# Urine and Plasma Galactitol in Patients With Galactose-1-Phosphate Uridyltransferase Deficiency Galactosemia

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**Urinary excretion of galactitol was determined in 95 normals (N/N), 67 galactosemic (G/G), and 39 compound heterozygotes for the Duarte and galactosemia genotype (D/G). Galactitol excretion is age-dependent in both normal individuals and patients with classic galactosemia on lactose-restricted diets. In galactosemic patients who are homozygous for the Q188R mutation, urinary galactitol levels were fivefold to 10-fold higher than those of normal subjects of comparable age. All but a few patients with classic galactosemia with the Q188R mutation and another mutant G allele had urinary excretion comparable to the Q188R homozygous patients. African-American galactosemic patients with the S135L mutation of the galactose-1-phosphate uridyltransferase (GALT) gene also excreted abnormal quantities of galactitol. Most subjects with a Duarte allele and a G allele excrete normal amounts of the sugar alcohol. There is a correlation between galactitol excretion and red blood cell (RBC) galactose-1-phosphate (gal-1-P). Plasma galactitol was also elevated in galactosemic patients (3.4 to 23.2 μmol/L; undetectable in normal individuals). In contrast to the decrease in urinary galactitol with age, plasma levels remain in a narrow concentration range with no significant difference with age. Urine and plasma galactitol distinguish galactosemic patients from normals. In addition, urinary galactitol excretion may be an important parameter for the assessment of steady-state galactose metabolism in galactosemia.**



THE REDUCTION OF GALACTOSE to galactitol has emerged as an important alternate pathway of galactose disposition when galactose metabolism is impaired by a defective function of galactose-1-phosphate uridyltransferase (GALT).[1] Galactitol has been found in the brain and other tissues of galactosemic patients,[2] as well as the tissues of rats fed a high-galactose diet.[3] A high level of the sugar alcohol is present in the cataractous lens of patients with galactosemia,[4] and is believed to be the prime etiologic factor for cataracts in the lens of rats fed a high-galactose diet.[5-7]

The excretion of abnormal quantities of galactitol in the urine of galactosemic patients is a characteristic of this disorder,[8-10] and is indeed a key element in the biochemical phenotype of affected individuals. The application of isotope-dilution methods with mass spectrometry has also revealed elevated plasma levels of galactitol.[10] These elevated levels in biological fluids occur in patients with impaired GALT activity despite their ingestion of galactose-restricted diets.

Here, we report our experience in quantifying galactitol in the urine of 67 patients with galactosemia (G/G) alleles, 39 patients with Duarte-galactosemia (D/G) alleles, and 95 normal subjects (N/N). The galactitol level was also measured in the plasma of 24 galactosemic (G/G) subjects and 10 normal individuals (N/N). An important feature is the relationship of the urinary excretion and plasma level with the genotype. The relationship of urinary galactitol excretion with red blood cell (RBC) galactose-1-phosphate (gal-1-P) and with the plasma galactitol concentration was also determined.

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## SUBJECTS AND METHODS

### *Subjects*

Single random urine specimens were obtained from 95 normal subjects with an age range from newborn to 45 years. Samples were collected from infants and children in general pediatric clinics and from healthy laboratory and medical personnel. All were on normal diets for their respective ages. No parameters of galactose metabolism other than galactitol excretion were assessed in this group.

Galactitol was quantified in random urine specimens from 67 galactosemic patients, of whom 60 are Caucasian and seven are African-American. While only a single analysis was available for some patients, many had multiple analyses from the time of detection, all of which are included. Thirty-two patients were homozygous for the Q188R mutation and 27 were compound heterozygotes for the Q188R and another mutation (G allele; Table 1). All patients who were homozygous for the Q188R mutation and all but two compound heterozygotes had no detectable RBC GALT activity and no observable GALT isoforms on isoelectric focusing, thus fulfilling criteria for the GALT biochemical phenotypes of G/G. Eight patients had a genotype with the S135L mutation, of whom seven were African-American and one was Caucasian with Q188R as the second allele (Table 1). All patients with the S135L mutation had absent RBC GALT activity.

Thirty-nine subjects were identified as compound heterozygotes for the N314D mutation, the Duarte allele, and a second galactosemic (G) allele. Thirty had the common mutation, Q188R, while the mutation was E320K in one, R148W in another, and unidentified in the remaining seven. These subjects with D/G biochemical phenotypes had RBC GALT activity at 15% to 30% of normal. Urine was also examined from two subjects who were homozygous for N314D. Patients were aged 9 days to 34 years.

Plasma galactitol determinations were performed on 24 subjects of various genotypes simultaneously with urine galactitol measurement, and plasma galactitol was also determined in 10 normal subjects aged 1 month to 45 years.

Galactose-loading studies were performed in three normal adults, one

**Table 1. Genotype of Galactosemic Patients**

| First G Allele | Second G Allele | No. |
|---|---|---|
| Q188R | Q188R | 32 |
| | H184Q | 2 |
| | ΔT2359 (stop codon) | 2 |
| | F171S | 2 |
| | M142K, Q344K, E308K | 1 each |
| | L195P, R123Q, Y209C | 1 each |
| | Unidentified | 13 |
| S135L | S135L | 4 |
| | H315H | 2 |
| | Q188R | 1 |
| | Unidentified | 1 |

woman and two men aged 28 to 45 years, after an 8-hour overnight fast. Urine was collected 2 and 4 hours after oral ingestion of 50 g D-galactose (Pfanstiehl Laboratories, Waukegon, IL).

These studies were approved by The Children's Hospital of Philadelphia and the Emory University Committee for the Protection of Human Subjects.

### *Molecular Analysis*

The Q188R and S135L mutations were identified using polymerase chain reaction (PCR) and restriction enzymes as previously described.[11,12] Molecular genotypes for all other mutations were established by screening single-stranded conformational polymorphisms and direct sequencing of PCR-amplified DNA on an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA).[12]

### *Galactitol Analysis*

Galactitol was routinely analyzed in random urine samples by capillary gas chromatography (GC). Somogyi filtrates of 200 μL urine were prepared after addition of the internal standards ribitol and perseitol (200 μL each of a 1-mmol/L solution). The filtrate was evaporated to dryness under a stream of nitrogen, and the trimethylsilyl derivatives were prepared. Capillary GC was performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a flame-ionization detector. Injections of 2 μL were made, and separation was achieved using a HP 5% phenylmethylsilicone capillary column (35-mm × 0.32-mm ID, 0.52-μm film thickness, Ultra II) with initial column temperature set at 70°C and a subsequent ramping rate of 4°C/min. Quantitation was performed using the response factors (amount/area) of the internal standards ribitol and perseitol prepared from pure compounds (Pfanstiehl Laboratories). Galactitol concentrations were calculated as millimoles per mole of creatinine by the Jaffé method. The amount was calculated independently based on each internal standard, and samples with an intersample variation of greater than 15% were reextracted and analyzed again. The sensitivity of this method is 2 mmol/mol creatinine.

In some instances, the galactitol level in urine was measured by proton nuclear magnetic resonance (NMR) spectroscopy and $^{13}$C-NMR spectroscopy on 10-fold concentrated urine using the methods described by Wehrli et al.[13] Spectroscopy was performed at 400 MHz on a Bruker AM400 wide-bore spectrometer (Bruker Instruments, Billerica, MA). Galactitol levels determined by NMR correlated well with those determined by GC.

Plasma galactitol was determined by GC–mass spectrometry (GC-MS) methods previously described using heptadecanoic acid as an internal standard.[14] A plasma sample (100 μL) was incubated with urease for 30 minutes to remove urea. After the addition of an internal standard (25 nmol heptadecanoic acid) and deproteinization with 0.9 mL ethanol, the supernatant was evaporated to dryness. The residue was trimethylsilylated with 0.2 mL each of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) and pyridine at 100°C for 1 hour. A 0.5-μL volume was analyzed on a HP 5890/5972 GC-MS. Gas chromatographic separation was performed with temperature programing on a fused capillary column (5% phenylmethylsilicone, 30-mm × 0.2-μm film thickness; J&W Scientific, Folson, CA). Identification of galactitol was based on a comparison of the retention time and mass spectra with a standard compound. Quantitation was based on the peak area of ion 217 of galactitol relative to the peak area of ion 327 of the internal standard.

RBC gal-1-P was determined by an enzymatic method.[15]

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

## RESULTS

### *Normal Urine Galactitol Concentration*

The normal range of urine galactitol was age-related in specimens analyzed from 95 subjects. These data are shown in Table 2, where three age groups are delineated (<1 year, 1 y to 6 years, and >6 years) as reported previously.[10,16] In many normal subjects, galactitol excretion was below the level of detection, ie, 2 mmol/mol creatinine. The highest rate of excretion was found in subjects aged less than 1 year, with a maximum value of 78 mmol/mol creatinine. Galactitol excretion decreased to a maximum of 36 in the group aged 1 to 6 years and to 19 in those over 6 years. These values are similar to those reported by Jansen et al,[16] who used a similar gas chromatographic technique.

Galactitol was not detected in the urine of three normal adult subjects prior to an oral bolus of 50 g galactose. Galactitol was found in the urine at both 2 and 4 hours after ingestion (Table 3). Galactitol excretion remained similarly elevated at both times while urinary galactose markedly declined, even to nondetectable levels in subject no. 3. Yamazaki et al[9] reported that galactitol excretion in a normal 12-year-old who received 1.25 g/kg orally, although elevated for 3 hours, could not be detected 24 hours later.

### *Urinary Galactitol Excretion by Galactosemics*

Figure 1 shows galactitol excretion by patients whose GALT biochemical phenotype is G/G. Some are homozygous for the

**Table 2. Relationship of Urinary Galactitol Excretion (mmol/mol creatinine, range and/or mean ± SD) With Age in Normal Subjects and Galactosemics Whose G Allele Is a Q188R GALT Mutation**

| Age (yr) | Normals | Q188R/Q188R | Q188R/Other |
|---|---|---|---|
| <1 | <2-78 (46) | 183-909 (38) | 170-566 (14) |
| | | 466 ± 166 | 296 ± 108¶ |
| 1-6 | <2-36 (28) | 194-620 (38) | 54-442 (32) |
| | | 344 ± 110‡ | 235 ± 91¶ |
| >6 | <2-19 (21) | 98-282 (32) | 76-282 (28) |
| | | 165 ± 40¶‖ | 183 ± 60*† |

NOTE. Since some normal values are below the level of detection, only the range is given. Values in parentheses are the number of normal subjects from whom single specimens were obtained, or the number of total specimens from galactosemic patients. The data include patients <1 month of age.

*$P < .0001$ *v* 1 day-1 year.

†$P = .01$ *v* 1-6 years.

‡$P < .0003$ *v* = 1 year.

§$P < .0001$ *v* < 1 year.

‖$P < .0001$ *v* 1-6 years.

¶$P = .001$ *v* Q188R/Q188R.

**Table 3. Effect of a Galactose Challenge (50 g orally) on Urinary Galactitol Excretion in Normal Adult Subjects**

| | Excretion (mmol/mol creatinine) | | | |
|---|---|---|---|---|
| | 2 Hours | | 4 Hours | |
| Subject no. | Galactose | Galactitol | Galactose | Galactitol |
| 1 | 5,500 | 109 | 150 | 77 |
| 2 | 2,850 | 55 | 330 | 82 |
| 3 | 690 | 69 | 0 | 65 |

NOTE. Neither galactose nor galactitol was detected (<2 mmol/mol creatinine) in urine obtained before galactose administration.

Q188R mutation (Fig 1A), some have the Q188R mutation plus another known G allele (1B), and some have the Q188R mutation and an unidentified G allele (1C). All of the specimens were obtained after diet therapy was initiated. In the newborn toxic period, levels as high as 12,000 mmol/mol creatinine have been observed.[10] With dietary lactose restriction, urinary galactitol excretion decreases with age, as most definitely shown in the group of Q188R homozygous patients (Fig 1A). In these patients during the first year, the observed levels varied from a low value of 183 mmol/mol creatinine to a value, in most patients, in the range of 300 to 600 mmol/mol creatinine. The highest level observed was 909 mmol/mol creatinine. In the group aged 1 to 6 years, the lowest value was 194 mmol/mol creatinine and the highest 620 mmol/mol creatinine. However, nearly all specimens in this age group showed values between 210 and 420 mmol/mol creatinine. The levels decreased after age 6, where the range is 98 to 282 mmol/mol creatinine, but it is apparent that most values are less than 200 mmol/mol creatinine. The difference between the means for the different age groups was highly significant (Table 2).

The pattern of galactitol excretion by 14 compound heterozygotes for the Q188R and a second known G allele is shown in Fig 1B. The pattern of excretion is similar to that of the homozygous Q188R patients, except for two patients in whom the second allele is R123Q and Y209C. In these two patients, urinary galactitol excretion is lower versus the Q188R patients. Values for 14 subjects in whom the second mutant G allele is yet unknown is shown in Fig 1C. Three subjects had lower excretion rates than the homozygous Q188R patients. Table 2 shows a comparison of the range and mean values for all compound Q188R heterozygotes and Q188R homozygotes. In the groups aged less than 1 year and 1 to 6 years, the mean excretion is significantly different in compound heterozygotes versus Q188R homozygotes. However, this is not the case in the group older than 6 years. The lower values for the range in compound heterozygotes reflects the few patients who have lower excretion rates than the homozygous patients. Galactitol excretion is higher in all G/G galactosemic patients, both Q188R homozygotes and compounds heterozygotes, versus the normal groups, with no overlap.

Figure 2 shows urinary galactitol excretion in patients with the S135L mutation. All S135L homozygous patients had lower galactitol excretion than the Q188R homozygous or Q188R/other genotypes. The lowest levels in galactosemics were found in S135L homozygote teenagers. However, their levels were still above the normal range (Table 2) and indicated impaired galactose metabolism. The S135L compound heterozygote adult patient with the S135L/H315H also had above-normal excretion, but it was low compared with patients having the Q188R mutation. Indeed, the patient with the S135L/Q188R genotype had a galactitol excretion rate in the same high range as other patients with the Q188R allele.

Figure 3 shows galactitol excretion in patients with the Duarte-galactosemic genotype. Most were discovered in newborn screening programs and placed on lactose-restricted diets during the first year. Urine from young infants aged less than 1 year was obtained while the diet was used. Except for four subjects in the 1- to 6-year age group, patients with the N314D/Q188R, as well as the N314D/other, genotype had galactitol excretion rates in the normal range (Table 2). The 2-year-old subject with an excretion rate of 100 mmol/mol

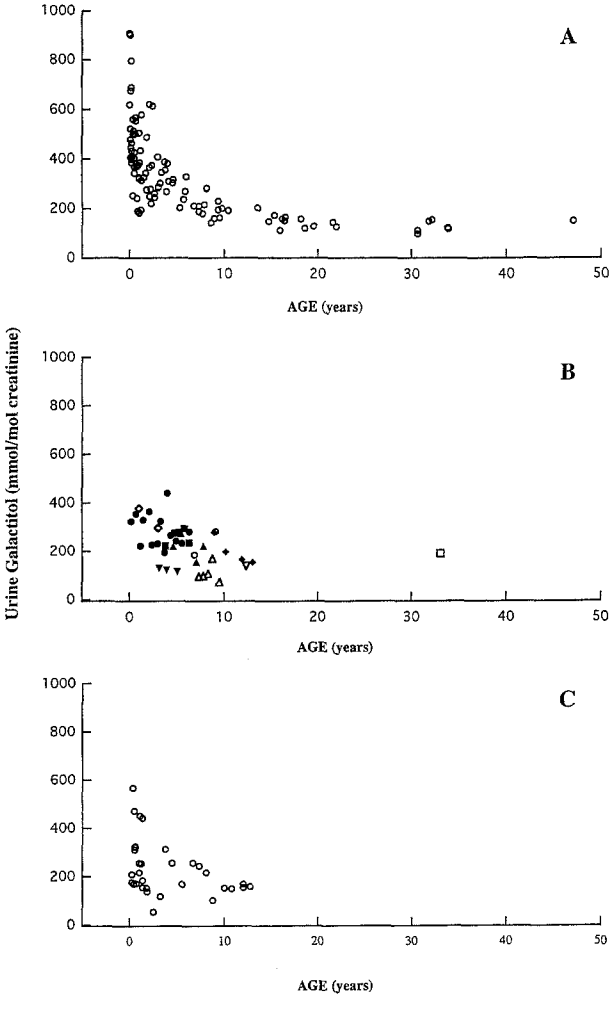


**Fig 1. Urinary galactitol excretion in subjects with the Q188R allele. (A) Patients with the Q188R/Q188R mutation (n = 100 [data sets], subjects = 32). (B) Subjects with the Q188R allele and an identified second G allele: ○, Q188R/H184Q (n = 2, subjects = 2); ●, Q188R/ΔT2359 (n = 14, subjects = 2); ▽, Q188R/M142K (n = 1, subjects = 1); ▼, Q188R/R123Q (n = 3, subjects = 1); □, Q188R/Q344K (n = 1, subjects = 1); ■, Q188R/E308K (n = 5, subjects = 1); △, Q188R/Y209C (n = 5, subjects = 1); ▲, Q188R/R259W (n = 4, subjects = 2); ◇, Q188R/L195P (n = 2, subjects = 1); and ◆, Q188R/F171S (n = 4, subjects = 2). (C) Subjects with the Q188R/unknown allele (n = 32, subjects = 14). Values are for patients aged >1 month.**

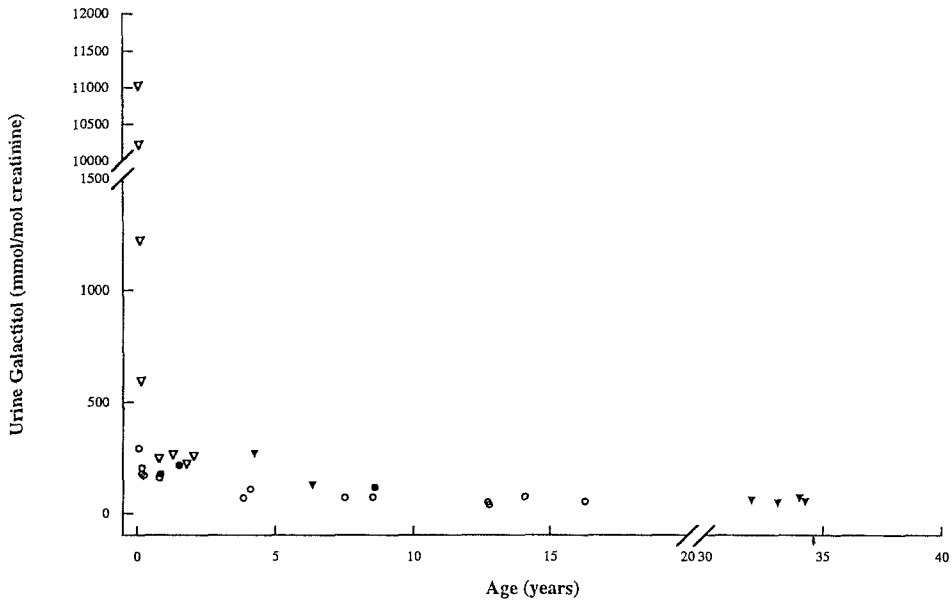


**Fig 2. Urinary galactitol in patients with the S135L allele(s). ○, S135L/S135L (n = 13 [data sets], subjects = 4); ▼, S135L/H135H (n = 6, subjects = 2); ▽, S135L/Q188R (n = 9, subjects = 1); and ●, S135L/unknown (n = 3, subjects = 2).**

creatinine also had an elevated gal-1-P level. Two subjects over age 6 also excreted normal amounts of the sugar alcohol. All of these subjects would be distinguishable from classic homozygous galactosemic subjects by measurement of urine galactitol. This is true for the N314D/N314D homozygote, who had a relatively high level of urinary galactitol without a lactose-free diet, but at 4 months of age on the diet, had normal galactitol excretion.

*Urinary Galactitol Excretion and RBC Gal-1-P Level*

Galactitol excretion was examined in relation to the RBC gal-1-P concentration, and Fig 4 shows the level of both of these metabolites in a Q188R/Q188R patient evaluated over a period of 4 years from the time a lactose-restricted diet was instituted. There appeared to be good agreement between the 2 parameters. The high levels of both compounds decreased from the newborn period, and a relatively constant level of each was established by 6 months of age. Galactitol levels were maintained close to 300 mmol/mol creatinine, while RBC gal-1-P alternated between 2 and 3 mg/dL. The trends for the values of both compounds appeared similar.

Figure 5 shows a plot of 112 simultaneous measurements of urinary galactitol and RBC gal-1-P in 47 classic galactosemics. For the group as a whole, there is a significant correlation

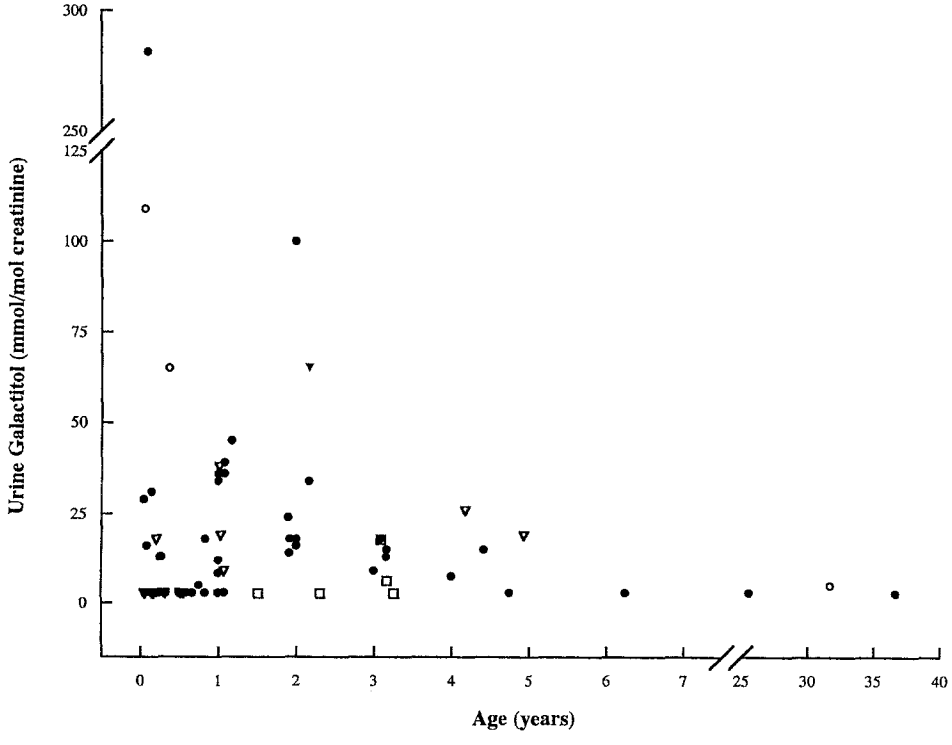


**Fig 3. Urinary galactitol in patients with the Duarte allele(s). ○, N314D/N314D (n = 4 [data sets], subjects = 2); ●, N314D/Q188R (n = 50, subjects = 30); ▼, N314D/E320K (n = 1, subjects = 1); □, N314D/R148W (n = 5, subjects = 1); and ▽, N314D/unknown (n = 13, subjects = 6).**

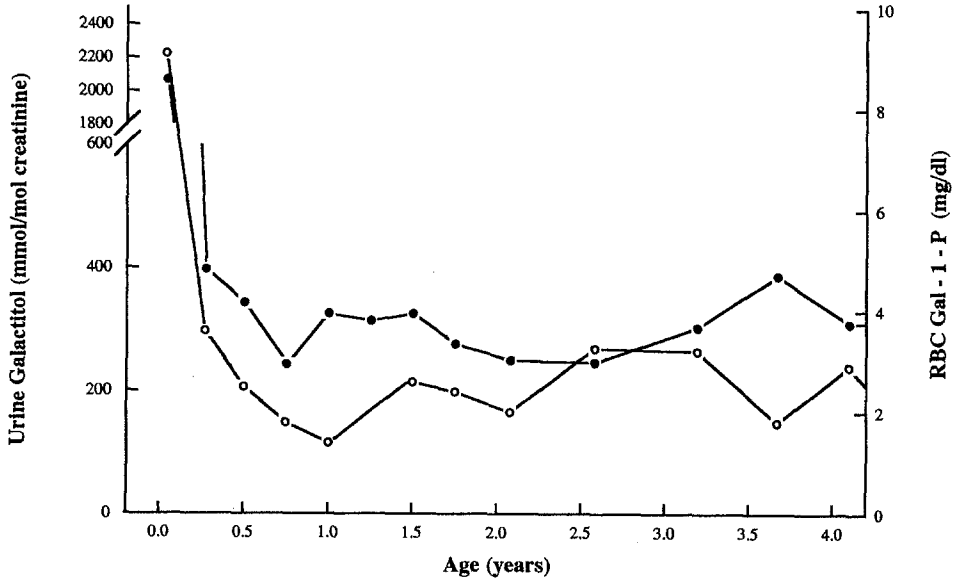


**Fig 4. Relationship of urinary galactitol to RBC Gal-1-P in a patient homozygous for the Q188R mutation over 4 years ($r = .94$, $P < .0001$). ○, RBC Gal-1-P; ●, urine galactitol.**

between the parameters ($r = .7$, with the slope of the line differing from no slope by $P < .0001$) but a wide scatter. For example, at 2 mg gal-1-P/dL, galactitol excretion varied between 100 and 400 mmol/mol creatinine. The same scatter is also exemplified at a RBC level of gal-1-P between 4 and 6 mg/dL.

*Plasma Galactitol Concentration*

No galactitol (<1 μmol/L) was detected in the plasma of 10 normal subjects of various ages, including infants. However, the sugar alcohol was found in the plasma of all 24 patients of various genotypes and ages in which it was assayed (Table 4). If the 15-day-old infant who was not yet under dietary control with a plasma galactitol level of 574 μmol/L is excluded, the plasma level is in a narrow range from 1 month to 19 years. In 13 patients less than 1 year old, the range was 8.4 to 23.2 μmol/L (12.3 ± 3.9, mean ± SD). In the group aged 1 to 6 years, the range for 11 patients was 4.9 to 15.1 μmol/L (9.5 ± 2.7), and in seven patients over 6 years, the range was 3.4 to 13.7 μmol/L (10.4 ± 3.6). There is overlap in the three groupings by age as used for urinary excretion in Table 2, with no significant difference between groups. Schweitzer et al[17] reported no difference with age and a similar mean of 9 ± 2.2 μmol/L (range, 4.7 to 20) in 75 treated patients. The plasma galactitol level was measured in several patients, no. 1, 4, and 5, over time (Table 4). The level in patient no. 1 decreased from 574 μmol/L at 15 days to 8.8 μmol/L at 15 months and 9.4 μmol/L at 18 months. In patient no. 4, it decreased from 23.2 μmol/L at over 1 month of age to 11 μmol/L at 7 months and 10.1 μmol/L at just over 1 year. There appears to be little

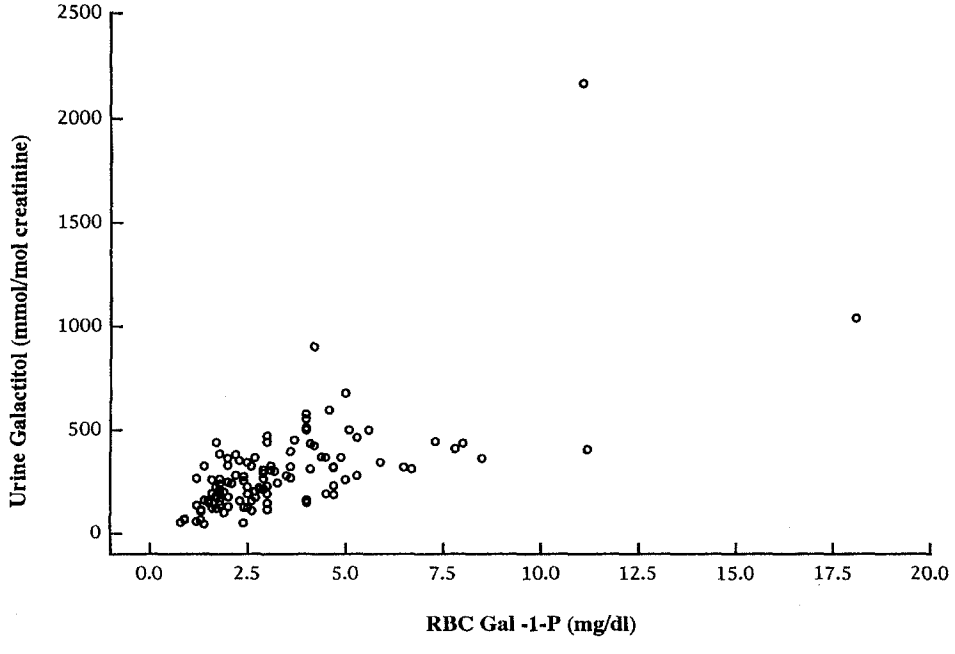


**Fig 5. Simultaneous samples of urine for galactitol and RBC Gal-1-P in galactosemic subjects (n = 112 [data sets], subjects = 47).**

relationship to genotype. Four patients had obviously low plasma values: patient no. 8 with a S135L/unknown genotype, 4.9 μmol/L at 1.5 years; patient no. 15 with the Q188R/R123Q genotype, 6.6 μmol/L at 5.25 years; patient no. 19 with Q188R/Y209C, 6.3 μmol/L at 9 years; and patient no. 23, who is not yet genotyped, with the lowest level of 3.4 μmol/L at age 13 years.

Urinary galactitol excretion measured in specimens obtained at the same time as plasma samples shows a range and mean values for this subset of subjects that resemble the data in Table 2, with values decreasing with age. There was no correlation of plasma levels with urinary excretion except in patients no. 15 and 19, who both had low plasma levels and low urinary excretion. Figure 6shows the relationship of the plasma galactitol concentration to urinary excretion in 31 parallel analyses. In the narrow range for the plasma concentration with age, there is a marked difference in urinary excretion.

## DISCUSSION

Abnormally high galactitol excretion in the urine characterizes the individual with an inability to metabolize galactose normally and serves to distinguish galactosemic patients from normals. For the aspects of urinary galactitol excretion we studied, ie, the age and genotype of the galactosemic patient, this appears to be the case. It is also evident that elevated plasma galactitol is the hallmark of the galactosemic patient.

**Table 4. Comparison of Plasma and Urine Galactitol in Galactosemics**

| Subject no.† | Genotype | Age (yr) | Plasma Galactitol (μmol/L) | Urine Galactitol (mmol/mol creatinine) |
|---|---|---|---|---|
| 1 | S135L/Q188R | 0.04 | 574 | 11,026 |
| 2 | Q188R/Q188R | 0.08 | 9.9 | 567 |
| 3 | Unknown/unknown | 0.08 | 10.3 | 286 |
| 4 | Q188R/unknown | 0.14 | 23.2 | 604 |
| 5 | Q188R/Q188R | 0.15 | 12.9 | 405 |
| 6 | Unknown | 0.5 | 14.1 | 431 |
| 7 | Unknown | 0.5 | 12.5 | 413 |
| 8 | S135L/unknown | 0.52 | 9.2 | 177 |
| 9 | Q188R/Q188R | 0.54 | 8.4 | 402 |
| 10 | IVSF/Y209C | 0.58 | 10.1 | 459 |
| 4 | Q188R/unknown | 0.63 | 11.0 | 358 |
| 5 | Q188R/Q188R | 0.75 | 12.9 | 371 |
| 4 | Q188R/unknown | 0.83 | 13.4 | 448 |
| 4 | Q188R/unknown | 1.08 | 10.1 | 266 |
| 1 | S135L/Q188R | 1.25 | 8.8 | 267 |
| 11 | Q188R/unknown | 1.25 | 8.8 | 253 |
| 1 | S135L/Q188R | 1.5 | 9.4 | 218 |
| 8 | S135L/unknown | 1.5 | 4.9 | 216 |
| 12 | Unknown/unknown | 3.5 | 10.3 | 313 |
| 13 | Q188R/ΔT2359 | 4.0 | 12.6 | 440 |
| 14 | S135L/T-C2749* | 4.25 | 8.5 | 269 |
| 15 | Q188R/R123Q | 5.25 | 6.6 | 116 |
| 16 | Q188R/unknown | 5.5 | 9.3 | 169 |
| 17 | Q188R/Q188R | 5.92 | 15.1 | 327 |
| 18 | Q188R/ΔT2359† | 6.25 | 12.0 | 281 |
| 19 | Q188R/Y209C | 9.42 | 6.3 | 76 |
| 20 | Unknown/unknown | 9.67 | 11.4 | 178 |
| 21 | Q188R/Q188R | 10.17 | 13.7 | 194 |
| 22 | Q188R/Q188R | 12.83 | 10.9 | 172 |
| 23 | Unknown/unknown | 13.33 | 3.4 | 130 |
| 24 | Q188R/Q188R | 19.58 | 10.8 | 129 |

*H315H.

†Stop codon exon.

‡Duplicate numbers show subjects at different ages.

Our findings and other studies[10,16] indicate that urinary galactitol excretion is age-dependent in both normals and galactosemics. At all ages, the classic galactosemic patient with the homozygous Q188R genotype and no detectable RBC GALT on a lactose-restricted diet excretes fivefold to 10-fold more urinary galactitol than the normal subject. Galactosemic patients with this genotype over age 6 excrete, on average, about half the amount of younger patients.

In nearly all G/G galactosemic subjects who are compound heterozygotes for Q188R and another G allele, the urinary excretion pattern with age does not differ versus subjects who are homozygous for the Q188R allele. However, the mean value at ages less than 6 years is lower (Table 2). This may be due to the fact that some of the compound heterozygotes have a lower excretion rate for the polyol. Two G/G patients with either R123Q or Y209C as the other abnormal allele appeared to excrete less galactitol than homozygote Q188R patients. Both of the patients have detectable RBC GALT levels between 2% and 4% of normal. A lower excretion rate was also observed previously in patients with some detectable RBC GALT activity.[10,17]

The African-American patient with galactosemia has been identified as having a characteristic mutation, S135L. Patients in the homozygous state[18] and those with S135L/H315H[19] can oxidize galactose in a near-normal fashion. Despite this ability and a residual 10% GALT activity in the intestine[20] and liver,[21] they still excrete abnormally high levels of galactitol, albeit not as high as Q188R homozygotes but high enough to identify them as having abnormal galactose metabolism.

Our data suggest that the measurement of urinary galactitol may be helpful in determining the extent of abnormal galactose metabolism in patients who are Duarte-galactosemic compounds. These patients should excrete normal amounts of galactitol on a lactose-restricted diet under 1 year of age. Any who showed elevated urinary levels on a lactose-containing diet at any age are candidates for diet therapy.

There is indeed a wide range of urinary galactitol excretion in classic galactosemics at any age. Our measurement of plasma galactitol surprisingly showed a very narrow range and no difference with age or genotype, except in two patients who are compound for Q188R and R123Q and Y209C. It appears that the high urinary content of galactitol in young patients and the variation from patient to patient may result, in part, from differences in renal handling of the compound. Urinary excretion of intravenously injected radiolabeled galactitol in humans appears to be the main route of galactitol disposition.[22] The maintenance of a similar plasma galactitol level in young patients with high excretion rates and in older patients with lower excretion rates suggests that younger patients have a higher rate of galactitol formation from the endogenous production of galactose.[23]

A measurable plasma galactitol concentration also serves to distinguish the galactosemic patient, since normal individuals have no detectable plasma galactitol by our method. The source of plasma galactitol in these patients on lactose-restricted diets

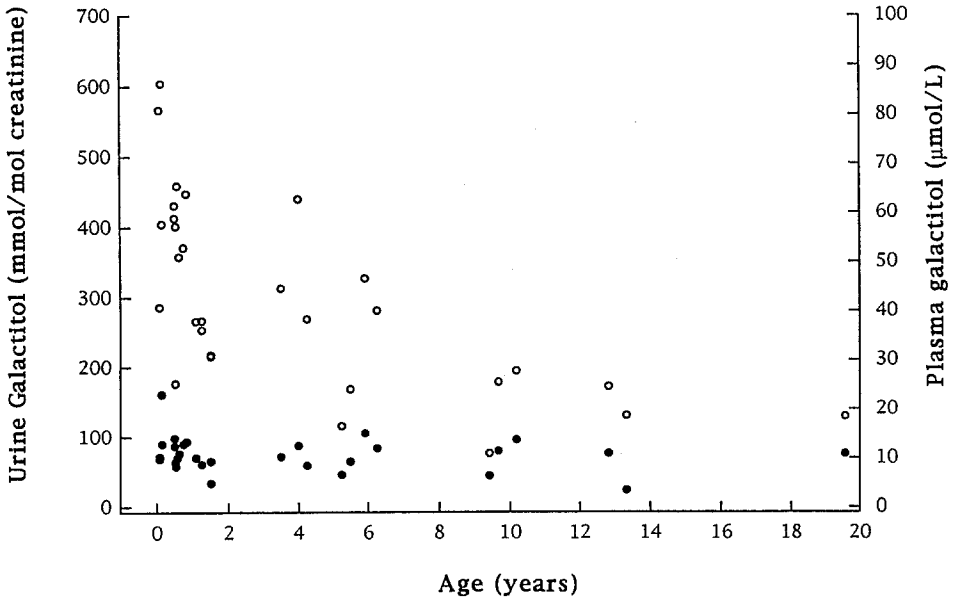


**Fig 6. Relationship of urine and plasma galactitol in subjects with galactosemia. ○, Urine galactitol; ●, plasma galactitol.**

is thought to be the extensive endogenous production of galactose.[23] With a blockage of galactose metabolism due to GALT deficiency, galactose is acted on by a NADH-dependent aldose reductase enzyme, with resulting galactitol formation.[7,24] Although the human aldose reductase gene has been cloned,[25,26] little is known about the genetic variation of the activity in humans. A difference in aldose reductase activity could contribute to the individual variation in galactitol formation by galactosemic individuals. Galactitol formation and urinary excretion is an important means of ridding the body of galactose. It cannot be further metabolized to any great extent.[27] The conversion of galactose to galactitol is a "dead-end" pathway. Reported estimates are that the daily excretion of galactitol by adult galactosemics accounts for about 20% of the endogenous production.[28]

We have routinely measured urinary galactitol excretion in our patients at follow-up visits for the past several years, which has permitted our comparison of the urinary galactitol level with the RBC gal-1-P concentration as parameters for evaluating dietary compliance and the overall state of galactose metabolism. Although Schweitzer et al[17] found no relation of RBC gal-1-P and urine galactitol excretion, Fig 5 indicates a statistical correlation between the two. However, there is a wide scatter for urinary galactitol values between the range of RBC gal-1-P of 2 and 5 mg/dL, which is the acceptable level for patients on well-maintained lactose-restricted diets. On the other hand, for a given individual (Fig 4), there appears to be a similar pattern for both metabolites. Which metabolite is better for evaluating the state of galactose metabolism is an important question. Administering a very large load of galactose to galactosemics increases both RBC gal-1-P and urinary galactitol.[29,30] Administration of 200 mg galactose daily to adult patients for a period of several weeks did not increase RBC gal-1-p but did increase urinary galactitol excretion.[28] Urinary galactitol excretion and perhaps plasma galactitol may be better parameters for evaluating breeches in the diet or the metabolic state of the patient. From a biochemical point of view, RBC gal-1-P may reflect the ambient plasma galactose concentration in response to sharp increases in galactose intake, while galactitol formed intracellularly by many tissues must leak into the plasma for excretion, thus being a more subtle indicator of overall steady-state body galactose metabolism. We favor the monitoring of patients with measurement of urinary galactitol. However, further studies are needed to correlate the levels in single urine specimens as reported here with 24-hour urine collections.[28]

Whether the level of urinary galactitol excretion or the plasma level may be a parameter predictive of long-term outcome remains to be determined. It should be noted that diet-independent complications may be related to an array of factors that characterize the galactosemic patient, such as the genotype[11,12] and residual tissue GALT activity, the extent of endogenous galactose production,[23] and the existence of alternate metabolic pathways, especially the activity of galactose oxidation via galactonate.[31,32] The latter is also excreted in abnormally high amounts in urine, usually at a level about one third that of galactitol.[13]

Although plasma and urinary galactitol levels are of importance in assessing abnormal galactose metabolism and galactitol formation provides a route for ridding the galactosemic patient of galactose, its presence is an etiologic factor in the toxic effects of galactose. It appears to be the cause of cataracts in galactokinase-deficient patients since it is the principal abnormal metabolite in that condition,[33] and in GALT-deficient patients, where a high galactitol content has been found in the cataractous lens.[4] In vitro studies have clearly shown that the lens readily forms galactitol, which acts as an osmolyte causing water imbibition accumulation and swelling of lens fibers.[5] In galactose-toxic animals, inhibitors of aldose reductase and galactitol formation will prevent cataracts,[34,35] and mice that have a low level of lens aldose reductase are resistant to galactose-induced cataracts.[36]

High levels of galactitol have been found in the amniotic fluid of affected fetuses[37] and in the liver and brain of galactosemic patients.[2] The latter may be the cause of pseudotumor cerebri observed in both GALT-deficient[38] and galactokinase-deficient[39] patients. Aside from cell swelling due to the accumulation of this inert compound, the cellular pathophysiology in cells exposed to galactose remains to be determined. The finding that galactose induces swelling of the endoplasmic reticulum of galactosemic fibroblasts incubated with galactose that may be related to galactitol accumulation has been reported.[40]

Although galactitol formation occurs in both galactokinase- and GALT-deficient patients, the latter also accumulate gal-1-P. It appears that the clinical phenotype of GALT deficiency depends on the accumulation of both metabolites. It may be that gal-1-P exerts a deleterious effect on the metabolism of tissues other than the lens that is disrupted by galactitol. Galactitol formation may be necessary but not sufficient for the emergence of the many complications of GALT deficiency. However, since aldose reductase inhibitors can prevent galactose-induced cataracts,[34,35] as well as other manifestations of galactose toxicity in animals,[41,42] an important new therapeutic strategy to prevent chronic diet-independent complications in classic galactosemia may be the prevention of galactitol formation by such compounds.

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