ENZYMOLOGY:

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Developmental Aspects and Some Characteristics of Mammalian Galactose 1-Phosphate Uridyltransferase

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SUMMARY

Galactose 1-phosphate uridyltransferase (EC 2.7.7.12: UDP-glucose: α-D-galactose-1-phosphate uridylyltransferase) activity in the developing rat liver, gut, and kidney is presented. The activity in liver tissue increased from 3 days before birth to a maximun in the 10-day-old animal and then declined to adult levels between 35 and 45 days. No observed rise in transferase activity occurred in gut and kidney from term until 10 days with a subsequent decrease until 35 days. Adult rat brain and diaphragm were also examined for enzyme activity. By comparison at any age, liver had a consistently higher enzyme rate than any of the other tissues studied, and brain had the lowest in the adult tissues studied. The enzyme activity in liver of female adult rats was significantly lower than in the same aged male animals.

By differential centrifugation, transferase from newborn and adult rat liver was located entirely in the soluble fraction. The enzyme in newborn liver was 3 times more active and had V_{max} values 3 times greater than in the adult liver, but the enzyme from both preparations had the same (*a*) stability characteristics, (*b*) K_m values for galactose-1-P (1.39×10^{-4} M) and for UDP-glucose (1.56×10^{-4} M), (*c*) pH optimum (8.3 to 8.6), and (*d*) sulfhydryl requirements. In addition, both enzyme preparations utilized UDP-galactose as a substrate with galactose-1-P with the same K_m (1.67×10^{-4} M), and neither was as sensitive to inhibition by this substrate as with galactose-1-P or UDP-glucose. The enzyme from both sources was more sensitive to UDP-glucose inhibition than galactose-1-P inhibition, but the enzyme from newborn liver was 3 times less sensitive to inhibition by either of these substrates than the enzyme from adult liver. Glucose-1-P caused marked inhibition of the enzyme from the adult liver.

The enzyme galactose 1-phosphate uridyltransferase is one in a series by which galactose enters the pathways of glucose metabolism. It catalyzes the reversible conversion,

α-D-Galactose-1-P + UDP-glucose ⇌

UDP-galactose + α-D-glucose-1-P

The presence of this enzyme has been demonstrated in galactose-adapted yeast (1), rat and calf liver (2), galactose-adapted *Escherichia coli* (3), human red and white blood cells (4, 5), and human liver (6). Kalckar, Anderson, and Isselbacher (7) have shown that this enzyme is absent in the inherited metabolic disorder congenital galactosemia. A partial purification and some of the enzyme properties have been studied in microorganisms (3). Results of purification of the enzyme in calf liver (8) and human red cells (9) have been reported, but little has been studied about the characteristics of the mammalian enzyme.

The present study has been undertaken to characterize further the role of galactose 1-phosphate uridyltransferase in the biological pathways of galactose and glucose metabolism, special emphasis being placed on enzyme activity changes with regard to animal age. In order to ascertain these developmental changes, certain kinetic properties of the crude enzyme preparations from newborn and adult rat liver were studied. A comparison of the enzyme activity in liver, gut, and kidney in the developing rat and activities in some other rat tissues are presented.

EXPERIMENTAL PROCEDURES

Materials—α-D-Glucose 1-phosphate, α-D-galactose 1-phosphate, uridine 5′-diphosphoglucose, uridine 5′-diphosphogalactose, uridine 5′-diphosphoglucuronic acid, uridine 5′-diphosphomannose, uridine 5′-diphosphoxylose, adenosine 5′-diphosphoglucose, cytidine 5′-diphosphoglucose, guanosine 5′-diphosphoglucose, guanosine 5′-diphosphomannose, thymidine 5′-diphosphoglucose, uridine 5′-triphosphate, β-diphosphopyridine nucleotide, uridine 5′-diphosphoglucose dehydrogenase (Type III), and Cleland's reagent (dithiothreitol) were products of Sigma or Calbiochem. α-D-Galactose 1-phosphate-^{14}C (uniformly labeled; specific activity, 13 to 20 mC per mmole) was purchased from Volk and the International Chemical and Nuclear Corporation. UDP-Glucose-^{14}C (uniformly labeled; specific activity, 12 mC per mmole) and UDP-galactose-^{14}C (uniformly labeled; specific activity, 30 mC per mmole) were products from the International Chemical and Nuclear Corporation. UDP-glucuronic-^{14}C acid (uniformly labeled; specific activity, 137 mC per mmole) and Liquifluor were from New England Nuclear. The bacterial alkaline phosphatase, BAP-C (approximately 30 enzyme units per mg), was a Worthington product. Whatman No. DE 81 was the DEAE-cellulose anion exchange paper used.

Animals—All animals were obtained from the animal colony of the National Institutes of Health. Male Sprague-Dawley rats were used and, unless otherwise specified, were between 35 and

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45 days old. These animals as well as those over 20 days old (weanlings) were fed a Purina rat chow diet and water *ad libitum* until killed by decapitation. Fetal rat age was estimated from the day of mating.

Enzyme Preparations—Adult liver specimens were usually taken from the right lobe; whole livers were pooled from a given litter of the fetal and newborn animals. All gut specimens were 3-cm segments pooled from different animals and taken from a level 3 to 4 cm below the ligament of Treitz. Whole kidneys from several animals in a single litter of fetal and newborn animals were used, and from the adult animals only the cortex was taken. Hemidiaphragms were the muscle specimens used, and brain tissue was taken from the frontal cortex.

About 200 to 500 mg of tissue were removed, dipped in cold 0.1 M KCl, blotted, weighed, and homogenized in ground glass grinders on ice with 4 volumes of KCl (0.10 M for liver; 0.02 M for other tissues). Homogenates prepared from adult liver were diluted 1:6 with quartz glass-distilled water. Those prepared from newborn livers were diluted 1:8; other tissue homogenates were not diluted. The homogenates were centrifuged at 30,000 × *g* for 30 min at 4°. The approximate protein concentration for 10 μl of the clear supernatants were 30 μg for adult liver; 20 μg for newborn liver; 80 μg for gut and kidney; 75 μg for brain; and 70 μg for diaphragm. All enzyme preparations were kept on ice and assayed between 2 and 3 hours after tissue removal. Proteins were determined by the method of Lowry *et al.* (10).

Differential centrifugations were done on the newborn and the adult liver preparations. From determinations on the crude preparations and supernatants of the same preparations centrifuged at 30,000 × *g* for 30 min and 100,000 × *g* for 60 min, it was observed that all the enzyme activity was in the soluble fraction.

Assay Procedure—The assay for galactose 1-phosphate uridyltransferase was adapted from a method developed by Sherman and Adler as an assay for galactokinase (11, 12). The principle of our assay procedure was based on the formation of ^{14}C-labeled UDP-galactose during the incubation of the enzyme with ^{14}C-labeled galactose-1-P. To separate the radioactive substrate and product, alkaline phosphatase was added to the system to hydrolyze the unreacted ^{14}C-galactose-1-P to galactose-^{14}C. On DEAE-cellulose anion exchange paper the UDP-galactose and galactose are readily separated when chromatographed with glass-distilled water.

The routinely used incubation mixture contained 68 mμmoles of ^{14}C-galactose-1-P (0.167 μC), 50 mμmoles of UDP-glucose, 20 μmoles of glycine buffer, pH 8.2, 2 μmoles of dithiothreitol, 10 or 20 μl of the enzyme preparation, and water to a final volume of 200 μl with a final pH of 8.0. The reactions were started with the addition of the enzyme preparation. Incubations were carried out at 37°, and the reaction was stopped by placing the tubes in boiling water for 90 sec. The pH was then adjusted by adding 20 μl of 1 M glycine buffer, pH 8.7, and 20 μl of bacterial alkaline phosphatase (approximately 0.30 enzyme units per μl) were added, bringing the total volume of the mixture to 240 μl. After a 15-min incubation with the phosphatase at room temperature, a 10-μl aliquot (about 7000 cpm) was transferred to DEAE-cellulose anion exhange paper strips, 1½ × 17 cm, and spotted in the area between 9 and 10 cm from the bottom. The strips were chromatographed in a closed tank with quartz glass-distilled water until the front reached the bottom of the strip (30 to 40 min). After drying with a jet of cold air, the strips were cut into three sections 4, 2, and 4 cm, respectively, from the origin. The paper sections were counted at 47% efficiency in a liquid scintillation spectrometer. A 1:20 dilution of Liquifluor with toluene was used for the counting system. The UDP-galactose-^{14}C formed and remaining in the upper section equaled the decrease in alkaline phosphatase-treated ^{14}C-galactose-1-P which moved to the bottom of the strip as galactose-^{14}C. The first two sections were taken as product, but the center section was counted separately to confirm complete separation. A product correction was applied from an analysis of the complete system, which was boiled immediately upon the addition of the enzyme preparation.

Product Identification—In order to confirm the methodology, it was necessary to show that (*a*) each of the reactants galactose-1-P and UDP-glucose was stable in the incubation mixture in the presence and absence of the enzyme and (*b*) the reaction product was indeed UDP-galactose and not UDP-glucose as a result of the reaction UDP-galactose ⇄ UDP-glucose, which is catalyzed by the enzyme UDP-galactose 4-epimerase (13, 14).

Each of these considerations was examined with enzyme preparations from both the adult and newborn rat liver. ^{14}C-galactose-1-P and UDP-glucose-^{14}C were each incubated in the system and were found to be stable in both the absence and presence of enzyme. The phosphatase-treated assays showed that the galactose-1-P was hydrolyzed as expected. After hydrolysis there is always 4 to 5% of the total counts applied which does not travel as galactose but remains in the first section, and this value was repeatedly the same in our product correction applied to the routine incubations. UDP-glucose was not hydrolyzed during (*a*) the incubation, (*b*) the short boiling time under slightly alkaline conditions, or (*c*) the alkaline phosphatase treatment. Alkaline phosphatase-treated assays with labeled UDP-glucose did not reveal any labeled glucose-1-P formed by UDP-glucose pyrophosphorylase, indicating that this enzyme was inactive in our system. To confirm that UDP-galactose pyrophosphorylase (15) was not active in the system even if minute amounts of UTP were formed from UDP-glucose by UDP-glucose pyrophosphorylase, we incubated 0.25 mM UTP with labeled galactose-1-P in the routine system and found no formation of labeled product under the conditions of our experiment.

UDP-galactose-^{14}C was shown to be the reaction product by treatment of incubation mixtures with UDP-glucose dehydrogenase and subsequent descending chromatography on Whatman No. 1 paper with ^{14}C-labeled and unlabeled galactose-1-P, UDP-glucose, UDP-galactose, and UDP-glucuronic acid as standards. All of the strips were run overnight in a closed tank with 1 M ammonium acetate, pH 3.8-95% ethyl alcohol (2:5, v/v) as the solvent (16). The chromatograms of the incubation mixtures and of the standard ^{14}C-labeled markers were cut into 1½ cm sections from the origin, and the papers, 2 × 1½ cm, were counted in a liquid scintillation spectrometer. The nucleotides on the chromatograms of the unlabeled standards were located by ultraviolet absorption and marked, and then the strips were stained with ferric chloride-ethanol-sulfosalicylic acid reagent (17) to detect the galactose-1-P as well. All of the UDP-glucose-^{14}C in a control system was coverted to UDP-glucuronic acid-^{14}C, but none of the reaction product appeared as UDP-glucuronic acid-^{14}C; all of it traveled as UDP-galactose-^{14}C. The addition of DPN to the incubation prior to stopping the transferase reaction caused approximately 30% of the product to be converted to

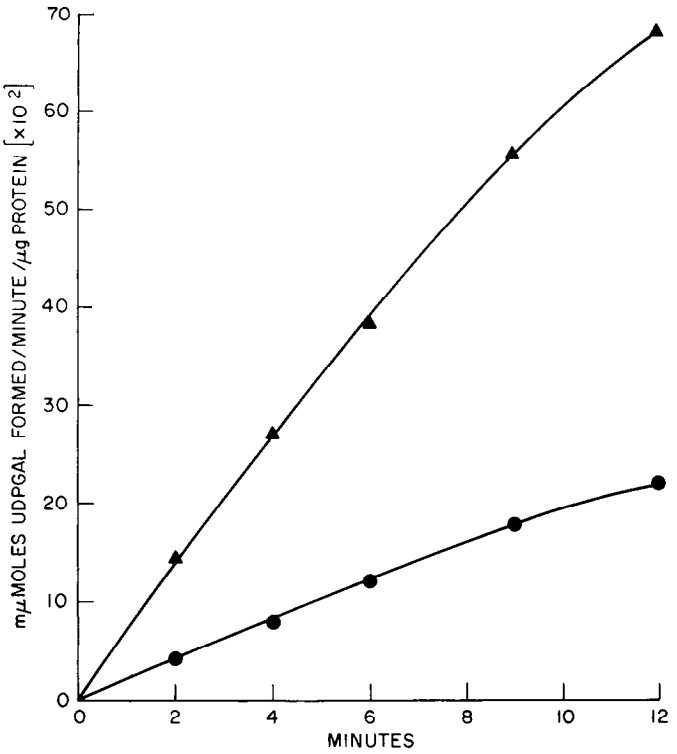

FIG. 1. Effect of time on galactose-1-P uridyltransferase velocities in adult (●——●) and newborn (▲——▲) rat liver. The experimental conditions are described in the text. *UDPGAL*, UDP-galactose.

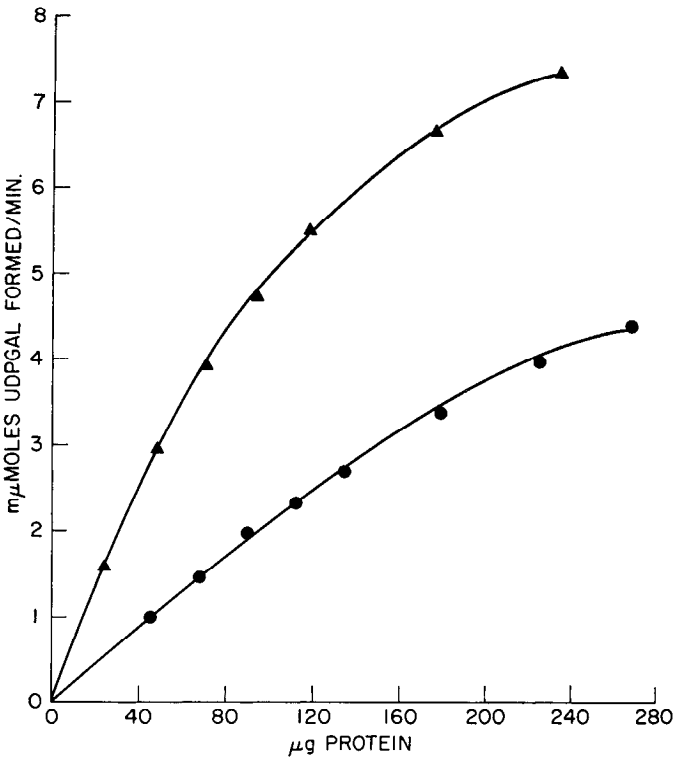

FIG. 2. Effect of protein concentration on galactose-1-P uridyltransferase velocities in adult (●——●) and newborn (▲——▲) rat liver. Experimental conditions are described in the text. *UDPGAL*, UDP-galactose.

UDP-glucuronic acid-^{14}C. This showed that UDP-galactose 4-epimerase was present in the enzyme preparation, but that it was not active in the absence of added DPN.

Enzyme Stability—Frozen tissue showed no apparent loss in enzyme activity for periods up to 1 month, but at 4° enzyme preparations gradually lost activity over a period of a week. When enzyme preparations were exposed to 50° and the activity was measured at 2-min intervals for 12 min, the rate of reduced activity was the same for the newborn and the adult liver extracts, showing a 50% loss in activity in a 3-min time period.

The omission of dithiothreitol from the reaction mixture caused a 50% reduction in activity for both the newborn and the adult liver preparations. This was in agreement with the finding of Kurahashi and Anderson (8), who showed that the activity of this enzyme in calf liver was partially protected by reduced glutathione and inhibited 50% by *p*-chloromercuribenzoate.

Initial Velocities—Fig. 1 shows that the amount of UDP-galactose formed by the newborn preparation (20 μg of liver protein) was proportional to the incubation time for 7 min, and that formed by the adult preparation (60 μg of liver protein) was proportional for 9 min. Five minutes was chosen as a convenient routine incubation time period. The specific activity of the enzyme is expressed as millimicromoles of UDP-galactose formed per min per μg of soluble protein.

Galactose-1-P uridyltransferase activity was proportional up to 150 μg of liver protein (50 μl) for the adult extract and 50 μg (25 μl) for the newborn (Fig. 2). Different volumes of the supernatants were used to obtain the desired protein concentration, and it was necessary to add proper concentrations of KCl to maintain uniform conditions of comparison. Controls, however, showed

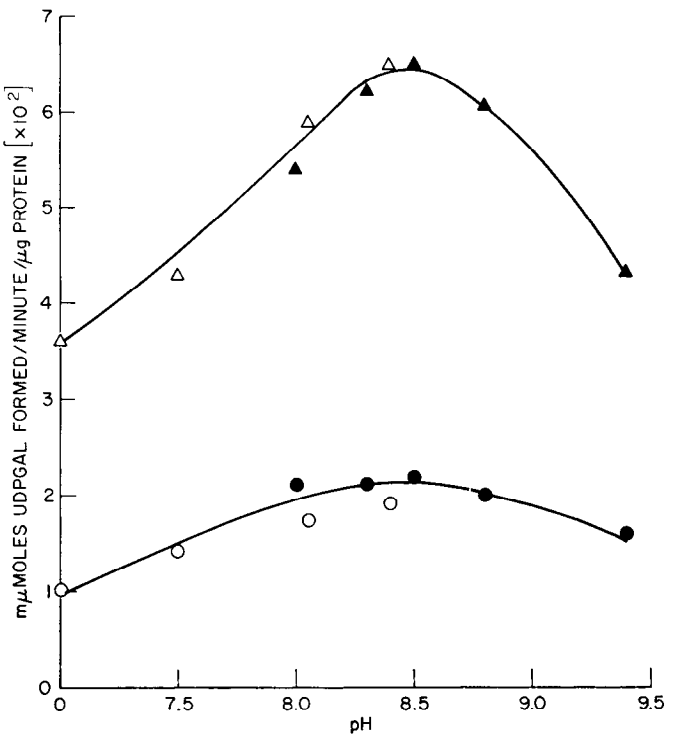

FIG. 3. Effect of pH on the enzyme reaction. Buffers for the pH adjustments were Tris-HCl (△——△) and glycine (▲——▲) for the newborn and Tris-HCl (○——○) and glycine (●——●) for the adult enzyme assays. Experimental conditions are described in the text. *UDPGAL*, UDP-galactose.

that the added KCl had no effect on the assay. For the routine assay, 10 and 20 μl, respectively, of the supernatants from the newborn (20 μg of protein) and the adult (60 μg of protein) liver preparations were used.

RESULTS

Characteristics of Rat Liver Galactose-1-P Uridyltransferase

pH Optimum—The relationship between pH and the rate of reaction is shown in Fig. 3. Final pH values of the reaction mixtures are shown. They were determined with a Beckman pH meter and a Radiometer, with the use of a scale expander and Leeds and Northrop electrodes. The reaction mixtures were adjusted with 20 μmoles of glycine or Tris-HCl buffer between pH 7.0 and 9.4. Galactose-1-P uridyltransferase has a pH optimum between 8.3 and 8.6. Time curves for the newborn preparations done at pH 8.5, however, indicated nonlinearity in the reaction rate for short incubation times; consequently, pH 8.0 was routinely used to insure measurement of initial velocities for all preparations under identical conditions.

Effects of Substrate Concentration—Lineweaver-Burk analyses

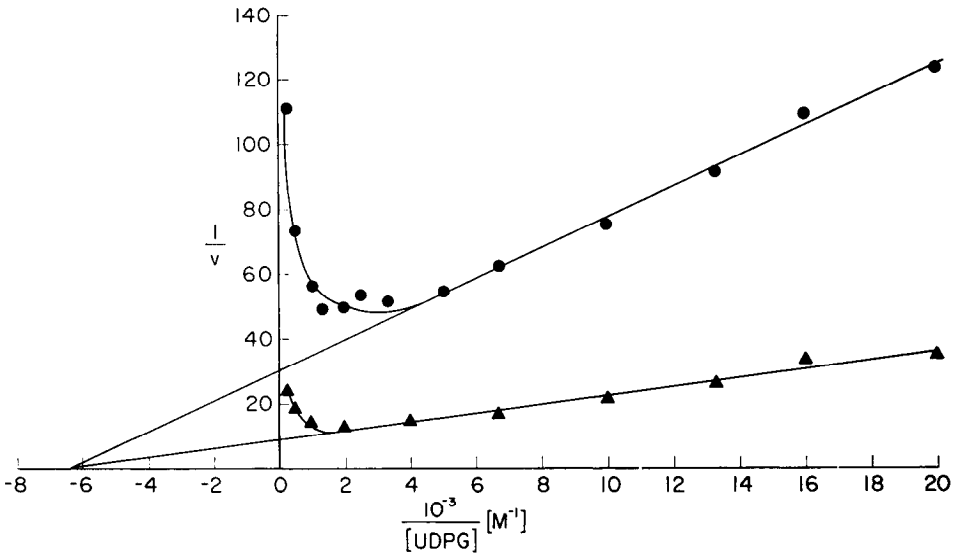

FIG. 4. Effect of UDP-glucose (*UDPG*) concentration on enzyme activity at 0.34 mM galactose-1-P. Initial velocities (v) are expressed as millimicromoles of UDP-galactose formed per min per μg of newborn (▲——▲) and adult (●——●) rat liver protein. Conditions are described in the text.

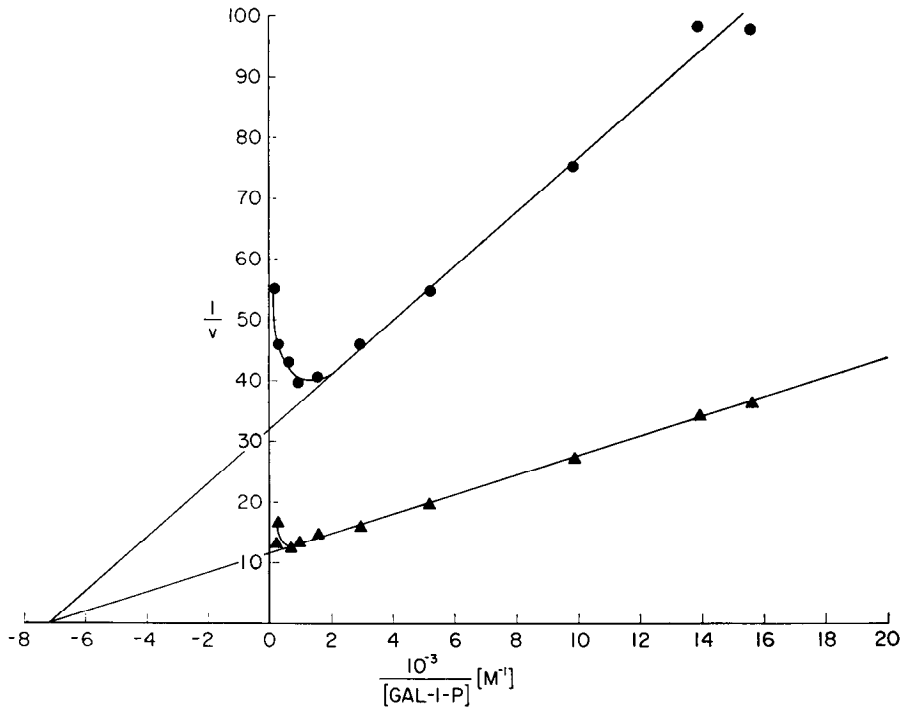

FIG. 5. Effect of galactose-1-P (*GAL-1-P*) on enzyme activity at 0.25 mM UDP-glucose. Initial velocities (v) are expressed as millimicromoles of UDP-galactose formed per min per μg of newborn (▲——▲) and adult (●——●) rat liver protein.

TABLE I

Effect of various product concentrations on adult rat liver enzyme activity

The reported values are specific activities × 10^3, and the conditions are those described in the text for the routine incubation. Two adult rat liver enzyme preparations were used.

Product concentration	Enzyme activity	
	With glucose-1-P	With UDP-galactose
mM		
0	17.92	24.75
0.5	12.71	28.86
1	7.79	28.01
2	3.38	26.54
4	0.75	24.20

showing the relationship between the concentration of UDP-glucose, galactose-1-P, and the enzyme activity in the adult and newborn liver are presented in Figs. 4 and 5. The K_m values for the adult and the newborn liver enzymes were the same, but the V_{max} for the newborn enzyme was 3 times that for the adult in both instances. The V_{max} for galactose-1-P at 0.25 mM UDP-glucose was 0.031 for the adult, and for the newborn it was 0.087. The V_{max} value for UDP-glucose at 0.34 mM galactose-1-P was 0.033 for the adult, and for the newborn it was 0.111.

Substrate Inhibition—Under the conditions of the experiments, the newborn enzyme was inhibited by substrate concentrations approximately 3 times greater than those for the adult enzyme. At 0.25 mM UDP-glucose, substrate inhibition was observed with 0.5 and 1.5 mM galactose-1-P, respectively, for the adult and the newborn enzymes. UDP-glucose concentrations of 0.3 and 0.8 mM for the adult and the newborn, respectively, showed inhibition with galactose-1-P at 0.34 mM.

Product Inhibition—Product inhibition was also examined. Table I shows the effect of various concentrations of glucose-1-P and UDP-galactose on the activity of the adult enzyme. From graphic analysis of this data, the enzyme activity appeared to be 50% inhibited at 0.8 mM glucose-1-P. The unexpected effect of UDP-galactose, indicating increased activities, led us to investigate UDP-galactose as a substrate instead of UDP-glucose in the routine incubation. We found that the decrease in ^{14}C-labeled galactose-1-P exactly equaled the formation of ^{14}C-labeled UDP-galactose, and that the rate of this stoichiometric reaction was the same as that observed for UDP-glucose. The labeled product was identified as UDP-galactose by the chromatographic procedure described with the use of UDP-glucose dehydrogenase, and the unlabeled substrate was found to be free of UDP-glucose contamination by a spectrophotometric analysis with UDP-glucose dehydrogenase. Fig. 6 shows the effect of UDP-galactose concentration on the activity of both the newborn and the adult enzyme extracts at 0.34 mM galactose-1-P. From the Lineweaver-Burk analysis, the UDP-galactose K_m was determined to be 1.67 × 10^{-4} M for both the enzyme preparations. This value is quite similar to that observed for UDP-glucose for the two preparations, and the V_{max} values are also approximately the same: 0.032 for the adult and 0.105 for the newborn. The only difference in the effect of the two substrates seemed to be that at 1 mM UDP-galactose there was no apparent inhibition whereas UDP-glucose showed substrate inhibition at even lower concentrations.

None of the following nucleotide sugars showed any significant activity with either the newborn or the adult enzyme extracts: ADP-glucose, CDP-glucose, GDP-glucose, GDP-mannose, TDP-glucose, UDP-xylose, and UDP-mannose.

Enzyme Activity Changes in Various Tissues in the Developing Rat

Table II lists enzyme activity levels in various adult and newborn rat tissues. The liver had the highest activity of any of the tissues studied in both the newborn and the adult, and the enzyme activities in all the newborn tissues were higher than in corresponding adult tissues. The transferase activity in adult female rat liver was significantly lower than that for the male

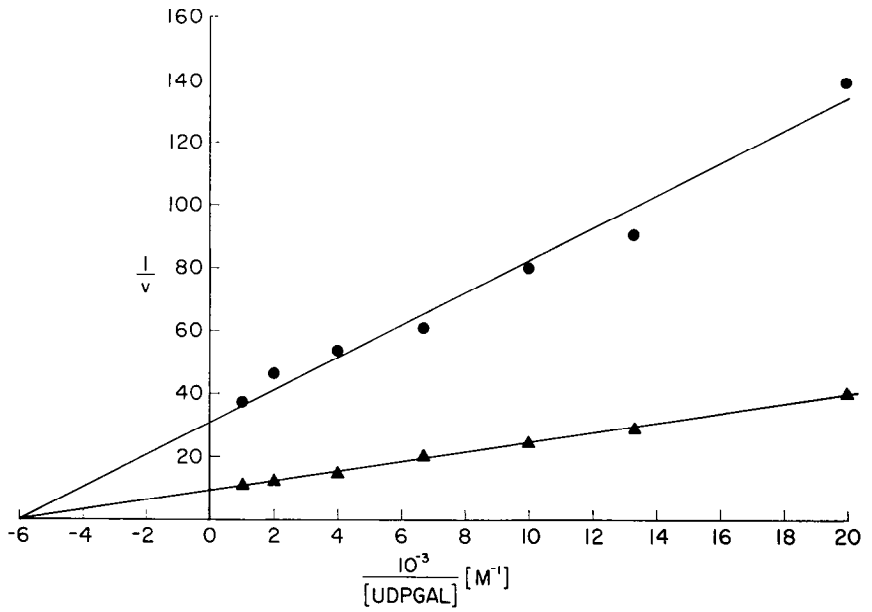

FIG. 6. Effect of UDP-galactose (*UDPGAL*) concentration on enzyme activity at 0.34 mM galactose-1-P. Initial velocities (v) are expressed as millimicromoles of galactose-1-P converted per min per μg of newborn (▲——▲) and adult (●——●) liver protein.

(20%). Galactokinase activity in liver of male adult rats is also significantly higher than in the female rat (18).

Galactose-1-P uridyltransferase activity varied in the developing rat tissues as is shown in Fig. 7. In male liver there was a continuous and striking increase in enzyme activity from 3 days before birth until a maximum level at 10 days after birth, followed by a gradual decrease in activity to adult levels in animals between 35 and 45 days old. In gut and kidney, the level of enzyme activity was approximately the same at each age, and neither of these tissues had a significant increase in activity from term to 10 days after birth, but there was subsequent gradual decrease to adult levels in the 35-day-old animal. The enzyme activity of mixtures of liver homogenates of newborn and 45-day-old rats with liver homogenates of 10-day-old animals was a summation of the individual enzyme activities, thus indicating that neither activators nor inhibitors are responsible for the changes in activity shown in Fig. 7.

TABLE II

Galactose 1-phosphate uridyltransferase activities in various rat tissues

Except as indicated for liver, all adult tissues were from male animals. The experimental conditions are those described in the text for the routine assay. The values given are (average specific activity ± standard error of the mean) × 10^3. Activities reported in the adult male and female liver differed significantly ($p < 0.01$) as determined with the t test.

Tissue	Specific activity	No. of homogenates	Animals per homogenate
Adult			
Liver			
Male	20.70 ± 0.94	9	1
Female	16.40 ± 0.89	9	1
Kidney	8.20 ± 0.54	6	1
Gut	6.50 ± 0.56	6	2
Diaphragm	5.50 ± 0.29	4	2
Brain	3.20 ± 0.11	4	1
Newborn			
Liver			
Male	63.70 ± 2.96	10	3
Kidney	15.30 ± 0.88	3	7
Gut	14.30 ± 0.67	3	10

DISCUSSION

The observations reported here clearly indicate striking variations in liver galactose-1-P uridyltransferase activity with respect to animal age. The developmental changes for transferase in liver follow a pattern similar to those reported for galactokinase (18) determined by similar methods of measurement. Galactokinase (4, 19, 20) and transferase (2, 4–6) activities have been demonstrated in various mammalian tissues, but the activities of these enzymes in liver are so much greater that it must be considered the key organ in controlling the conversion of galactose to glucose. The maximum activity for liver galactokinase (18) occurs in the 5-day-old animal whereas the transferase activity is maximum in the 10-day-old animal. Whether or not this observation may be significant in the sequential development of the enzymes remains to be examined.

The decrease in activity of both these enzymes in liver with increasing age is in agreement with some of our earlier studies which suggested that the adult liver had a lower capacity to utilize galactose than the newborn liver (21). In order to correlate the decreased galactose utilization in the adult with an increase in transferase activity which Isselbacher reported (22), it was proposed that galactokinase may be the rate-determining enzyme in the series of reactions converting galactose to glucose (21). Even though transferase activities have now been shown to be decreased in the adult, the enzyme activities are considerably greater than galactokinase activities at any given age, and the theory for the rate-limiting step is still applicable. The 8-fold higher activities of transferase over galactokinase reported by Isselbacher *et al.* (23) in erythrocytes would also support this theory.

Our data on developmental changes are in contrast to the preliminary data published by Isselbacher (22), who reported a higher transferase activity in adult liver than in the newborn. Isselbacher used the UDP-glucose consumption test to determine transferase activities in liver, and there are no data available on the applicability of that method for measuring the enzyme in liver. In view of the different methods employed, it is difficult to evaluate the discrepancies in our findings.

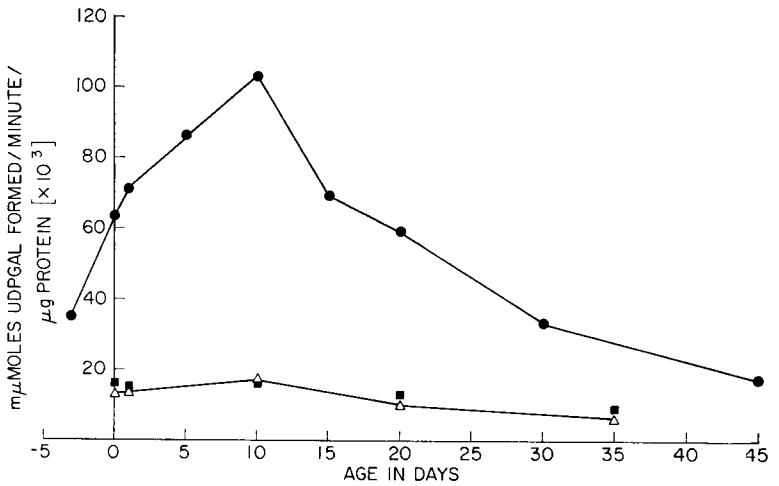

FIG. 7. Developmental changes in galactose-1-P uridyltransferase activity in rat liver (●), gut (△), and kidney (■). Each *point* represents an average of three to six determinations. Experimental conditions are described in the text. *UDPGAL*, UDP-galactose.

The similarity in the kinetic properties of transferase from the newborn and adult liver preparations strongly suggests that the enzyme from both sources is the same. Both had the same K_m values, sulfhydryl requirements, and stability characteristics. The higher V_{max} values with the newborn extracts may be due to actual increases in the amount of enzyme protein, but this explanation remains to be examined after purification of the enzyme.

The results of our studies of substrate and product inhibition suggest several possible factors contributing to the control of galactose-1-P uridyltransferase activity in the biological system. Transferase is inhibited by relatively low concentrations of UDP-glucose; consequently those factors regulating the tissue levels of UDP-glucose such as the rate of its production by UDP-glucose pyrophosphorylase and its utilization in either glycogen synthesis or glucuronic acid synthesis, would also have an effect on transferase.

The effect of galactose-1-P concentration on transferase activity certainly must be considered in relation to galactokinase activity, which supplies the main source of this substrate. Galactokinase in the newborn is more sensitive to product inhibition by galactose-1-P than that in the adult, and transferase in the newborn is less sensitive to substrate inhibition by galactose-1-P than that in the adult. In addition, in the newborn, transferase activities are 1½ times greater than galactokinase activities, and, in the adult, transferase is 3 times more active than galactokinase. It appears that liver may provide a natural regulatory system to prevent an accumulation of galactose-1-P in the normal animal, especially in the newborn, which ingests large amounts of galactose. Prevention of galactose-1-P accumulation may be of great importance, since this compound may be one of the etiological factors in the galactose toxicity syndrome.

The product glucose-1-P appeared to be an extremely effective inhibitor of the transferase in the adult. The source and many fates of glucose-1-P in the system may also contribute to controlling the transferase at various stages of development.

UDP-galactose did not inhibit transferase activity as expected; in fact, it seemed to increase the enzyme activity as measured in the forward reaction. The possibility that we were measuring another transferase led us to examine a number of nucleotide sugars as substrates with labeled galactose-1-P. UDP-Galactose was the only nucleotide sugar other than UDP-glucose to cause any appreciable formation of labeled product. The similarity of the K_m and V_{max} values determined for UDP-galactose and UDP-glucose suggests that they are both substrates for the same enzyme. It is quite possible that this reaction partially contributes to the decreased rate of enzyme activity observed with respect to both time and increased protein concentration. In our routine system with UDP-glucose as the substrate, the accumulating labeled UDP-galactose would react with the labeled galactose-1-P, and in time the rate for the latter reaction would approach the reaction rate of UDP-glucose, causing an over-all reduced rate of labeled product formation.

Galactose is a major nutrient for the young of most animal species. The high rate of over-all galactose utilization (21) accompanied by high levels of galactokinase (18) and transferase activity found in the liver of the young rats may also occur in the human. Indeed, Vink and Kroes (24) reported that the elimination rate of intravenously administered galactose was higher in young children than in adults, and Haworth and Ford (25) have found that large amounts of orally administered galactose cause much greater increases in blood glucose in infants and young children than in adults. The galactokinase level of human erythrocytes has been found to be highest after birth with subsequent progressive decrease to constant low levels at age 2 or 3 years (26).

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