Hum. Genet. 37, 261—270 (1977)

Human Genetics

The Chicago Variant of Clinical Galactosemia

Claramma M. Chacko, Rebecca S. Wappner, Ira K. Brandt, and Henry L. Nadler*

Division of Genetics, Children's Memorial Hospital, Department of Pediatrics, Northwestern University Medical School, 2300 Children's Plaza, Chicago, Illinois 60614, U.S.A.
Indiana University School of Medicine, James Whitcomb Riley Hospital for Children, Indianapolis, Indiana, U.S.A.

Summary. A new variant of clinical galactosemia with two hitherto unidentified alleles on the transferase locus in one family is described. This new clinical variant of transferase has 25% of normal control activity in blood and in skin fibroblasts, and the patient accumulates galactose-l-phosphate in blood on an unrestricted galactose diet. Using starch gel electrophoresis on the hemolysate of the family members, a fast-moving transferase with mobility in between those of the normal control and of the Duarte variant is identified. This new allele is designated as $GALT^{C_1}$ (fast-moving Chicago variant). In addition, a second new allele was documented in this family by studying the instability of the transferase enzyme in hemolysates of family members at 50° C for various time intervals. This new allele is designated as $GALT^{C_2}$ (heat-labile Chicago variant). On the basis of these studies, the transferase genotype of this patient is thought to be a double heterozygote compound, $GALT^{C_1}/GALT^{G}$.

Introduction

Classical galactosemia is a familial metabolic disorder characterized by vomiting, diarrhea, jaundice, failure to thrive, hepatic dysfunction, mental retardation, and cataracts (Hsia and Walker, 1961; Holzel, 1961; Donnell et al., 1961). This disorder is inherited as an autosomal recessive condition in which the basic biochemical defect is the deficiency of the enzyme, galactose-l-phosphate uridyl transferase (E.C. 2.7.7.12; uridine diphosphoglucose: α-D-galactose-l-phosphate uridyl transferase) which will be referred to simply as transferase (Kalckar et al., 1956; Holzel and Komrower, 1955).

A number of variants of clinical galactosemia and of transferase have previously been described (Table 1). In this study, we provide evidence for a new variant of galactosemia in which red blood cell and skin fibroblast transferase is

* To whom offprint requests should be sent

Table 1. Variants of clinical galactosemia and of transferase

	Red blood cell transferase Activity (% of control)	Red blood cell transferase Mobility	Red blood cell transferase Stability
Classical galactosemia	essentially 0	—	—
Symptomatic transferase variants			
a. Rennes variant (Schapira and Kaplan, 1969)	7	slow	not tested
b. Indiana variant (Chacko et al., 1971)	40	—	unstable (4°C)
Asymptomatic transferase variants			
a. Duarte variant (Beutler et al., 1965; Beutler and Mathai, 1968; Mathai and Beutler, 1966)	50	fast	stable
b. Los Angeles variant (Ng et al., 1973)	100+	fast	not tested

approximately 25% of normal, electrophoretic mobility is altered, and three transferase alleles are present in one family. The two new alleles of transferase described in this family are designated as $GALT^{C_1}$ (fast-moving Chicago variant) and $GALT^{C_2}$ (heat-labile Chicago variant).

Case Report

C. B. is the second son of unrelated young parents with an essentially negative family history. Pregnancy was complicated by the mother's exposure to nonicteric hepatitis during the first trimester for which she received gamma globulin. Delivery was induced but otherwise normal; the birth weight was 3.6 kg and physical examination revealed no abnormalities. Breast feeding was instituted and the patient did well with the exception of unusual fussiness and wakefulness. These symptoms persisted even when breast feedings were changed to Similac at 6 weeks of age.

At 7 weeks, the patient developed anorexia and restlessness. By the 8th week of life, light-colored stools and mild jaundice were noted. Physical examination was normal with the exception of a mildly enlarged liver, 4 cm below the right costal margin.

At 9 weeks of age, the patient was admitted to the James Whitcomb Riley Hospital for Children for evaluation of jaundice. Except for jaundice and mild hepatomegaly, the patient appeared to be normal. Slit-lamp examination was negative.

The fluorescence screening test (Beutler and Baluda, 1966) revealed no erythrocyte galactose-l-phosphate uridyl transferase activity on two occasions.

On the basis of the clinical and laboratory findings, the diagnosis of galactosemia was entertained and the patient was started on Isomil; his lifelong fussiness soon disappeared. After 7 days, his abnormal laboratory tests had almost completely reverted to normal and he was discharged. Since that time, he has continued to thrive on a galactose-free diet. Growth and development have been normal.

At 2 years of age, the patient was challenged with galactose. He was given 20 g of galactose daily in four divided oral doses. After 5 days, his red blood cell galactose level was 1.0 mg/100 ml blood and the galactose-l-phosphate level was 0.4 mg/100 ml blood. The patient was then

placed on an unrestricted galactose diet. After 1 week of normal galactose intake, the galactose-1-phosphate level in the red blood cells had risen remarkably to 7.9 mg/100 ml blood. The galactose level at that time was 0.7 mg/100 ml blood. On the basis of these results, the patient has been restarted on a galactose restricted diet.

Material and Methods

Blood was collected in heparin from the patient, family members, and control subjects and stored at 4° C until processed. To prepare the hemolysate, the red blood cells were sedimented by centrifugation and the plasma and buffy coat were removed. Hemolysis was achieved by addition of one volume of deionized water, freezing in a dry ice-acetone bath, and thawing in a water bath at 37° C. The hemolysates were stored at —70° C. Transferase activity determinations, partial purification of the enzyme, the Michaelis-Menten constant determinations and starch gel electrophoresis were carried out as previously described (Chacko et al., 1971). Skin biopsies were obtained from the patient, his parents and controls. The methods used for cultivation and processing of fibroblasts to study the properties of transferase were those routinely used in our laboratory (Nadler et al., 1970). The blood from a Duarte variant homozygote, T.V., was obtained through Dr. William J. Mellman; fibroblasts from a Duarte variant were obtained from the Human Genetic Mutant Cell Repository (cell line GM-264); and blood from a known normal/Duarte compound and skin fibroblasts from a known Duarte/galactosemic compound were obtained from Dr. George Donnell.

Heat Treatment at 50° C. The effect of heat treatment at 50° C on the transferase levels in the hemolysate was determined as follows: 0.5 ml of each hemolysate was placed in a water bath at 50° C for 0, 5, 10, 20, 30, or 40 min. At the end of the desired time, the sample was removed and placed in an ice bath and UDPG consumption assays carried out on each sample.

All chemicals were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A., with the exception of dithiothreitol (DTT, Cleland's reagent) which was obtained from Calbiochem, and hydrolyzed starch, from Connaught Medical Research Laboratories, Toronto, Canada.

Results

The levels of transferase activity in the red blood cell hemolysates of: control subjects, obligate heterozygotes, classical galactosemia patients, the patient (C. B.), family members, and a Duarte variant (T.V.), are shown in Table 2. These values represent an average of 16 separate determinations.

The patient (C. B.) had approximately one-fourth normal transferase activity in his red blood cells and the mother (M. B.) had values very similar to the galactosemia heterozygotes. The father (G. B.) and grandfather (F. B.) had activities in the upper heterozygote or low normal range. The patient's brother (A. B.) and grandmother (Q. B.) had normal levels of activity.

Transferase activity levels in the supernatant fractions of skin fibroblasts obtained from normal controls, the patient, his parents, a Duarte/galactosemia compound, and a Duarte variant are also shown in Table 2. The patient had approximately 25% of the control transferase activity, his mother had 50% and the father had near normal levels of activity in the fibroblasts. The Duarte variant fibroblasts had approximately 40% and the Duarte/galactosemic compound fibroblasts had about 12.5% of control level of transferase in skin fibroblasts.

Table 2. Transferase activity in heparinized red blood cells and skin fibroblasts

Subject	Red blood cells[a] (μmoles UDPG consumed/hr/gm hemoglobin)	Fibroblasts[a] (nmoles glucose-l-phosphate produced/hr/mg protein)
Normal controls (30)[b]	25.4 ± 4.9	164 ± 3.5
Obligate heterozygotes for galactosemia (33)	12.6 ± 6.3	—
Galactosemia patients (20)	1.4 ± 1.2	—
Family of C. B.		
Patient (C. B.)	6.9 ± 0.4	39 ± 1.1
Father (G. B.)	19.5 ± 1.2	132 ± 2.0
Mother (M. B.)	10.8 ± 0.7	79 ± 0.7
Brother (A. B.)	21.9 ± 0.6	—
Paternal grandfather (F. B.)	17.4 ± 0.9	—
Paternal grandmother (Q. B.)	28.9 ± 0.8	—
Duarte variant (T.V.)	10.5 ± 0.6	68 ± 1.0
Duarte/galactosemic compound	—	20 ± 0.2

[a] Values are expressed as mean ± standard deviation
[b] Numbers in parentheses refer to numbers of patients (Nadler et al., 1969)

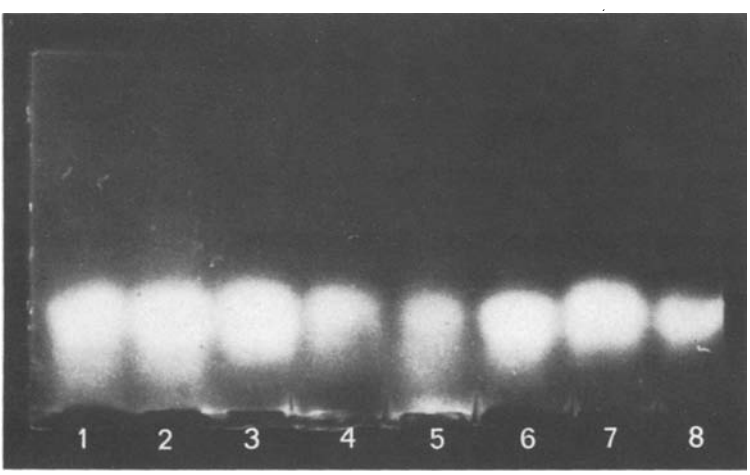


Fig. 1. Starch gel electrophoresis of red blood cell transferase from control (slots 1, 2, 6, 8), father (slots 3, 7), Duarte variant (slot 4), and the patient (slot 5) demonstrating the difference in mobility of transferase. Electrophoresis was carried out at pH 7.0 as previously described (Mathai and Beutler, 1966)

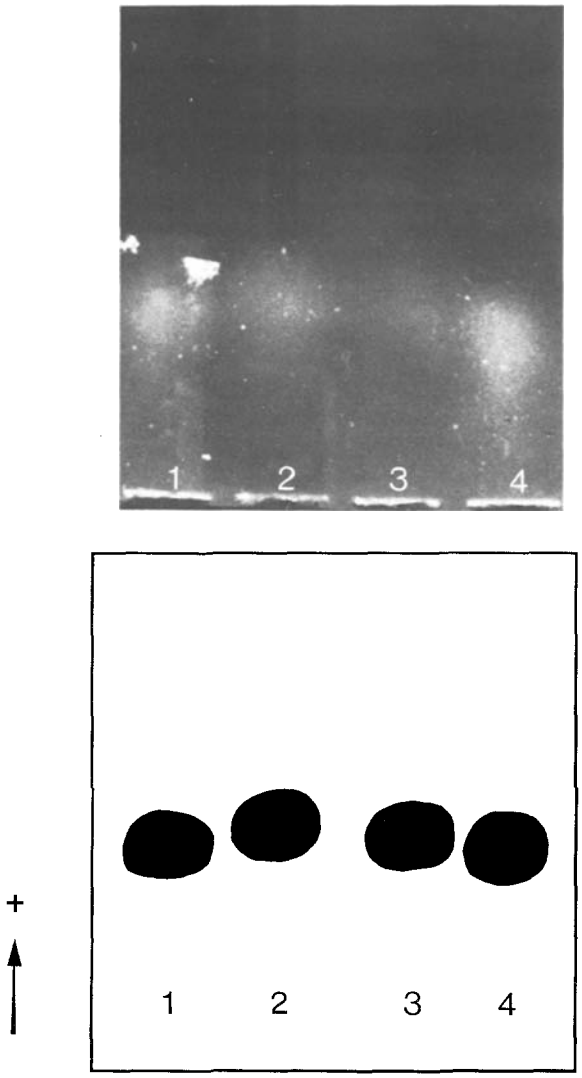
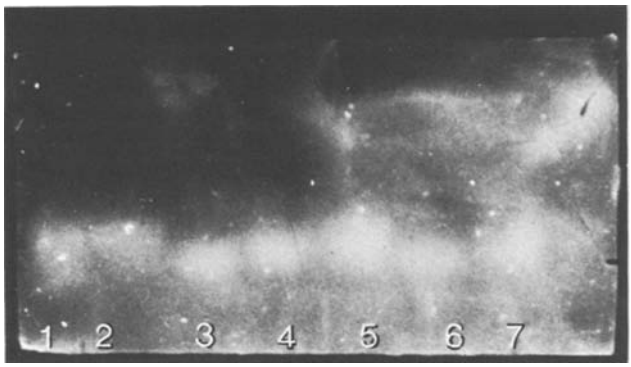


Fig. 2. Starch gel electrophoresis of red cell transferase from control (slots 1, 4), Duarte variant (slot 2), and patient (slot 3) demonstrating the intermediate mobility of transferase of the patient. Electrophoretic conditions were as in Figure 1

The results of starch gel electrophoresis on the hemolysate from the father, patient, controls, and Duarte variant are shown in Figures 1 and 2. The patient (C. B.) and his father had faster mobility than the normal. The fast-moving transferase in this family has an intermediate mobility between normal and the Duarte variant. The electrophoretic patterns of the enzyme from the mother and grandmother were similar to those of the control, whereas the enzyme from the grandfather and the brother of the patient had mobilities similar to those of the father and the patient.



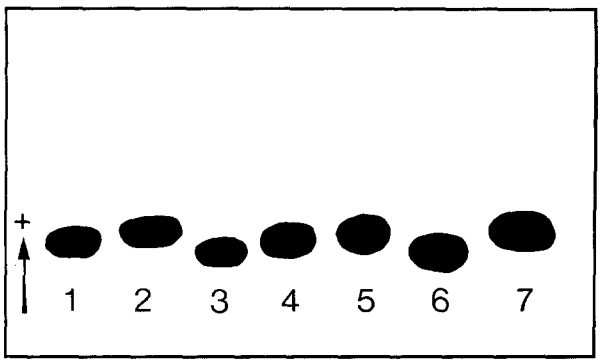


Fig. 3. Starch gel electrophoresis of skin fibroblast transferase from control (slots 1, 4, 7), Duarte variant (slot 2), patient (slots 3, 6), and Duarte/galactosemic compound (slot 5) demonstrating slower than normal electrophoretic mobility of the skin fibroblast transferase of patient, C. B., and identical and faster mobility of the Duarte variant and Duarte/galactosemic compound than the normal control. Electrophoresis was carried out at pH 7.0 in phosphate buffer (Mathai and Beutler, 1966)

Table 3. Percentage of transferase activity remaining after heat treatment at 50° C for 5, 10, and 20 min

Subject	Time of heat treatment at 50° C			
	0	5	10	20
Controls	100	94	98	99
Father G. B.	100	85	65	48
Mother M. B.	100	97	120	97
Patient C. B.	100	111	93	91
Brother A. B.	100	—	97	91
Paternal grandfather F. B.	100	97	89	91
Paternal grandmother Q. B.	100	92	76	52
Duarte variant T.V.	100	92	104	90

All numbers are an average of three experiments in duplicate

The results of starch gel electrophoresis of the supernatants prepared from skin fibroblasts of control, the patient, a Duarte/galactosemic compound, and a Duarte variant are shown in Figure 3. The electrophoretic mobility of the transferase of the patient's fibroblasts was slightly slower than the control. The Duarte variant transferase and the Duarte/galactosemic compound transferase had identical and faster mobility than control transferase. The mother's transferase had mobility similar to that of the control and the father had slightly faster mobility than the control.

The results of heat treatment studies at 50° C are shown in Table 3. The patient, his mother, brother, and paternal grandfather had essentially no loss of activity when heated at 50° C for 20 min or longer whereas the father and paternal grandmother had lost about 50% activity by 20 min of heating. Continued heating for 30 or 40 min did not have any further effect on the transferase levels of these individuals. The Duarte variant and control samples had essentially no loss of activity at 50° C. Storage of the prepared hemolysate at —70° C for up to four months did not have any effect on the level of transferase activity in any of the family members.

The Km values of red blood cell transferase for galactose-l-phosphate (at 0.6 mM UDPG at pH 8.7) were as follows: normal control subjects, 0.4 mM; Duarte variant, 0.32 mM; patient C. B., 0.53 mM; C. B.'s father, 0.54 mM; mother, 0.53 mM; grandfather, 0.45 mM; and grandmother, 0.40 mM. The Km values of red blood cell transferase for UDPG at 2.0 mM galactose-l-phosphate were similar in all instances; normal control subjects, 0.16 mM; Duarte variant, 0.14 mM; patient C. B., 0.19 mM; father, 0.14 mM; mother, 0.14 mM; grandmother, 0.18 mM; and grandfather, 0.14 mM.

The Km value of skin fibroblast transferase of normal controls for galactose-l-phosphate at 0.6 mM UDPG was 0.40 mM. The father's, mother's, and patient's fibroblast transferase Km values were similar to that of the control. The Km for UDPG at 1.5 mM galactose-l-phosphate for control fibroblast transferase was 0.07 mM. The Km's for UDPG of transferase of the patient, his father and mother were similar to that of the normal control value.

Discussion

These studies indicate that the patient, C. B., represents a new variant of clinical galactosemia. C. B.'s red blood cells have approximately 25% of normal levels of transferase and faster than normal electrophoretic mobility. These findings resemble those seen in Duarte variants; however, our patient differs in that (1) he developed symptoms of galactosemia, (2) galactose-l-phosphate accumulates in his red blood cells, and (3) the electrophoretic mobility of transferase in his skin fibroblasts is slower than normal control in contrast to Duarte variant transferase which is faster than normal control. The electrophoretic mobility patterns of the hemolysate transferase of the family members are interesting. C. B., his father, grandfather, and brother have fast-moving bands, whereas his mother and paternal grandmother have normal mobility. The level of transferase activity in the hemolysates of the family members, the instability of transferase at 50° C in

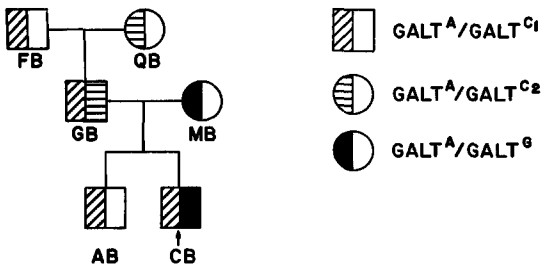


Fig. 4. Pedigree of the family as interpreted from the data collected in this study. The alleles are indicated as follows: □ $GALT^A$, ▨ $GALT^{C_1}$, ◍ $GALT^{C_2}$, ● $GALT^G$

certain members (G. B. and Q. B.) and the difference in electrophoretic mobility suggest that at least three different alleles are present at the transferase loci (Fig. 4). The mother has about one-half normal activity, and her transferase behaves as normal with respect to electrophoretic mobility and heat treatment at 50° C, suggesting that she is a heterozygote for classic galactosemia. C. B's father is a double heterozygote (compound) for the fast-moving and the unstable alleles of transferase. C. B. is a double heterozygote (compound) for the fast-moving and classic galactosemia alleles of transferase.

The alleles responsible for the fast-moving band can be differentiated from the Duarte variant allele. The combination of the fast-moving allele with the normal allele (grandfather and brother of C. B.) resulting in fast electrophoretic mobility is in contrast to the normal/Duarte heterozygotes whose mobility is undistinguishable from the normals at pH 7.0 (Mathai and Beutler, 1966). In order to prove that this is not the Duarte allele, hemolysates from a known normal/Duarte heterozygote, the father (G. B.) of the patient, and controls were subjected to electrophoresis at pH 8.7 in 0.05 M glycine buffer as described previously (Hammersen et al., 1975). The electrophoretic mobility of the three samples differed from one another with multiple bands. Normals had the fastest mobility, normal/Duarte heterozygote samples had intermediate mobility, and G. B. had the slowest mobility (Fig. 5).

The electrophoretic mobility of transferase in skin fibroblasts appears to differ from that in hemolysates. The reason for this discrepancy is not clear. In our experience, the electrophoretic mobility of transferase from different tissues varies. Since crude homogenates of different tissues vary in their composition, they may contribute different substances for binding to the transferase molecule and alter its apparent mobility. The Duarte and the Duarte/galactosemia compound transferase of skin fibroblasts have similar and faster mobility than C. B.'s fibroblast transferase. This clearly establishes that this patient is different from Duarte/galactosemic compounds.

The Michaelis-Menten constants for UDPG and galactose-l-phosphate of transferase of the hemoglobin-free preparations and of skin fibroblasts were similar to those of the normals and the Duarte variant. The Km's of many of the variants have not been reported; however, those studied, e.g., the Duarte variant and the Indiana variant, are not different from those observed for the normal controls.

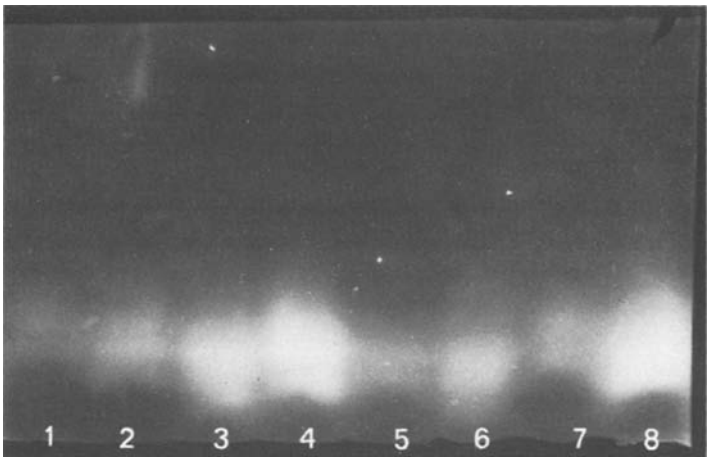


Fig. 5. Starch gel electrophoresis of red cell transferase from controls (slots 1, 4, 7, 8), Normal/Duarte heterozygote (slots 2, 5), and G. B., the father of the patient (slots 3, 6) in 0.05 M glycine buffer at pH 8.7 (Hammersen et al., 1975) demonstrating the difference in mobility of all three samples from one another

The patient, C. B., should not be confused with the previously described Indiana variant (Chacko et al., 1971). The Indiana variant enzyme is extremely unstable even at cold temperatures, whereas the red cell enzyme of this patient is stable at 50° C. C. B.'s skin fibroblasts contain about 25% of normal transferase level, whereas we were unable to demonstrate any transferase activity in fibroblasts for the Indiana variant patient. In addition, C. B.'s symptoms are significantly less severe than those described in the Indiana variant.

The Beutler spot test is an efficient method of mass screening for galactosemia. In this procedure, individuals with partial enzyme deficiency including the heterozygote for galactosemia, heterozygotes for Duarte variant, and the homozygotes for the Duarte variant show delayed appearance of fluorescence. Since the screening procedure also depends on the internal levels of phosphoglucomutase and glucose-6-phosphate dehydrogenase for the production of fluorescing NADPH, the deficiency of any of these enzymes will lead to false positive tests in the screening. It is of interest that our patient with 25% of normal transferase activity demonstrated no fluorescence.

The finding of two new alleles in this patient is a highly improbable event. We have no explanation for this observation. The experiments have been repeated numerous times and the results were always reproducible.

This family as well as the previously described Indiana variant are examples of galactosemia in which patients are presumably double heterozygotes (compounds) for different mutant alleles at the transferase locus. It is not unreasonable to believe that the phenomenon of double heterozygosity is much more common than appreciated in all rare autosomal disorders.

Acknowledgement. These studies were supported by grants from The National Institutes of Health, HD 00036 and HD 04252, The National Foundation—March of Times, and The Chicago Community Trust. H. L. Nadler is the Irene Heinz Given and John La Porte Given Research Professor of Pediatrics.

References

Beutler, E., Baluda, M. C.: A simple spot screening for galactosemia. J. Lab. Clin. Med. **68**, 137—141 (1966)

Beutler, E., Baluda, M. C., Sturgeon, P., Day, R.: A new genetic abnormality resulting in galactose-l-phosphate uridyl transferase deficiency. Lancet **1965I**, 353—354

Beutler, E., Mathai, C. K.: Genetic variations in red cell galactose-l-phosphate uridyl transferase. Reprinted from: Hereditary Disorders of Erythrocyte Metabolism, pp. 66—86. New York: Grune and Stratton 1968

Chacko, C. M., Christian, J. C., Nadler, H. L.: Unstable galactose-l-phosphate uridyl transferase. A new variant of galactosemia. J. Pediatr. **78**, 454—460 (1971)

Donnell, G. N., Collado, M., Koch, R.: Growth and development of children with galactosemia. J. Pediatr. **58**, 836—844 (1961)

Hammersen, G., Mandell, R., Levy, H.: Galactose-l-phosphate uridyl transferase in fibroblasts: Isozymes in normal and variant states. Ann. Hum. Genet. **39**, 147—150 (1975)

Holzel, A.: Galactosemia. Br. Med. Bull. **17**, 213—216 (1961)

Holzel, A., Komrower, G. M.: A study of the genetics of galactosemia. Arch. Dis. Child. **30**, 155—159 (1955)

Hsia, D. Y.-Y., Walker, F. A.: Variability in the clinical manifestations of galactosemia. J. Pediatr. **59**, 872—883 (1961)

Kalckar, H. M., Anderson, E. P., Isselbacher, K. J.: Galactosemia, A congenital defect in a nucleotide transferase. Biochim. Biophys. Acta **20**, 262—268 (1956)

Mathai, C. K., Beutler, E.: Electrophoretic variations of galactose-l-phosphate uridyl transferase. Science **154**, 1179—1180 (1966)

Nadler, H. L., Chacko, C. M., Rachmeler, M.: Interallelic complementation in hybrid cells derived from human diploid strains deficient in galactose-l-phosphate uridyl transferase activity. Proc. Natl. Acad. Sci. USA **67**, 976—982 (1970)

Nadler, H. L., Inouye, T., Hsia, D. Y.-Y.: Classical galactosemia: A study of fifty-five cases. In: Galactosemia. D. Y.-Y. Hsia, Ed., pp. 127—139. Springfield: Charles C. Thomas Publ. 1969

Ng, W. G., Bergren, W. R., Donnell, G. N.: A new variant of galactose-l-phosphate uridyl transferase in man: The Los Angeles variant. Ann. Hum. Genet. **37**, 1—8 (1973)

Schapira, F., Kaplan, J. C.: Electrophoretic abnormality of galactose-l-phosphate uridyl transferase in galactosemia. Biochem. Biophys. Res. Commun. **35**, 451—455 (1969)

Received February 10, 1977