Y. EDWARD HSIA*
IRA K. BRANDT**

Department of Pediatrics, Yale University School of Medicine

GLUCOSE-1-PHOSPHATE URIDYLYL TRANSFERASE ACTIVITY IN DEVELOPING RAT LIVER†

Glucose-1-phosphate (G-1-P) uridylyl transferase, E.C. 2.7.7.9,[1] catalyzes the reversible synthesis of uridinediphosphoglucose (UDPG) from G-1-P and uridine triphosphate with the liberation of pyrophosphate. This reaction appears to be ubiquitous in nature.[2] The importance of this enzyme is evident from its participation in the metabolic pathways of glycogen synthesis,[3] galactose conversion to glucose,[4] detoxication of certain compounds by glucuronosylation[5,6] and synthesis of mucopolysaccharides,[7] among others. In rat liver homogenate the enzyme is present in the soluble fraction exclusively.[8,9]

Previously published data on the activity of this enzyme in rat liver at various ages have differed widely and have not been comprehensive (Table 1). The present studies demonstrate a striking developmental pattern for this enzyme.

METHODS

A colony of Sprague-Dawley rats was maintained on "D. & G. Research Laboratory Diet" (Price-Wilhoite Co., Frederick, Md.). Mating was allowed to take place overnight, the following day was considered the first day of gestation and birth occurred on the twenty-second day of gestation.

Animals were sacrificed by decapitation or by exsanguination after a blow on the head. Subsequent procedures up to the assay were carried out at 0 to 4°C. A 1 per cent suspension of liver in 0.25 M sucrose was made with the use of a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 12,000 x *g* for ten minutes, the overlying fat was removed, and the supernatant fluid was assayed immediately for enzyme activity.

G-1-P uridylyl transferase was determined by a modification of the spectrophotometric technique of Munch-Petersen.[10] The reaction mixture contained trishydroxymethylaminomethane-HCl 0.2 M at pH 8.65, $MgCl_2$ 0.005 M, sodium pyrophosphate 0.01 M, cysteine 0.0014 M, nicotinamide adenine dinucleotide phosphate (NADP)

* Instructor in Pediatrics. Present address: Department of Medicine, University of Malaya, Kuala-Lumpur.
** Associate Professor of Pediatrics.
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0.0002 M, phosphoglucomutase 0.03 mg/ml., glucose-6-phosphate dehydrogenase (G-6-PDH) 0.4 Kornberg units/ml., UDPG 0.0006 M, and the 12,000 x *g* supernatant fluid corresponding to 0.1 mg. of liver homogenate (0.01 ml.) in a final volume of 0.50 ml. UDPG was added last after the reaction mixture had been equilibrated to 37°C. and there was a constant absorbance at 340 mμ. After addition of UDPG, readings were taken every 30 seconds for at least five minutes, from which the initial velocity was determined. Activity was expressed as enzyme units (EU), one EU being equal to one μmole NADP reduced per minute at 37°C., using the molar absorption coefficient of Horecker and Kornberg.[11]

TABLE 1. G-1-P URIDYLYL TRANSFERASE ACTIVITY OF RAT LIVER NOTED BY OTHERS

Authors	*Age*	*Activity (EU)*	
		per gm. liver	*per mg. protein*
Isselbacher[15]	Fetus, 18 days		0.017
	1 day		0.018
	60 days		0.005
Reid[8]	Adult male	0.86	
Villar-Palasi and Larner[9]	Adult male	59	0.43
Ballard and Oliver[13]	Fetus, 16 days	1.7	
	Fetus, 18 days	4.3	
	Fetus, term	5.3	
Ballard[16]	2 days	10.7	
	10 days	11.5	
	18 days	11.5	
	27 days	12.0	
	Adult	13.5	

Protein was measured by an adaptation of the bromosulfophthalein dye adsorption technique of Bonting and Jones.[12] This method was calibrated for the 12,000 x *g* supernatant fluid of 1 per cent rat liver homogenate using nine different representative samples. At 580 mμ a decrease of 0.22 absorbancy units per mg. protein per ml. (as determined by the biuret reaction using albumin as standard) was found with good linear correlation. Neither nucleic acids nor glycogen adsorbed the dye to a significant degree.

RESULTS

All of the activity of the crude liver homogenate was found to be present in the supernatant fluid of both the 12,000 × *g* and the 100,000 × *g* centrifugates. No reduction of NADP occurred in the absence from the reaction mixture of UDPG, of pyrophosphate, or of the liver preparation. The G-6-PDH used (type V, Sigma Chemical Company, St. Louis, Mo.)

was found to be free of any G-1-P uridylyl transferase activity, and it was not found necessary to add glucose-1,6-diphosphate to catalyze the phosphoglucomutase (Sigma Chemical Company) in the reaction mixture, even in the absence of the liver preparation. In the assay system, conditions were adjusted to those found to be optimal for adult male rat liver. A pH of 8.4 to 8.65 resulted in up to three times the activity found at pH 7.4. This is in agreement with the statement of Ballard and Oliver.[18]

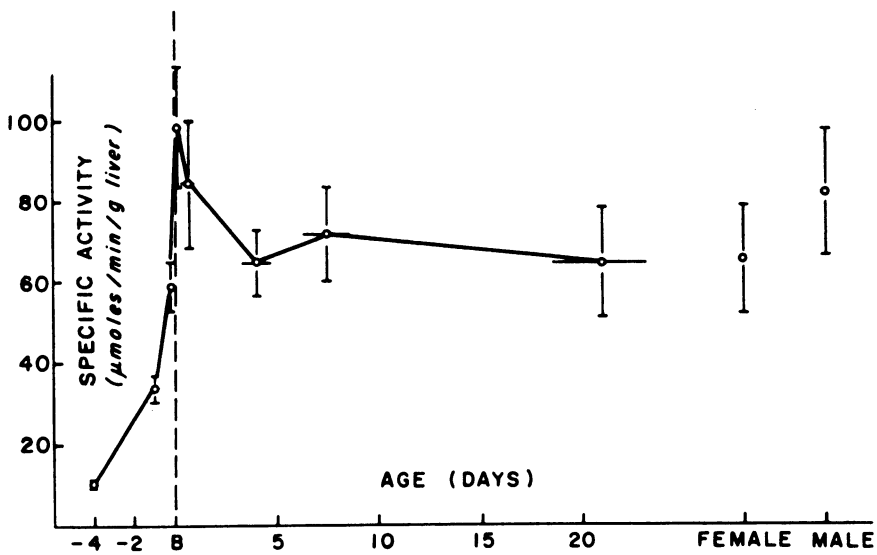

FIG. 1. G-1-P uridylyl transferase in developing rat liver: activity per gm. of liver. The vertical line represents one standard deviation above and below the mean enzyme activity. The horizontal line represents one standard deviation either side of the mean age for each group (except that of the fetuses and adults). The numbers of animals in each group are given in Table 2. The male adults were 80-242 days of age and the female adults 53-280. No variation with age was noted within the adult groups. Animals within each age group were from different litters except for the fetal groups in which all came from the same litter except that two litters contributed to the full-term fetuses.

The mean G-1-P uridylyl transferase activity in liver from rats of different ages is presented in Figure 1. Adult female rats had 66 ± 13 (S.D.) EU per gm. liver, males had 82 ± 15; this sex difference is statistically significant ($P < .01$). Peak enzyme activity of 98 ± 15 EU per gm. occurred within the first two hours after birth. This was significantly more than adult male activity ($P < .05$) and more than the activity of any other age group ($P < .01$) except the group aged 3 hours to 2 days ($P < .1$). When calculated as EU per mg. protein (Table 2), however,

TABLE 2. VARIATION WITH AGE OF LIVER WEIGHT IN RELATION TO BODY WEIGHT, OF PROTEIN IN 12,000 x *g* SUPERNATANT FLUID OF RAT LIVER, AND OF G-1-P URIDYLYL TRANSFERASE ACTIVITY (MEAN ± S.D.)

Age	*Number of animals*	*Liver weight (gm.)*	*Liver weight (per cent of body wt.)*	*12,000 x g supernatant protein (mg. per gm. liver)*	*G-1-P uridylyl transferase (EU per mg. protein in 12,000 x g supernatant fluid)*
Fetus, 18 days	6	0.07 ± 0.01	8.3 ± 0.01	54 ± 8	0.17 ± 0.04
Fetus, 21 days	6	0.31 ± 0.05	8.0 ± 1.0	49 ± 3	0.72 ± 0.13
Fetus, 22 days	7	0.30 ± 0.05	5.6 ± 0.8	48 ± 5	1.24 ± 0.12
Birth to 3 hrs.	9	0.28 ± 0.04	4.9 ± 1.7	61 ± 8	1.62 ± 0.32
4 hrs. to 2 days	6	0.28 ± 0.04	4.3 ± 0.4	66 ± 6	1.27 ± 0.05
3 to 5 days	7	0.37 ± 0.01	3.8 ± 0.5	65 ± 5	0.99 ± 0.12
6 to 12 days	8	0.60 ± 0.26	3.3 ± 0.7	75 ± 8	0.87 ± 0.24
15 to 23 days	7	2.21 ± 0.37	3.6 ± 0.5	85 ± 14	0.77 ± 0.17
Adult female	12	8.30 ± 1.3	3.1 ± 0.2	83 ± 13	0.80 ± 0.12
Adult male	15	10.40 ± 2.54	3.0 ± 0.4	83 ± 11	1.00 ± 0.20

the values for the latter group are significantly different ($P < .05$). Later age groups did not differ significantly from each other or from adult female activities.

When activity was calculated per mg. protein of the supernatant fraction of rat liver (Table 2) the statistically significant differences between the sexes remained, and the significant immediate postnatal peak became more definite because of the lower protein concentration of neonatal liver (Table 2).[14]

DISCUSSION

The G-1-P uridylyl transferase activity of liver from adult male rats in this study was slightly higher than that found by Villar-Palasi and Larner.[9] This may be a result of the higher pH and temperature which we employed. Isselbacher,[15] Reid,[8] and Ballard and Oliver[13] found much less activity (Table 1).

The developmental pattern found by Ballard and Oliver[13] and by Ballard[16] was similar in outline to that reported here except that they did not study sex differences and did not study newborn animals during the first postnatal day, when peak activity was found to occur.

The great magnitude of the change in enzyme activity during *fetal* life (as shown in Fig. 1) and the rather inbred nature of our animal colony indicate that the significance of the data is not appreciably reduced by the fact that the groups of *fetal* animals were littermates. The newborn and older groups did not contain littermates, however, so that the tests of significance applied to the more subtle changes in enzyme activity which occurred from birth on were of the highest strength.

The striking increase in activity in late fetal life coincides with the rapid accumulation of glycogen by rat liver noted by others.[17] Immediately after birth, however, liver glycogen rapidly diminishes, whereas the activity of G-1-P uridylyl transferase reaches the maximum value which we observed.

The developmental patterns of some other enzymes of carbohydrate metabolism in rat liver as reported by Burch, *et al.*[14] are quite unlike that of this enzyme. Glucuronide conjugation, which depends upon uridine diphosphate glucuronic acid as a substrate, however, is more active in rat liver slices from 11 to 20-hour old animals,[18] and the remainder of the developmental pattern of glucuronide conjugation resembles that of G-1-P uridylyl transferase as well.

The sex difference noted here is not as marked as that found for glucuronide conjugation in microsomes of adult rat liver by Inscoe and Axelrod.[19]

A developmental variation for G-1-P uridylyl transferase has been reported for guinea pig liver by Kornfeld and Brown.[20] There is not the same striking late fetal rise to a postnatal peak of activity in the guinea pig, though its liver glycogen is increased prenatally in the same way as in the rat. This may be related to the longer gestation period of the guinea pig and its greater maturity at birth.

The reaction catalyzed by G-1-P uridylyl transferase in the liver participates in many metabolic pathways, many of which are of vital significance, particularly to the newborn animal. The developmental pattern therefore may be an expression of the metabolic changes occurring at that time. The *in vitro* activity of this enzyme, as compared to that of enzymes catalysing related reactions, is so great that it is not likely to be a rate-limiting step in any of these pathways.

SUMMARY

Glucose-1-phosphate uridylyl transferase activity was assayed in the soluble fraction of rat liver in animals ranging in age from 18 fetal days to adult. The activity in adult males was 82 ± 15 (S.D.) μmoles per minute per gm. liver, or 1.0 ± 0.2 μmoles per minutes per mg. soluble protein. Adult females had significantly lower activity than males: 66 ± 13 or 0.8 ± 0.1 respectively ($P < 0.01$ for both). On the 18th fetal day the activity was 12 per cent of that of the adult male, but rose rapidly thereafter. Maximal activity was found in the liver of newborn animals during the first two hours after birth: 98 ± 15 or 1.6 ± 0.3, respectively. From the second day to the time of weaning, enzyme activity was not significantly different from that of the adult female. The sex difference in adults and the postnatal peak in activity of this enzyme, both significant statistically, have not been noted previously.

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