Galactose Elimination Capacity in the Rat

S. KEIDING
Division of Hepatology, Medical Dept. A,
Rigshospitalet, Copenhagen, Denmark

Keiding, S. Galactose Elimination Capacity in the Rat. *Scand. J. clin. Lab. Invest. 31,* 319–325, 1973.

The elimination of galactose infused in rats was found to follow saturation kinetics with estimated maximal elimination rate 122 μmol per h and 200 g body weight and estimated half saturation concentration 0.4 mmol/l. The galactose elimination capacity (GEC) is defined as the elimination rates determined at blood concentrations between 6 and 20 time K_m. Multiple regression analysis demonstrates that both body weight and liver weight are determining for GEC. The relation between elimination rate and concentration of galactose metabolites in liver tissue indicates that the phosphorylation of galactose is rate limiting in the metabolism of galactose.

Key-words: Galactose, diagnostic use; galactose metabolism; liver function tests; rat metabolism

Susanne Keiding, M.D., Medical Dept. A, Division of Hepatology, Rigshospitalet, Copenhagen 2100, Denmark

Different tests of galactose elimination have been used as liver function tests for more than 60 years (4). On the assumption that the elimination rate of galactose from the blood represents the hepatic capacity for uptake of galactose, this has been suggested as a measure of the 'functional liver mass' (23). The purpose of the present work was to quantitate the rate of galactose elimination in relation to blood concentrations of galactose in the intact rat in order to define the conditions for estimation of the galactose elimination capacity, and to study the metabolic changes in the liver during saturated and unsaturated galactose elimination.

MATERIAL AND METHODS

Female Wistar rats were fed on Altromin® pills ad lib. In experiments with fasting animals, food was withdrawn for 18 hours but there was free access to water. Anaesthesia induced by intraperitoneal injection of 100 mg per kg of Pentothal® was followed by tracheotomy and intubation with Intramedic polyethylene tubing PE 240.

Galactose was administered via an Intramedic polyethylene tubing PE 50 catheter in a jugular vein as single injections of 0.10 to 0.15 ml of a 0.4 to 3 mmol/l solution of galactose (Kabi), given by a constriction pipette, followed by continuous infusion of 1.20 to 1.35 ml/h of a 15 to 120 mmol/l solution of galactose for 60 min by means of an LKB ReCyChrom type 4912 A peristaltic pump. Control rats were primed and infused with isotonic saline.

Nephrectomy was performed immediately before the experiment by retroperitoneal incision, ligation of the vessels, and removal of the kidneys.

Sampling procedures. Blood was drawn into a 50 μl constriction pipette via an Intramedic polyethylene tubing PE 50 catheter from a common carotid artery after withdrawal of 400 μl to flush the catheter. This volume was reinjected after the sampling. Duplicate samples were taken before the infusion was started and at intervals of 7 min during the experiment after an equilibration period of 20 min. In rats without nephrectomy urine was obtained after the infusion experiment by suprapubic puncture of the bladder and flushing 3 times with 2 ml of isotonic saline.

Liver tissue was taken at the end of the experiment through a midline incision by freeze-clamping in situ with an aluminium clamp precooled in liquid nitrogen. Carcass samples were prepared immediately after the last blood sample by passing the animal through a mincing machine at 4 °C directly into 1 litre of 3 mol/l perchloric acid.

Analytical procedures. The blood samples were precipitated immediately by 400 µl 0.3 mol/l perchloric acid and centrifuged at 4 °C. The supernatant was stored at 4 °C for 1 to 2 days or at − 20 °C for 2 to 7 days. The concentration of galactose was determined by galactose dehydrogenase (18). Galactose was determined in the urine as for blood samples after dilution of the acid extract. Liver tissue was stored for 24 hours in liquid nitrogen, and about 500 mg was ground in liquid nitrogen and homogenized by a Sonifier Cell Disruptor B–12 in 3100 µl 3 mol/l perchloric acid, cooled in ice and ethanol. After centrifugation at 4 °C the supernatant was neutralized with 3 mol/l KOH and the content of galactose (18), galactose-1-phosphate (27), UDP-galactose (14), UDP-glucose (15), ATP (19), ADP and AMP (13), lactate (10), pyruvate (7), and glucose (5), were determined by the methods quoted. Minced carcass in perchloric acid was homogenized for 5 min at 4 °C by an Ultra-turrax T45. The further procedure was the same as for liver tissue.

Calculations. The relative volume of distribution of galactose (D) is estimated by the linear regression through zero of c_b on c_c

$$c_b = \frac{1}{D} c_c \qquad [1]$$

where c_c is the concentration of galactose in carcass and c_b the concentration of galactose in blood.

The galactose elimination rate (GE) in the nephrectomized rat is estimated by

$$GE = I - \frac{dc}{dt} D\,W \qquad [2]$$

where I is the rate of infusion, dc/dt the regression coefficient of the linear regression of galactose concentration in blood on time, D the relative volume of distribution, and W is the weight of the animal.

In the non-nephrectomized rats in which urinary galactose was measured, the galactose elimination rate is estimated by

$$GE = I - \frac{dc}{dt} D\,W - U\frac{A}{P + A} \qquad [3]$$

whereI, D, W, and dc/dt are the same as above, U the amount of galactose measured in the urine, A the amount of galactose infused during the experiment, and P the priming dose. The correction is based on the assumption that identical fractions of the priming dose and the infusion dose are excreted.

The maximal elimination rate, Vmax, and the half saturation concentration, K_m, are estimated according to the Michaëlis-Menten kinetics by the linear regression

$$\frac{1}{V} = \frac{1}{Vmax} + \frac{K_m}{Vmax} \cdot \frac{1}{S} \qquad [4]$$

where V is the elimination rate of galactose (GE) and S is the substrate concentration. The estimates of the standard errors of K_m and Vmax are calculated as described by Hald (9).

The galactose elimination capacity (GEC) is arbitrarily defined as the elimination rate of galactose at concentrations in the blood between 6 and 20 times K_m, ensuring that the elimination rate is between 85 and 95 per cent of the Vmax.

RESULTS

Fig. 1 shows the relation between galactose concentrations in blood and carcass. The linear regression has an intercept of the ordinate, which is not significantly different from zero ($P > 0.7$), and the relative volume of distribution estimated by the linear regression through zero (equation [1]) is 0.41 ± 0.01 (estimate ± standard error of the estimate). No difference is seen between fed and fasted animals, and the calculated volume of distribution also seems to be independent of the

concentration of galactose and the duration of infusion (between 30 and 90 minutes).

Fig. 2 gives the relation between the mean arterial galactose concentration and the galactose elimination rate in 42 fasted and nephrectomized rats. At concentrations higher than about 1.5 mmol/l, the elimination rate appears to be practically unrelated to the concentration, presumably due to saturation of the elimination mechanism. The estimated Vmax is 131 μmol/h $\pm$ 1 (estimate $\pm$ standard error of the estimate) and the apparent K_m 0.37 mmol/l (equation [4]). The Michaëlis-Menten curve calculated from these data is shown in the Figure.

The galactose elimination capacity (GEC) is thus defined as the elimination rate at blood concentrations between 2 and 7 mmol/l (see 'Calculations'). GEC measured in rats weighing from 182 to 216 g is 138 μmol/h $\pm$ 14 (N = 10, mean $\pm$ S.E.M.) for fed and nephrectomized rats, 102 μmol/h $\pm$ 13 (N = 10) for fed and non-nephrectomized rats, 100 μmol/h $\pm$ 7 (N = 9) for fasted and non-nephrectomized rats, and 126 μmol/h $\pm$ 8 (N = 27) for fasted and nephrectomized rats. Neither fasting nor nephrectomy has any effect on the GEC (each $P > 0.5$, t-tests) and GEC is, on the average, 118 μmol/h $\pm$ 30 ($\pm$ S.D.) for these rats weighing about 200 g (N = 56). In the non-nephrectomized rats the urinary excretion of galactose ranged from 2 to 16 per cent of the amount of galactose given, the average being 9 per cent $\pm$ 1 ($\pm$ S.E.M., N = 19).

The relation of GEC to body weight and liver weight was studied in all rats, which weighed from 80 to 308 g (N = 67), by multiple regression analysis (9) (Table I). Both single regression coefficients are significantly different from zero (each $P < 0.001$). The fit is further improved when GEC is related to both body weight and liver weight ($P < 0.005$), which means that GEC depends on both of these weights independently. (None of the y-intercepts are significantly different from zero, and the single regressions through zero are given in the Table.)

Table II shows the concentrations in liver tissue of metabolites involved in the turnover of galactose in groups of rats with different blood concentrations of galactose. In animals with 'saturated' metabolism (i.e. blood galactose

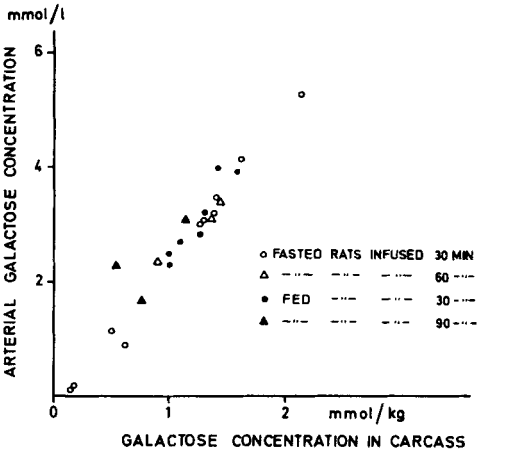

Fig. 1. Relation between galactose concentration in blood and carcass. The volume of distribution of galactose is estimated by the linear regression through zero.

Fig. 2. Relation between galactose elimination rate and galactose concentration in blood in 42 fasted and nephrectomized rats weighing 196 g $\pm$ 12 ($\pm$ S.D.). The curve gives the relation for

$$GE = \frac{131 \cdot S}{0.37 + S} [\mu mol/h]$$

where GE is the galactose elimination rate, S the arterial concentration of galactose. The estimated Vmax is 131 μmol/h and K_m 0.37 mmoles/l.

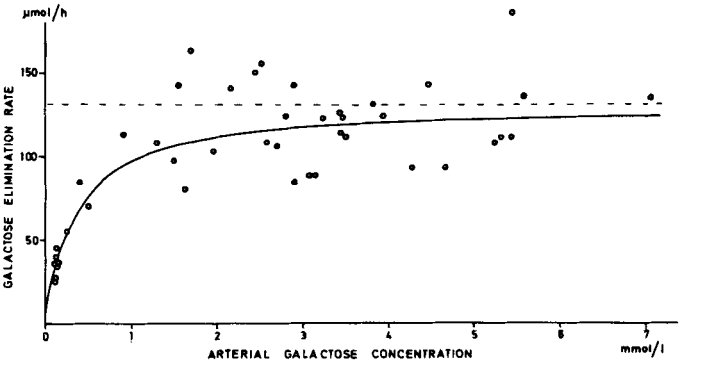

Table I. Galactose elimination capacity (GEC, μmol/h) in 67 rats weighing from 80 to 308 g in relation to g body weight and g liver weight

	GEC	Regression of GEC on body weight (BW)	Regression of GEC on liver weight (LW)	Regression of GEC on BW and LW
μmol/h	126	$10 + 0.58 \cdot BW$	$24 + 15 \cdot LW$	$-45 + 0.47 \cdot BW + 11 \cdot LW$
Residual variance	1691	1185	1302	987
S.D. μmol/h	41	34	36	31
		Regression through zero of GEC on BW	Regression through zero of GEC on LW	
μmol/h		$0.63 \cdot BW$	$19 \cdot LW$	
S.D. μmol/h		34	36	

concentrations > 2 mmol/l), the liver concentrations are significantly increased in relation to the control animals for galactose-1-phosphate, UDP-galactose, lactate, and pyruvate, and a significant reduction is seen for UDP-glucose, ATP, and the sum of adenine nucleotides ($P < 0.05$). For the groups with lower galactose concentrations the hepatic concentrations of galactose, galactose-

Table II. Liver and blood metabolites in rats weighing from 185 g to 227 g, fasted, nephrectomized, and infused with galactose at different concentrations (Mean ± S.E.M.)

		'Saturated' galactose metabolism ($S \geq 10 \cdot K_m$)	'Partly saturated' galactose metabolism ($2\frac{1}{2} \cdot K_m < S < 10 \cdot K_m$)	'Unsaturated' galactose metabolism ($S \leq 2\frac{1}{2} \cdot K_m$)	Control
	No. of rats	10	5	10	7
BLOOD mmol/l	Galactose	4.7–2.0	1.6–0.9	0.5–0.1	0
LIVER, mmol/kg wet weight	Galactose	3.20 ± 0.22	0.92 ± 0.31	0.11 ± 0.02	0
	Galactose-1-P	1.15 ± 0.29	0.32 ± 0.07	0.21 ± 0.04	0.23 ± 0.06
	UDP-gal	0.17 ± 0.02	0.21 ± 0.03	0.10 ± 0.01	0.06 ± 0.01
	UDP-glu	0.15 ± 0.01	0.15 ± 0.02	0.17 ± 0.06	0.22 ± 0.01
	Glucose	6.04 ± 0.85	7.26 ± 0.57	6.97 ± 0.60	5.72 ± 0.91
	ATP	2.29 ± 0.17	1.48 ± 0.25	2.13 ± 0.22	2.72 ± 0.10
	ADP	1.52 ± 0.08	1.64 ± 0.11	1.61 ± 0.08	1.64 ± 0.08
	AMP	0.46 ± 0.10	0.54 ± 0.09	0.37 ± 0.06	0.47 ± 0.08
	Lactate	1.16 ± 0.28	1.66 ± 0.37	0.83 ± 0.18	0.44 ± 0.05
	Pyruvate	0.17 ± 0.04	0.28 ± 0.03	0.19 ± 0.03	0.10 ± 0.01
LIVER	ATP + ADP + AMP	4.09 ± 0.15	3.66 ± 0.37	4.17 ± 0.18	4.83 ± 0.08
	ATP/ADP	1.60 ± 0.22	0.90 ± 0.14	1.42 ± 0.23	1.70 ± 0.14
	ATP AMP/ADP[2]	0.37 ± 0.05	0.30 ± 0.08	0.34 ± 0.06	0.48 ± 0.07
	UDP gal + UDP glu	0.31 ± 0.03	0.34 ± 0.04	0.28 ± 0.02	0.28 ± 0.02
	L/P	8.7 ± 1.7	6.9 ± 2.0	4.2 ± 0.6	5.4 ± 0.8
	Gal-1-P/gal	0.36 ± 0.08	0.61 ± 0.22	2.46 ± 0.43	
	UDP gal/gal-1-P	0.34 ± 0.16	0.68 ± 0.06	0.61 ± 0.12	0.34 ± 0.10
	UDP glu/UDP gal	0.89 ± 0.10	0.69 ± 0.12	1.57 ± 0.24	4.07 ± 0.39

Table III. Estimates of apparent K_m and Vmax calculated from the linear regressions of galactose elimination rate^{-1} on concentrations^{-1} of galactose in blood and of galactose metabolites in the liver, respectively. The material consists of 23 rats weighing from 186 to 227 g, fasted for 18 hours, nephrectomized, and infused for 60 min with galactose at steady-state concentrations of galactose in blood ranging from 0.01 to 5 mmol/l

	Vmax*	K_m*
Blood	μmol/min · g liver	mmol/l
galactose	0.33 ± 0.04	0.30 ± 0.06
Liver tissue	μmol/min · g liver	mmol/kg
Galactose	0.29 ± 0.05	0.15 ± 0.07
Galactose-1-P	0.36 ± 0.14	0.27 ± 0.17
UDP-galactose	0.82 ± 0.88	0.45 ± 0.59

* Estimate ± standard error of the estimate.

1-phosphate, and UDP-galactose increase with increasing blood concentrations of galactose.

The ratio galactose-1-phosphate/galactose, given in Table II, represents the product/substrate ratio of the galactokinase step, and UDP-galactose/galactose-1-phosphate and UDP-glucose/UDP-galactose similarly represent the ratios for the transferase and the epimerase steps, respectively. The galactose-1-phosphate/galactose ratio is reduced by, on the average, a factor 7 from unsaturated to saturated metabolism, the UDP-galactose/galactose-1-phosphate and UDP-glucose /UDP-galactose by a factor 2. This indicates that the kinase reaction is the rate-limiting step in the hepatic conversion of galactose to UDP-glucose. If the kinetic constants are calculated for all three metabolic steps from available data of velocities and substrate concentrations, the Vmax of the transferase and epimerase steps are found to be higher than that of the kinase step (Table III).

DISCUSSION

In the intact rat the relation between blood concentration and removal rate from the blood of galactose is found to be compatible with Michaëlis-Menten kinetics. Accordingly the conditions for measurements of galactose elimination rates approximating to the elimination capacity may be defined. For practical purposes the galactose elimination capacity (GEC) is defined as elimination rates which are between 85 and 95 per cent of the estimated maximal velocity. To obtain this the concentration should be between 6 and 20 times the apparent K_m or, in the preparation studied, between 2 and 7 mmol/l.

The GEC estimated in the present study (63 μmol per h and 100 g body weight of the animals, Table I) is consistent with the value found by Salaspuro & Salaspuro (22) in unanaesthetized rats by the single injection technique (73 μmol/h and 100 g). Those authors also found that the elimination of galactose was temporarily blocked by penthotal anaesthesia. This was not found in the present experiments, but a systematic influence of anaesthesia on the results obtained cannot be excluded. It is assumed that the surgical trauma was of minor importance, since nephrectomy and splenectomy (unpublished) were without detectable influence. GEC per g liver (29 μmol/min and g, Table III) is considerably higher than that of liver slices (0.04 μmol/min and g liver) (24), presumably because of a low metabolic activity of slices.

The estimation of the galactose elimination rate involves a correction which depends on the volume of distribution of galactose and the slope of the blood galactose concentration curve (equations [3] and [4]) .The volume found in the present study (41 per cent of body weight) is of the same order of magnitude as that found for rats by Levine, Goldstein, Huddlestun & Klein (20), 45 per cent, and by Waterman & Hetenyi (26), 30 per cent. Galactose seems to equilibrate in the volume of distribution in less than 30 min. The error of the estimate is relatively small (S.D. = 5 per cent of body weight), which justifies the use of a common estimate of this volume instead of individual determinations. To minimize the influence of the volume of distribution, the blood concentrations were kept as constant as possible by adjusting the infusion dose to the expected elimination rate. The deviation was never greater than 25 per cent, and on the average it was near to zero. It is therefore improbable that the volume of distribution has a significant influence on the results obtained.

In rats with nephrectomy both urinary ex-

cretion and renal metabolism of galactose are avoided, but it cannot be determined from these experiments whether there is a quantitatively significant conversion of galactose in other extrahepatic tissues.

The concentrations in liver tissue (Table II) of UDP-glucose and UDP-galactose (16) and ATP, ADP, and AMP (2, 11) are of the same order of magnitude as found by the investigators mentioned.

The Michaëlis-Menten kinetics of enzyme reactions (21) are usually examined in vitro by studying initial reaction velocities in order to have constant and optimal conditions. The implications of using this system for the description of reactions studied under steady-state conditions in intact organisms have not been fully elucidated. However, in so far as they give a reasonably accurate description of the observations, the calculated Vmax and K_m are acceptable as empirical measures when used to describe and compare experiments performed under similar conditions. Furthermore, the description is based on the assumptions that galactose enters the liver cell freely or with a very high Vmax (8), that the reaction velocity of all three steps during steady-state conditions is identical, and that no significant alternative pathway exists between galactose and UDP-glucose.

'K_m' for galactose in blood (Table III) is the arterial concentration of galactose at which the elimination rate is half maximal. This value is higher than 'K_m' calculated on the basis of galactose concentrations in liver tissue owing to the decreasing galactose concentration as the blood flows through the liver (3). The 'K_m' for galactose in liver corresponds very well to the K_m measured for rat liver galactokinase (0.15 mmol/l (6), and 0.14 mmol/l (25)).

The hepatic product/substrate concentration ratios of the three initial steps in the metabolism of galactose (Table II) invite the hypothesis that the first step is rate limiting. This is supported by the Michaëlis-Menten relation between galactose elimination rates and substrate concentrations of the three steps as described by the Vmax of the processes (Table III), that of the first step being the slowest one.

Isselbacher & McCarthy (12) have proposed that NAD exerts a controlling effect on the galactose metabolism in rat liver homogenates via the reversible epimerase step. Keppler, Rudiger & Decker (16) supposed that this step is rate limiting in the intact rat. However, this implies an increase in the concentration of galactose-l-phosphate during galactose metabolism, which then inhibits the kinase step non-competitively (1).

Cuatrecasas & Segal (6) and Walker & Khan (25) measured the uptake of radioactive galactose by rat liver homogenates, and from their data Vmax can be calculated to be about 2 and 0.8 μmol/min and g liver, respectively. These values are higher than the in vivo value of the present study (0.29 μmol/g min, Table III), indicating that the rate-limiting factor is not the enzyme activity per se.

The decrease of ATP in the animals with 'saturated' galactose metabolism corresponds to that found in brain tissue by feeding chicks galactose-rich diets (17). However, the measured concentrations of ATP are high in relation to K_m for ATP of rat liver galactokinase, which is about 0.11 mmol/l (6, 25), and therefore the availability of ATP is probably not rate-limiting for the reaction under normal conditions.

In animals with intact liver the GEC depends both on body weight and liver weight individually (Table II). This is interpreted as indicating that the GEC reflects not only the mass of functioning liver tissue, but also the amount of 'hepatic metabolic activity' required by the body of the animal. In other words, a liver with a given tissue mass is more active in a big than in a small animal.

ACKNOWLEDGEMENTS

My thanks are due to Mrs. Aase Vesterby and Mrs. Liselotte Hansen for expert technical assistance. The work was supported by grants from the Danish Medical Research Council and the Danish Foundation for the Advancement of Medical Science.

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Received 25 August 1972
Accepted 10 March 1973