Eur J Pediatr (1995) 154 [Suppl 2] : S65–S71

Stanton Segal

Defective galactosylation in galactosemia: is low cell UDPgalactose an explanation?

S. Segal
Division of Biochemical Development
and Molecular Diseases,
Children's Hospital of Philadelphia,
34th Street and Civic Center Boulevard,
Philadelphia, PA 19104, USA
Tel: (215) 590–3372
Fax: (215) 590–3364

Abstract There is circumstantial evidence that defective galactosylation of complex glycoconjugates exists in tissues from galactosemic patients. Whether this is an etiologic factor in the long-term complications of the disorder is not known. Also not evident is the basis for the impaired galactosylation. The hypothesis that abnormally low cellular uridine diphosphate galactose (UDPgal) content is responsible has not been established. There is a tendency for galactosemic red cell UDPgal to be in the low normal range with a high uridine diphosphate glucose to UDPgal ratio. This may reflect an inability of red cell UDPgal-4′-epimerase to maintain a normal ratio and consequently higher levels of UDPgal. In the more complex white blood cells and cultured fibroblasts, the UDPgal content and the uridine diphosphate glucose to UDPgal ratio of galactosemics are normal. Therefore, defective galactosylation observed in galactosemic fibroblasts must result from a defect in the transfer of galactose from UDPgal to these moieties.

Key words Galactosylation · Uridine diphosphate galactose · Red blood cells

Abbreviations *GALT* galactose-1-phosphate uridyltransferase · *UDPgal* uridine diphosphate galactose · *UDPglc* uridine diphosphate glucose

Introduction

Galactose is an important constituent of the complex polysaccharides which are part of cell glycoconjugates, key elements of immunologic determinants, hormones, cell membrane structures, endogenous animal lectins and numerous other glycoproteins. In addition galactose is incorporated in galactolipids, important structural elements of the central nervous system. An example of the position of galactose in the complex structure of N-linked glycoconjugates is shown in Fig. 1. In the branches of the polysaccharides, galactose is linked to N-acetylglucosamine in the near terminal position. Attached to the galactose is N-acetylneuraminic or sialic acid. Without the insertion of galactose there would be no addition of the sialic acid, an important determinant of glycoconjugate function. In addition, galactose can serve as a substrate for sulfation which endows the characteristics of a number of hormonal substances. It is not difficult to assume that the abnormal galactose metabolism in the galactosemic patient could have profound and widespread effects on glycoconjugate structures and their biological function. This idea is supported by the fact that uridine diphosphate galactose (UDPgal) is the donor of the galactose via galactosyltransferase reactions to form the polysaccharide structure. Low cellular levels of UDPgal or impaired function of the specific galactosyltransferase involved in the process might compromise the synthesis of glycoconjugates.

UDPgal is formed by the reactions shown in Fig. 2. The primary route ubiquitous in mammalian cells involves two steps, the formation of uridine diphosphate glucose (UDPglc) from uridine triphosphate and glucose-1-phosphate by UDPglc pyrophosphorylase and the conversion of UDPglc to UDPgal by the enzyme UDPgal-4'-epimerase. Both enzymes involved are very active. In ad-

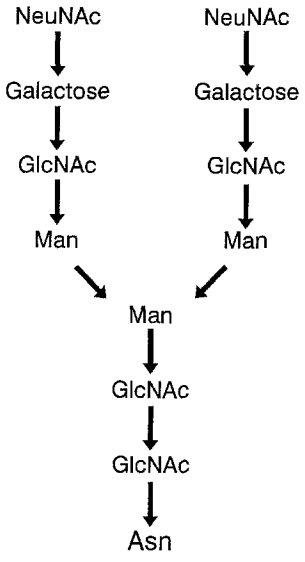

Fig. 1 Simplified structure of an oligosaccharide component of glycoconjugate showing sites of galactosylation. *NeuNAc* N-acetylneuraminic acid, *GlcNAc* N-acetylglucosamine, *Man* mannose, *Asn* asparagine

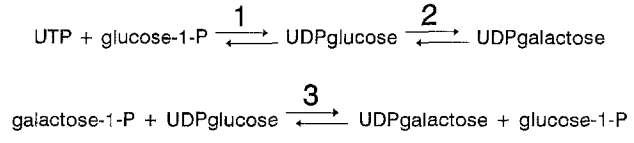

Fig. 2 Principal reactions for the synthesis of UDPgal. *1* UDPglc pyrophosphorylase; *2* UDPgal-4′-epimerase; *3* GALT

dition, the epimerase maintains a cellular equilibrium of UDPglc to UDPgal in a ratio of about 3: 1. Via this route, cells not exposed to galactose form the sugar from glucose in adequate amounts to satisfy normal growth and development. A second route of UDPgal formation is the reaction which occurs when cells are exposed to galactose. After phosphorylation of galactose, the resulting galactose-1-P, via galactose-1-phosphate uridyltransferase (GALT) reacts with UDPglc to form UDPgal. The latter undergoes epimerization to UDPglc to maintain the equilibrium. It is the GALT reaction which is defective in classic galactosemia. To a small extent UDPglc pyrophosphorylase can react uridine triphosphate with galactose-1-phosphate to form UDPgal but this is probably a minor metabolic route.

In 1989, Ng et al. [15] published data obtained by enzymatic analysis showing that UDPgal in red cells, cultured fibroblasts and liver of galactosemics was consistently and markedly lower than in corresponding normal cells. On the basis of these findings they postulated that the etiology of the poor long-term outcome in galactosemics was related to the low cell UDPgal which impaired the synthesis of glycoproteins and other glycoconjugates. Since that time several publications have appeared which support the idea that there is defective galactosylation by galactosemic cells [2, 17, 19]. On the other hand, the data from three different laboratories where enzymatic [13] and HPLC [1, 4, 5] analysis of UDPgal and UDPglc were employed have failed to confirm the consistent and large decrease of UDPgal in galactosemic red cells reported by Ng et al. [15, 16]. In addition, no abnormality has been shown in the UDPgal level in cultured skin fibroblasts [6, 12] or blood leukocytes [6] of galactosemics. This report has a dual purpose: to summarize the evidence for defective galactosylation in galactosemia and the findings which demonstrate that abnormally low UDPgal does not exist in the cells of most galactosemics as an explanation of the galactosylation defect.

Evidence for a galactosylation defect

The first suggestion for a galactosylation defect in galactosemia was made by Haberland et al. [7] in 1971 who examined the brain of a galactosemic at autopsy and found an abnormal pattern of glycoproteins. More recently, analysis of the brain of a galactosemic infant who had died of *Escherichia coli* sepsis revealed a decrease in galactolipid content compared to the brains of two infants dead of other causes [19]. Jaeken et al. [9] has shown that in the plasma of a galactosemic infant exposed to milk there are abnormal electrophoretic isoforms of glycoproteins such as plasma transferrins. These isoforms correspond to those seen in the plasma of patients with the complex carbohydrate deficiency syndrome where there is a known defect in the formation of glycoconjugates [9].

Additional evidence has come from the analysis of cultured galactosemic fibroblasts. Tedesco and Miller [22] reported defective incorporation of 35S sulfate into galactosemic fibroblasts which could be explained by a decrease in galactose residues available to be sulfated in glycoproteins. Dobbie et al. [2] found an abnormal mannose to galactose ratio in the hydrolyzed glycoproteins of galactosemic fibroblasts that were grown on glucose-containing medium, again evidence for abnormal glycoprotein galactose content.

The situation was recently examined with a different approach by Ornstein et al. [17]. They cultured galactosemic and normal cells in media containing only glucose and examined whether the glycoconjugates of those cells had a difference in available sites that could be galactosylated (Fig. 1). The reasoning was that if there were a deficit in galactosylation, exposed N-acetylglucosamine sites would be waiting to receive external galactose. Cell extracts were incubated with radiolabeled UDPgal and a purified milk galactosyl transferase that transferred galactose from UDPgal to cell glycoconjugates. The result of such a study, shown in Fig. 3, in which the vacant sites on nine individual galactosemic cell lines were compared with seven normals, revealed a significantly increased number of vacant receptors sites for the

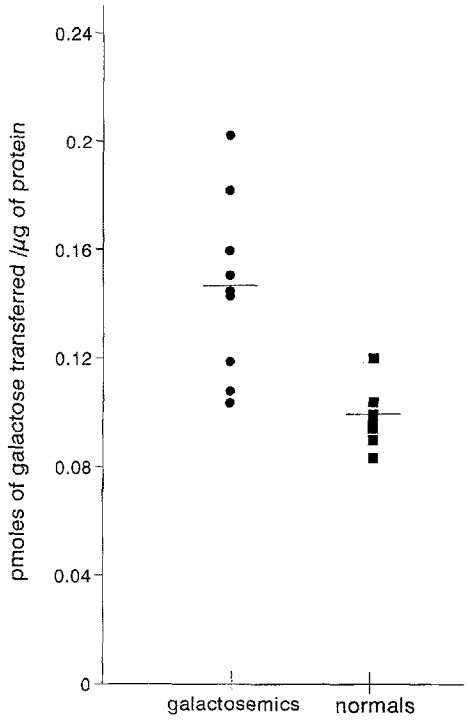

Fig. 3 Galactosylation acceptor activity of galactosemic and normal cultured fibroblasts [improved from Ref. 17]

radioactive galactose in six of the nine galactosemic cell lines.

At the present time it is not clear why some cell lines give normal results. There seems to be no correlation with the genetic mutation and residual GALT activity. The genotype of eight of the nine patients studied by Ornstein et al. [17] shown in Fig. 3 has been analyzed by Dr. Louis Elsas whose paper on the molecular genetic abnormalities appears in this journal [3]. Of the galactosemic cell lines exhibiting galactosylation in the normal range one is homozyous for the Q188R mutation, one is compound heterozygous for Q188R and an unknown mutation, and the third has two as yet unidentified mutant alleles. None of these cell lines have any detectable GALT activity. On the other hand, two of the five cell lines with abnormal galactosylation whose genotype is known are homozygous for the Q188R mutation and have no detectable GALT activity, one is compound heterozygous for Q188R and two have mutant alleles as yet unidentified. The last two, one of which has the largest number of vacant galactose acceptor sites and has been well characterized metabolically, are cells from Afro-Americans with residual GALT activity.

It should be emphasized that the evidence for a galactosylation defect in galactosemic cells is circumstantial and presumptive until glycoconjugates are isolated and the polysaccharide structure is shown to be abnormal. Whether such defective galactosylation is an etiologic factor in the long-term complications is then open for speculation and remains to be determined.

Normal values for red cell UDP-hexoses

The report of Ng et al. in 1989 [15] and 1993 [16] that galactosemic red cell, liver and fibroblast UDPgal was abnormally low while UDPglc was normal suggested that GALT activity was vital to the maintenance of a normal cell UDPgal pool. A publication by Kaufman et al. [10] supported this assumption with the finding that red cells of galactosemic patients who had detectable red cell GALT activity also had normal levels of UDPgal. These data were puzzling in light of the UDPgal-4′-epimerase reaction by which UDPgal could easily be maintained by its formation from UDPglc. The concern over this prompted Kirkman [13], my colleagues and myself [1, 18] to assay these compounds in red cells to verify the results of Ng et al. [15] and to determine the true levels of UDPgal and UDPglc in red cells of both normal and galactosemic patients.

In the method of Ng et al. [15] a coupled reaction was utilized in which UDPglc was measured by the fluorescence produced from the NADH generated when the nucleotide sugar reacted with UDPglc dehydrogenase. Following the conversion of UDPgal by added epimerase to UDPglc which was assayed by the same reaction, the UDPgal was calculated by the difference between the total and the original UDPglc found.

The same assay system was set up by Kirkman [13] who utilized a more purified preparation of UDPglc dehydrogenase and found levels of both UDPglc and UDPgal about 25% of those reported by Ng et al. [15] (Table 1). My own group considered alternative methods of measuring nucleotide sugars which were direct and independent of enzymatic conversions employed by Ng et al. [15] and Kirkman [13] . An HPLC system was devised [18] and gave results (Table 1) very similar to those reported by Kirkman [13] and 25% of those reported by Ng et al. [15]. In addition, an age dependence was found with children's red cells having higher levels of UDPglc than adults. Initially, age 18 was used to separate the concentrations [1, 18] but we [5] and others [11] have found that age 10 marks an apparent transition in concentrations.

In order to substantiate the HPLC values reported by Palmieri et al. [18] and Berry et al. [1], UDPgal and UDPglc were also measured by quantitative ^{31}P-NMR [23]. The red cell concentration by this technique was essentially the same as that measured by Kirkman's enzymatic [13] and the Palmieri et al. [18] HPLC methods on the same specimens [23] (Table 1). In addition, Wehrli et al. [24] found that intact red cells analyzed directly by ^{31}P-NMR without preparation of protein-free filtrates gave values for the sum of UDPgal and UDPglc which were similar to those found when red cell filtrates were assayed by HPLC and ^{31}P-NMR.

To evaluate the difference between the results of the Ng et al. [15] and the Kirkman enzymatic procedures [13]

Table 1 Concentration of nucleotide sugars in red blood cells of healthy controls

	Method		UPDgalactose μmoles/ 100 g HB $\bar{x} \pm$ SD	UPD-glucose μmoles/ 100 g Hb $\bar{x} \pm$ SD
Shin [20, 21]	Enzymatic[a]	All ages	35–65	
Ng et al. [15]	Enzymatic[b]	Not specified	11.2 ± 2.5	38.1 ± 7.1
Kirkman [13]	Enzymatic[b]	Adults	2.1 ± 0.5	5.5 ± 1.0[c]
Berry et al. [1]	HPLC	> 18 years	2.8 ± 0.5	7.6 ± 1.1
		< 18 years	4.5 ± 1.2	10.2 ± 1.6
Gibson et al. [5]	HPLC	> 10 years	2.5 ± 1.2	7.4 ± 1.4
		< 10 years	3.7 ± 0.9	9.3 ± 2.0
Keevill et al. [11]	HPLC	> 10 years	3.1 ± 0.6	6.9 ± 1.0
		< 10 years	3.8 ± 0.6	8.8 ± 2.9
Wehrli et al. [23]	NMR	> 10 years	2.1 ± 0.6	6.6 ± 1.4
Gibson et al. [4]	HPLC-GA	> 10 years		5.4 ± 1.0

[a] Uridyltransferase
[b] UDPglucose dehydrogenase
[c] Converted from μmoles/l packed cells by using an average hemoglobin concentration per l of cells

for UDPglc, where NADH production is assayed, Gibson et al. [4] developed a combined HPLC and UDPglc dehydrogenase method. In this procedure the unique product of the dehydrogenase reaction, UDPglucuronic acid, was quantitated instead of the generation of NADH which can result from other reactions in an impure system. In the assay the UDPglc peak disappeared on incubation with UDPglc dehydrogenase with the stoichiometric conversion of the UDPglc to UDPglucuronic acid. The value for UDPglc by this method was 5.4 ± 1.0 μmoles/100 g Hb which is similar to that obtained by the enzymatic method of Kirkman and those obtained by HPLC [11, 18] and 31P-NMR [23] analyses and only 15% of the normal value for UDPglc reported by Ng et al. [15].

Table 1 summarizes the array of values for red cell UDP-hexoses. It should be evident that by HPLC methods used in two different laboratories [5, 11, 18], ^{31}P-NMR [23], combined HPLC and glucuronic acid assay [4] and Kirkman's enzymatic method [13], the true normal values for UDPgal are in the range of 2–4 μmoles/100 g Hb and for UDPglc 5–10 μmoles/100 g Hb and not the four- six-fold higher values reported by Ng et al. [15]. The conclusion by Holton et al. [8] is that HPLC is the practical method of choice for measurement of red cell UDP-hexoses. At this point it is important to mention the UDPgal method of Shin [20] whose normal values have recently been published [21]. Her method using reagent GALT and radiolabeled glucose-1-P to convert red cell UDPgal to radiolabeled UDPglc give values 20 times the true norm and obviously measured compounds other than UDPgal.

UDP-gal in the red blood cells of galactosemic patients

The study of UDP-hexoses in red blood cells of galactosemic patients by Ng et al. [15, 16] showed a reduction of UDPgal from a normal of 11.2 ± 2.5 to 3.9 ± 1.6 μmoles/100 g Hb with no overlap of the 40 galactosemic values into the normal range. Schweitzer et al. [21] employing Shin's method reported recently that all of 73 classic galactosemics had UDPgal levels below the lowest normal value of 35 μmoles/100 g Hb. Both of these reports cannot be looked upon as reliable estimates of the true UDPgal levels in red cells of galactosemic patients since they are based on the use of flawed analytical procedures.

What then is the true situation? Kirkman reported that there was no difference in UDPgal levels in red cells of galactosemic children when compared with those of children with phenylketonuria who were considered controls [13]. A similar finding was also reported by Berry et al. [1] when galactosemic children's red cells were compared to those of patients with other metabolic disorders who, like phenylketonuria patients, were on protein restricted and consequently low lactose diets. When, however, the galactosemic values were compared with correspondingly aged normal children or adults the average UDPgal was significantly lower in the galactosemic cells but there was considerable overlap. A similar finding has been reported by Keevill et al. [11] who employed a modification of the Palmieri et al. [18] HPLC method of analysis.

With HPLC established as an accurate means of determining red blood cell UDP-hexoses, Gibson et al. [5] have utilized this method to study over 100 normal subjects and over 70 galactosemics of various ages. The results for UDPgal are shown in Fig. 4 and Table 2. There is extensive overlap of values for many of the galactosemic patients with normals. While there are a number of subjects who have low normal or lower than normal levels, most, however, are within 2 SD of the normal range. There is no difference in UDPglc between galactosemics and normals. A comparison of the ratio of UDPglc to

Table 2 Red blood cell UDP-galactose and UDPglucose levels in normals and patients with galactosemia [5]

	n	UPDgalactose µmoles/ 100 g Hb $\bar{x} \pm$ SD	UPD-glucose µmoles/ 100 g Hb $\bar{x} \pm$ SD	Ratio UDPglu/ UDPgal $\bar{x} \pm$ SD
Children (< 10 years)				
Normals	34	3.71 ± 1.24	9.33 ± 1.99	2.65 ± 0.54
Galactosemics	47	2.62 ± 0.88**	10.40 + 3.11	4.16 ± 1.01***
Adults (> 10 years)				
Normal	80	2.54 ± 0.88	7.35 ± 1.70	2.54 ± 0.88
Galactosemics	26	2.07 ± 0.94*	7.58 ± 2.57	3.91 ± 0.89***

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

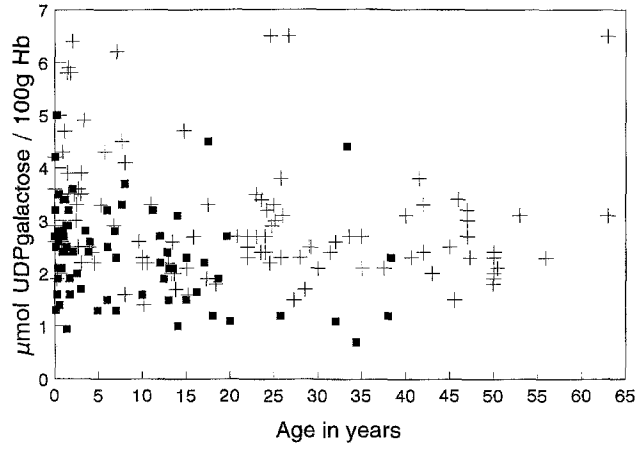

Fig. 4 Levels of UDPgal in erythrocytes of normals (+) and galactosemics (▪) [5]

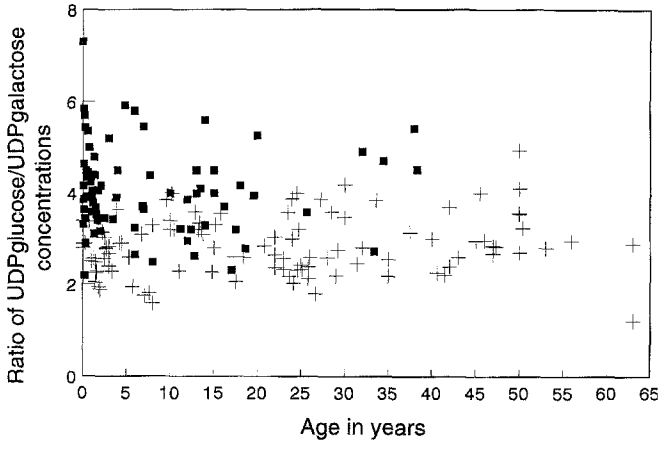

Fig. 5 The ratio of UDPglc to UDPgal in erythrocytes of normals (+) and galactosemics (▪) [5]

UDPgal revealed that many of the galactosemic red cells have ratios higher than the normal range (Fig. 5).

The data of Gibson et al. [5] are summarized in Table 2. On the average there is a significant difference between the red blood cell level of UDPgal in normals and galactosemics and this holds for children below age 10 ($P < 0.01$), for children over age 10 and for adults ($P < 0.05$). There is no difference in UDPglc. There is, however, as found previously, a highly significant difference ($P < 0.001$) between the ratios of UDPglc to UDPgal in normal and galactosemic subjects with many patients' values beyond 2 SD of the normal mean. This difference in ratio has also been found by Keevill et al. [11].

UDP-hexose levels in cultured fibroblasts

A pertinent question is whether the tendency to lower UDPgal and an abnormal ratio of UDPglc to UDPgal observed in galactosemic red cells reflect the findings in other tissues when they are analyzed by HPLC. Ng et al. [15] had reported that galactosemic fibroblasts and long-preserved liver specimens showed low UDPgal levels by what must now be considered a faulty enzymatic method. In addition, they presented no data on the stability of UDP-hexoses in frozen tissues nor on the handling of the tissue specimens. Groups in England [12] and the USA [6], measuring the sugar nucleotide content of cultured galactosemic fibroblasts by HPLC both report that there is no deficit of UDPgal and UDPglc in cultured fibroblasts from galactosemics compared to normals. Moreover, Gibson et al. [6] reported that there was no difference in UDPgal values between cells from patients with the homozygous Q188R genotype and those heterozygous for this mutation. In addition, they found that galactosemic white blood cell UDPgal concentration also did not differ from that of normals [6].

Discussion

The tendency to low normal levels of UDPgal and a significantly abnormal ratio of UDPglc to UDPgal in galactosemic red cells remains to be explained. The data suggest that the basis is an altered equilibrium between UDPglc and UDPgal that is normally maintained by UDPgal-4′-epimerase rather than a lack of UDPgal formation because of defective GALT. The epimerase activity is regulated by a number of factors the most important of which is the ratio of NAD to NADH. Investigation of metabolic flux rates of the epimerase reaction appear to be warranted. The finding of Kaufman et al. [10], based on

the flawed enzymatic method, that UDPgal is normal in galactosemic red cells which have detectable GALT activity in contrast to the very low UDPgal where there is no activity appears to be questionable. Gibson et al. [5] have found no statistical difference between UDPgal content of red cells from patients who are homozygous for the Q188R GALT mutation and have no residual activity and those of compound heterozygotes who may possess some activity.

The alteration in ratio of UDPglc to UDPgal and low average UDPgal in galactosemic red blood cells are not found in cultured fibroblasts [6, 12], or circulating leukocytes [6]. At the present time it seems that this is a unique feature of the erythrocyte. It may be related to the truncated metabolic pathways of the red cell and not a property of other tissues. On the basis, however, of erythrocyte data showing that the incubation with uridine increased UDPgal levels and their observation of low UDPgal in galactosemic red cells, Ng et al. [15] advocated a trial of uridine administration to patients as a new approach to prevent long-term complications. This experimental treatment is ongoing. In face of the overwhelming data of other laboratories that red cell UDPgal of galactosemics is not outside the normal range in most patients, and that leukocyte and fibroblast levels are normal, the rationale for uridine administration is questionable.

An important consideration is whether the proposed defect of galactosylation in galactosemic tissues is related to abnormal UDPgal levels as Ng et al. [15] indicated. The data in fibroblasts demonstrate that this is not the case. The abnormal galactosylation found by Ornstein et al. [17] occurs in the presence of normal UDPgal levels. An etiology of defective galactosylation other than by low UDPgal has to be considered. One possibility is that galactose-1-P which is known to accumulate in galactosemic fibroblasts inhibits endogenous UDPgal galactosyltransferase, the enzyme that transfers the galactose to the glycoconjugate moiety. Such an inhibition has been described for milk galactosyltransferase [14]. Another possibility involves the synthesis of complex glycoconjugates which occurs in the Golgi and requires that UDPgal be translocated from the cytoplasm into the Golgi. It may be that in galactosemic cells there is defective intracellular trafficking and that UDPgal does not reach the correct location for glycoconjugate synthesis. These alternatives deserve future investigation.

Acknowledgement This work was supported by grant PO1 29847 from the National Institute of Child Health and Human Development.

References

1. Berry GT, Palmieri MJ, Heales S, Leonard JV, Segal S (1992) Red blood cell uridine sugar nucleotide levels in patients with classic galactosemia and othr metabolic disorders. Metabolism 41: 783–787
2. Dobbie JA, Holton JB, Clamp JR (1990) Defective galactosylation of proteins in cultured skin fibroblasts from galactosemic patients. Ann Clin Biochem 27: 274–275
3. Elsas LJ (1994) The GALT gene and its mutation. Eur J Pediatr
4. Gibson JB, Reynolds RA, Rogers S, Palmieri MJ, Segal S (1993) The uridine disphosphoglucose content of human erythrocytes: assessment by conversionn to UDPglucuronate. J Pediatr 123: 906–914
5. Gibson JB, Reynolds RA, Palmieri MJ, Berry GT, Elsas LJ, Levy HL, Segal S (1994) Comparison of erythrocyte uridine sugar nucleotide levels in normals, classic galactosemics and patients with other metabolic disorders. Metabolism (in press)
6. Gibson JB, Reynolds RA, Palmieri MJ, States B, Berry GT, Segal S (1994) UDPHexoses in leukocytes and fibroblasts of classic galactosemics and patients with other metabolic diseases. Pediatr Res 36: 613–618
7. Haberland C, Perou M, Brunngraber EG (1971) The neuropathology of galactosemia: a histopathological and biochemical study. J Neuropathol Exp Neurol 30: 431–437
8. Holton JB, Cruz F de la, Levy HL (1993) Galactosemia: the uridine diphosphate galactose deficiency-uridine treatment controversy. J Pediatr 123: 1009–1013
9. Jaeken J, Kint J, Spaaken L (1992) Serum lysosomol enzyme abnormalities in galactosemia. Lancet 340: 1472–1473
10. Kaufman FR, Xu YK, Ng WG, Donnell GN (1988) Correlation of ovarian function with galactose-1-phosphate uridyltransferase levels in galactosemia. J Pediatr 112: 754–756
11. Keevill NJ, Holton JB, Allen JT (1993) The investigation of UDPGlucose and UDPGalactose concentration in red blood cells of patients with classical galactosaemia. Clin Chim Acta 221: 135–142
12. Keevill NJ, Holton JB, Allen JT (1994) UDPglucose and UDPgalactose concentrations in cultured skin fibroblasts of patients with classic galactosemia. J Inherited Metab Dis 17: 23–26
13. Kirkman HN (1992) Erythrocyte uridine diphosphate galactose in galactosemia. J Inherited Metab Dis 15: 4–16
14. McGuire EJ, Kerlin R, Cerba JJ, Roth S (1989) A milk galactosyltransfrase is specific for secreted but not plasma IgA. J Immunol 143: 2933–2938
15. Ng WG, Xu YK, Kaufman FR, Donnell GN (1989) Deficit of uridine diphosphate galactose in galactosemia. J Inherited Metab Dis 12: 257–266
16. Ng WG, Xu YK, Kaufman FR, Donnell GN (1993) Uridine diphosphate galactose depletion. In: GN Donnell (ed) Galactosemia: new frontiers in research. NIH Publication # 90–3438, pp 119-159
17. Ornstein KS, McGuire EJ, Berry GT, Roth S, Segal S (1992) Abnormal galactosylation of complex carbohydrates in cultured fibroblasts from patients with galactose-1-phosphate uridyltransferase deficiency. Pediatr Res 31: 508–511
18. Palmieri MJ, Berry GT, Player DA, Rogers S, Segal S (1991) The concentration of red blood cell UDPglucose and UDPgalactose determined by high-performance liquid chromatography. Anal Biochem 194: 388–393

19. Petry K, Greinix HT, Nudelman E, Eisen H, Hakomori S-I, Levy HL, Reichardt JKV (1991) Characterization of a novel biochemical abnormality in galactosemia: deficiency of glycolipids containing galactose or N-acetylgalactosamine and accumulation of precursors in brain and lymphocytes. Biochem Med Metab Biol 46: 93–104
20. Shin YS (1991) Galactose metabolites and disorders of galactose metabolism. In: Hommes FA (ed) Techniques in diagnostic human biochemical genetics. Wiley-Liss, NY, pp 267–283
21. Schweitzer S, Shin YS, Jacobs C, Brodehl J (1993) Long-term outcome in 134 patients with galactosemia. Eur J Pediatr 132: 36–43
22. Tedesco TA, Miller KL (1979) Galactoemia: alterations in sulfate metabolism secondary to galactoe-1-phosphate uridyltransferase deficiency. Science 205: 1395–1397
23. Wehrli SL, Palmieri MJ, Berry GT, Kirkman HN, Segal S (1992) 31P-NMR analysis of red blood cell UDPglucose and UDPgalactose: comparison with HPLC and enzymatic methods. Anal Biochem 202: 105–110
24. Wehrli SL, Palmieri MJ, Reynolds RA, Segal S (1993) 31P-NMR spectra of intact red blood cells: Quantitation of UDPglucose and UDPgalactose. Magnetic Res Med 30: 494–497