Problems in the Diagnosis of Transferase and Galactokinase Deficient Galactosemia

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ABSTRACT

Galactose in serum and galactose-1-phosphate in erythrocytes were measured in six transferase deficient children to determine if these metabolites could be used in detecting transferase deficient galactosemia. In all six children the galactose levels were normal and the galactose-1-phosphate elevated. The galactose level depends on diet and the rate of metabolism to galactose-1-phosphate and, therefore, should not be used to predict transferase deficient galactosemia. The galactose-1-phosphate level was elevated in all the transferase deficient children because once formed it cannot be metabolized. Measurement of galactose-1-phosphate is difficult and is usually requested to determine whether or not the child is following the galactose restricted diet. In transferase deficient galactosemia, the enzyme hexose-1-phosphate uridylyltransferase is absent. The diagnosis should be determined by measurement of the activity of the enzyme hexose-1-phosphate uridylyltransferase in erythrocytes.

In galactokinase deficient galactosemia, the enzyme galactokinase is absent. Galactose levels are elevated but the amount present depends on diet and how soon the blood was collected after the ingestion of galactose containing foods. The diagnosis of galactokinase deficient galactosemia is based on the measurement of the enzyme galactokinase in erythrocytes.

Introduction

Transferase and galactokinase deficient galactosemia are inborn errors of galactose metabolism. The main source of galactose is from the disaccharide lactose which is found in milk. Lactose is hydrolyzed in the intestine by the enzyme lactase to galactose and glucose. Galactose is metabolized to uridine diphosphoglucose by the series of reactions shown in table I. In transferase deficient galactosemia, the enzyme hexose-1-phosphate uridylyltransferase* is absent. In this disorder, vomiting, diarrhea, jaundice and failure to thrive are the earliest and most

* This enzyme is frequently referred to as galactose-1-phosphate uridylyltransferase. Hexose-1-phosphate uridylyltransferase (EC 2.7.7.12) is the name designated by the Commission On Biochemical Nomenclature and will be used throughout this article.
Normal Metabolic Pathway for Galactose

<table>
<thead>
<tr>
<th>Reaction Scheme</th>
<th>Enzyme</th>
<th>Product 1</th>
<th>Product 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose + ATP</td>
<td>Galactokinase</td>
<td>Galactose-1-phosphate + ADP</td>
<td>Galactose-1-phosphate + ATP</td>
</tr>
<tr>
<td>Galactose-1-phosphate + uridine diphosphoglucose</td>
<td>Hexose-1-phosphate uridylyltransferase</td>
<td>Glucose-1-phosphate + uridine diphosphogalactose</td>
<td>Glucose-1-phosphate + uridine diphosphoglucose</td>
</tr>
<tr>
<td>Uridine diphosphogalactose</td>
<td>Uridine diphosphogalactose-4-epimerase</td>
<td>Uridine diphosphoglucose</td>
<td>Uridine diphosphoglucose</td>
</tr>
<tr>
<td>Uridine diphosphoglucose + PP</td>
<td>Uridine diphosphoglucose pyrophosphorylase</td>
<td>Glucose-1-phosphate + UTP</td>
<td>Glucose-1-phosphate + PP</td>
</tr>
</tbody>
</table>

common symptoms and usually appear after milk is given to the infant. If milk feedings are continued, hepatic failure results, infection may occur, cataracts and mental retardation may develop and death can result. In galactokinase deficient galactosemia, the enzyme galactokinase* is absent. In this disorder, cataracts are the only symptom. None of the severe symptoms associated with transferase deficient galactosemia are observed.

Treatment for both of these disorders is a galactose restricted diet. If initiated in the newborn period, the clinical problems can usually be prevented. Therefore, it is important that the diagnosis be determined as soon as possible after birth.

The diagnosis of transferase and galactokinase deficient galactosemia is usually confirmed by measuring the activity of the enzymes hexose-1-phosphate uridylyltransferase and galactokinase in erythrocytes. It has been suggested that elevated levels of galactose5,7 or galactose-1-phosphate3 could be used to detect transferase deficient galactosemia because their rate of metabolism would be slow and, therefore, would remain in the blood for a considerable length of time after the consumption of milk. To determine if this assumption was correct, hexose-1-phosphate uridylyltransferase activity, galactose and galactose-1-phosphate levels were measured in normal and transferase deficient children. These results and some of the pitfalls that can be encountered in the diagnosis of transferase and galactokinase deficient galactosemia will be presented.

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TABLE II

Reaction Scheme for Measurement of Hexose-1-phosphate Uridylyltransferase Activity

<table>
<thead>
<tr>
<th>Reaction Scheme</th>
<th>Enzyme</th>
<th>Product 1</th>
<th>Product 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose-1-phosphate + uridine diphosphoglucose</td>
<td>Hexose-1-phosphate uridylyltransferase</td>
<td>Glucose-1-phosphate + uridine diphosphogalactose</td>
<td>Glucose-1-phosphate + uridine diphosphoglucose</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>Phosphoglucomutase</td>
<td>Glucose-6-phosphate</td>
<td>Glucose-6-phosphate</td>
</tr>
<tr>
<td>Glucose-6-phosphate + NADP⁺</td>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>6-Phosphogluconate + NADPH</td>
<td>6-Phosphogluconate + NADPH</td>
</tr>
<tr>
<td>6-Phosphogluconate + NADP⁺</td>
<td>Phosphogluconate dehydrogenase</td>
<td>Ribulose-5-phosphate + NADPH + CO₂</td>
<td>Ribulose-5-phosphate + NADPH + CO₂</td>
</tr>
</tbody>
</table>

* (EC 2.7.1.6).
Methods

Hexose-1-phosphate uridylyltransferase activity was measured in erythrocytes by a kinetic enzymatic system based on the reaction scheme shown in Table II. Blood was collected in ammonium heparinized microhematocrit tubes, the erythrocytes lysed with water and the hexose-1-phosphate uridyl transferase activity determined by mixing the hemolysate with a reagent consisting of galactose-1-phosphate, uridine diphosphoglucose, NADP+, ethylene diaminetetraacetate (EDTA) and the enzymes phosphoglucomutase and glucose-6-phosphate dehydrogenase. Phosphogluconate dehydrogenase was not added to the reagent system because it is present in sufficient quantities in the hemolysate to convert all of the 6-phosphogluconate produced to ribulose-5-phosphate. EDTA activates the enzyme hexose-1-phosphate uridylyltransferase and must be added to the reagent system. This reaction mixture was incubated for 30 minutes at 37°C and the activity of hexose-1-phosphate uridylyltransferase determined by measuring the rate of increase in the absorbance of the NADPH for ten minutes. Enzyme activity is expressed as units per gram of hemoglobin. One unit of activity is defined as the number of micromoles of substrate consumed per hour at a temperature of 37°C.

Galactose in serum was determined by measuring the increase in absorbance of the NADH formed from the reaction of galactose and NAD+ catalyzed by the enzyme galactose dehydrogenase.

Galactose-1-phosphate levels in erythrocytes were measured by a new fluorometric method. With this assay, blood was collected in ammonium heparinized microhematocrit tubes, the packed cells lysed with water and immediately deproteinized in tris buffer at 100°C. Galactose-1-phosphate in the supernatant was measured by the series of reaction shown in Table II. The NADPH formed was measured fluorometrically and related to the concentration of the galactose-1-phosphate in the sample. Galactose-1-phosphate concentration is reported as µg of hemoglobin.

Results

Hexose-1-phosphate uridyl transferase activity, galactose and galactose-1-phosphate values were measured in ten normal children who had been fasting from 8 to 12 hours and in six transferase deficient children. The results for the normal group are shown in Table III. The galactose levels in serum ranged between 0.0 and 0.6 mg per dl, the galactose-1-phosphate levels in erythrocytes were from 14 to 42 µg per g of hemoglobin and the hexose-1-phosphate uridylyltransferase activity was between 18 and 28 units per gram of hemoglobin.

The results for the transferase deficient group are shown in Table IV. In all six children, the galactose-1-phosphate level was elevated, as compared to individuals with normal hexose-1-phosphate uridylyltransferase activity, and ranged between 84 and 422 µg per g of hemoglobin. All these children were supposedly following a galactose restricted diet. Galactose-1-phosphate levels in transferase deficient patients who are adhering to their galactose restricted diet should be about 100 µg per g of hemoglobin. In all the transferase deficient children, the galactose level in serum was normal. The first four children were following their diet and, therefore, their galactose level in serum was expected to be normal. The last two children were consuming galactose containing foods, as indicated by their galactose-1-phosphate level, but their galactose level in serum was also normal.

The data in Table IV indicate that whether or not the transferase deficient child is on a galactose restricted diet, the galactose level in serum will be normal.
and the galactose-1-phosphate level in erythrocytes will be elevated.

**Discussion**

**MEASUREMENTS WHICH PROVIDE DIAGNOSIS OF TRANSFERASE DEFICIENT GALACTOSEMIA**

*Hexose-1-phosphate uridylyltransferase activity.* Since the enzyme hexose-1-phosphate uridylyltransferase is absent in transferase deficient galactosemia, the diagnosis should be based on the estimation of the activity of this enzyme in erythrocytes. However, measurement of hexose-1-phosphate uridylyltransferase activity is not an easy task and is subject to various problems. For example, the enzyme activity in normal individuals is low as compared to other erythrocyte enzymes. The activity of hexose-1-phosphate uridylyltransferase in normal children is about 25 times lower than the normal activity of glucose-6-phosphate dehydrogenase. Therefore, a sensitive method must be employed to measure transferase activity. With the kinetic system described in the Method Section, sufficient sensitivity for measuring hexose-1-phosphate uridylyltransferase activity is achieved because two moles of NADPH are formed for each mole of glucose-6-phosphate oxidized.

It was recently shown that the analytical method employed for measuring hexose-1-phosphate uridylyltransferase activity can influence the results because some enzyme activity was detected in the erythrocytes of a transferase deficient infant when measured by the uridine diphosphoglucose (UDPG) consumption assay. The UDPG consumption assay is based on the series of reactions shown in Table V. With this method, the hemolysate is incubated with galactose-1-phosphate and uridine diphosphoglucose for a specified time and the reaction stopped by precipitation of the proteins. The uridine diphosphoglucose in the supernatant is measured by reaction with uridine diphosphoglucose dehydrogenase and NAD+. The activity of hexose-1-phosphate uridylyltransferase is related to the amount of uridine diphosphoglucose consumed. In patients with transferase deficient galactosemia, no uridine diphosphoglucose should be consumed and the amount of uridine diphosphoglucose found in the supernatant should be the same as that added into the hemolysate. The reason for detection of transferase activity in this patient was the absence of NADase in the erythrocytes.

**TABLE III**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Galactose (mg/dl)</th>
<th>Galactose-1-phosphate (µg/gHb)</th>
<th>Hexose-1-phosphate Uridyltransferase Activity (U/gHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.5</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>F</td>
<td>0.4</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>M</td>
<td>0.4</td>
<td>42</td>
<td>28</td>
</tr>
<tr>
<td>F</td>
<td>0.6</td>
<td>40</td>
<td>26</td>
</tr>
<tr>
<td>M</td>
<td>0.5</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>F</td>
<td>0.0</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>M</td>
<td>0.4</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>M</td>
<td>0.4</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>F</td>
<td>0.3</td>
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<td>18</td>
</tr>
<tr>
<td>F</td>
<td>0.6</td>
<td>40</td>
<td>26</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4</td>
<td>33</td>
<td>22</td>
</tr>
</tbody>
</table>

**TABLE IV**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Galactose (mg/dl)</th>
<th>Galactose-1-phosphate (µg/gHb)</th>
<th>Hexose-1-phosphate Uridyltransferase Activity (U/gHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>M</td>
<td>0.6</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>*</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>0.3</td>
<td>114</td>
<td>1</td>
</tr>
<tr>
<td>Newborn</td>
<td>M</td>
<td>0.4</td>
<td>185</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>0.0</td>
<td>422</td>
<td>1</td>
</tr>
</tbody>
</table>

*There was not enough serum to determine a galactose level.*
As shown in table V, uridine diphosphogluco- 
ose can also be converted to uridine di-
phosphogalactose by the erythrocyte 
enzyme uridine-diphosphate galactose-
4-epimerase. This reaction is catalyzed by 
the NAD⁺ present in the hemolysate. 
NADase inactivates NAD⁺; therefore, no 
uridine diphosphoglucose can be con-
sumed. Because of the absence of 
NADase in the erythrocytes of this pa-
tient, uridine diphosphoglucose was con-
verted to uridine diphosphogalactose, er-
roneously indicating that there was some 
hexose-1-phosphate uridylyltransferase 
activity. The absence of NADase in the 
erythrocytes will not interfere with the 
kinetic assay for measuring hexose-1-
phosphate uridylyltransferase activity be-
cause sufficient uridine diphospho-
glucose has been added to assure that the 
small amount of uridine diphospho-
glucose converted to uridine diphospho-
galactose will not alter zero order kinetics.

For accurate enzyme results, hexose-
1-phosphate uridylyltransferase activity 
should be measured soon after collection 
of the blood. It was recently shown that 25 
percent of the enzyme activity was lost if 
the blood or erythrocytes were stored at 
4°C or at room temperature for one day.⁹ A 
loss of about 10 percent in enzyme activity 
was observed if the erythrocytes were 
stored at −20°C for one day.⁹ If the deter-
mination cannot be performed immedi-
ately, the packed cells can be stored for 
one day at −20°C without producing a 
significant change in enzyme activity.

**Galactose in Serum.** Galactose is usu-
ally absent or present in small quantities 
in the serum of normal individuals. As 
shown in table IV, the galactose level in 
serum was normal in the transferase defi-
cient individuals, including those chil-
dren who had consumed galactose con-
taining foods, because the blood was 
drawn several hours after the diet viola-
tion. During this time, galactose was 
metabolized to galactose-1-phosphate by 
the enzyme galactokinase which is the 
first enzyme in the normal metabolic 
pathway of galactose (table I). Elevated 
galactose levels will be found in the 
serum of transferase deficient individuals 
only if the blood is drawn soon after the 
child has ingested galactose containing 
foods. Galactose levels should not be used 
to detect transferase deficient galacto-
semia because the results depend on diet 
and rate of metabolism.

**Galactose-1-phosphate in Erythro-
cytes.** Galactose-1-phosphate is present in 
small quantities in the erythrocytes of in-
dividuals with normal transferase activity, 
the upper limit of normal is about 42 µg 
per g of hemoglobin. As shown in table IV, 
galactose-1-phosphate levels are elevated 
in all the transferase deficient children 
whether or not they have deviated from 
their diet because galactose-1-phosphate 
one formed by the phosphorylation of 
galactose cannot be metabolized. Galactose-1-phosphate levels of greater 
than 120 µg per g of hemoglobin are found 
in transferase deficient individuals who
are consuming galactose containing foods. Galactose-1-phosphate levels of about 100 μg per g of hemoglobin are observed even in individuals who are following their galactose restricted diet because they are probably ingesting, either by chance or knowingly, small quantities of galactose containing foods.

An elevated galactose-1-phosphate level does not necessarily indicate transferase deficient galactosemia because in patients with erythrocyte uridinediphosphate galactose-4-epimerase deficiency the galactose-1-phosphate level was markedly elevated. Uridinediphosphate galactose-4-epimerase is the third enzyme involved in the normal pathway of galactose metabolism and converts uridine diphosphogalactose to uridine diphosphoglucose (table I). All these patients were asymptomatic with normal hexose-1-phosphate uridylyltransferase activity and with either normal or slightly elevated galactose levels. The diagnosis was confirmed by measuring the activity of the enzyme uridinediphosphate galactose-4-epimerase in erythrocytes.

Galactose-1-phosphate measurements are difficult to perform and are generally used to determine if the transferase deficient individual has deviated from the galactose restricted diet.

**Conclusion.** The possibility of transferase deficient galactosemia should be considered based on the clinical symptoms of the patient and the finding of elevated galactose and galactose-1-phosphate levels. However, the diagnosis must be confirmed by measurement of the activity of the enzyme hexose-1-phosphate uridylyltransferase in erythrocytes.

**Diagnosis of Galactokinase Deficient Galactosemia**

Galactokinase deficient galactosemia is a rare disorder and is defined as absence of the enzyme galactokinase (table I). Cataracts are the only clinical symptom. There is none of the vomiting, diarrhea, jaundice, liver damage or mental retardation that is associated with transferase deficient galactosemia. In this disorder, galactose cannot be metabolized and is usually elevated in serum. However, the galactose level depends on when the blood was taken after the patient had consumed milk. It was reported that the galactose level in the serum of a galactokinase deficient individual who has fasted overnight was only 3 mg per dl.10 In galactokinase deficient individuals, galactose-1-phosphate is absent because it cannot be formed.

The diagnosis of galactokinase deficient galactosemia should be determined by measuring the activity of the enzyme galactokinase in erythrocytes. In order to measure galactokinase activity, a very sensitive method is required because the activity of galactokinase in the erythrocytes of normal individuals is about 100 times lower than the activity of glucose-6-phosphate dehydrogenase. An indirect way to determine the possibility of galactokinase deficiency is to measure galactose, galactose-1-phosphate levels and hexose-1-phosphate uridylyltransferase activity. If the patient has cataracts, elevated levels of galactose with the absence of galactose-1-phosphate and normal hexose-1-phosphate uridylyltransferase activity, the possibility of galactokinase deficient galactosemia should be considered. However, the only way to confirm the diagnosis of this disorder is to measure the activity of the enzyme galactokinase in erythrocytes.

**References**


