ARTICLE:

Mammalian Galactokinase: DEVELOPMENTAL AND ADAPTIVE CHARACTERISTICS IN THE RAT LIVER

Pedro Cuatrecasas and Stanton Segal

J. Biol. Chem. 1965, 240:2382-2388.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 240, No. 6, June 1965
Printed in U.S.A.

Mammalian Galactokinase

DEVELOPMENTAL AND ADAPTIVE CHARACTERISTICS IN THE RAT LIVER

PEDRO CUATRECASAS AND STANTON SEGAL

From the Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland 20014

(Received for publication, February 28, 1965)

Galactokinase catalyzes the formation of α-D-galactose 1-phosphate from adenosine triphosphate and D-galactose (1, 2) and is the first enzyme in a series by which galactose enters the varied pathways of glucose metabolism (3, 4). Although galactokinase has been studied extensively in various microorganisms, in mammalian tissues investigations have been limited to confirmation of its presence in liver, brain, and erythrocytes (3, 5–7).

Studies of mammalian galactokinase have been undertaken in order to evaluate the participation of the enzyme in the regulation of galactose metabolism, especially in the young in which there is a critical dependence on dietary galactose. The present work describes certain properties of young and adult rat liver galactokinase and suggests the presence of at least two different hepatic enzymes capable of phosphorylating galactose. Galactokinase activity was also measured in several other rat tissues. Changes occurring during development and those induced by dietary treatment are described.

EXPERIMENTAL PROCEDURE

Animals—Sprague-Dawley rats weighing about 200 g were fed standard Purina chow (galactose-free) unless specified otherwise and were given water *ad libitum*. Groups of animals on special diets were always accompanied by paired control groups which were tested the same day. Young animals were ordinarily kept with their mother until 40 days of age. A 40% galactose diet was prepared by mixing 400 g of galactose with 600 g of the control diet. Gestational age of pregnant animals was estimated from the dates of mating.

Enzyme Preparations—The rats were killed by stunning with a blow to the head, decapitation, and immediate exsanguination. About 100 to 200 mg of the appropriate tissue was rapidly removed, weighed, and homogenized. Liver specimens were taken from the right lobe of the liver except in fetal and newborn animals where several lobes were used. Gut specimens were from the proximal jejunum, just distal to the ligament of Treitz. From fetal and newborn animals the whole kidney was used, and in adults a wedge was obtained from the cortex. Sartorius and diaphragm were used for muscle specimens, and brain tissue was excised from the frontal cortex.

The tissues were homogenized at 0° in ground glass grinders with 4 volumes of 0.01 M sodium phosphate buffer, pH 6.7, containing 0.01 M mercaptoacetic acid, 0.001 M disodium Versenate, and 0.01% recrystallized bovine serum albumin. The crude extracts were centrifuged at 30,000 × *g* for 30 min, and the supernatant fractions were diluted 1:4 with the same buffer to obtain enzyme preparations containing approximately 50 μg of protein per 10 μl. Protein determinations were performed by the method of Lowry *et al.* (8) with crystallized bovine serum albumin for standards. All enzyme preparations were assayed within 2 hours of tissue removal and were kept at 5° until that time.

Galactokinase Assay—The galactokinase assay procedure was modified from that described by Sherman and Adler (9, 10). The incubation mixture contained 40 mμmoles of D-galactose-1-^{14}C, 600 mμmoles of ATP, 500 mμmoles of NaF, 500 mμmoles of $MgCl_2$, 1 μmole of mercaptoethanol, 20 μmoles of Tris-hydrochloride buffer at pH 7.8, and 10 μl of enzyme preparation (approximately 50 μg of protein) in a total volume of 100 μl with a final pH of 7.1.

Incubations were carried out at 37°, and the reactions were stopped at the proper time by spotting 30-μl aliquots onto DEAE-cellulose paper strips (1.5 × 15 cm). The latter were chromatographed with glass-redistilled water until the front reached the bottom of the strips. After drying, the lowermost 4-cm section (galactose-1-^{14}C) and the 4-cm section above this (galactose-1-P-^{14}C) were counted separately at 50% efficiency in a liquid scintillation spectrometer. The solvent consisted of 0.010% *p*-2′-(5′-phenyloxazolyl)benzene and 0.457% 2,5-diphenyloxazole in toluene.

The decrease in galactose radioactivity was exactly equaled by the increase in the galactose-1-P region of the paper strips. No galactose-1-P was detected if ATP was omitted from the reaction mixture or if the enzyme preparation was boiled for 5 min. With both adult and newborn liver enzyme preparations, the reaction product was completely hydrolyzed by heating for 2 min at 90–95° in 0.3 N HCl, as expected for galactose-1-P (11).

The presence of only two reaction products, galactose and galactose-1-P, was confirmed by paper chromatography with 95% ethanol and M ammonium acetate, pH 3.8 (7.5:3, v/v) (12). The sugars were detected with ferric chloride-ethanol-sulfosalicylic acid reagent (13), and the chromatographs were examined for fluorescence and studied with a windowless gas flow paper strip scanner with 4 π counting geometry.

The amount of galactose phosphorylated by adult and newborn rat liver supernatants was proportional to the length of incubation during the first 10 min, after which a fall in rate was observed (Fig. 1). Hence, initial velocities were obtained with 6-min incubation periods. Specific activity was defined as micromoles of galactose-1-P formed per min per g of tissue

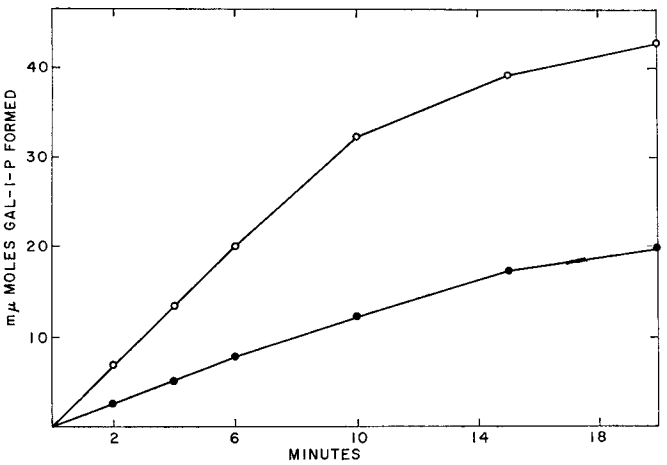

FIG. 1. Time course of liver galactokinase assay for adult (●——●) and newborn (○——○) rats. Measurements were made from 300-μl reaction mixtures containing galactose-^{14}C, 0.4 mM; ATP, 6.0 mM; NAF, 5 mM; mercaptoethanol, 10.0 mM; Tris-hydrochloride buffer, 0.2 M, pH 7.8; and 30 μl of dilute 30,000 × *g* supernatant fraction. Incubations were at 37°. Values given are for 100 μg of liver protein.

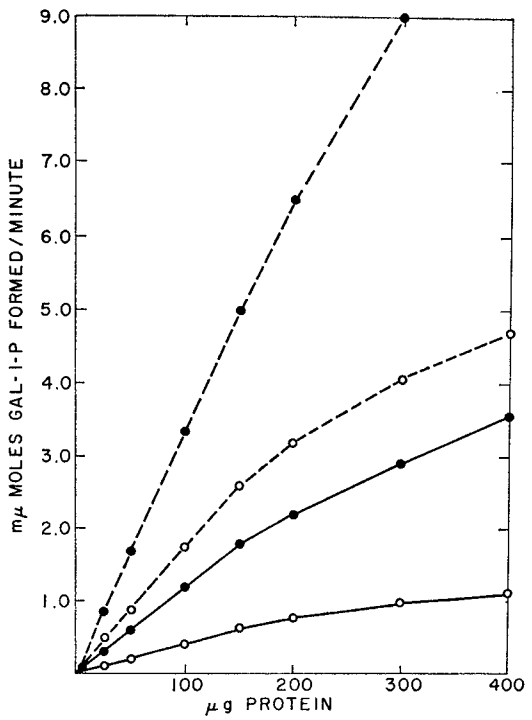

FIG. 2. Effect of protein concentration of supernatant fraction of adult (——) and newborn (– – –) liver (●) and gut (○) on initial velocity of the galactokinase reaction. Incubation mixtures were as given in Fig. 1 except that the total volume was 100 μl. Varying amounts of protein were contained in 10-μl aliquots of the supernatant preparations. Incubations were carried out at 37° for 6 min.

protein. Galactokinase activity was proportional to supernatant protein up to 150 μg (per 10 μl) for adult and newborn liver and gut (Fig. 2). In all subsequent experiments supernatant enzyme preparations contained 40 to 60 μg of protein per 10 μl.

Reagents—Glucose-free D-galactose, galactose-1-P, ATP, and UDP-glucose were obtained from Sigma Chemical Company. Regular insulin and protamine zinc insulin were purchased from Eli Lilly and Company. D-Galactose-1-^{14}C (specific activity, 1.32 to 3.05 mC per mmole) was obtained from Nuclear Research Chemicals, Inc., Nuclear-Chicago Corporation, and from the National Bureau of Standards. Uniformly labeled D-galactose-1-P-^{14}C (specific activity, 13.0 mC per mmole) was purchased from Volk Radiochemical Company. D-Galactose for use in diets was purchased from Pfanstiehl Laboratories, Inc. Whatman No. DE80 was used as the DEAE-cellulose anion exchange paper.

RESULTS

Characteristics of Liver Galactokinase—The effect of galactose and ATP concentration on galactokinase activity was determined on fetal, newborn, and adult liver. Lineweaver-Burk analyses for adult and newborn are presented in Fig. 3. Substrate inhibition of galactokinase activity was first detected at galactose concentrations of 3.2 mM for adult liver, 0.8 mM for newborn liver (Fig. 3), and 0.4 mM for 18-day-old fetal liver. Increasing the ATP concentration above 20 mM at a constant galactose concentration (0.4 mM) also caused marked inhibition of galactokinase activity in adult liver. High ATP concentrations did not reverse the galactose inhibition, and galactose did not decrease the inhibition caused by ATP. Magnesium did not affect the inhibition caused by either substrate.

The Michaelis constants (K_m) for galactose from Lineweaver-Burk plots (ATP, 6 mM) were 1.5×10^{-4} M for adult, 6.5×10^{-4} M for newborn (Fig. 3), and 9.1×10^{-4} M for 18-day fetal liver. The K_m for ATP (galactose, 0.4 mM) was 1.2×10^{-4} M for adult

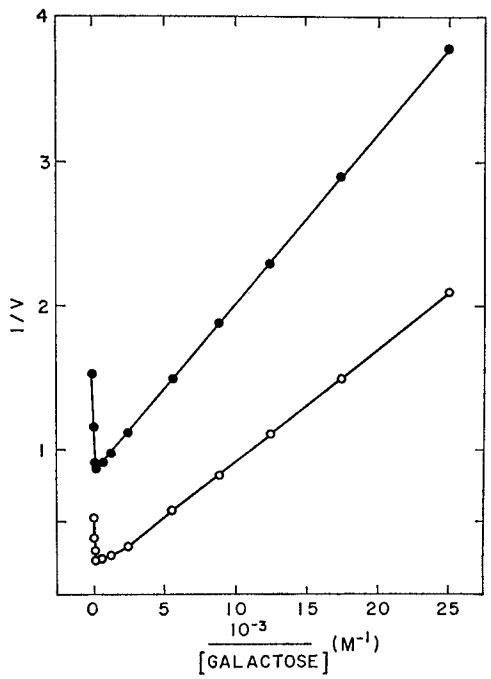

FIG. 3. Plot of reciprocal initial velocity with respect to reciprocal galactose concentration for galactokinase system of adult (●——●) and newborn (○——○) rat liver. Incubation procedures were as described in Table I except that galactose concentration was varied from 20 to 0.04 mM. Initial velocity is expressed as millimicromoles of galactose-1-P formed per min per 100 μg of liver protein.

liver. The V_{max} values for galactose at 6 mM ATP were 1.2 for adult, 6.7 for newborn, and 6.4 for 18-day fetal liver and were expressed as micromoles of galactose-1-P produced per g of protein. The enzyme preparation from newborn animals therefore has a K_m 4 times greater and a V_{max} 6 times greater than the preparations from adult animals, and it is inhibited by galactose at concentrations which are 4 times lower than those which affect the adult preparation. This inhibiting concentration of galactose is near the K_m for the newborn preparation, but it is 20 times greater than the K_m for the adult preparation.

Galactose-1-P caused inhibition of both young and adult liver galactokinase activity, but there were some important differences between the two (Fig. 4). The preparation from the 2-day-old animal, which exhibited a much greater initial velocity and V_{max} compared with the adult, was about 60% inhibited at all galactose concentrations by a galactose-1-P concentration (1 mM) which caused only a 15% inhibition of the adult extract

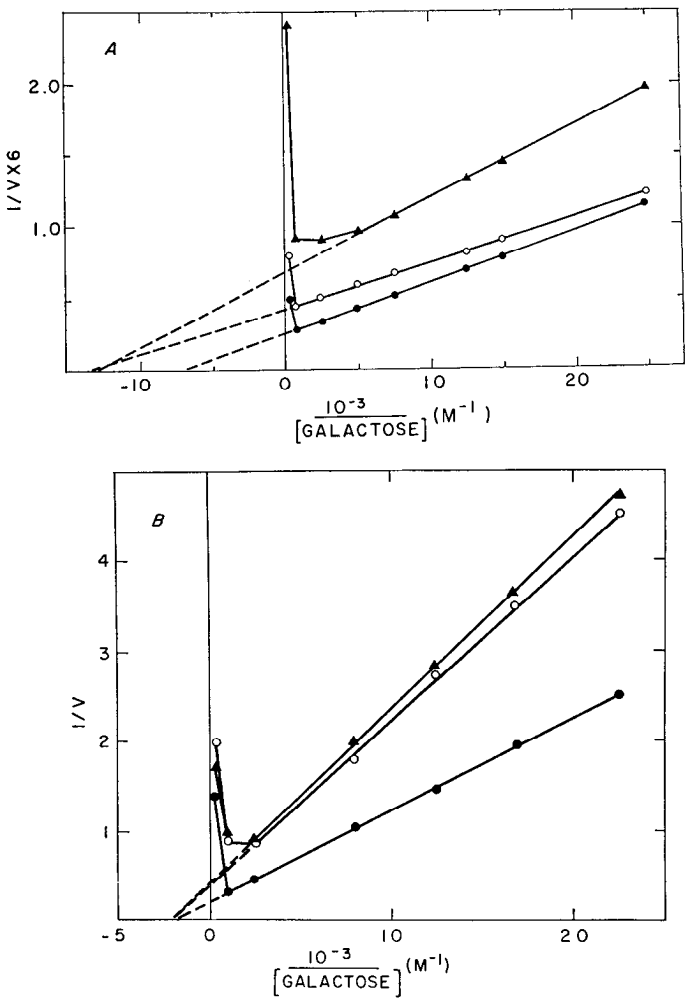

FIG. 4. Lineweaver-Burk analysis of the effects of galactose-1-P on adult (*A*) and 2-day-old (*B*) rat liver galactokinase. Incubations were as described in Table 1 (●——●) except for the additional presence of 1 mM (○——○) and 5 mM (△——△) galactose-1-P in those two plots. For all incubations in *A* and *B*, 10-μl aliquots (approximately 50 μg of protein) of the same enzyme preparations were used. Initial velocity is expressed as millimicromoles of galactose-1-P produced per min per 10 μl of supernatant preparation.

TABLE I

Galactokinase activity of various adult rat tissues

All animals were male except as indicated for liver. Activity is expressed as the average specific activity ± s. e. of the mean as described in the text. The incubation mixture contained galactose-^{14}C, 0.4 mM; ATP, 6.0 mM; NaF, 5 mM; $MgCl_2$, 5 mM; mercaptoethanol, 10.0 mM; Tris-hydrochloride buffer, pH 7.8, 0.2 M; and 10 μl of tissue supernatant (about 50 μg of protein) in a total volume of 100 μl. Incubations were for 6 min at 37°.

Tissue	No. of animals	Galactokinase activity
Liver		
Male	11	12.00 ± 0.13
Female	11	9.54 ± 0.18*
Gut	4	4.14 ± 0.10
Kidney	4	4.66 ± 0.19
Brain	2	3.24 ± 0.07
Muscle		
Sartorius	2	0.88 ± 0.07
Diaphragm	3	2.03 ± 0.16

* Male and female differ with $p < 0.001$; level of significance determined from t test for two sets of nonpaired data with homogeneous variance, the latter confirmed by F test of variance analysis (14).

activity. Increasing the galactose-1-P from 1 to 5 mM had essentially no further effect on the activity obtained with the 2-day-old liver preparation but now clearly caused a 50% inhibition of the adult enzyme activity. In the presence of both 1 and 5 mM galactose-1-P the galactose inhibition seen with the enzyme of the 2-day-old animal first appeared at 0.4 mM galactose rather than at 0.8 mM when galactose-1-P was not present. However, with the adult extract this change in galactose inhibition was seen only with 5 mM galactose-1-P, and the inhibition was first manifest at 0.2 mM galactose compared with 3.2 mM in the absence of galactose-1-P. Furthermore, both 1- and 5-mM concentrations of galactose-1-P altered the adult enzyme K_m for galactose from 1.5×10^{-4} to 8×10^{-5}, but both failed to affect the K_m of the enzyme obtained from the younger animals. Thus product inhibition in the newborn preparation is noncompetitive while in the adult extracts it is uncompetitive. Galactose-6-P at 5 mM concentration produced no significant inhibition of activity of either adult or newborn liver preparations.

The highest galactokinase activity of the five tissues studied was found in liver preparations (Table I). The activity of livers of adult males (12.00) was significantly higher than that of adult females (9.54), and the activity of diaphragm was significantly higher than that of sartorius muscle. Differential centrifugation studies of these five tissues were performed. The total and specific enzyme activities were determined on crude preparations and on 30,000 × *g* and 100,000 × *g* supernatants. Galactokinase activity was located entirely in the cytoplasmic (soluble) fraction in all adult tissues as well as in newborn liver and gut.

Developmental Changes in Galactokinase Activity—Assessment of the changes in liver galactokinase activity with increasing age were complicated by the differences in kinetic properties of young and adult liver tissues. All initial velocity measurements for developmental studies therefore were done at three widely different galactose concentrations (0.04, 0.4, and 4.0 mM). The results at these three concentrations were similar, and the follow-

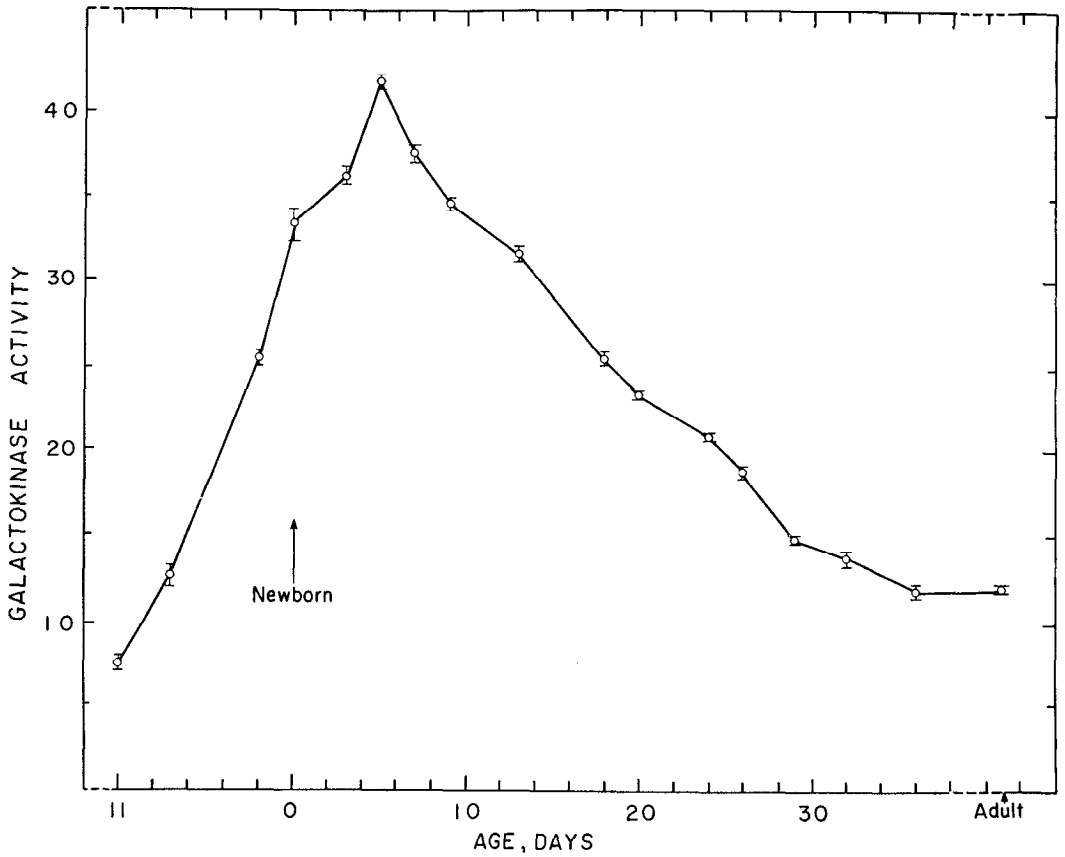

FIG. 5. Changes in galactokinase activity in the developing rat liver. All animals older than newborn were male. Each *point* represents the average specific activity ± s. e. of the mean of 2 to 11 animals (total of 70 rats). Livers from an entire litter were pooled for the 11- and 14-day fetus determinations, two separate litters being used in each case. All animals were kept with their mothers until 40 days of age. Fetuses are represented by gestational age. Assay procedures are described in Table I.

ing studies are presented for 0.4 mM galactose; this concentration was for all tissues near the K_m value and near that concentration which resulted in highest measured initial velocities.

The galactokinase activity of embryonic liver increased sharply during the last 4 days of gestation and continued to rise after birth, reaching a maximum of 42.0 in animals 5 days old (Fig. 5). The activity then decreased slowly to reach the adult level of 12.0 in males 36 days old.

The possibility that the age differences in activity were due to changes in nonspecific phosphatases was investigated. The previous assay procedures were used, except that galactose-1-^{14}C was replaced by uniformly labeled galactose-1-P-^{14}C in various concentrations. The radioactivity which migrated down the DEAE-cellulose paper strips with deionized water resulted from hydrolysis of galactose-1-P to free galactose. With 60 μM galactose-1-P the adult liver preparation hydrolyzed 0.081 mμmoles per min per 100 μg of protein compared with 0.195 mμmoles per min per 100 μg of protein for the 5-day-old liver. Hence, if galactose-1-P hydrolysis played a role in the galactokinase assays, it would have been to diminish rather than magnify the measured differences between young and adult animals. Furthermore, these hydrolysis rates were 15 to 20 times lower than the rates obtained for phosphorylation. This suggests that galactose-1-P hydrolysis was a relatively insignificant factor in the galactokinase assays. This is especially true since the hydrolysis rates were measured with initial galactose-1-P concentrations which were 2 to 3 times greater than were found at the end of the usual 6-min incubations.

The kinetic data suggest that there are at least two liver enzymes capable of phosphorylating galactose. Further evidence for this comes from apparent differences in the stability of newborn and adult liver preparations. After storage at 10° for 24 hours the adult and newborn liver preparations had lost 62 and 27%, respectively, of their original galactokinase activities (averages of five experiments). From mixing experiments, it did not appear that these differences were due to greater proteolytic activity in the adult liver preparation causing more rapid breakdown of galactokinase (Table II). The differences in stability must be largely due to factors inherent in the enzyme systems. Furthermore, since both immediately and 24 hours after mixing the resultant activities were as predicted from the individual specific activities, it is unlikely that inhibitors or activators were responsible for the measured differences in young and adult liver galactokinase activities.

The galactokinase activity of gut and kidney tissue from young animals was also higher than that of adults although the differences were less striking than for liver (Table III). The 18-day fetal and newborn gut had almost twice as much galactokinase activity as was found for kidney at these two ages. By 5 days, however, the activities of gut and kidney were nearly the same. Immediately before and after birth, neither tissue

TABLE II

Galactokinase activity obtained by mixing adult and young rat liver enzyme preparations

Assays were performed with the enzyme preparations immediately upon preparation and after storage at 10° for 24 hours. Values represent specific enzyme activities. The predicted activity of the mixture of adult and 3-day-old rat liver supernatants is based on the proportions of mixing and the specific activity of each. The incubation procedures are described in Table I.

Time of assay	Adult rat	3-day-old rat	Mixture	
			Predicted	Measured
Initially	11.2	37.0	26.8	27.8
24 hours	5.12	24.8	16.5	16.3

TABLE III

Galactokinase activity of developing rat gut and kidney

All animals older than newborn were male. The two tissues in each age group were obtained from the same animals. The incubation procedures are described in Table I. Activity is expressed as the average activity ± s. e. of the mean.

Age of animals	No. of animals	Galactokinase activity	
		Gut	Kidney
−3 days	1	20.08	10.35
Newborn	2	17.79 ± 0.26	10.07 ± 0.12
5 days	3	10.68 ± 0.58	10.69 ± 0.20
18 days	3	6.45 ± 0.01	6.23 ± 0.20
24 days	3	3.81 ± 0.08	5.44 ± 0.23
36 days	2	4.16 ± 0.07	4.72 ± 0.18
Adult	4	4.14 ± 0.10	4.66 ± 0.19

showed the sharp rise in activity which was observed for liver, and both had fallen to the adult level earlier than liver.

Adaptive Aspects of Liver Galactokinase Activity—There was no change in liver galactokinase activity in male or female adult rats after 5 days of feeding *ad libitum* on a 40% galactose diet (Table IV). However, after 20 days of such a diet there was a significantly higher activity compared with the animals receiving the control diet.

Changes in liver galactokinase activity in response to dietary modifications were more easily shown in developing rats (Table V). The normal fall in activity with increasing age could be readily accelerated by separating the young animals (16 or 18 days) from their mothers and by feeding them the standard control (galactose-free) diets. However, the activity did not fall below the level seen in adults, and if such treatment were started at 24 days the accelerated fall could no longer be seen. Animals younger than 14 days when separated from their mothers failed to grow and thrive as the controls. Eight days of feeding a 40% galactose diet to 16-day-old rats not only prevented the usual fall in galactokinase activity but also resulted in higher activity levels than were found in nursing 16-day-old rats. This increase did not occur if 18-day-old rats were used, although the usual fall in activity could be prevented. The high galactose diets, started at age 16 or 18 days, did not prevent a fall in activity if continued longer than 8 days. But when these animals reached 36 to 37 days of age the activity was nevertheless higher than found in adults or control animals kept with their mothers. Although the galactokinase activity in adulthood of all these young galactose-fed animals was higher than that found in the control rats or normal adults, the ones which began the diets at 16 days of age had activities similar to those which had begun the diets at 24 days of age.

Fasting for 24 or 48 hours and insulin administration *in vivo* or *in vitro* to fasted, normal, or galactose-fed animals caused no significant changes in the galactokinase activity of liver or muscle of adult animals. Twenty-four hour fasting of 2-day-old animals caused no changes in liver galactokinase activity. There was no significant change in liver galactokinase activity associated with pregnancy.

TABLE IV

Effects of 40% galactose diet on galactokinase activity of adult rat livers

Activity in each case is given as the average specific activity of three animals ± s. e. of the mean. The incubation procedures are described in Table I.

Length of diet period	Diet	Sex	Galactokinase activity
5 days	Control	F	9.68 ± 0.16
		M	12.03 ± 0.10
	Galactose	F	10.01 ± 0.20
		M	11.90 ± 0.13
20 days	Control	F	8.91 ± 0.41
		M	11.01 ± 0.17
	Galactose	F	12.34 ± 0.15*
		M	13.54 ± 0.54†

* Comparison with control groups shows $p < 0.01$ by analyses as explained in Table I.

† Comparison with control groups shows $p < 0.02$ by analyses as explained in Table I.

TABLE V

Effects of 40% galactose and galactose-free diets on liver galactokinase activity in developing rats

Rats that were 16, 18, and 24 days old were given three different diets for periods ranging from 5 to 18 days, as indicated by the age at the end of each study period. Control animals were kept with their mothers on Purina chow; Purina chow animals were separated from their mothers and given only this diet, and galactose-fed animals were separated from their mothers and given a 40% galactose diet. All animals were male. Assay conditions are described in Table I. Activity is expressed as the average specific activity of from three to six rats ± s. e. of the mean. Comparisons are with control groups; analyses are as in Table I.

Age at beginning	Age at end	Control	Purina chow	Galactose
days				
16	24	20.92 ± 0.27	16.51* ± 0.26	27.27* ± 0.44
18	26	18.86 ± 0.21	15.22† ± 0.38	23.73* ± 0.36
24	29	14.92 ± 0.28	14.54‡ ± 0.22	17.52* ± 0.26
16	37	12.78 ± 0.48	12.87‡ ± 0.12	15.84† ± 0.12
18	36	11.68 ± 0.24	11.15‡ ± 0.34	14.73* ± 0.08
24	45	11.85 ± 0.13	11.90‡ ± 0.11	14.93* ± 0.09

* $p < 0.001$.

† $p < 0.01$.

‡ Not significant.

DISCUSSION

The findings presented here clearly indicate that the liver of young rats has a much greater capacity than the adult liver for galactose phosphorylation. Isselbacher (15) reported that the activities of rat liver galactose-1-P uridyltransferase and UDP-galactose pyrophosphorylase increased 6- and 8-fold, respectively, with increasing age from the late fetal and newborn to the adult animal. It was then suggested that the capacity for galactose metabolism improves with age and that maturation of one or both of these enzymes could explain the generally accepted observation that with age there is an apparent amelioration of human galactosemia, a disease noted for absent or very low activity of galactose-1-P uridyltransferase (16, 17). However, Segal *et al.* found that over-all *in vitro* metabolism of galactose by liver, as measured by rates of uptake from the medium and oxidation to CO_2, was considerably greater for fetal and newborn than for adult animals (18). The changes in galactokinase activity reported here parallel closely the changes in galactose utilization and support the notion that the initial phosphorylation of galactose is the rate-limiting step in its utilization. Further evidence for this comes from studies of adult human hemolysates where galactokinase activity was found to be much lower than that of the other galactose enzymes (19). The role of galactokinase as the rate-limiting enzyme can explain the observation that galactosemic individuals improve symptomatically with age even though they are unable to metabolize intravenously administered galactose-1-^{14}C (18, 20). The accumulation of galactose-1-P in the tissues, which is thought to be responsible for the toxic effects in this disease (21–23), might be expected to decrease with age. An alternative explanation, however, is that older galactosemic subjects are actually exposed to very little galactose after milk ingestion because of a secondary loss of intestinal β-galactosidase which results from abstention from lactose ingestion for prolonged periods (24).

The differing properties of adult and newborn liver galactokinase suggest that at least two different molecular species of the enzyme exist. The fetal and newborn enzyme appears to have lower galactose affinity but greater V_{max} than the adult enzyme. This could be of some significance since the young animal must metabolize large amounts of dietary galactose.

Young animals are particularly apt to accumulate galactose-1-P in their tissues as a result of high galactose diets (25–27) as well as the relative immaturity of galactose-1-P uridyltransferase (15). Galactose-1-P accumulation, which is considered to be the cause of galactose toxicity in galactose-fed animals, has been shown to inhibit phosphoglucomutase (22, 23, 28), UDP-glucose pyrophosphorylase (29), glucose-6-P dehydrogenase (30), and glucose 6-phosphatase (31). UDP-galactose also accumulates following high galactose feedings (26, 32, 33), and this compound can inhibit UDP-glucose dehydrogenase (34). The young animal, therefore, needs to utilize large amounts of galactose without accumulating galactose-1-P even in the presence of low activity of galactose-1-P uridyltransferase. Two features unique to fetal and newborn galactokinase could serve as checks on the galactokinase reaction and therefore act as safeguards. First, the concentration of galactose at which substrate inhibition appears is much lower for the newborn than for the adult preparation and is lower still for the fetal extracts. Second, galactose-1-P acts as a noncompetitive inhibitor for galactose phosphorylation in the newborn and is effective at low concentrations. The effects of galactose-1-P on the adult galactokinase are quite different and show an apparent increase in affinity for galactose with a decrease in V_{max}. This effect has been referred to as "uncompetitive inhibition" (35, 36).

It appears that the decrease in galactokinase activity with development is at least in part determined by changes in the quantity of the enzyme. Further definition of this matter must await separation and further characterization of the enzymes.

The decrease appears to be only in part the result of lack of substrate (galactose) in the diet. Rats ordinarily begin to share their mothers' food at about 16 days of age, and at this age a considerable fall in liver galactokinase activity has already occurred. Withdrawal of galactose at this stage accelerates the rate of fall of activity, but levels lower than those found in adults are not reached. The liver galactokinase activity of animals 16 to 24 days of age is quite responsive to changes in dietary galactose, but changes occur with age that modify and limit the capacity to adapt to this carbohydrate. Animals which begin high galactose diets at 24 days of age have liver galactokinase activities at 45 days which are similar to those of animals which begin at 16 days, even though the latter develop a striking early rise in activity. Adult liver galactokinase activity is also responsive to administration of galactose, but a considerably longer time is required. It is quite possible that only one of the liver enzymes, that type found predominantly in the newborn and fetal animals, is capable of adaptation in response to dietary changes.

SUMMARY

The galactokinase activity of extracts of newborn rat liver and gut, and of adult rat liver, gut, kidney, muscle, and brain was found entirely in the soluble cellular fraction. Liver had the greatest activity, and adult male rats had significantly higher liver activity than females. Galactokinase activity of liver tissue rises sharply soon before birth, reaches a maximum at 5 days of age, and declines slowly to reach adult levels at 36 days. A fall in activity with age was also observed for gut and kidney although the developmental patterns differed from those of liver.

The adult and newborn liver enzyme preparations showed the following different properties. (*a*) The newborn is considerably more stable; (*b*) galactose inhibition of galactokinase activity in the newborn is first manifest at a concentration 4 times lower than that which affects the adult, and the inhibiting concentration is near its K_m in the newborn while in the adult it is 20 times greater than its K_m; (*c*) the newborn has a galactose K_m 4 times greater and a V_{max} 6 times greater than the adult; (*d*) the galactose inhibition caused by galactose-1-P is noncompetitive in the newborn, "uncompetitive" in the adult, and much more effective in the former.

Changes in galactokinase activity in response to dietary changes were readily shown in the livers of young developing rats. In adult rats prolonged galactose administration produced a slight but significant rise in liver galactokinase activity.

REFERENCES

1. Trucco, R. E., Caputto, R., Leloir, L. F., and Mittleman, N., *Arch. Biochem.*, **18**, 137 (1948).
2. Wilkinson, J. F., *Biochem. J.*, **44**, 460 (1949).
3. Kalckar, H. M., *Advances in Enzymol.*, **20**, 111 (1958).

4. Cardini, C. E., and Leloir, L. F., in E. J. King and W. M. Sperry (Editors), *Biochemists' handbook*, D. Van Nostrand Company, Inc., New York, 1961, p. 404.
5. Cardini, C. E., and Leloir, L. F., *Arch. Biochem. Biophys.*, **45,** 55 (1953).
6. Schwarz, V., Golberg, L., Komrower, G. M., and Holzel, A., *Biochem. J.*, **62,** 34 (1956).
7. Cleland, W. W., and Kennedy, E. P., *J. Biol. Chem.*, **235,** 45 (1960).
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193,** 265 (1951).
9. Sherman, J. R., *Anal. Biochem.*, **5,** 548 (1963).
10. Sherman, J. R., and Adler, J., *J. Biol. Chem.*, **238,** 873 (1963).
11. Kosterlitz, H. W., *Biochem. J.*, **37,** 318 (1943).
12. Paladini, A. C., and Leloir, L. F., *Biochem. J.*, **51,** 426 (1952).
13. Wade, H. E., and Morgan, D. E., *Nature*, **171,** 529 (1953).
14. Snedecor, G. W., *Statistical methods*, Ed. 4, The Collegiate Press, Inc., Ames, Iowa, 1946, pp. 75, 249.
15. Isselbacher, K. J., *Science*, **126,** 652 (1957).
16. Kalckar, H. J., Anderson, E. P., and Isselbacher, K. J., *Biochim. et Biophys. Acta*, **20,** 2262 (1956).
17. Isselbacher, K. J., Anderson, E. P., Kurahashi, K., and Kalckar, H. M., *Science*, **123,** 635 (1956).
18. Segal, S., Roth, H., and Bertoli, D., *Science*, **142,** 1311 (1963).
19. Kirkman, H. N., and Kalckar, H. M., *Ann. N. Y. Acad. Sci.*, **75,** 274 (1958).
20. Segal, S., Blair, A., and Topper, Y. J., *Science*, **136,** 150 (1962).
21. Holzel, A., Komrower, G. M., and Schwarz, V., *Am. J. Med.*, **22,** 703 (1957).
22. Ginsburg, V., and Neufeld, E., *Abstracts of the American Chemical Society Meeting, New York, September 1957*, p. 27C.
23. Sidbury, J. B., in L. I. Gardner (Editor), *Molecular genetics and human disease*, Charles C Thomas, Springfield, Illinois, 1960, p. 61.
24. Cuatrecasas, P., Lockwood, D. H., and Caldwell, J. R., *Lancet*, **1,** 14 (1965).
25. Schwarz, V., Golberg, L., Komrower, G. M., and Holzel, A., *Biochem. J.*, **62,** 34 (1956).
26. Schwarz, V., and Golberg, L., *Biochim. et Biophys. Acta*, **18,** 310 (1955).
27. Kosterlitz, H. W., *Biochem. J.*, **37,** 318 (1943).
28. Sidbury, J. B., *Abstracts of the American Chemical Society Meeting, New York, September 1957*, p. 27C.
29. Oliver, I. T., *Biochim. et Biophys. Acta*, **52,** 75 (1961).
30. Lerman, S., *Nature*, **184,** 1406 (1959).
31. Kalckar, H. M., and Maxwell, G. S., *Physiol. Revs.*, **33,** 77 (1958).
32. Klethi, J., and Mandel, P., *Biochim. et Biophys. Acta*, **57,** 379 (1962).
33. Hansen, R. G., Freedland, R. A., and Scott, H. M., *J. Biol. Chem.*, **219,** 391 (1956).
34. Salitis, G., and Oliver, I. T., *Biochim. et Biophys. Acta*, **81,** 55 (1964).
35. Ebersole, E. R., Guttentag, C., and Wilson, P. W., *Arch. Biochem.*, **3,** 399 (1943).
36. Dixon, M., and Webb, E. C., *Enzymes*, Ed. 2 Longmans, Green and Company, Inc., London, 1964, p. 318.