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*A PROCEDURE FOR DETECTING CARRIERS OF GALACTOSEMIA**

BY R. K. BRETTHAUER, R. G. HANSEN, GEORGE DONNELL, AND W. R. BERGREN

DEPARTMENT OF AGRICULTURAL CHEMISTRY, MICHIGAN STATE UNIVERSITY, EAST LANSING, AND CHILDRENS HOSPITAL OF LOS ANGELES

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Introduction.—Galactose-1-phosphate-uridyl transferase (transferase) catalyzes a reversible hexose exchange which results in the formation of uridine diphosphate galactose (UDPGal) and glucose-1-phosphate (Glu-1-P) from galactose-1-phosphate (Gal-1-P) and uridine diphosphate glucose (UDPGlu):

$$\text{Gal-1-P} + \text{UDPGlu} \rightleftharpoons \text{UDPGal} + \text{Glu-1-P}.$$

This enzyme is absent or deficient in the erythrocytes of galactosemics,[1] thus partially blocking the normal metabolism of galactose. As much of the ingested galactose is not excreted by galactosemics,[2,3] the possibility of an incomplete metabolic block or of an alternative pathway of galactose metabolism must be considered. Such an alternative pathway has been demonstrated in liver.[4]

Methods have been devised to detect galactosemics, based upon measuring (1) galactose tolerance, (2) the Gal-1-P which accumulates in the red cells,[5] or (3) the transferase level in the red cells.[1] Standard galactose tolerance tests have revealed that one of the parents of galactosemic children usually show a lower tolerance to galactose.[6] It might be expected therefore, that the transferase level of red cells of these parents should also be lower. Nevertheless, Anderson *et al.*[7] were unable to detect any difference (in the enzyme level of the red blood cells) between parents of galactosemic children and normal individuals. Presuming that this result may be due to limitations of the enzyme assay employed, the quantitative aspects of the procedure have been investigated.[8] Some important modifications have resulted in a more satisfactory procedure, which, when applied to a study of known galactosemics and their families, reveals that heterozygotes indeed have a transferase level intermediate between the affected and the normal individuals. Hsia *et al.*[9] also have recently presented evidence that transferase levels tend to be lower in the parents of galactosemics as compared to normal individuals.

The description and application of the modified transferase assay are the subject of this communication.

Methods.—The initial assay for transferase was based on the original procedure of Kalckar *et al.*[1] and consisted of incubating hemolyzates of red cells with Gal-1-P

and UDPGlu. The residual UDPGlu was determined spectrophotometrically by relating the quantitative reduction of diphosphopyridine nucleotide (DPN) to UDPGlu in the dehydrogenase catalyzed oxidation of UDPGlu to UDPGlucuronic acid. The UDPGal formed was determined by the addition of a purified UDPGal-4-epimerase to the same reaction mixture after the dehydrogenase catalyzed reaction had ceased. This assay resulted in the qualitative detection of the transferase, but was not suitable for further studies requiring more quantitative enzyme levels.

The modified assay, as now employed, is similar to the most recent enzymatic assays published by Anderson *et al.*[7] and Maxwell *et al.*[10] with the following principal exceptions: the present procedure employs a higher concentration of UDPGlu and a higher ratio of Gal-1-P to UDPGlu, less hemolyzate, and a shorter incubation period. A fourfold excess of Gal-1-P over that of UDPGlu was necessary for optimum transferase activity. While using these assay conditions, namely, 0.20 μM UDPGlu and 0.80 μM Gal-1-P, the amount of hemolyzate (equal volume of water and red cells) was varied in a 15-min incubation period to determine the optimum enzyme concentration. In similar experiments, the substrate concentration and the incubation time were varied while the amount of hemolyzate was held constant at 0.2 ml (Fig. 1). Reasonably good linearity was achieved until the

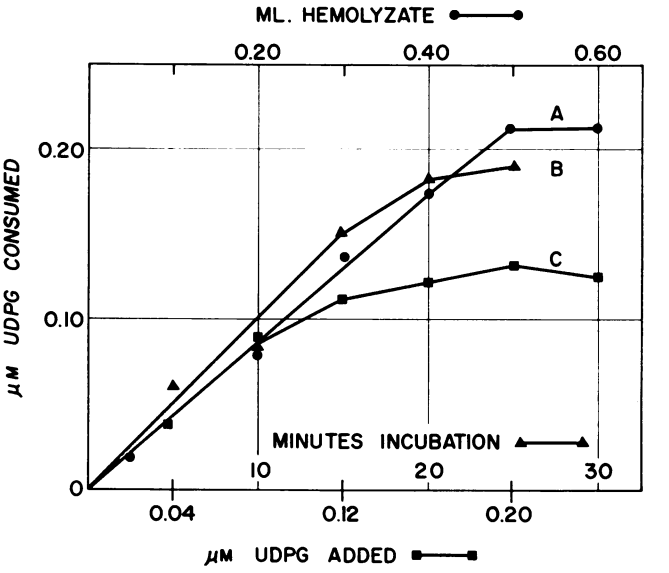

FIG. 1.—Three separate experiments are shown. In *A*, the volume of hemolyzate was varied and all other conditions were held constant. In *B*, the time and in *C*, the substrate level was varied. The following are the reaction conditions unless otherwise indicated: 0.10 ml of 1 *M* glycine, pH 8.7; 0.20 μM UDPGlu and 0.8 μM Gal-1-P (always added in a 1:4 ratio); and 0.20 ml. hemolyzate. Incubation time was 15 min.

UDPGlu became limiting at approximately 0.50 ml hemolyzate (curve A) or at 20 min incubation time (curve B). In the presence of excess substrate (more than 0.12 μM UDPGlu), incubation time and/or hemolyzate concentration became limiting (curve C). The difference in slopes of these 3 curves resulted from using different blood samples for each of the three experiments. For routine determinations, a 10-min incubation period and 0.20 ml of hemolyzate were selected, affording a wide range of measurable values with the same assay conditions. Adequate care was taken to insure excess of substrate for any given sample (usually 0.20 μM UDPGlu and 0.80 μM Gal-1-P).

The spectrophotometric comparisons were carried out in a Beckman DU spectro-

photometer with a photomultiplier attachment and a pinhole filter. The microcuvettes contained 0.20 ml DPN (0.10 μM) and 0.01 ml filtrate from the incubation mixture (omitted in control cell), final volume being made to 0.48 ml with glycine buffer (0.1 *M*, pH 8.7). Having the control cell set at zero absorption, the absorption at 340 mμ of the assay cell was recorded. UDPGlu dehydrogenase (0.01 ml) was then added to the control cell and reset to a zero absorption before a similar addition to each assay cell. After the increase in optical density had ceased, 0.01 ml of UDPGal-4-epimerase was added to each cell in the same manner as described for the dehydrogenase and any further reduction of DPN was noted. The difference in optical density increases before the addition of the epimerase was calculated as UDPGlu consumed in the transferase catalyzed reaction. The increase in optical density after addition of epimerase was calculated as UDPGal formed in the same reaction. The UDPGal recovered was 85 to 105 per cent of the UDPGlu consumed.

Results and Discussion.—In the application of the method, the levels of the transferase in red cells of galactosemics, their families, and normal individuals were determined. Results for three typical families and appropriate controls are shown (Table 1). A more detailed genetic study involving several more families will be published elsewhere. Enzyme levels are expressed as μM UDPGlu consumed per hour of incubation both per milliliter of red cells and per gram of hemoglobin. Before positive identification of the subjects, the values were categorized as follows: 0, galactosemics; 2.9–3.7, parents or carriers; 4.8–8.1, normal. Subsequent work with a greater population will define the expected range of transferase levels for patients, carriers, and normals more precisely.

Anderson *et al.*[7] reported normal transferase levels in the range of 2.4–3.5 μM of UDPGlu utilized per gram of hemoglobin per hour. The levels reported by Hsia *et al.*[9] are also in the same low category and consequently, when applied to a study of galactosemics and their families, there was marked overlapping in the various categories listed above, particularly in the carrier and normal groups. This is in part due to limitations of the assay employed since the high values were underestimated. The more quantitative procedure described herein yields values of greater magnitude, thus extending the over-all range with no overlapping between galactosemics, carriers, and normals. The carrier category is intermediate between the other two groups.

It has been suggested that galactosemia is inherited as a simple recessive characteristic.[6] Data presented here substantiate this concept. The results of the application of an improved enzymatic method to a study of transferase levels indicate that it is possible to detect parents who are likely to give birth to galactosemics and to survey a larger population of galactosemic and normal individuals to determine more precisely the genetics and also the incidence of this metabolic disorder. As galactosemia and other metabolic diseases may in some cases limit mental development, a consideration of transferase levels in mentally retarded patients should reveal if a significant number of patients have an abnormal galactose metabolism. Such a study is presently in progress.

Conclusions.—Existing procedures have been modified to determine galactose-1-phosphate uridyl transferase quantitatively in human erythrocytes. This method has been applied to galactosemics, their families, and appropriate normal individuals

TABLE I

GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE LEVELS IN RED CELLS

		μM UDPGlu Consumed per Hour	
		Per Ml Red Cells	Per Gm Hb
Family 1	Galactosemic	0	0
	Mother	2.9	8.7
	Father	3.5	10.3
	Sister	3.0	9.0
	Brother	7.0	21.2
	Sister	6.0	...
Family 2	Galactosemic	0	0
	Mother	3.7	10.8
	Father	3.6	10.7
Family 3	Galactosemic	0	0
	Mother	3.0	9.4
	Father	3.6	10.7
Controls			
9	Child—male	7.6	23.0
10	Child—male	6.9	21.5
11	Adult—male	8.1	25.4
12	Adult—female	5.9	18.8
25	Adult—male	6.0	17.7
51	Adult—female	6.0	18.3
52	Adult—female	5.0	14.9
53	Child—female	6.5	21.7
58	Child—male	5.0	15.8
60	Adult—male	4.8	14.7
61	Adult—female	5.0	14.7
Mean	Galactosemics	0	0
	Carriers	3.33	9.94
	Normal	6.14	19.0
Range	Galactosemics	0	0
	Carriers	2.9–3.7	8.7–10.8
	Normal	4.8–8.1	14.7–25.4

Incubation conditions were as follows: 0.20 μM UDPGlu, 0.80 μM Gal-1-P, 0.10 ml glycine buffer (1 *M*, pH 8.7), and 0.20 ml hemolyzate (1:1 ratio of red cells and water), made to final volume of 0.45 ml with water. Incubation time was 10 min at 37°C. The incubation mixture was denatured by placing in a boiling water bath for 2 min. After centrifugation, UDPGlu and UDPGal were determined as described in the text.

Results for three typical families are reported. As parents have a transferase level which is intermediate between the normal and the galactosemic, carriers of this genetic disorder may be detected by determination of transferase in the red cells. This procedure should be useful in a more detailed consideration of the genetics of this disorder.

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