Glucose-6-phosphate Dehydrogenase in the Developing Human Liver (36236)

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(Introduced by E. Beutler)

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Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase) (EC 1.1.1.49) (G-6-PD) is the initial enzyme in the pentose phosphate shunt. This enzyme catalyzes the oxidation of glucose-6-phosphate generating NADPH. The NADPH formed may be involved in the formation of certain amino acids and is essential for the biosynthesis of lipids, steroids, and purines.

Previous studies of G-6-PD in human fetal material have dealt primarily with the delineation of its specific activity. Clausen and Hurstrulid (1) failed to demonstrate any significant change in the activities of G-6-PD or other enzymes of the hexosemonophosphate shunt in striated muscle obtained from human fetuses of 13 to 25 weeks of gestational age. In contrast they were able to demonstrate a linear increase in total lactic dehydrogenase activity. Mino *et al.* (2) reported an increase in total activity of G-6-PD in human fetal liver and red cells from 4 to 6 months of gestation. This activity decreased to the adult level of activity by 7 months gestation. Because of its critical role in intermediary metabolism and the relative sparsity of data a study was undertaken to determine the developmental pattern of G-6-PD in human fetuses. This manuscript reports the developmental patterns of G-6-PD in human liver from 12 weeks of gestation to adult life with regards to its specific activity, thermal stability, Michaelis-Menten constants, pH optima, and electrophoresis on starch gel.

Materials and Methods. Eleven human fetuses and four placentas were obtained at the time of therapeutic abortion. Samples of five adult and four newborn livers were obtained at postmortem examination immediately after death. Tissues were either used immediately or frozen at −20° until ready for use. G-6-PD from fetal, newborn, and adult livers were studied for pH optima, Michaelis-Menten constants, thermal stability, electrophoretic mobility, and specific activity.

All biochemical reagents used in the study were purchased from Sigma Chemical Co. (St. Louis, MO), with the exception of the hydrolyzed starch which was purchased from Connaught Medical Research Laboratories of Toronto, Canada.

Preparation of enzyme. Homogenates (20%) of all tissues used were prepared using a tissue homogenizer in dialysis solution consisting of 0.05 *M* Tris buffer (pH 8.0), which contained 2.7 m*M* EDTA, 0.01 m*M* NADP, and 7 m*M* β-mercaptoethanol (3). The homogenates were centrifuged at 64,000*g* for 30 min in a Model L-265B Beckman ultracentrifuge. All preparations were carried out at 4° unless stated otherwise. The supernatant obtained was dialyzed against the dialysis solution using negative pressure dialysis, in a collodion bag to half the original volume. After dialysis, the volume was brought up to the initial level with dialyzing solution and the enzyme preparation stored at 4° until assay. This preparation was stable for 3 days at 4° but demonstrated progressive loss in activity upon further storage at this temperature.

Assay. G-6-PD was assayed according to Kirkman's (4) modification of the method of Glock and McLean (5). The assay system contained 0.1 ml of 1.0 *M* Tris buffer (pH 8.0); 0.1 ml of 0.1 *M* $MgCl_2$; 0.1 ml of 6 m*M* G-6-P; 0.1 ml of 2.0 m*M* NADP; and

TABLE I. Biochemical Characteristics of G-6-PD.

Age (weeks)	No.	Sp act[a] (mean ± SD)	Michaelis-Menten Constants	
			G-6-P (M) at 0.25 mM NADP	NADP (M) at 0.6 mM G-6-P
Fetal 10–16	5	13.9 ± 2.4	$6.5 \times 10^{-5} \pm 3.3$	$5.5 \times 10^{-5} \pm 4.2$
Fetal 18–24	6	42.7 ± 7.5		
Newborn	4	22.0 ± 3.0	5.5×10^{-5}	8.5×10^{-5}
Adult	5	26.1 ± 3.6	$4.6 \times 10^{-5} \pm 4.0$	$3.8 \times 10^{-5} \pm 1.3$

[a] Micromoles of NADPH per minute per gram of protein.

0.1 ml of 6-PGD (0.49 units, yeast type V, Sigma). The final volume was adjusted to 1.0 ml. G-6-P was omitted from the blank. The rate of NADP reduction was followed at 37° at 340 mμ in a Gilford Model 2000 spectrophotometer. Excess of 6-PGD (0.49 units) was added to the assay system to insure that all the 6-PGA formed during the reaction was oxidized. To account for the oxidation due to 6-PGD activity, one-half the rate of NADP reduction was taken as G-6-PD activity. In all instances the reaction was started with the addition of the enzyme preparation.

Specific activity. Specific activity determinations of all samples were carried out using 0.6 mM G-6-P and 0.2 mM NADP in 0.1 M Tris buffer at pH 8.0. Specific activity was expressed as micromoles of NADPH produced per minute per gram of protein. Protein determinations were carried out using the method of Lowry *et al.* (6).

pH optimum. The pH optimum studies of all samples were carried out in a previously described buffer system (Tris–phosphate–glycine) at pH values ranging from 5.5 to 11.0 (3).

Thermal stability. Heat inactivation studies of all enzyme preparations were carried out at 45 and 55° for 0, 2, 5, 10, and 15 min. For these studies, the protein concentrations of the samples were adjusted to 2.0 mg/ml.

Electrophoresis. Electrophoresis of G-6-PD of all samples was carried out at pH 7.0, using a previously described method (7); and at pH 9.0 in a Tris–phosphate–glycine buffer system. In the latter system the gel was prepared in 0.01 M Tris–phosphate–glycine buffer and the bridge buffer concentration was 0.05 M.

Michaelis-Menten constants. The Michaelis-Menten constant of G-6-P at 0.25 mM NADP concentration was determined at pH 8.0 using concentrations of G-6-P ranging from 0.5 to 15 mM. The K_m values of NADP at 0.6 mM G-6-P were also determined at pH 8.0 with concentrations ranging from 0.5 to 15 mM.

Specific activity. The specific activity was determined for all samples. The results of these studies are shown in Table I. G-6-PD activity at 10–16 weeks gestation was approximately one-half the adult ($p < .001$) and newborn ($p < .01$) levels. In contrast, G-6-PD activity in 18–24 week fetuses was twice as high as the newborn ($p < .01$) and adult ($p < .01$) levels. No significant changes in specific activities were observed in preparations from frozen material as compared to fresh tissue preparations. Since it is known that the liver contains two enzymes (8, 9) which oxidize the substrate glucose-6-phosphate all enzyme preparations were assayed for microsomal hexose-6-phosphate dehydrogenase activity using galactose-6-phosphate as substrate. The rate of NADP reductions with galactose-6-phosphate was less than 10% the rate of reduction when G-6-P was the substrate.

Thermal stability at 45 to 55°. Thermal stability studies at 45 and 55° at 0, 2, 5, 10, and 15 min were carried out on fetal, newborn, and adult liver preparations. The fetal liver G-6-PD showed a continuous decrease in activity at 45°. At the end of 15 min incubation, the fetal liver preparation re-

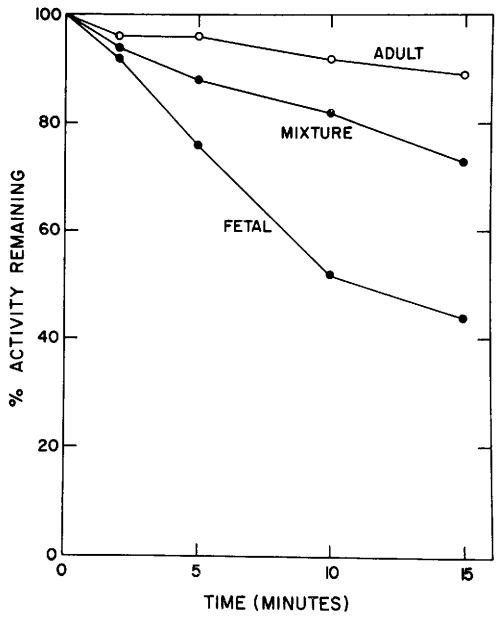

FIG. 1. Thermal stability studies of G-6-PD from an adult and a fetal liver preparation and their mixture at 45° for 0, 2, 5, 10, and 15 min. The percentage of activity remaining is plotted against time of incubation (min): (●—) 16-week-old fetus; (○—) adult; (●—) mixture of the two preparations in equal volumes having the same protein concentrations. Similar results were obtained with several other preparations of adult and fetal liver.

tained about 40% of the initial activity. In contrast, the newborn and adult liver preparations retained more than 75% of the initial activity at the end of 15 min. Results of the heat stability studies of G-6-PD from an adult and a fetal liver preparation and their mixture are shown in Fig. 1. Heating of the mixture at 45° resulted in losses of activity, which were intermediate between those of the adult and fetal G-6-PD. When heat inactivation studies were carried out at 55°, the fetal liver preparation demonstrated no G-6-PD activity after 5 min; whereas, approximately 35% of the G-6-PD activity remained in newborn and adult liver preparations. Continued heat inactivation at 55° resulted in complete inactivation of G-6-PD in newborn and adult.

pH optimum. Results of the pH studies are shown in Fig. 2. Each curve is a composite of at least 4 pH curves from fetal, newborn, and adult material. The G-6-PD enzyme from adult and newborn had similar flat curves with broad plateaus of activity between pH 7.0 and 9.0. The curves from fetal livers of 18–24 weeks gestation showed two sharp peaks of activity: the first peak between pH 6.5–8.0; and a second peak between pH 9.0 and 10.0, while the pH curve from fetal livers of 10–16 weeks showed a small peak between pH 6.0 and 7.0 and another small peak between pH 9.0 and 10.0.

Thermal inactivation with respect to pH. Studies of fetal liver G-6-PD at various pH values before, and after, thermal inactivation at 45° for 10 min showed a 30–50% drop in specific activity at all pH values after heat treatment. In contrast, G-6-PD enzyme preparations from adult and newborn demonstrated no substantial change in specific activity at any pH after heat inactivation.

In one instance, liver from a 20-week-old fetus exhibited a significant drop in activity

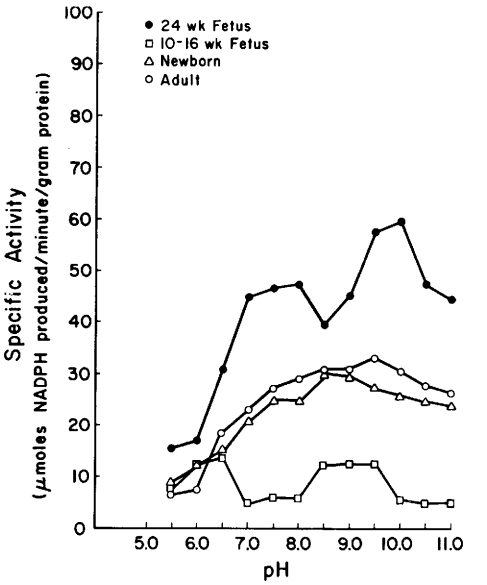

FIG. 2. Specific activityof G-6-PD with respect to pH at various stages of development. Activity determinations from pH 5.5 to 11.0 were carried out using Tris–phosphate–glycine buffers as described in the text: (□—) represents enzyme preparation from fetal livers of 10–16 weeks gestation; (●—) represents enzyme from livers of 20–24 week old fetuses; (△—) represents newborn liver enzyme; and (○—) represents adult liver enzyme.

only between pH 9.5 and 10.5, while the remainder of the pH curve was essentially the same as observed prior to heat treatment.

Michaelis-Menten constants. The results of the determination for the Michaelis-Menten constants are given in Table I. The K_m for G-6-P at 0.25 m*M* NADP concentration was similar in fetal, newborn, and adult liver. The K_m for NADP at 0.6 m*M* G-6-P concentration was of the same order of magnitude in all samples studied.

Electrophoretic mobility. Enzyme preparations from all fetuses, newborns, and adults, demonstrated two bands which migrated toward the anode at both pH 7.0 and 9.0. The substitution of galactose-6-phosphate for glucose-6-phosphate yielded two faint bands which were identical to those observed when G-6-P was used. These bands could be observed only after prolonged staining.

Discussion. These data indicate that G-6-PD from human liver demonstrates quantitative and perhaps qualitative differences during development. Our observations are in agreement with the studies of Mino *et al.* (2), who reported low G-6-PD activity in early human fetal development, an abrupt increase in activity at 4 and 5 months gestation, and a decrease in activity to adult and newborn levels after 6 months of gestation. The increase in activity observed in our studies at 18–24 weeks gestation was much higher than the activity seen in either newborn or adult liver. The lack of fetal material after 24 weeks gestation made it difficult to determine precisely the time at which G-6-PD activity begins to fall.

Studies of G-6-PD activity, with respect to pH, demonstrate clear differences at different stages of development. The most significant change occurs at 18–24 weeks gestation, where a biphasic curve with a sharp increase in activity between pH 9.5 and 10.0 is observed. It is tempting to postulate a correlation between the increased specific activity of the enzyme and the appearance of the prominent second peak on the pH curve between pH values 9.5 and 10.0 at the 18–24 weeks gestational age. The higher G-6-PD activity observed in the 18–24 week gestational period may be due to an increased requirement for NADPH production to meet the demand for lipogenesis in fetal liver. Villee *et al.* (10) have shown that lipogenesis in fetal liver and placenta occurs as early as week 20 of gestation. In addition, Roux *et al.* (11) have shown that fetal liver is capable of synthesizing lipids utilizing carbohydrates as precursors by 28 weeks gestation.

Our studies on the electrophoresis of G-6-PD from placentas obtained at 10 weeks of gestation to full term, demonstrate the presence of two distinct bands with similar migration. This observation is in contrast to the findings of Von Bogaert *et al.* (12), who have reported two G-6-PD bands from early placentas, but only a single enzyme band at term. This discrepancy is probably due to the use of different methods in the preparation of enzyme for electrophoresis. In our studies, the enzyme was protected by using a dialysis solution which contained NADP, a known stabilizer of G-6-PD; whereas Von Bogaert *et al.* (12) used water in the preparation of their homogenates. A second possible explanation is the media used for electrophoresis. Starch gel was used in our studies, while polyacrylamide gel was used by Von Bogaert *et al.* (12).

The differences in specific activity, thermal stability, and pH optima suggest that G-6-PD from fetal liver is different from newborn or adult liver. This suggestion is made, despite the fact that no significant differences were observed in substrate requirements and electrophoretic mobility. Our studies do not distinguish the stage of development or the mechanism by which the fetal enzyme changes to the adult-type G-6-PD.

Summary. G-6-PD was studied in human liver at various stages of development. Distinct differences were observed in specific activity, pH optimum, and thermal stability of the fetal enzyme compared to newborn and adult enzyme. In contrast, no differences were observed in Michaelis-Menten constants or electrophoretic mobility.

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