COMPARISON OF GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE IN FETAL AND ADULT TISSUES

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Summary

Galactose-1-phosphate uridyl transferase, the enzyme deficient in galactosemia, is demonstrated to be present in erythrocytes from fetuses of 12-30 weeks gestation. The specific activity and starch gel electrophoretic pattern of this enzyme in fetal erythrocytes is virtually identical to that found in erythrocytes postnatally. This enzyme was also studied in cultured fibroblasts from skin and amniotic cells and found to have similar specific activity and identical electrophoretic mobility in tissues from both sources. There does not appear to be a fetal isozyme for galactose-1-phosphate uridyl transferase in either erythrocytes or cultured fibroblasts. Thus, with fetal erythrocytes obtained by fetoscopy, it is likely that prenatal diagnosis of galactosemia can be rapidly and reliably accomplished.

Introduction

Galactose-1-phosphate uridyl transferase (transferase) is an enzyme in the intermediary metabolism of galactose. Deficiency of transferase activity results in an inborn error of carbohydrate metabolism known as galactosemia [1]. Since this enzyme is present in erythrocytes, among other tissues, its deficiency in galactosemia can readily be studied.

Though dietary therapy is available for galactosemia, there still may be indications for prenatal diagnosis [2,3]. For the latter purpose, transferase activity has been shown to be present in cultured amniotic cells from normal fetuses but deficient in such cells from a fetus with galactosemia [2]. However, the prenatal diagnosis of galactosemia by the study of cultured cells is delayed by the length of time necessary for the growth of such cells. If fetal erythro-
cytes contain transferase and if methods for obtaining such cells are proven to be feasible [4], the intrauterine diagnosis of galactosemia could be greatly facilitated.

We have studied fetal transferase in both cultured amniotic cells and fetal erythrocytes. Our results indicate that transferase is present in fetal erythrocytes and, furthermore, that in activity and in electrophoretic mobility the differences between transferase in erythrocytes and cultured fibroblasts in the fetus are identical to the differences between transferase in erythrocytes and cultured skin fibroblasts found in adults [5].

Materials and Methods

Fetal blood was obtained from the placental side of the transected umbilical cord after delivery and fetal death following therapeutic abortion induced by either hysterotomy or intraamniotic prostaglandin injection. Gestational ages of the nine fetuses were between 12 and 19 weeks with the exception of a 30 week fetus who had died in utero some days before the pregnancy was terminated. Liquid whole blood samples were available on four fetuses and filter paper dried blood specimens were available on the remaining five fetuses. Control specimens consisted of venous blood obtained from healthy individuals and an infant with reticulocytosis.

Skin biopsies and amniotic cells were obtained from healthy individuals or patients with disorders unrelated to galactosemia. Fibroblasts were grown in Eagle's medium, supplemented with 15% fetal calf serum and "nonessential" amino acids [6]. After 10 days of subculture the confluent monolayers were washed with phosphate-buffered saline and trypsinized. Cells were counted, centrifuged, washed twice with cold isotonic saline, and resuspended in distilled water at $2 \times 10^7$–$6 \times 10^7$ cells/ml. They were then alternately frozen in a dry ice bath and thawed three times. The lysate was centrifuged 20 minutes at 700 g and the supernates were used for the determination of transferase activity and for starch gel electrophoresis.

Transferase activity in erythrocytes was determined by the uridine diphosphoglucose (UDPG) consumption method of Beutler and Baluda [7]. Fetal hemolysates were preincubated with NADase (50 μl NADase/ml hemolysate; NADase; 0.63 units/ml) to avoid artifacts due to galactose-4-epimerase activity [8]. In the fetal filter paper blood specimens transferase activity was demonstrated by use of the enzyme spot screening test [9]. In fibroblast lysates transferase activity was measured by the UDPG consumption method of Tedesco and Mellman [10]. To inactivate galactose-4-epimerase the lysates were preincubated with 0.1 M dithiothreitol [11].

Electrophoretic mobility of transferase from erythrocytes and cultured fibroblasts was determined by starch gel electrophoresis [12]. For this determination in filter paper blood specimens, rectangular pieces of the dry filter paper were directly applied to the starch gel. We have found this method to be suitable for filter paper blood specimens [13].

Results and Discussion

By the enzyme spot screening test, all fetal blood specimens yielded evi-
TABLE I
RESULTS OF TRANSFERASE ACTIVITY BY UDPG CONSUMPTION ASSAYS IN ERYTHROCYTES AND CULTURED FIBROBLASTS

<table>
<thead>
<tr>
<th></th>
<th>Erythrocytes (units)*</th>
<th>Cultured fibroblasts (units)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal cells</td>
<td>22.0 ± 4.0 (N = 4)</td>
<td>34.7 ± 14.3 (N = 4)</td>
</tr>
<tr>
<td>Adult cells</td>
<td>22.0 ± 3.7 (N = 20)</td>
<td>38.9 ± 10.6 (N = 14)</td>
</tr>
</tbody>
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* One unit of erythrocyte activity equals one μmol UDPG consumed/h/g hemoglobin.
** One unit of fibroblast activity equals one μmol UDPG consumed/h/10⁶ cells.

dence of transferase activity with fluorescence equal to that of normal postnatal blood. Transferase activity as measured by UDPG consumption is indicated in Table I. This activity in fetal erythrocytes (22.0 ± 4.0 units) and in fetal fibroblasts (34.7 ± 14.3 units) was not significantly different from the activity found in adult erythrocytes (22.0 ± 3.7 units) and adult fibroblasts (38.9 ± 10.6 units), respectively. The difference between fetal and adult tissues, either erythrocytes or fibroblasts, was insignificant, p > 0.1, for each comparison.

By starch gel electrophoresis transferase from fetal erythrocytes showed two brightly fluorescing bands and a single poorly fluorescing and slower moving third band (Fig. 1). This electrophoretic pattern differs from the normal postnatal erythrocyte transferase in the presence of three rather than only two bands. However, the pattern of three bands is identical to that found Dr. W.G. Ng (personal communication) in individuals with reticulocytosis and confirmed by us (Fig. 1). There was no difference in the electrophoretic mobility of

Fig. 1. Patterns of transferase following starch gel electrophoresis of fetal and adult erythrocytes and cultured fibroblasts. Slots 1, normal postnatal erythrocyte transferase; 2, erythrocyte transferase from infant with reticulocytosis (20%); 3, erythrocyte transferase from fetus; 4, transferase from cultured skin fibroblasts of adult; 5, transferase from cultured amniotic fibroblasts of fetus.
transferase from fetal and adult fibroblasts. Fibroblast lysates from both sources showed four activity bands, each slower moving than any erythrocyte band. Fibroblast bands I and IV were less intensely fluorescent than bands II and III (Fig. 1).

At least by the gestational age of 12 weeks, transferase activity is present in fetal erythrocytes. Unlike several other enzymes, including galactokinase [14] and hexokinase [15], and unlike certain non-enzymatic proteins such as hemoglobin [16], there appears to be no distinct fetal isozyme of transferase in either erythrocytes or cultured fibroblasts. Since reticulocytes comprise 30–90% of all fetal red blood cells at the gestational ages of 12–24 weeks [17], it is probable that the third transferase band in the fetal hemolysate is due to this reticulocytosis of fetal blood and thus is a reticulocyte band rather than a specific fetal transferase isozyme.

Since fetal transferase appears to be identical to transferase present post-natally, absent transferase activity in fetal hemolysates should be consistent with the presence of galactosemia. Consequently, when fetal erythrocytes uncontaminated by maternal blood become readily obtainable by fetoscopy [4] or by other means, the prenatal diagnosis of galactosemia could be far more easily and rapidly made than is now possible.

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