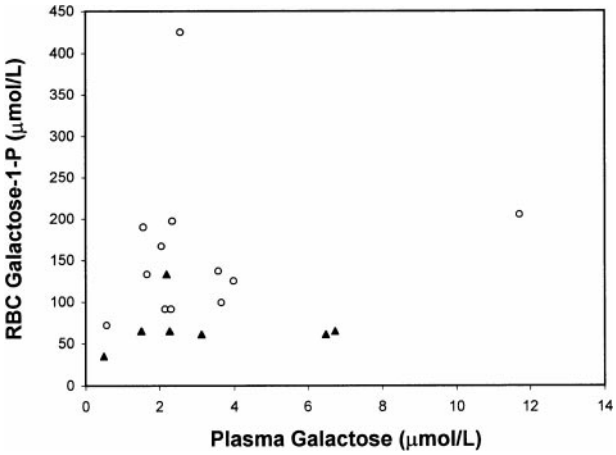

Fig 4. Relationship between plasma galactose and galactose-1-phosphate in galactosemic patients. ○, Q188R/Q188R homozygotes; (▲) other genotypes.

There is a significant difference in red blood cell galactose-1-phosphate in the Q188R homozygous subjects and the others with different mutations if the 13-day-old and 1-month-old patients are excluded, assuming they were still galactose-toxic, with values of 161 ± 27.25 and 69.29 ± 13.37 μmol/L in red blood cells, respectively.

DISCUSSION

Although some studies have measured urinary galactose[7] and galactitol[7,8] excretion and plasma galactitol levels in galactosemic patients, little is known of their ambient plasma galactose levels. To this end, we devised a GC/MS isotope dilution method for quantitation of the sugar in plasma. The mean level found in normal infants and children aged 6 days to 11.3 years with 1 young adult included was 1.48 ± 0.32 μmol/L, with a range of 0.11 to 6.33 and no relationship to age. Plasma galactose levels in galactosemics varied widely, but the mean value in the 27 patients was less than 2-fold higher versus the normal subjects. However, they were about 4-fold higher than the levels in nongalactosemic infants on a galactose-restricted diet.

There was no difference in the mean level of galactose in Q188R homozygous patients and Q188R compounds. The two S135L homozygous patients had the lowest levels. These patients were African-American and are known to be able to oxidize galactose in a near-normal fashion.[14,15] The other African-American patient, the 4-year-old S135L/H315H, who can also metabolize the sugar, has a level much higher than the S135L homozygous subjects. The variations in plasma levels may be due to different amounts of dietary galactose ingested even though the galactose-restricted diet was maintained,[16] and the fact that the plasma samples were obtained nonfasting

during follow-up clinic visits. They also may reflect varying differences in endogenous galactose production rates in the patients.[17]

The GC/MS method with the hexacetate derivative was also used to determine the plasma galactitol concentration. The values determined by this method corresponded to those determined with the trimethysilyl (TMS) derivative previously reported.[8] The disadvantage of the TMS derivative is that the gas chromatographic procedure is not adequate to determine galactose, due to the difficulty in separating the high levels of glucose from the small galactose content. The galactitol concentration was in a narrow range in the Q188R homozygous patients, with a larger variation in patients with other mutations. The mean galactitol concentration was about 4-fold higher than the galactose level. As a group, there was no correlation between plasma galactose and plasma galactitol levels. There was a suggestive relationship for the ratio of the two to the galactose content, that is, subjects with the lowest galactose levels tended to have the highest galactitol to galactose ratio, while those with the highest galactose tended to have the lowest ratio of the two.

As noted previously,[8] galactitol was not detected in the plasma of normals with galactose levels comparable to galactosemic subjects, and plasma galactitol was not found in galactosemic heterozygotes or patients with the N314D Duarte allele. The elevated levels of plasma galactitol serve to distinguish the galactosemic patient from individuals without impaired galactose metabolism. The persistent high level of galactitol and low level of galactose in the plasma explains why galactose is low or undetectable in the urine of galactosemics while galactitol is excreted in large quantities.[7,8]

The lack of a relationship of plasma galactose to plasma galactitol suggests the possibility that they are derived from different sources. Galactose may be of exogenous dietary origin or endogenously produced from the turnover of complex carbohydrates in the various organs from which it would exit into the plasma. Galactitol is formed intracellularly by the action of aldose reductase on galactose entering the cell, and is generated by glycoconjugate breakdown. Also, galactitol formation may vary in different organs dependent on the activity of aldose reductase with variable leakage into the plasma. Indeed, aldose reductase activity is genetically regulated[18] and may vary in each patient.

Red blood cell galactose-1-phosphate had no relationship to the ambient plasma galactose in which the cells were circulating, nor any relationship to plasma galactitol. If the red blood cell galactose-1-phosphate is derived from plasma galactose, a correlation would be expected. The fact that the concentration of galactose-1-phosphate in red blood cells is manyfold higher than plasma galactose may also be due to the fact that galactose-1-phosphate is formed endogenously and accumulates from UDP-galactose as described by Gitzelmann.[19] The variable galactose-1-phosphate level in relation to plasma galactose also may be due to different activity levels for red blood cell galactokinase in each of the patients.

There is fundamental question as to why the circulating galactose pool is so small compared with the galactitol pool and the galactose-1-phosphate pool. It may be that the galactose pool is maintained at its steady-state level because there is a high rate of conversion to red blood cell galactose-1-phosphate and to alternate-pathway metabolites such as galactitol and galactonate in tissues. The latter compound is below the level of detection in plasma but is excreted in significant amounts in urine.[9] Since galactonate formation is the first step in the conversion of galactose to CO_2 and xylulose, the operation of this alternate pathway offers a means of disposal of galactose.[20] Its function in galactosemia has been demonstrated[21] and may indeed be responsible for the low plasma galactose levels we describe in galactosemic patients. In contrast, galactose conversion to galactitol and galactose-1-phosphate are in essence dead-end pathways.

Until now, little has been known about plasma galactose levels in galactosemic patients on a galactose-restricted diet, despite the fact that a block in its metabolism causes the clinical disorder. It was to this end that we devised a GC/MS isotope dilution method for quantitation of the sugar in plasma. Our report amplifies the single published value obtained in the plasma of an 11-year-old Japanese patient, whose plasma level determined by an enzymatic galactose dehydrogenase method was found to be 55 μmol/L.[22] Normal plasma values in 5 Japanese infants were 50 to 239 μmol/L, and in 5 children, 6 to 17 μmol/L. Normal young adults have been reported to have a mean of 30 μmol/L.[23] The much lower levels obtained with the GC/MS method reported here are based on an identified specific chemical species determined with precision and accuracy. We attribute the difference in the results between the GC/MS isotope dilution and enzymatic methods to the detection of substances in the plasma other than galactose by the latter techniques which measure NADH formation. This would appear to be the case, since fasting plasma in infants analyzed by a galactose oxidase method was reported to have a mean of 59 μmol/L.[24] Galactose oxidase is known to detect substances other than galactose in blood.[25]

We report here a single value for plasma galactose in a group of galactosemics of varying genotype. It behooves us now to begin to prospectively follow the levels in galactosemics over time to discern variations and factors that determine circulating galactose, such as diet or endogenous production. Is it more important to measure the plasma galactose concentration versus the red blood cell galactose-1-phosphate or urinary galactitol to evaluate dietary compliance? Are the levels related to outcome and the presence of long-term complications? The availability of a sensitive method of plasma galactose quantitation provides a new way to characterize the galactosemic patient. The ability to routinely measure the sugar in the plasma of galactosemics may be a valuable aid in unraveling the enigma of this disorder.[1,2]

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