Comparison of Erythrocyte Uridine Sugar Nucleotide Levels in Normals, Classic Galactosemics, and Patients With Other Metabolic Disorders

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By limiting galactosylation mechanisms, a cellular deficiency of the uridine sugar nucleotide, UDPgalactose, has been implicated as a cause of the long-term complications seen in patients with classic galactosemia despite dietary treatment. As a result, great interest has been generated in the accurate assessment of UDPgalactose, as well as UDPglucose, from which UDPgalactose may be derived by the function of a ubiquitous, active UDPgalactose-4-epimerase. Since several series of values for the concentration of these compounds in red blood cells (RBCs) of galactosemics have been flawed by the use of methods subsequently shown to be unsuitable, we have quantified erythrocyte UDPgalactose and UDPglucose levels by an accurate high-performance liquid chromatography (HPLC) assay in 116 normals, 76 galactosemics, and 39 patients with other metabolic disorders. These large groups have permitted the evaluation of age, diet, and genotype as influential factors in the steady-state RBC levels of the sugar nucleotides. The data show that age is an important determinant of RBC levels, with children younger than 10 years having higher values than individuals older than 10 years. Mean UDPgalactose levels in galactosemic children younger than 10 years and those older than 10 years were 30% and 18% lower, respectively, than levels in comparable normals. Although the mean differences were highly significant, there was considerable overlap of individual values. There was no difference in UDPglucose levels between galactosemics and normals. Diet plays a role in that erythrocytes of children on special metabolic diets low in protein and therefore also low in lactose show significantly lower levels of both UDPgalactose and UDPglucose than normal children, with the average UDPgalactose level similar to that in galactosemics. An analysis of UDPgalactose content with respect to genotype in galactosemics did not show any correlation with homozygosity of the Q188R allele. The average ratio of UDPglucose to UDPgalactose in RBCs of galactosemics under 10 years of age was 62% higher than the ratio in cells of comparable normals, whereas in older galactosemics there was a 29% increase. In 55% of galactosemics, the ratio was more than 2 SD above the mean of the normals, indicating that an increase in the erythrocyte UDPglucose to UDPgalactose ratio is a characteristic of the majority of galactosemic patients. The difference in the ratio, which deviates from the expectation for the equilibrium normally established by UDPgalactose-4-epimerase, implies a perturbation in epimerase function in galactosemic RBCs, the nature of which remains to be determined by examining the functional cellular pools and flux of hexoses not only in erythrocytes but in other model cell systems.

EVIDENCE HAS BEEN MOUNTING that the dietary treatment of classic galactosemia initiated over 50 years ago[1] has not prevented many long-term complications. Analysis of follow-up data in more than 300 patients in the United States and elsewhere by Waggoner et al,[2] in 173 German patients by Schweitzer et al,[3] and in 69 patients in The Netherlands by Bakker and Boelen[4] has shown that many adequately treated galactosemics, even those in whom galactose restriction is initiated at birth, have speech defects, mental disability, and neurologic abnormalities. In addition, most females show evidence of ovarian failure. The German data[3] suggest a distressingly high incidence of cerebellar ataxia and that mental capacity is progressively lost.

At the present time, the etiology of these clinical abnormalities is enigmatic.[5] Despite strict lactose restriction, galactosemics maintain abnormally high levels of galactose-1-phosphate in erythrocytes[6] and excrete excessive galactitol,[7] the product of an alternate pathway of galactose metabolism. Gitzelmann and Steinmann[8] have postulated that there is a continuous "self-intoxication" via the production of galactose-1-phosphate from the breakdown of UDPgalactose formed, in turn, from UDPglucose. More recently, interest has centered on UDPgalactose as a pivotal compound, stimulated by the proposal of Ng et al[9] that there is a deficiency of UDPgalactose in tissues of galactosemics and that the complications result from a decreased capacity to synthesize complex carbohydrates and galactolipids, for which UDPgalactose is an essential precursor.

The postulate of Ng et al[9] was based on their finding with an enzymatic assay that the red blood cells (RBCs) of galactosemics have much lower UDPgalactose levels than normals. Using a direct high-performance liquid chromatography (HPLC) analysis, we subsequently showed in a small group of galactosemics that they had a significantly lower average RBC UDPgalactose level, but with considerable overlap with normal values.[10] However, using an enzymatic method, Kirkman[11] reported that RBC UDPgalactose levels in galactosemics did not differ from those in metabolic patients on a diet restricted in protein and milk. We observed a similar finding.[10] More recently, Schweitzer et al,[3] using a radioactive assay method, reported that erythrocyte UDPgalactose levels were below the normal range in a large group of classic galactosemics. However, these observations have been attended by a marked discrepancy about

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the normal erythrocyte concentrations of UDPgalactose and UDPglucose. The normal UDPgalactose values determined by the enzymatic method reported by Ng et al[9] and the radioactive method reported by Shin[12] used in the German study[3] were, respectively, four and 10 times the values reported by Kirkman using an enzymatic assay.[11] This confusion has recently been resolved by quantitative ^{31}P–nuclear magnetic resonance (NMR) analysis of RBC extracts[13] and intact erythrocytes,[14] and by quantitation of the unique product of the enzymatic assay, UDPglucuronic acid.[15] The results of these analyses indicate that the values reported by both Ng et al[9] and Schweitzer et al[3] are erroneous and that the direct HPLC assay of nucleotide sugars devised by us accurately and reliably quantifies these substances in erythrocytes.

We now report an analysis of erythrocyte uridine sugar nucleotide levels in 116 normal individuals, 76 galactosemics, and 39 patients with other metabolic disorders. The large number of samples from normal individuals ranging in age from birth to 64 years has enabled us to determine the age-related change in RBC concentrations of these compounds and thereby appropriately assess their alteration in cells of galactosemics and other metabolic patients. We were able to confirm our previous findings from a smaller series of patients, ie, there is a statistical difference in average UDPgalactose levels between galactosemics and normal individuals, but individual values may overlap between these two populations. Additionally, a clear distinguishing feature of the two populations is the magnitude of the ratio of UDPglucose to UDPgalactose. With multiple sampling of normal individuals and galactosemics and with the increased sample size, the values can be shown to vary around an individual mean, as well as an age-determined population mean. Furthermore, using newly available genotype information on the mutation underlying deficient galactose-1-phosphate uridyltransferase activity, we have been able to demonstrate that there is no correlation of erythrocyte UDPgalactose level or the ratio of UDPglucose to UDPgalactose with homozygosity or heterozygosity for the most common mutation.

SUBJECTS AND METHODS

Subjects

Blood samples were obtained from 116 normal individuals aged from birth to 64 years, 76 classic galactosemics aged 14 days to 44 years, and 39 patients with other metabolic disorders aged 2 days to 16 years. Informed consent for the venipuncture was obtained from either the donors or their guardians in accordance with the study approval by the Children's Hospital of Philadelphia Institutional Review Board. A number of the specimens were obtained by colleagues at other institutions. Prandial state was not determined before venipunctures. Some individuals had repeated venipunctures during a period of up to 3 years. For the determination of population statistics, the first sample collected from each individual was used.

The normal population consists of healthy children who were having blood studies performed as preoperative evaluations for elective surgery or as part of ongoing well-child care, or for some of the adolescents and all adults, as uncompensated volunteers. For 114 of the normal individuals, exact ages were available. The sex of the individual was included in the information received with the specimens in 105 cases: 46 females and 59 males.

Samples from galactosemics and from individuals with other metabolic diseases on a low-protein diet were obtained in the Metabolism Clinic at Children's Hospital of Philadelphia or in similar clinics at other institutions. At the time of venipuncture, galactosemics were on a galactose-restricted diet and had acceptable levels of erythrocyte galactose-1-phosphate. All had markedly reduced or absent erythrocyte uridyltransferase activity. The patient's sex was known to us in 66 of 76 subjects, with 34 females and 32 males.

Individuals with other metabolic disorders included 19 with maple syrup urine disease, four with urea cycle disorders, one with phenylketonuria, and 15 with organic acidurias. Age and sex information was available on all these subjects, all of whom were judged to be compliant with their prescribed low-protein diets at the clinic visit when blood samples were obtained.

Methods

Five to 10 mL venous blood was placed into sodium heparin–containing tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) at 4°C. The cellular fraction was pelleted at 4°C at 900 × *g*. The plasma- and leukocyte-containing layers were removed by aspiration. One volume of packed cells was added to 2 vol 10% trichloroacetic acid (TCA). For samples collected over the last half of the study, 2 nmol UDPxylose was added to the TCA as an internal standard. After centrifugation, the supernatant was filtered and extracted with water-saturated ethyl ether to remove the TCA, as described by Palmieri et al.[16] Erythrocyte extracts were stored at −40°C until HPLC was performed. Samples from other institutions were sent as frozen TCA preparations. Stability of UDPhexoses in such material was demonstrated by Palmieri et al.[16]

UDPgalactose, UDPglucose, and TCA were obtained from Sigma Chemical (St Louis, MO). The purity and concentration of UDPgalactose and UDPglucose were confirmed by ^{31}P-NMR and by spectrophotometric analysis. Other reagents for the HPLC techniques were of the highest commercially available purity.

HPLC was performed on 20 μL of each sample with a Dionex CarboPac PA-1 anion-exchange column (Dionex, Sunnyvale, CA) using the system described by Palmieri et al[16] (first half of samples), or with the following modifications: The eluant gradient was increased by a concave gradient from 100 to 200 mmol/L KH_2PO_4, pH 5.7, and then by a series of convex gradients from 200 to 500 mmol/L KH_2PO_4, pH 4.5. The flow was also increased from 0.6 to 1.0 mL/min during the convex gradient series. Peaks were detected at 254 nm, and retention times of unknowns were compared with commercially obtained material. The sensitivity of the detector was 0.025 to 0.015 absorbance units, full scale, adjusted to give peaks of adequate size for reproducibility.

A series of external chromatographic standards was used to calculate concentrations of UDPgalactose and UDPglucose. Standard curves for linearity of response and recovery were constructed with the commercially obtained compounds, taking into account the purity, water of hydration, counter ions, and other solvents of each lot. The area of the UDPxylose peak was used to check on recovery and volume loss during the extraction.

Hemoglobin (Hgb) determinations were performed on packed erythrocytes using the commercially available kit (no. 625-A) from Sigma. Genotype analysis for the Q188R mutation was performed by a rapid polymerase chain reaction–based assay as previously described.[17]

All values are expressed as micromoles of UDPhexoses per 100 g of Hgb. Statistical analysis was performed with Student's *t* test, with the minimal level of significance set at P less than .05.

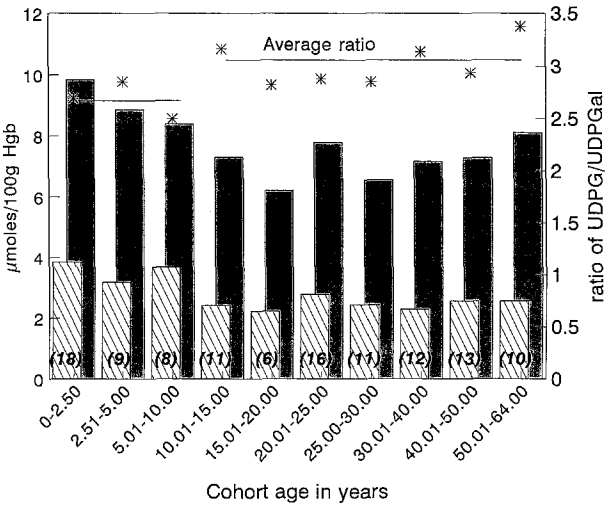

Fig 1. RBC (■) UDPglucose (UDPG) and (▨) UDPgalactose (UDPGal) levels and (*) their ratio in groups of normals according to age.

RESULTS

Age-Related Erythrocyte Sugar Nucleotide Levels

In our previous analysis of erythrocyte sugar nucleotides in a small cohort, we arbitrarily separated the values into two groups: individuals under and over 18 years of age. Differences in sugar nucleotide levels within that normal group of 35 subjects were readily apparent, since the group included 19 adults aged 22 to 51 years and 16 children aged 10 months to 7 years. In the present study of 114 normal subjects in whom precise ages were known, an adequate spectrum was available to make an analysis of the relationship of uridine sugar nucleotide levels to age. After an iterative analysis of UDPgalactose, UDPglucose, and their ratio between adjacent age cohorts, the stratification shown in Fig 1 was established. It was apparent that UDPgalactose values in the three groups aged 10 years and younger did not differ from each other, but the levels in each group differ from the levels in the groups over age 10 years. Therefore, we decided to separate the cohorts into two age groups: those under and over 10 years of age. In the 35 individuals under age 10 years, the average erythrocyte UDPgalactose content was 45% higher than that of the 77 older individuals (>age 10 years, $P < .001$): the mean ±SD of the younger group's values was 3.71 ± 1.24 μmol/100 g Hgb versus 2.54 ± 0.80 (Table 1). The UDPglucose content of RBCs from the younger individuals (those <age 10 years) was also 25% to 30% higher than in older subjects: 9.33 ± 1.99 versus 7.35 ± 1.70 μmol/100 g Hgb, respectively, $P < .001$. The total UDPhexose content was also approximately 30% higher in younger individuals. The ratio of UDPglucose to UDPgalactose was also different (2.65 ± 0.54 for < age 10 *v* 3.02 ± 0.54 for older groups, $P < .01$).

Sugar Nucleotides in Normals and Galactosemics

UDPgalactose levels. Levels of erythrocyte UDPgalactose with regard to age for each normal and galactosemic subject are shown in Fig 2. Values ranged from 1.60 to 6.40 μmol/100 g Hgb (mean, 3.71 ± 1.24) in the 35 normal children and from 0.94 to 5.00 (mean, 2.61 ± 0.93) in the 51 galactosemic children. This 30% reduction in the average UDPgalactose value in galactosemic children was highly significant ($P < .001$). Nevertheless, despite this significant difference, as shown in Fig 2, there was considerable overlap in UDPgalactose levels among individual galactosemic and normal children. Although almost 90% of the younger galactosemics have a UDPgalactose value less than the mean for the normal age-related population, only 10% of the values are below 2 SD of this mean.

In the 81 normal individuals over age 10 years, erythrocyte UDPgalactose levels ranged from 1.10 to 6.50 μmol/100 g Hgb (mean, 2.54 ± 0.88), whereas this level in 25 galactosemic individuals over age 10 years ranged from 0.70 to 4.50 (mean, 2.08 ± 0.95) (Table 1). This 19% reduction in the average value in galactosemic individuals was statistically significant at P less than .05.

As in normal subjects, there was an age-related difference in the RBC UDPgalactose level in younger and older galactosemic patients. The 21% reduction in the mean (2.07 ± 0.94 *v* 2.61 ± 0.83 μmol/100 g Hgb) was smaller than the difference between mean UDPgalactose values of younger and older normal subjects, but was still significant ($P < .05$).

Table 1. RBC UDPgalactose and UDPglucose Levels (μmol/100 g Hgb) and Their Ratio in Normal Subjects and Patients With Galactosemia or Other Metabolic Disorders

	No. of Subjects	UDPgalactose	UDPglucose	Ratio
<10 years of age				
Normals	35	3.71 ± 1.24	9.33 ± 1.99	2.65 ± 0.54
Galactosemics	51	2.61 ± 0.93*	10.45 ± 3.22	4.29 ± 1.37*
Other disorders	26	2.67 ± 0.71*	8.67 ± 1.62¶	3.42 ± 0.88*¶
>10 years of age				
Normals	81	2.54 ± 0.88*	7.35 ± 1.70*	3.02 ± 0.64†
Galactosemics	25	2.07 ± 0.94§#	7.58 ± 2.57‖	3.91 ± 0.89§
Other disorders	13	2.32 ± 0.74	7.43 ± 2.53	3.20 ± 0.63**

NOTE. Results are the mean ± SD.

*$P < .001$, †$P < .01$: *v* normals <10 years.

‡$P < .001$, §$P < .05$: *v* normals >10 years.

‖$P < .001$, ¶$P < .01$, #$P < .05$: *v* galactosemics <10 years.

**$P < .01$ *v* galactosemics >10 years.

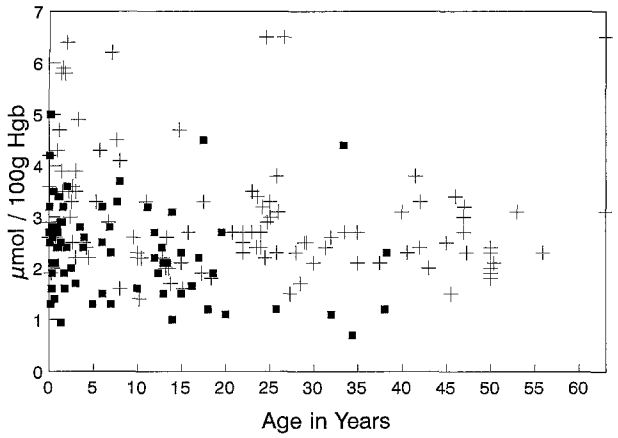

Fig 2. UDPgalactose levels in erythrocytes of (+) normals and (■) galactosemics of various ages.

UDPglucose levels. Figure 3 shows individual values for erythrocyte UDPglucose in relation to age. It is readily apparent that there is overlap between galactosemics and normal individuals regardless of age. In normal individuals under age 10 years, UDPglucose ranged from 5.30 to 13.50 μmol/100 g Hgb (mean, 9.33 ± 1.89), and in age-comparable galactosemic patients, this range was 4.5 to 19.70 (mean, 10.45 ± 03.22). The difference between normal and galactosemic individuals was not significant. In normal individuals older than 10 years, UDPglucose levels ranged from 4.10 to 15.10 μmol/100 g Hgb (mean, 7.35 ± 1.70) whereas in galactosemics older than 10 years, the range was 3.30 to 14.40 (mean, 7.58 ± 2.57). Again, this difference between normal and galactosemic individuals was not significant. As with UDPglucose values in normal subjects of the two age groups, there was a significant decrease (27%, $P < .001$) in the mean value for galactosemic subjects older than 10 years as compared with those younger than 10 years.

Total UDPhexoses. The total erythrocyte uridine nucleotide sugar content in normal subjects under age 10 years, 13.12 ± 3.06 μmol/100 g Hgb, was not significantly different from that in comparably aged galactosemic subjects, 12.77 ± 3.86. The total erythrocyte UDPhexose content of each of the older groups also did not differ between normal and galactosemic individuals. However, there was a significant decrease in the total erythrocyte UDPhexose content of each older group as compared with their respective younger group ($P < .001$ for normals, $P < .01$ for galactosemics).

Ratio of UDPglucose to UDPgalactose in erythrocytes. The ratio of UDPglucose to UDPgalactose values provides another parameter by which an assessment of a sugar nucleotide abnormality can be made, since the ratio can serve to define the equilibrium of the epimerization reaction of the two compounds. These values in erythrocytes were calculated for each patient, with mean values shown in Table 1 and the array of data with respect to subject age shown in Fig 4. In normal subjects under age 10 years, the ratios ranged from 1.60 to 3.85 (mean, 2.65 ± 0.54), whereas values for similar-aged galactosemic subjects varied from 2.20 to 7.30 (mean, 4.29 ± 1.37). These means were significantly different ($P < .001$). The same degree of difference was observed between the means of 3.02 ± 0.64 and 3.91 ± 0.89 for the older normal and galactosemic groups. In younger galactosemics, the ratio of UDPglucose to UDPgalactose was less than the normal mean in only 6% but greater than 2 SD of the normal mean in 65%. In older galactosemics, this ratio was greater than 2 SD of the normal mean in only 20%. There was no significant difference between the mean ratio of younger and older galactosemics, whereas values between the two age groups of normal subjects were different at P less than .01.

Repetitive Determinations in Normals and Galactosemics

From two to 12 repeat measurements were performed in 16 normal individuals over age 10 years and 24 galactosemic subjects, 16 of whom were under age 10, over a period of 3 to 36 months. Figure 5 shows the replicate values for erythrocyte UDPgalactose, with the solid horizontal line denoting the population means of the normal groups as determined earlier. The broken lines mark the average value for single determinations of the two galactosemic groups. Considerable variation of the values occurred in both galactosemic and normal subjects in specimens drawn from the same individual over a period of months. In the 16

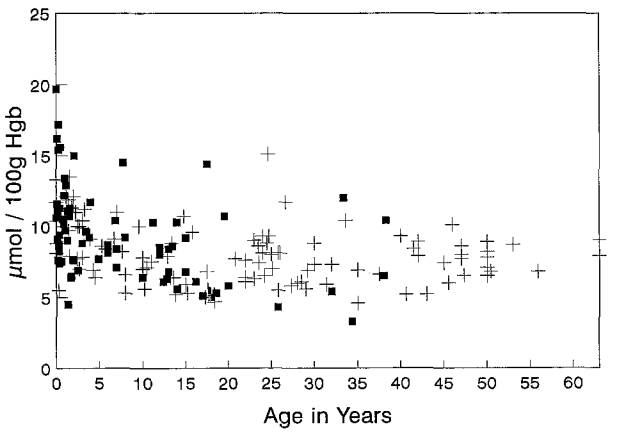

Fig 3. UDPglucose levels in erythrocytes of (+) normals and (■) galactosemics.

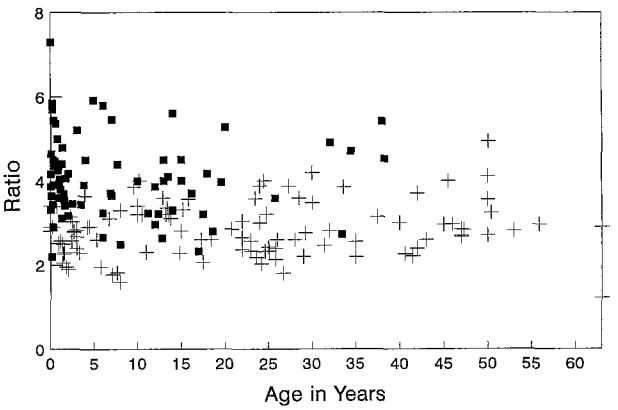

Fig 4. UDPglucose to UDPgalactose ratio in erythrocytes of (+) normals and (■) galactosemics.

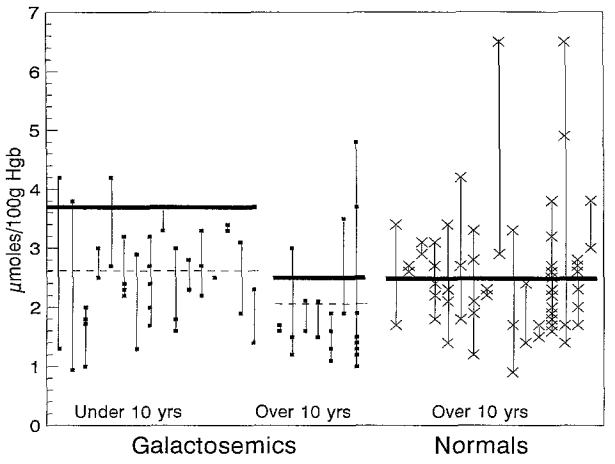

Fig 5. RBC UDPgalactose levels in repeated blood samples of (×) normals and (■) galactosemics at varying intervals over a 3-year period. (▬) Normal population mean (See Table 1). (---) Galactosemic population mean.

younger galactosemic individuals, only three had a UDPgalactose value slightly greater than the normal mean. The variation in values took place about the mean for the entire 51 patients whose initial values are shown in Fig 2. The mean of each younger galactosemic patient's average erythrocyte UDPgalactose level was 2.36 ± 0.56, which is not different from the value of 2.61 ± 0.93 μmol/100 g Hgb for the entire group's mean. The same pattern exists in the data for repeated sampling in galactosemic subjects over age 10 years, with variation being found below the normal mean. In contrast, the array of UDPgalactose values in repeated sampling of the 16 normal subjects over age 10 shows the variation to center about the mean of the entire 81 normal subjects. The repeat UDPglucose values, in both galactosemic and normal individuals who were sampled more than once, varied about the mean of the age-appropriate normal groups.

The distinguishing difference between the ratio of erythrocyte UDPglucose to UDPgalactose in galactosemic and normal subjects seen in Fig 4 is also observed in this population. The range of ratios found in multiple independent samples from galactosemic individuals varied by age about means of 3.87 ± 0.96 in younger subjects and 4.41 ± 1.19 in older patients, considerably higher than the means of the age-matched normal populations. The variation of the ratio in multiple samples in the 16 normals was about the mean of the entire population of 81 normals (data not shown).

Genotype Correlation

The most common genotype associated with classic transferase-deficient galactosemia is the single base mutation at codon 188 in the structural gene for galactose-1-phosphate uridyltransferase, which results in a substitution of arginine for glutamine (Q188R).[17] Over 65% of the alleles sequenced have this mutation.[17] To determine if the presence or absence of this allele affected erythrocyte UDPgalactose levels, the subset of data on galactosemics with known genotypes was separated by homozygosity or heterozygosity for the Q188R allele (Fig 6). An insufficient number of identified patients without any Q188R alleles prevented analysis of that group.

In younger patients with one or two Q188R alleles, there was no difference in the mean UDPgalactose (2.29 *v* 2.40), UDPglucose (10.23 *v* 10.37), or ratio (4.73 *v* 4.48). In the older galactosemics, the differences approach significance only in the ratio ($P = .05$ for 4.31 ± 0.90 *v* 3.38 ± 0.28), but the small sample size precludes rigorous analysis.

UDPhexose Levels in Individuals on a Protein- and Lactose-Restricted Diet

Current therapy for galactosemia is the elimination, so far as possible, of galactose from the diet. The role of this restriction, via lactose exclusion from the diet, in altering UDPhexose concentrations in erythrocytes is unknown. To examine a population of individuals who ingest low amounts of lactose, samples from 26 children under age 10 years and 13 older individuals on protein-restricted diets for dietary therapy for other metabolic disorders were obtained. This population was selected because the exclusion of dairy products, a mainstay of a low-protein diet, also removes the major sources of lactose. As shown in Table 1, the erythrocyte UDPgalactose content of younger individuals who are moderately protein-restricted (age-appropriate intake, 0.8 to 1.6 g protein/kg body weight/d) was 2.67 ± 0.71 μmol/100 g Hgb, which was not different from the value of 2.61 ± 0.93 in comparably aged galactosemic subjects but was significantly different from the 3.71 ± 1.24 of normal children ($P < .001$). In protein-restricted older individuals, erythrocyte UDPgalactose content was 2.32 ± 0.74 μmol/100 g Hgb, which was not different from the values in either normal or galactosemic individuals of similar ages.

The erythrocyte UDPglucose content of the 26 protein-restricted patients under age 10 years was significantly lower than that of the younger galactosemic subjects, 8.67 ± 1.67 μmol/100 g Hgb versus 10.45 ± 3.22 ($P < .01$), and was also lower than that of the normal group ($P < .05$). The UDPglucose content of the older population with other

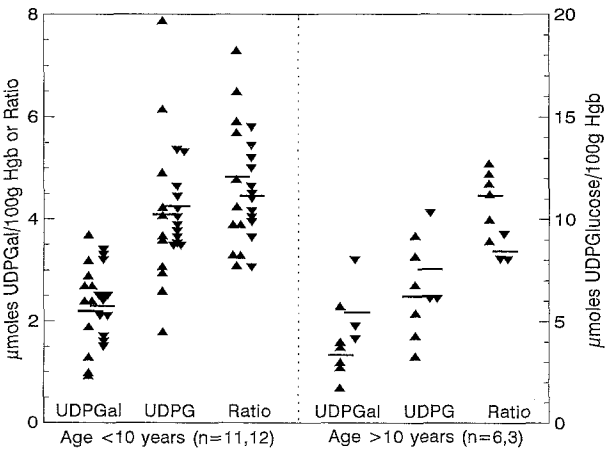

Fig 6. A comparison of RBC UDPgalactose (UDPGal) and UDPglucose (UDPG) content and their ratio in normals and galactosemics (▼) homozygous and (▲) heterozygous for the Q188R galactose-1-phosphate uridyltransferase mutation.

metabolic diseases was not different from that of either the older normal or galactosemic individuals.

The total UDPhexose content of younger patients on protein-restricted diets, 11.35 ± 1.94 μmol/100 g Hgb, was lower than that of similar-aged normal (13.12 ± 3.06, $P < .05$) or galactosemic (12.77 ± 3.86, $P < .05$) individuals. The total UDPhexose content of older individuals was the same in all three groups.

In younger patients with other metabolic disorders, the UDPglucose to UDPgalactose ratio was 3.42 ± 0.88, significantly different ($P < .01$) from that in normal or galactosemic populations of similar ages. The value of 3.20 ± 0.63 for the ratio in older metabolic patients was different at the P less than .01 level from the value in older galactosemic subjects. However, it did not differ from the ratio in normal individuals over age 10 years.

DISCUSSION

It is clear from our data that, as a group, erythrocytes of classic galactosemic patients have an average UDPgalactose level that is significantly lower than levels in normal individuals, but there is no difference in the concentration of UDPglucose. On the other hand, there are only a small number of galactosemics whose levels of UDPgalactose are less than the normal range. Our findings in this large group of normals and galactosemics substantiate our prior conclusion[10] and that of Keevill et al[18] based on a smaller amount of data.

It is important to consider our results in relationship to others and place them all in a proper perspective. Three other published reports on this subject from other laboratories all used different methods of analysis, which initially created a problem in interpreting the results. Ng et al[9] and Schweitzer et al[3] both reported an average lower level of erythrocyte UDPgalactose in galactosemics, which we confirm. A major difference is the fact that they reported no overlap of values in galactosemics and normals, with galactosemics clearly less than the normal range, whereas we find great overlap. Since it is now clear that both Ng et al[9] and Schweitzer et al[3] have used flawed methods of analysis,[19] their findings in this regard should be discounted. On the other hand, the data of Kirkman,[11] based on enzymatic analysis of UDPgalactose levels in erythrocytes, are substantially in agreement with our own HPLC analysis and our ^{31}P-NMR assessment of comparable groups. However, he concluded that there was no UDPgalactose decrease in galactosemic RBCs. But Kirkman did not examine a group of normal children on unrestricted diets. We have examined erythrocyte UDPgalactose levels in such children and found that the average of 3.71 μmol/100 g Hgb is different from the mean of 2.61 ($P < .001$) in galactosemic children (Table 1). Kirkman also did not compare a group of galactosemic adults with normal adults. Here, too, average values differ significantly. To sort out accurately the real difference in erythrocyte UDPgalactose between normal and galactosemic individuals has required both accurate methods and a proper comparison of levels with regard to age and diet. However, the striking thing is that both Ng et al[9] and Schweitzer et al,[3] on the basis of faulty analytical procedures, highlighted a derangement of sugar nucleotides in erythrocytes of galactosemic individuals, which we believe does exist. Nevertheless, we do not yet know the abnormality these investigators may have uncovered.

Our results indicate that the factors controlling erythrocyte steady-state levels of uridine sugar nucleotides are complex. Age is clearly one of these factors, which was an earlier impression. The clear separation in the mean erythrocyte UDPgalactose level in normal and galactosemic cohorts above and below age 10 years dictates that analyses of all other groups must be in comparison to appropriate age-matched control groups. This dividing age is a lower cut-off than used by Berry et al,[10] but erythrocyte UDPglucose values and the ratio of UDPglucose to UDPgalactose are similar in both younger populations.

The reason for the decrease in erythrocyte UDPhexoses with age is not obvious. It is possible that the age-related difference in normal individuals is due to diet, since adults generally consume less milk and therefore less galactose per body weight than children. Differences in lactose intake would not explain why both UDPgalactose and UDPglucose were also lower in galactosemic adults as compared with affected children. One possible explanation for these differences is hormonal control related to puberty. The possibility that diet plays a role was examined by determining erythrocyte sugar nucleotide levels in patients with other metabolic diseases on low-protein diets in which milk and consequently galactose ingestion was limited. The average erythrocyte UDPgalactose level in such children was virtually the same as in galactosemic children. However, the average UDPglucose level and the total UDPgalactose and UDPglucose level in these metabolic patients were significantly less than in normal or galactosemic children, suggesting that the etiology of the lower UDPgalactose level in children on protein-restricted diets is different from that in the other groups. Protein intake is a known positive affector of uridine synthesis and tissue UDPglucose levels,[20] making it reasonable to suspect that the limited protein intake in these patients could be responsible for the lower UDPglucose and UDPgalactose levels. Of course, in these individuals with normal galactose-1-phosphate uridyltransferase activity, dietary galactose may also play a role. The patients on low-protein diets appear to lose the age-dependent decrease in erythrocyte sugar nucleotide levels.

Random repeat analyses of both normal and galactosemic erythrocytes in a subset group over a period of months for the past 3 years show up to twofold changes in values for some individuals. Despite these wide differences of the repeated values, the mean of erythrocyte UDPgalactose levels of a given subject was the same as the appropriate population mean, with the values varying about the population mean. Consequently, the repeated-sampling data give credence to the overall population mean. Since we do not know the etiology of individual variations in both normal and galactosemic individuals, it does not appear reasonable at this time to measure levels of erythrocyte nucleotide sugars on a routine basis as a means of making an assessment of dietary control or clinical outcome in galactosemia.

The altered ratio of UDPglucose to UDPgalactose[10,18] appears to be a key finding specific to individuals with

galactosemia. A majority of galactosemic children and adults can be distinguished from normal subjects by this ratio. The mean ratios in normal children and adults are close to the equilibrium value of 3 for UDPgalactose-4-epimerase, the enzyme that catalyzes their interconversion.[21] Despite the differences in erythrocyte sugar nucleotide levels between normal adults and children, this ratio is maintained. However, the average ratios of 4.29 and 3.91 for galactosemic children and adults, respectively, differ significantly from the norm ($P < .001$). Moreover, a majority of galactosemic patients have ratios outside the normal range, even though most erythrocyte UDPgalactose values in these patients are within the normal range and their total UDPhexose content is normal. In most instances of repeated measurements, this abnormal ratio was maintained. Although the ratio is also different in younger individuals on protein-restricted diets as compared with normals, the mechanisms appear to be different. Both the numerator and denominator of the ratio are smaller, perhaps due to the combination of protein restriction and coincidental low lactose intake.

A vexing question is why the ratio is abnormal in galactosemia. The steady-state erythrocyte level of UDPgalactose is determined by formation from galactose-1-phosphate via galactose-1-phosphate uridyltransferase and from UDPglucose via UDPglucose-4-epimerase and pyrophosphorylase activities. The concentration of UDPglucose is determined by synthesis from uridine triphosphate and glucose-1-phosphate and by degradation via pyrophosphorolytic cleavage. The reason for the altered ratio of UDPglucose to UDPgalactose in galactosemic RBCs is difficult to comprehend in the face of normal UDPglucose levels, since their interconversion is an equilibrium reaction of UDPglucose-4-epimerase. Even in the absence of transferase activity and elimination of UDPgalactose formation via galactose-1-phosphate, formation of UDPglucose should be maintained. The abnormal ratio suggests that a characteristic derangement in galactosemic erythrocytes is an alteration or failure to achieve the epimerase-mediated equilibrium as a result of altered epimerase activity.

The UDPhexose ratio abnormality we describe could not be linked to the genotype of the patient. Elsas et al[17] have made a preliminary estimate of the relationship of genotype to outcome by comparing homozygosity with heterozygosity for the Q188R mutation. This comparison was chosen because the Q188R mutation is associated with no transferase enzyme activity in a yeast expression system. Thus, an individual homozygous for this mutation would be expected to have no cellular enzyme activity. They concluded that homozygous patients had a poorer outcome than patients with compound heterozygosity who might have some residual enzyme. Our present analysis shows that the level of erythrocyte UDPgalactose and the ratio of UDPglucose to UDPgalactose is the same in individuals who are homozygous as in those who are compound heterozygotes for the Q188R mutation. It seems that although age and diet may be determinants of RBC sugar nucleotide levels, there is no genotype-phenotype relationship for the UDPgalactose level within the galactosemic population.

Whether the low UDPgalactose level or the abnormal UDPglucose to UDPgalactose ratio in galactosemic RBCs is related to long-term complications as a result of defective galactosylation, as postulated by Ng et al,[9] is unknown. That there is indeed defective galactosylation in galactosemic cells is becoming more apparent. There is evidence that galactosemic fibroblasts have altered galactosylation,[22,23] and there are suggestive reports of differences in other complex galactose-containing compounds in the brain.[24,25] At the present time, the etiology of this phenomenon cannot be attributed with certainty to abnormal sugar nucleotide metabolism. Our erythrocyte UDPhexose data neither support nor refute the hypothesis that impaired galactosylation is a mechanism underlying the long-term consequences of galactosemia. The static levels of UDPgalactose and UDPglucose in erythrocytes represent an incomplete picture of the UDPhexose pools and their bioavailability in metabolically more-active tissues. Conclusions about UDPglucose and UDPgalactose differences in other tissues from data in erythrocytes and, in turn, hypotheses of their significance are not warranted unless confirmed by HPLC assays or ^{31}P-NMR determinations.

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