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Michaelis-Menten kinetics of galactose elimination by the isolated perfused pig liver

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KEIDING, S., S. JOHANSEN, K. WINKLER, K. TØNNESEN, AND N. TYGSTRUP. *Michaelis-Menten kinetics of galactose elimination by the isolated perfused pig liver.* Am. J. Physiol 230(5): 1302–1313. 1976.—The relation between galactose elimination rates and blood concentrations in the isolated perfused pig liver was analyzed by a mathematical kinetic model. It assumes that the substrate, under steady-state conditions, is removed from the blood that flows through the sinusoids by an irreversible process which follows Michaelis-Menten (i.e., saturation) kinetics. The experiments consisted of successive periods with constant infusions of galactose. The model fitted the data to within the experimental uncertainty. The estimated maximal rate (V_{max}) ranged from 0.34 to 0.57 mmol·min^{-1}·kg^{-1} liver, and the Michaelis constant, K_m, ranged from 0.12 to 0.30 mmol·liter^{-1} plasma water in nine experiments. The ratio between the galactose concentration in hepatocyte water and plasma water was not significantly different from 1.0, indicating that membrane transport is not rate limiting for the elimination of galactose. In experiments with increasing concentrations of galactose in hepatocyte water and approximately saturated elimination rates, the concentrations of galactose 1-phosphate, UDPgalactose, and UDPglucose remained essentially constant. This indicates that the phosphorylation of galactose to galactose 1-phosphate is the rate-determining process.

enzyme kinetics; liver circulation; liver metabolism

ENZYMATIC PROCESSES IN VITRO are normally characterized by Michaelis-Menten kinetics. This model is not directly applicable to processes in intact organs because of the complex metabolic and physiologic conditions in vivo. The purpose of the present study was to implement the Michaelis-Menten kinetics for the intracellular enzymatic reaction in the intact liver, taking into account the effect of the sinusoidal perfusion.

The relation between in vivo and in vitro enzyme kinetics of ethanol elimination was discussed in 1958 by Lundquist and Wolthers (35). They assumed the in vivo elimination in humans to occur as a process with Michaelis-Menten kinetics in a homogeneous system of constant volume and compared the results with estimates obtained in vitro for alcohol dehydrogenase. Their model was modified by Dedrick and Forester (13), who studied the elimination kinetics of ethanol in rats based on a two-compartment model and discussed the influence of changes in the hepatic blood flow rate. The model, however, does not take into consideration the effect of the decreasing concentration in the sinusoid from the inflow to the outflow. The concentrations used in these and in other studies (24, 40) concerned with elimination kinetics in the whole body are usually measured in the mixed-venous or arterial blood. This corresponds roughly to the concentration in the inflow to the liver. Studies of elimination kinetics in perfused livers have used the concentration in either inflow (20), outflow (5), or liver tissue (39), although some studies (29) have used an "average" of inflow and outflow concentrations.

The concentration profile throughout the proximal tubules of the kidney was studied by Burgen (9), who assumed Michaelis-Menten kinetics for the reabsorption of glucose at each point along the tubule. This model, however, was complicated by the fact that large amounts of water are reabsorbed in the proximal tubules. This is not the case for the liver. Goresky et al. (17) studied hepatic galactose elimination kinetics in the intact dog by the indicator-dilution technique. They obtained estimates of V_{max} and K_m for the membrane carrier transport, as well as for the metabolic conversion. The estimates of K_m, however, are in terms of inflow concentration, although computed steady-state concentration profiles do allow for approximate estimation of K_m for the processes in the hepatocytes.

In the present investigation we have used a model which takes into consideration the sinusoidal flow and permits estimation of K_m of the elimination mechanism in the intact liver. The model assumes that the substrate under steady-state conditions is removed from the blood that flows through the sinusoids by an irreversible process which follows Michaelis-Menten (i.e., saturation) kinetics (4a, 43).

The elimination of galactose at concentrations above 2 mM is used as an estimate of hepatic galactose V_{max} in the quantitative evaluation of the function of the liver (37). In the present study the model is evaluated experimentally by the elimination of galactose by the isolated perfused pig liver.

MATERIAL AND METHODS

Female Danish country-breed pigs weighing from 31 to 48 kg (mean 37 kg) were fasted for 48 h with free access to water. After premedication with 2 mg/kg of Sernylan, the pig was intubated and anesthetized with

halothane (0.8%) and N_2O (70%) in oxygen administered by a respirator. The abdomen was opened and packs of ice were placed in the peritoneal cavity until a rectal temperature of 32°C was obtained. Then the hepatic artery was cannulated and perfused with isotonic saline containing 10,000 IU of heparin per liter at a rate of 10 ml/min. The caudal end of the caval vein was ligated below the liver, the cranial end was cut, and the portal vein was clamped and cut. The liver was removed, weighed, and transferred to the perfusion system without interruption of the hepatic arterial perfusion.

The perfusion system was primed with approximately 2.5 liters of fresh pig blood obtained from a sibling pig and 1.5 liters of isotonic saline with heparin (12,500 IU/liter blood) and $CaCl_2$ (0.1 mmol/liter blood). The perfusate, called blood, was oxygenated by a Rygg-Kyvsgaard bubble oxygenator aerated with 5 liters/min of atmospheric air with 5% carbon dioxide. The blood was recirculated by a roller pump; the inflow to the liver was divided into the portal inflow via a reservoir and into the arterial inflow via a pulsatile pump.

The liver was placed on gauze distended over a circular metal frame, below which any fluid exuding from the surface of the liver (0–25 ml/min) was collected and returned to the blood. The portal vein and the hepatic arterial catheter were connected to the perfusion system. A fenestrated, large-bore tube was inserted in the caval vein, returning the blood to the oxygenator via a reservoir. The common duct was cannulated and the cystic duct ligated.

The time from the clamping of the portal vein to the start of the portal perfusion was from 1.2 to 4 min (mean, 2.3 min).

The temperature of the blood, heated by a thermostated heat exchanger, was 32°C when the liver was connected and was raised to 38°C in the course of 20 min. During the experiments the temperature was 38.0°C (range 37.7–38.3).

The total blood flow rate was adjusted to and kept constant at ca. 1 liter·min^{-1}·kg^{-1} liver. The flow rates were recorded continuously by calibrated electromagnetic flowmeters (Nycoton), and the pressures by electromanometers (Elema). Final adjustments of the flow rates were performed in order to obtain a portal pressure below 20 mmHg (mean ± SD: 13 ± 4 mmHg, n = 14; in *expt 6* the mean portal pressure was 24 mmHg) and a mean arterial pressure about 100 mmHg. The ratio between the arterial and the portal flow rates was 0.25 ± 0.08 (mean ± SD, n = 14).

The pH was adjusted to 7.40 with 25–40 ml of 1 meq/ml of $NaHCO_3$ and was kept between 7.36 and 7.44 during the experiment by means of an automatic titrator (Radiometer). The amount of bicarbonate used was, on the average, 0.33 ± 0.18 ml/min (n = 14). Mixing of the blood in all reservoirs was ensured. Sodium taurocholate (0.012 mmol/min) was infused by a calibrated pump during the whole experiment. The liver was covered with a plastic sheet.

Experimental Procedure

The experiment, starting 30 min after the perfusate was reheated, lasted for 180–210 min. Nine experiments consisted of four to five periods with different galactose concentrations, from which the elimination kinetics were evaluated. Appropriate galactose elimination rates and perfusate concentrations were obtained by continuous infusions of 0.05–0.6 mmol/min of galactose (Kabi) in isotonic saline (2 ml/min) and priming doses of 0–80 mmol in galactose. Each period consisted of 15 min of equilibration and 25–32 min of sampling.

In three experiments constant amounts of galactose (0.38 mmol/min in *expt 10;* 0.87 mmol/min in *expt 11*; 0.70 mmol/min in *expt 12*) were given continuously. In two experiments isotonic saline was given instead of galactose (*expts 13* and *14*). Furthermore, metabolic changes taking place in the medium were tested in two liverless perfusions (*expts 15* and *16*) primed with 8 mmol of galactose and with five increasing doses from 0.06 to 10 mmol, respectively.

Inflow (portal) and outflow (caval) blood samples were drawn from collecting catheters close to the liver after flushing of catheters. Bile was collected during each period.

Tissue samples, weighing about 0.5 g, were taken in duplicate in each period from two different lobes, 3–7 cm from the edge, by a 10-mm-bore cannula. The samples were freeze-clamped in less than 5 s (mean 3.1) and stored in liquid nitrogen for 24–72 h. Hemostasis was ensured by occlusion by a closely fitting test tube. No physiologic and metabolic effects of the biopsy procedure were detected.

Analytical Procedure

A) Blood. Galactose (30) and glucose (36) concentrations were determined in 7–10 inflow and outflow samples during each period, after deproteinization with 0.3 M perchloric acid, by the methods cited.

Lactate (21) and pyruvate (12) were determined twice during each period in neutralized 1.0 M perchloric acid supernatants by the methods cited.

Oxygen saturation (Osm1, Radiometer) Po_2, Pco_2, and pH (Radiometer), and hemoglobin concentration were measured twice during each period in samples taken in airtight syringes.

Once during each period the concentrations of free hemoglobin (19), Na^+, K^+, Mg^+ (flame photometry), and inorganic phosphate (15) were determined. The hematocrit (Hct) was measured in microcapillary tubes after centrifugation for 5 min in a Crist microhematocrit centrifuge.

The volume of erythrocyte-free perfusate, called plasma, was determined during the first and last experimental period by injection of ^{131}I-labeled human serum albumin (Kjeller, Norway).

The concentration of solids in plasma was determined by drying the samples to a constant weight at 50°C for 5 days.

B) Liver tissue. The frozen samples were pulverized in liquid nitrogen in a porcelain mortar and transferred to weighed test tubes with 3.1 ml of 3 M perchloric acid cooled to −14°C. After being weighed, the samples were homogenized at −14°C by ultrasound (Sonifier B-12) and centrifuged (4,500 × g for 10 min) at 4°C. The supernatant was neutralized by 3 M KOH and centri-

fuged at 4°C. The supernatant was analyzed enzymatically for galactose (30), galactose 1-phosphate (14), UDPgalactose (27), UDPglucose (26), UTP (28), glucose (6), ATP (31), and ADP and AMP (22) by the methods cited. The concentrations were corrected for recovery of reference substances added to the pulverized tissue powder. Reference substances, coenzymes, and enzymes were obtained from Boehringer Mannheim, except galactose and glucose which were from Kabi and Merck, respectively.

The intrahepatic volume of plasma was determined in six liver biopsies taken simultaneously with the plasma samples for determination of the total volume by ^{131}I-labeled serum albumin. The tissue was homogenized in isotonic saline and was counted by the same procedure as used for the plasma. The intrahepatic volume of plasma was estimated as the counts per minute per gram tissue/counts per minute per milliliter plasma.

Physiologic Data[1]

The average liver weight was 1.2 kg (Table 1). The total blood flow rate was 1.0 ± 0.2 liter blood·min^{-1}·kg^{-1} liver (mean ± SD, n = 14). The mean of the arterial flow rate was 0.26 liter·min^{-1}·kg^{-1}; transhepatic resistance, 13 mmHg/liter, hepatic arteriolar resistance, 433 mmHg/liter; blood volume, 3.9 liters. The mean of the bile flow rate was 0.32 ml/min.

No systematic changes were seen during the experimental period for the following data, as evaluated by analyses of variance (each $P > 0.1$). The mean values for the plasma concentrations were: K^+, 2.6 mM; Na^+, 150 mM; Mg^{2+}, 0.46 mM; inorganic phosphate, 3.0 mM. The Po_2 in the inflow blood was 103 mmHg; in the outflow, 47 mmHg; Pco_2 was 39 and 50 mmHg, respectively.

The oxygen uptake decreased 10–15% in the course of the experiment. This was statistically significant, as evaluated by means of the slopes of regression analyses (each $P < 0.05$). The oxygen uptake was calculated for each experiment as a mean value of the measurements. There was no significant difference between the values from experiments with either successive galactose infusions, constant galactose infusions, or infusions of isotonic saline. The estimate of the common mean was 0.97 ± 0.17 mmol·min^{-1}·kg^{-1} liver (±SD, n = 14). The concentration of ATP in liver tissue decreased, on average, 0.12 ± 0.22 mmol/kg (±SD, n = 12) ($P > 0.05$) during the course of the experiment. ADP decreased 0.12 ± 0.07 mmol/kg ($P < 0.05$), and AMP showed a nonsignificant decrease of 0.001 ± 0.04 mmol/kg. There was no significant difference between the experimental groups for either ATP, ADP, or AMP. The common mean values were, for ATP, 2.03 ± 0.25 mmol·kg^{-1} wet liver; ADP, 0.92 ± 0.15 mmol·kg^{-1}; AMP, 0.20 ± 0.08 mmol·kg^{-1} (±SD, n = 12).

[1] For more detailed information and individual values of the physiologic and biochemical control data and derivation of the estimates of v, $\hat{c}$, $s(v)$, and $s(\hat{c})$, see NAPS Document No. 02814 for supplementary material. Order from ASIS/NAPS c/o Microfiche Publications, 440 Park Avenue South, New York, N. Y. 10016. Make checks payable to Microfiche Publications and remit in advance. Photocopies are $5.00; microfiche, $3.00. Outside the United States and Canada, postage is $2.00 for a photocopy or $1.00 for a fiche.

Glucose was not eliminated to a detectable extent in the liverless perfusions. In the experiments with a constant galactose infusion, the glucose concentration increased linearly, and accordingly, no time dependence was seen for the glucose production. The glucose production rate amounted to ca. 30–70% of the galactose elimination.

Lactate in blood increased in experiments with successive galactose infusions from 0.21 ± 0.13 to 1.58 ± 0.65 mM (±SD, n = 9) ($P < 0.01$). Pyruvate showed no significant change in the course of any of the experiments; the average was 0.07 ± 0.05 mM (±SD, n = 43). The concentration ratio of lactate to pyruvate in the outflow blood increased from 3.8 ± 1.4 (n = 7) to 21 ± 11 (n = 10).

Symbols Used

Concentrations (in mM)

- c_B blood (≡ perfusate)
- c_p plasma water (≡ erythrocyte-free perfusate water)
- c_i inflow plasma water
- c_o outflow plasma water
- $\hat{c} \equiv (c_i - c_o)/(\ln c_i - \ln c_o)$, logarithmic average concn
- $\bar{c} \equiv \int_{x=0}^{1} c_x dx$, space-average concn, where c_x is concn within sinusoid at location x
- $\bar{\bar{c}} \equiv (c_i + c_o)/2$, arithmetric average concn
- c_H hepatocyte water

Volumes of

- B blood, liters
- T solids in plasma, liter/liter
- P intrahepatic plasma water, liters/kg liver
- D intracellular volume of distribution, liters/kg liver
- L intrahepatic volume of distribution of galactose = P + D for galactose, liters/kg liver

Infusions

- i infusion rate of galactose, mmol/min
- h sum of infusion flow rates of galactose, taurocholate, and bicarbonate, minus evaporation of water in oxygenator, liters/min

Flow

- F total blood flow rate, liters/min
- W velocity of flow in sinusoids, cm/min

Elimination of galactose

- v elimination rate, mmol/min
- V_{max} maximal elimination rate, mmol/min
- K_m half-saturation concentration, mM

Calculations

Concentrations of galactose and glucose in plasma water (i.e., in erythrocyte-free perfusate water), c_p. These concentrations were calculated as

$$c_p = c_B/(1 - \text{Hct} - \text{T}) \quad (1)$$

where c_B is the concentration measured in the blood (mM), Hct is the hematocrit corrected for trapped plasma by the factor 0.96 (33), and T is the concentration of solids in the plasma (liter/liter).

The method presumes that galactose does not penetrate pig erythrocytes during the experiment as demonstrated in Fig. 1, in agreement with Laris (32). The density of the plasma was 1.005 ± 0.001 kg/liter (mean ± SE, n = 18) and the concentration of solids, 0.023 ± 0.001 kg/liter (n = 18), corresponding to 0.02 liter solids per liter plasma (T).

Concentration of galactose in hepatocyte water, c_H. This was obtained from

$$c_L = P \cdot \hat{c} + D \cdot c_H \qquad (2)$$

where c_L is the measured galactose concentration per kilogram liver tissue and P is liters of plasma water per kilogram liver tissue. The logarithmic average, $\hat{c}$, of the galactose plasma concentrations in inflow (c_i) and outflow (c_o), calculated according to *equation 5* is used as an approximate estimate of the space-average concentration, $\bar{c}$ *(equation 7)*. D is the intracellular volume of distribution of galactose per kilogram liver tissue.

The measured intrahepatic volume of plasma water (P) was 0.312 ± 0.02 liter/kg liver tissue (mean ± SE, n = 6).

The intracellular volume of distribution of galactose per kilogram liver tissue (D) was calculated from an equation similar to *equation 2* using the glucose concentrations for both $\hat{c}$ and c_H. This presumes that this volume is identical for galactose and glucose, and that $\hat{c}$ and c_H of glucose are identical as shown by Williams et al. (42) for the rat liver. The mean of D, 0.384 ± 0.005 liter/kg liver tissue (± SE, n = 60), was used in the calculations.

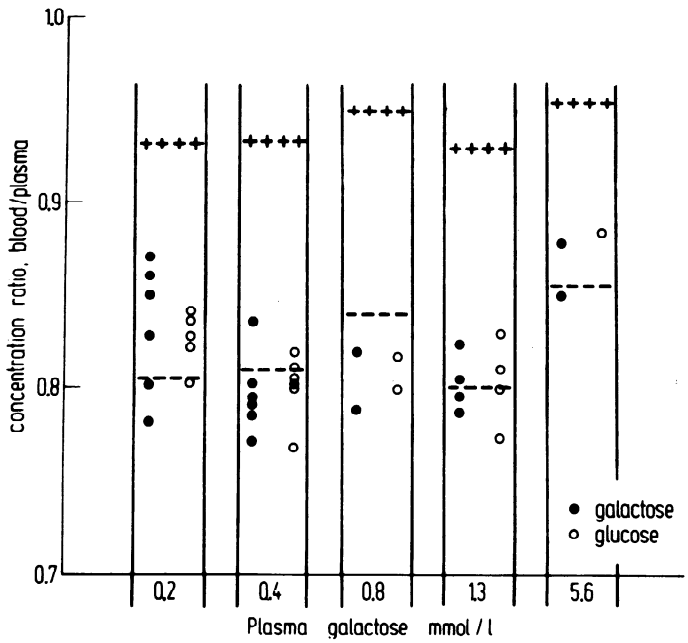

FIG. 1. Penetration of galactose and glucose into pig erythrocytes. Concentrations were measured both in blood and plasma, and blood-plasma concentration ratio is given in relation to measured concentrations of galactose in plasma. Glucose concentrations were between 3 and 10 mM. Ratios estimated on assumption of no penetration into red cells (- -) and of total equilibration between red cell and plasma water (+ +) are indicated. Calculation is based on measured values of hematocrit (corrected for trapped plasma by factor 0.96), called Hct, concentration of solids in plasma and red cells, T, and of water concentration in erythrocytes, E, (measured 0.65 liter/liter, unpublished). Amount of galactose or glucose in blood (concn c_B) equals amount in plasma water (concn c_P) plus amount in erythrocyte water (concn c_E): $c_B = c_P \cdot (1 - Hct - T) + c_E \cdot Hct \cdot E$. Free penetration (i.e., $c_P = c_E$) gives blood-plasma concentration ratio = $(1 - Hct - T + Hct \cdot E)/(1 - T)$. No penetration (i.e., $c_E = 0$) gives blood-plasma ratio = $(1 - Hct - T)/(1 - T)$.

Concentrations of galactose 1-phosphate, UDPgalactose, UDPglucose, and UTP in hepatocyte water. These metabolites were not detectable in the perfusate. *Equation 2,* therefore, is reduced to

$$c_H = c_L/D \qquad (3)$$

This assumes that the intrahepatic volume of distribution is identical for the four metabolites and galactose.

Galactose elimination rate, v. This was calculated as an average value in each period as further discussed in APPENDIX I *(equation A4)*.

$$v = i - B \cdot d\bar{c}/dt - \bar{c} \cdot h \qquad (4)$$

where i is the infusion rate (mmol/min) of galactose; B (liters) is the sum of the plasma water volume (calculated as a mean value in each period from T and the regression of the measured plasma volumes on time) and the average hepatic intracellular volume of distribution of galactose (calculated as D·LW, where D is 0.384 liter/kg and LW is the liver weight); $d\bar{c}/dt$ is the slope of the regression of the galactose concentrations in plasma water on time (mmol·liter^{-1}·min^{-1}); $\bar{c}$ is the average galactose concentration in plasma water (mM); h is the sum of the infusion fluid flow rates (liters/min) of galactose, taurocholate, and bicarbonate, minus the calculated rate of evaporation of water in the oxygenator (0.024 liter/min). Excretion of galactose in the bile was not included in *equation 4* because it always was less than 1.0% of v, the galactose concentration being about 25% of the concentration in plasma water.

Glucose and lactate uptake rates. These were calculated from *equation 4* with i = 0.

Oxygen uptake rate. This uptake rate was calculated as blood flow rate multiplied by the difference between the blood concentrations of oxygen in inflow and outflow.

Elimination kinetics of galactose. This was analyzed by the kinetic model (see APPENDIX I, *equation A9*).

$$v = V_{max} \cdot \hat{c}/(K_m + \hat{c}) \qquad (5)$$

where

$$\hat{c} = (c_i - c_o)/(\ln c_i - \ln c_o)$$

V_{max} and K_m were estimated from the nonlinear regression

$$\ln v = \ln V_{max} + \ln \hat{c} - \ln(\hat{c} + K_m) \qquad (6)$$

as described in APPENDIX II with v estimated from *equation 4*, and $\hat{c}$ estimated as the mean in each period of the values calculated from *equation 5* using the plasma water concentrations in inflow (c_i) and outflow (c_o), calculated from *equation 1*.

The accuracy of the estimates was evaluated as described in APPENDIX II.

RESULTS

Elimination Kinetics

Galactose was not eliminated to a detectable extent in the two liverless perfusions.

In the experiments with a constant infusion of galactose, no time-dependent change of the galactose elimination rate was seen during the experimental period (Table 1), as evaluated by analyses of variance (each $P > 0.05$).

In the experiments consisting of successive periods with increasing infusions of galactose, steady-state conditions were achieved within each period with good approximation; see Fig. 2 for an experiment. The change in the blood concentration with time was always less than ± 0.05 mmol·liter^{-1}·min^{-1} (see APPENDIX I). The galactose elimination rate (v) and mean plasma water concentrations in inflow (c_i) and outflow (c_o) in each period are given in Table 1. Figure 3 gives the plots of the reciprocals of the galactose elimination rate (v) and the logarithmic average concentration ($\hat{c}$) *(equation 5)* for the kinetic experiments.

The relative uncertainty of the estimate of v, estimated from double determinations of the measurements, $s(v)/v = s(\ln v)$, ranged from 2 to 7% (Table 2) (see APPENDIX II). The model was tested on the data by comparison of the variance of ln v from the measurements and the residual variance of ln v around the regression curve of the model (see APPENDIX II). As shown in Table 2, F tests gave P values above 0.5 for four of the experiments, and the model was accepted at the 10% level for all but two experiments. In both these experiments one aberrant set of v and $\hat{c}$ gave rise to the large residual variance (*expt 5, period B,* and *expt 7, period D;* see Table 1), and the deviations from the model were not systematic, as evaluated by plots of $1/v$ against $1/\hat{c}$. The common estimate of the variance of ln v was 0.0013 (df = 53) and that of the residual variance was 0.00157 (df = 22), which gives $F = 1.38$; $0.3 > P > 0.1$. The model thus fits within the error of the measurements.

Table 3 shows the estimates of V_{max} and K_m and their standard errors for each experiment. It can be seen that the individual errors of V_{max} ranged from 2 to 13% and those of K_m ranged from 3 to 18%. The estimates obtained from different livers were significantly different, as evaluated by analyses of variance ($P < 0.01$ for both V_{max} and K_m). Figure 4 shows the relation between the estimates of K_m and V_{max} and the 95% confidence ellipses (18). As shown on Fig. 4, the correlation between V_{max} and K_m was large within each experiment. Figure 4 also shows that the ratios of V_{max} to K_m were estimated with even greater accuracy than V_{max} and K_m.

The mean values were estimated (Table 3) as common estimates for V_{max} and K_m, since Fig. 4 also shows that the values obtained from different experiments were uncorrelated. The standard errors of these estimates were 6% for V_{max} and 10% for K_m.

Tissue Metabolites

In the experiments without galactose administration (*expts 13* and *14*) neither galactose nor galactose 1-phosphate was detectable in the liver tissue, i.e., the concentrations were less than 0.05 mmol/kg. The concentration in hepatocyte water of UDPgalactose was 0.35 ± 0.02 mM (mean ± SE, $n = 9$); UDPglucose, 0.61 ± 0.04 mM ($n = 9$); UTP, 0.98 ± 0.07 mM ($n = 5$). The concentrations were unchanged throughout the experiment, as evaluated by analyses of variance.

When the same infusion of galactose was given

TABLE 1. *Galactose elimination kinetics in isolated perfused pig liver: mean plasma water concentrations of galactose in inflow (c_i, mM) and outflow (c_o, mM) and elimination rates (v, mmol·min^{-1}·kg^{-1} liver)*

Expt No.	Liver, kg	Flow Rate of Plasma Water,* liters·min^{-1}	Parameter	Period A	B	C	D	E
Succesive periods with increasing infusion rates of galactose								
1	0.85		c_i:	0.25	0.81	1.31	27.9	
			c_o:	0.03	0.29	0.65	26.3	
			v:	0.20	0.40	0.47	0.57	
2	1.03	1.03	c_i:	0.14	0.43	0.46	1.69	3.69
			c_o:	0.04	0.20	0.23	1.30	3.20
			v:	0.09	0.22	0.22	0.32	0.37
3	1.04		c_i:	0.35	0.86	2.31	27.4	
			c_o:	0.07	0.41	1.73	26.0	
			v:	0.23	0.34	0.43	0.42	
4	1.08	0.94	c_i:	0.08	0.15	0.28	0.43	1.65
			c_o:	0.02	0.04	0.09	0.17	0.95
			v:	0.05	0.10	0.16	0.22	0.32
5	1.10	1.10	c_i:	0.16	0.53	20.0		
			c_o:	0.04	0.27	19.6		
			v:	0.17	0.35	0.41		
6	1.12	0.96	c_i:	0.36	0.63	2.63	26.5	
			c_o:	0.09	0.26	1.92	26.0	
			v:	0.23	0.32	0.49	0.51	
7	1.14		c_i:	0.39	1.06	2.96	24.1	
			c_o:	0.05	0.62	2.35	23.3	
			v:	0.20	0.30	0.34	0.43	
8	1.38	1.14	c_i:	0.23	0.36	0.80	1.47	4.12
			c_o:	0.08	0.13	0.48	1.14	3.78
			v:	0.11	0.15	0.26	0.30	0.30
9	1.75	1.22	c_i:	0.16	0.35	2.00	18.4	24.4
			c_o:	0.04	0.13	1.53	18.0	23.6
			v:	0.09	0.19	0.38	0.40	0.42
Same infusion rate of galactose throughout experiment								
10	1.35	1.31	c_i:	0.52	0.55	0.61		
			c_o:	0.17	0.19	0.25		
			v:	0.28	0.28	0.28		
11	1.36	1.42	c_i:	7.34	10.6	13.7	16.7	19.5
			c_o:	6.65	10.0	13.0	16.1	19.2
			v:	0.62	0.62	0.61	0.60	0.59
12	1.56	1.60	c_i:	11.4	11.6	12.7	13.4	
			c_o:	10.7	11.5	12.1	12.9	
			v:	0.36	0.35	0.36	0.35	

* Calculated as: blood flow rate·(1 − Hct − T), where Hct is the hematocrit corrected for trapped plasma by the factor 0.96, and T is the concentration of solid substances in plasma, 0.02 ml/ml.

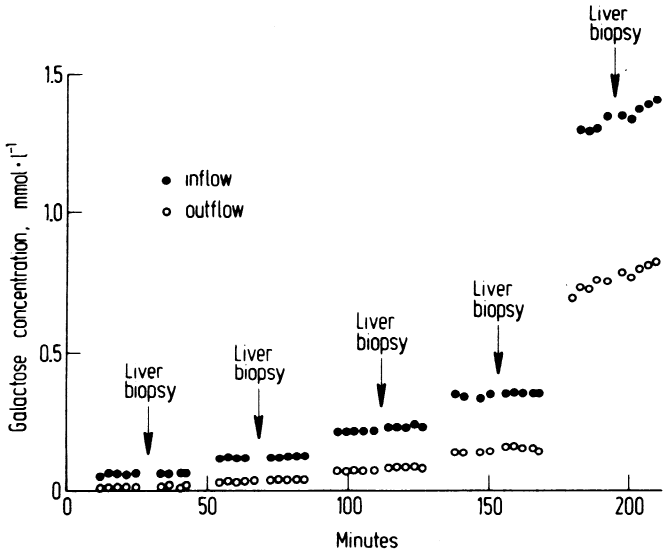

FIG. 2. *Experiment 2.* Galactose concentrations during 5 quasi steady-state periods which were used for estimation of kinetic constants. Infusion rates of galactose during successive periods were 0.023, 0.032, 0.035, 0.046, and 0.071 mmol·min^{-1}. *Periods 3–5* were initiated by a single injection (0.3, 0.4, and 0.6 mmol, respectively).

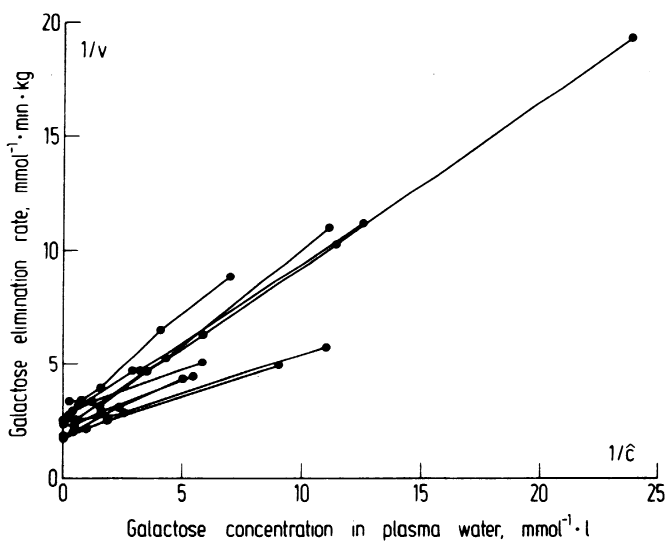

FIG. 3. Lineweaver-Burk plot of galactose elimination rate-concentration relation in isolated perfused pig liver. Each point represents one experimental period, and periods from same experiment are connected. *Abscissa:* reciprocal of logarithmic mean, $\hat{c} = (c_i - c_o)/(\ln c_i - \ln c_o)$, where c_i and c_o are galactose concentrations in plasma water in inflow and outflow, respectively.

throughout the experiment, the concentrations in hepatocyte water of galactose, galactose 1-phosphate, UDPgalactose, and UDPglucose were not changed significantly, whereas UTP decreased approximately 20%.

The metabolite pattern was examined during increasing galactose concentrations at low or high concentrations. In *experiment 9* (Fig. 5) five increasing galactose elimination rates were obtained by administration of five increasing levels of galactose infusions. In *experiment 11* (Fig. 6) a constant galactose elimination rate approximating V_{max} was ensured by a continuous infusion of galactose. Figures 5 and 6 show that there was a tendency to a lower galactose concentration in hepatocyte water than in plasma water at low concentrations and a higher concentration in hepatocyte water than in plasma water at high concentration. This tendency was also seen in the other galactose experiments, but it

TABLE 2. *Galactose elimination in isolated perfused pig liver*

Expt No.	$s^2(\ln v)$	df	s^2_{res}	df	F = $s^2res/s^2(\ln v)$	P
1	0.00069	6	0.00049	2	<1	$P>0.5$
2	0.00108	7	0.00020	3	<1	$P>0.5$
3	0.00058	6	0.00127	2	2.2	$0.3>P>0.1$
4	0.00061	6	0.00012	3	<1	$P>0.5$
5	0.00035	4	0.00411	1	11.7	$0.05>P>0.01$
6	0.00046	6	0.00046	3	1	$0.5>P>0.3$
7	0.00062	6	0.00583	2	9.4	$0.05>P>0.01$
8	0.00457	7	0.00214	3	<1	$P>0.5$
9	0.00041	5	0.00218	3	5.3	$0.3>P>0.1$

Test of the fit of the data to the model $v = V_{max}\cdot\hat{c}/(K_m + \hat{c})$, where $\hat{c} = (c_i - c_o)/(\ln c_i - \ln c_o)$. V_{max} is the maximal elimination rate; K_m, the half-saturation concentration; v, the elimination rate; c_i and c_o, the concentration in inflow and outflow, respectively. The F test compares the variance of ln v, calculated from the experimental measurements, $s^2(\ln v)$, with the residual variance of the data around the functional relationship given by the model, s^2_{res}.

TABLE 3. *Kinetic parameters of galactose elimination in isolated perfused pig liver*

Expt No.	$V_{max} \pm s(V_{max})$, mmol·min^{-1}·kg^{-1} liver	$K_m \pm s(K_m)$ mmol·liter^{-1}
1	0.57 ± 0.01	0.19 ± 0.01
2	0.39 ± 0.01	0.25 ± 0.01
3	0.44 ± 0.03	0.17 ± 0.02
4	0.40 ± 0.01	0.26 ± 0.01
5	0.43 ± 0.06	0.12 ± 0.02
6	0.53 ± 0.02	0.25 ± 0.01
7	0.39 ± 0.05	0.17 ± 0.03
8	0.34 ± 0.04	0.27 ± 0.03
9	0.43 ± 0.03	0.30 ± 0.02
Mean	0.43	0.23

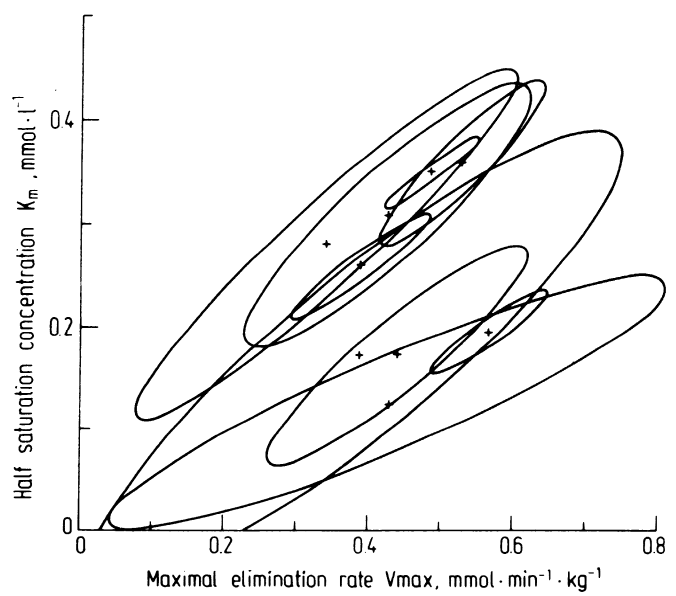

FIG. 4. Relation between estimates of V_{max} and K_m (+) of galactose and 95% confidence ellipses in 9 isolated perfused pig livers.

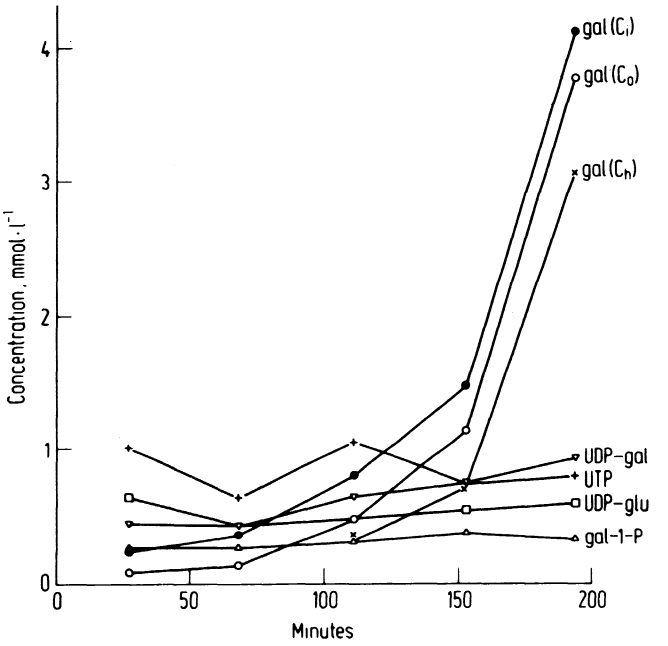

FIG. 5. *Experiment 9*. Concentrations of galactose and metabolites during 5 quasi steady-state periods. Plasma water concentrations in inflow and outflow (c_i and c_o) are mean values during period; other concentrations are in hepatocyte water, calculated from measured concentrations in liver tissue, taken in middle of each period. Infusion rates of galactose during successive periods were 0.08, 0.18, 0.36, 0.42, and 0.42 mmol·min^{-1}. Each period was initiated by a single injection (3.5, 7, 6, 7, and 14 mmol, respectively).

could not be substantiated statistically; the regression analysis of hepatocyte water concentration against plasma water concentration gave a y intercept which was not significantly different from zero ($P > 0.2$) and a slope which was not significantly different from 1.0 ($P > 0.2$).

Figures 5 and 6 also show that the concentration of galactose in hepatocyte water increased, in *experiment 9*, from less than 0.05 mM to 3 mM and in *experiment 11* from ca. 7 to 20 mM, whereas the concentrations of the four metabolites were essentially unchanged. Galactose 1-phosphate increased from 0.19 to 0.50 mM in *experiment 9* and from 0.48 to 0.80 mM in *experiment 11*. Also UDPgalactose nearly doubled, whereas the concentration of UDPglucose remained constant, and UTP decreased about 20%.

DISCUSSION

The present study shows that galactose elimination in the isolated perfused pig liver may be described by a mathematical kinetic model based on the assumption that the liver consists of sinusoidal tubes lined by hepatocytes which eliminate the substrate from the blood by an irreversible process described by Michaelis-Menten kinetics. The irreversible process corresponds to an impermeable-wall removal model, the process may be either the membrane transport or one of the intracellular enzymatic steps. For the elimination of galactose by the perfused pig liver, the rate-determining process seems to be the phosphorylation of galactose to galactose 1-phosphate.

The continuous-infusion technique was chosen for the following reasons. First, it reduces distribution phenomena of galactose and other metabolites in the circulatory system as well as in the intracellular spaces of the liver. Simultaneous steady-state estimates of the galactose elimination rate and blood and tissue concentrations were ensured, and this facilitates the comparison of the estimates.

The mathematical kinetic model *(equation 5)* assumes a time-independent concentration of galactose, i.e., $dc/dt = 0$. As illustrated in Fig. 2 this was almost achieved and, as shown in APPENDIX I, the term depending on dc/dt *(equation A5)* was negligible compared with the steady-state terms.

The Michaelis-Menten relation presumes that the concentration of the enzyme-substrate complex is unchanged during the enzymatic reaction (8). This is obtained, only approximately, by in vitro "initial-rate" reactions, mostly used for studies of enzymatic processes, from the small time-dependent change in the enzyme-substrate complex concentration in comparison with the change in substrate concentration. However, in the present study the continuous-infusion technique yields almost time-independent concentrations of galactose metabolites in liver tissue, indicating that exact steady-state enzyme kinetics was ensured throughout the experiment.

For these reasons the present quasi steady-state ex-

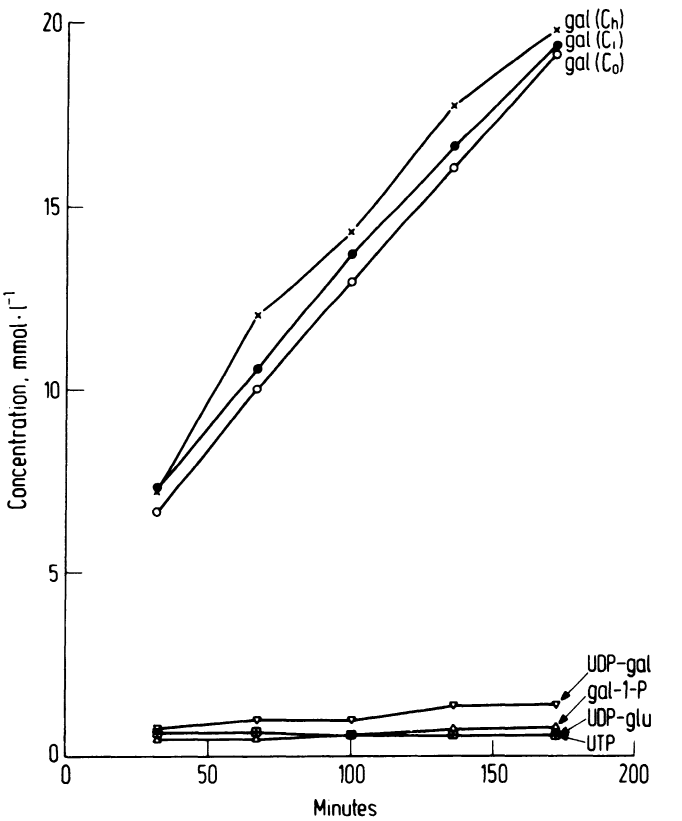

FIG. 6. *Experiment 11*. Concentrations of galactose and metabolites during a continuous infusion of 0.87 mmol·min^{-1} of galactose. Plasma water concentrations in inflow and outflow (c_i and c_o) are mean values of samples taken around time indicated by abscissa. Other concentrations are measured in liver tissue taken at time indicated.

periments are assumed to provide reliable estimates of the kinetic parameters.

The transient time for in vitro reactions with diluted enzyme solutions is of the order of magnitude of seconds·10^{-5} to 10^{-1} (4). In the intact liver the enzyme concentrations are several orders of magnitude higher, but still the transient time is of the order of magnitude of seconds. Accordingly, the enzymatic reaction in the perfused liver will most probably go through a continuous succession of steady states when the concentration changes with time, even if the single-injection technique is used.

The mathematical kinetic model requires that the total enzymatic activity for the rate-determining process is approximately equal in the sinusoids and that the blood flow rates of the sinusoids are approximately equal. During the nearly physiological conditions in the present study this probably is the case, as the number of normal inflow-outflow shunts is so small that the measured total flow rate corresponds closely to the flow rate through well-perfused sinusoids (17). In this case the estimation of V_{max} and K_m according to *equation 5* is not influenced by changes in the flow rate.

The substrate is offered the hepatocytes at the concentration in the space of Disse. According to Goresky (17), there is probably a rapid equilibrium perpendicular to the direction of the flow between the vascular water space and the water space of Disse. This partly is due to the bolus type of blood flow through the sinusoids with well-mixed spaces between the erythrocytes and partly to the fenestrated structure of the sinusoidal endothelia. Accordingly, the estimated concentration of galactose in plasma water was used in the calculations.

The model describes the relation between the galactose elimination rate, v, and the inflow concentration, c_i, and the outflow concentration, c_o, by the Michaelis-Menten relation, in which the concentration is the logarithmic average of c_i and c_o, called $\hat{c}$. This $\hat{c}$ is a derived concentration which allows for estimation of the K_m of the process in the hepatocytes lining the sinusoids; $\hat{c}$ lies between c_i and c_o, it is smaller than $(c_i + c_o)/2$ and larger than the space-average concentration, $\bar{c}$, defined as $\int_{x=0}^{x=1} c_x dx$. From the model we have

$$\bar{c} = [(c_i + c_o)/2 + K_m]\cdot F(c_i - c_o)/V_{max} \qquad (7)$$

At high concentrations both $\hat{c}$ and $\bar{c}$ approximate $(c_i + c_o)/2$, and the concentration profile of the sinusoid approximates a straight line (Fig. A1, APPENDIX I); at low concentrations the concentration decays exponentially (Fig. A1) and $\hat{c}$ approximates $\bar{c}$. In contrast to $\bar{c}$, $\hat{c}$ does not depend on the blood flow rate, F, and therefore no simple relation between $\hat{c}$ and $\bar{c}$ exists in the intermediate concentration interval.

The model was tested by plotting $1/v$ against $1/\hat{c}$. According to the model, this should give points scattered randomly around straight lines, and as shown in Fig. 3, this was also seen in the experiments. Fit of the polynomial $1/v = b_o + b_1\cdot(1/c) + b_2\cdot(1/c)^2$ to the data in which $\hat{c}$ was used for c gave positive values for b_2 in five of the nine experiments.

As a supplement to this preliminary evaluation of the fit of the model, the numerical test comparing the residual variance of ln v around the curve of the model (*equation A12*, APPENDIX II) with the variance of ln v estimated from the error of the measurements was performed; this test also supported the fit (Table 2).

Many kinetic studies use plots of the reciprocals of elimination rates and inflow concentrations and fit straight lines for calculation of V_{max} and K_m (20, 40). This corresponds to the Michaelis-Menten relation between v and c_i and would be the correct model if all cells and enzymes were offered the substrate at the inflow concentration. Other studies use a multiple of the outflow concentration, c_o (5). This would be the proper procedure if all the cells were mixed with the blood in a pool. According to our model, the relations between $1/v$ and $1/c_i$ and between $1/v$ and $1/c_o$ should not be straight lines but curves. Figure 7 gives an example of the good agreement between the predicted curves, calculated on the basis of the estimated kinetic parameters, and the experimental data. Fits of the above-mentioned second-degree polynominal using c_i for c gave negative values for b_2 in eight of the nine experiments ($P < 0.02$), and using c_o for c gave positive values for b_2 in seven of the nine experiments ($P < 0.09$). This supports our model as opposed to the models in which a Michaelis-Menten relation between v and c_i or c_o is assumed. Using $(c_i + c_o)/2$ would lead to an overestimation of K_m since $\hat{c} < (c_o + c_i)/2$. Also, use of the concentration measured in liver tissue biopsies (39) or of the concentration recalculated to that in hepatocyte water in the Michaelis-Menten equation, would give, systematically, estimates of K_m that are too low since $\bar{c} < \hat{c}$; this consideration presumes that the fall in concentration from the vascular bed to the hepatocytes that is perpendicular to the direction of flow is small compared with that along the sinusoid so that the space-average concentration within the hepato-

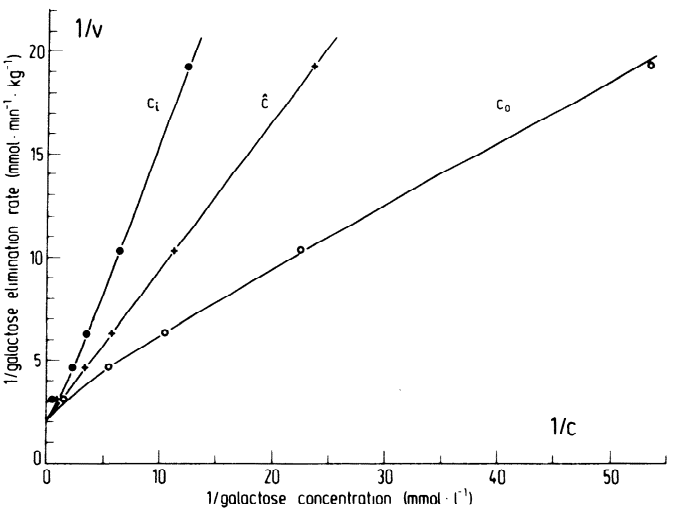

FIG. 7. Lineweaver-Burk plot of galactose elimination rate-concentration relation in *experiment 4*. Elimination rates, v, are related to plasma water concentrations in inflow, c_i, and outflow, c_o, and to logarithmic mean concentration $\hat{c} = (c_i - c_o)/(\ln c_i - \ln c_o)$. Curves are calculated from mathematical model, using estimated values of V_{max} and K_m and measured flow rate of plasma water.

cytes is equal to that in the vascular space of the sinusoid.

Estimates of Kinetic Parameters V_{max} and K_m

These estimates were determined with relatively small errors and showed significant individual differences. The interindividual variation includes probably both individual differences between the enzymatic reactions in different livers and variation between unknown experimental factors. It should be pointed out that the parametrization of the model by V_{max} and K_m implies that the estimates of the parameters become highly correlated (Fig. 4), and therefore caution should be exercised when the estimates are used for testing various hypotheses about the parameters.

The maximum rate of galactose elimination in the present study (mean 0.43 mmol·min^{-1}·kg^{-1} liver) is in good agreement with previous results from our group (38) in which the average galactose elimination rate was 0.45 mmol·min^{-1}·kg^{-1} at blood concentrations of ca. 5 mM. Our estimates are lower than those found by Welch and Parbhoo (41) in three pig-liver perfusions in which the average was 0.8 mmol·min^{-1}·kg^{-1}.

The half-saturation substrate concentration, K_m, (Table 3) is in terms of millimoles per liter plasma water. For pig blood with a hematocrit of 0.45 it would be about two-thirds of the stated values, i.e., an average of 0.19 mmol/liter blood.

Rate-Determining Process

In the hepatic elimination of galactose this process is described kinetically by the parameters V_{max} and K_m of the model, and could be either carrier transport of galactose across the hepatocyte membrane or one of the enzymatic steps in the metabolic conversion of galactose. In the present study the membrane transport does not seem to be rate determining since the ratio between the galactose concentrations in blood and plasma was close to unity. This is in agreement with Goresky et al. (17) who studied the galactose transport in the intact dog liver by indicator-dilution technique. They found that the membrane transport could be described by a carrier mechanism in which the V_{max} of the transport is about 50 times higher than the V_{max} for the hepatic conversion of galactose. K_m for the inward transport was about 30 mM and that of the outward transport was exceedingly high. This is taken to indicate that the metabolism is saturated at concentrations far below those that saturate the transport process.

The main pathway of the hepatic conversion of galactose is assumed to involve phosphorylation to galactose 1-phosphate with consumption of ATP (galactokinase, EC 2.7.1.6), conversion of galactose 1-phosphate and UDPglucose to UDPgalactose and glucose 1-phosphate (uridylyltransferase, EC 2.7.7.12), and epimerization of UDPgalactose to UDPglucose (UDPglucose 4-epimerase, EC 5.1.3.2) (23, 34). During infusion of galactose at a rate below V_{max}, the tissue concentrations of these metabolites were unchanged, and accordingly the unidirectional flows of galactose through the three steps can be assumed to be identical. At experimental conditions in which the concentration of galactose in hepatocyte water increased, either because of an infusion rate of galactose above V_{max} and a constant galactose elimination rate, or because of successive infusions of galactose at increasing rates, the concentrations of the above-mentioned intracellular galactose metabolites also remained essentially constant. The thermodynamic equilibration constant for galactokinase is 26 (2), which shows that the phosphorylation of galactose to galactose 1-phosphate is practically irreversible. The results, therefore, indicate that the galactokinase step is rate limiting in the elimination of galactose by the perfused liver.

In vitro estimates of the enzyme activities support this conclusion. Gordon et al. (16) found that the lowest activity of the three enzymes in chick liver was the activity of galactokinase. The lowest activity for galactokinase is also seen in the adult rat liver: 2.5 mmol·min^{-1}·kg^{-1} liver, as recalculated from Cuatrecasas and Segal (11); for transferase (7) it was 6.2 mmol·min^{-1}·kg^{-1} and for epimerase (10), 3.0 mmol·min^{-1}·kg^{-1}. As discussed by Atkinson (1), it is a prevailing phenomenon that the first step in a metabolic sequence is generally rate determining with the consequence that the intermediary metabolites are kept at relatively low and nontoxic concentration.

The in vivo kinetics of the galactokinase step is thus described by the estimates of V_{max} and K_m. K_m was, on the average, 0.22 mmol/liter hepatocyte water calculated from the galactose concentration ratio 1.0.

For purified galactokinase from pig liver Ballard (3) found the V_{max} for galactose to be ca. 1.0 mmol·min^{-1}·kg^{-1} wet liver and the K_m to be 0.1–0.6 mM. In studies of the galactose metabolism in the 40 samples of the cytosol fraction of pig liver tissue, we found (unpublished data) the average V_{max} to be 0.7 mmol·min^{-1}·kg^{-1} wet liver and the average K_m 0.1 mM. The estimate of K_m in the perfused liver is of the same order of magnitude as the in vitro values, whereas V_{max} tends to be smaller in vivo than in vitro. In the perfusion experiments no substrate inhibition was detectable, whereas this was seen both in our and in Ballard's in vitro studies at concentrations above 2 mM. This probably reflects the difference between the conditions for the enzymes examined in vitro, in which pH and the concentrations of coenzymes, inhibitors, and salts are kept as biochemically optimal as possible, and the conditions in vivo, in which the enzymes are attached to cytoplasmatic structures.

APPENDIX I

Mathematical Kinetic Model

The mathematical kinetic model comprises *1)* a model for the substrate in the circulatory system, consisting of a circular blood stream, the circulation pump, the infusion pump, the sampling outlets, and the liver; *2)* a model for the metabolism of the substrate in the liver. According to the latter, the liver is assumed to consist of sinusoidal tubes lined by sheets of hepatocytes. It is assumed that the substrate is removed from the blood by the hepatocytes according to irreversible Michaelis-Menten kinetics.

Since the galactose concentration in the blood stream was changing slowly during an experimental period, this has to be incorporated in the model. The equation for each model is a simple consequence of the law of mass conservation.

1) For the circulatory system the change in the amount of galactose is

$$d/dt(B(t)\cdot\bar{c}(t))$$

where $B(t)$ is the volume of distribution of galactose in the circulatory system, and $\bar{c}(t)$ is the concentration of galactose in the system. This equals the influx, i.e., the rate of galactose infusion, i, minus the efflux, f, due to sampling, minus the galactose elimination rate, $v(t)$. Omitting the argument t and noticing that $f = s\cdot\bar{c}$, where s is the flow rate of sampling, we get

$$d/dt(B\cdot\bar{c}) = i - s\cdot\bar{c} - v \qquad (A1)$$

or

$$v = i - s\cdot\bar{c} - B\cdot d\bar{c}/dt - \bar{c}\cdot dB/dt \qquad (A2)$$

Since

$$dB/dt = g + t + o - e - s \qquad (A3)$$

where g, t, and o are the infusion fluid flow rates of galactose, taurocholate, and bicarbonate, respectively, and e is the flow rate of evaporation of water from the oxygenator, *equation A2* becomes

$$v = i - B\cdot d\bar{c}/dt - \bar{c}\cdot h \qquad (A4)$$

with h defined as $(g + t + b - e)$. *Equation A4* was used to estimate v, using $(dc_i/dt + dc_o/dt)/2$ as an estimate of $d\bar{c}/dt$ (calculations, *equation 4)*.

2) For the liver we get

$$v = F\cdot(c_i - c_o) - L\cdot dc/dt \qquad (A5)$$

where F is the flow rate; c_i and c_o, the concentration in inflow and outflow, respectively; L, the intrahepatic volume of distribution of galactose.

Similarly, for a part of a sinusoidal tube we get, on the assumption of an irreversible Michaelis-Menten elimination process

$$P\cdot\left[\frac{\partial c}{\partial t} + W\frac{\partial c}{\partial x}\right] + V_{max}\cdot\left[\frac{c}{K_m + c}\right] = 0 \qquad (A6)$$

where P is the volume of plasma in the hepatic sinusoids, W is the velocity of flow in the sinusoids, and $c = c(x,t)$.

If the liver was in steady state, i.e., $dc/dt = 0$, *equation A6* reduces to (4a)

$$v = F\cdot K_m\cdot(\ln c_o - \ln c_i) + V_{max} \qquad (A7)$$

and *equation A5* to

$$v = F\cdot(c_i - c_o) \qquad (A8)$$

Equations *A7* and *A8* give

$$v = V_{max}\cdot\hat{c}/(K_m + \hat{c}) \qquad (A9)$$

where $\hat{c} = (c_i - c_o)/(\ln c_i - \ln c_o)$

This is the Michaelis-Menten relation where $\hat{c}$ is the logarithmic average of c_i and c_o. Fig. A1 gives an example of the concentration profile in the sinusoid, calculated from the model.

Obviously, the steady-state relation *(equation A9)* is not exact, in general, in the model that describes the liver *(equations A5* and *A6)*. However, since the terms depending on dc/dt proved experimentally to be small, in *equation A5* less than 6% of v, relation *A9* was used to describe the relation between v and $\hat{c}$ and to estimate V_{max} and K_m with v calculated from *equation A4*.

APPENDIX II

Statistical Evaluation of Acceptability of Model

The functional relationship (*equation A9*) gives a statistically adequate description of the physiological processes within the present experimental setup provided deviations from it may be explained from uncertainties in the measurements only. We shall here first describe how these uncertainties were evaluated, and second, how V_{max} and K_m were estimated and the acceptability of the model checked.

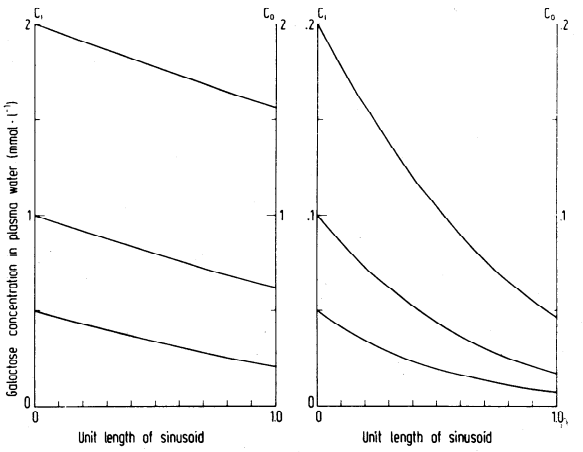

FIG. A1. Concentration profiles of galactose along sinusoids at different inflow concentrations, calculated from average values of V_{max} (0.43 mmol·min^{-1}·kg^{-1}), K_m (0.23 mM), and flow rate (0.83 liter·min^{-1}·kg^{-1}).

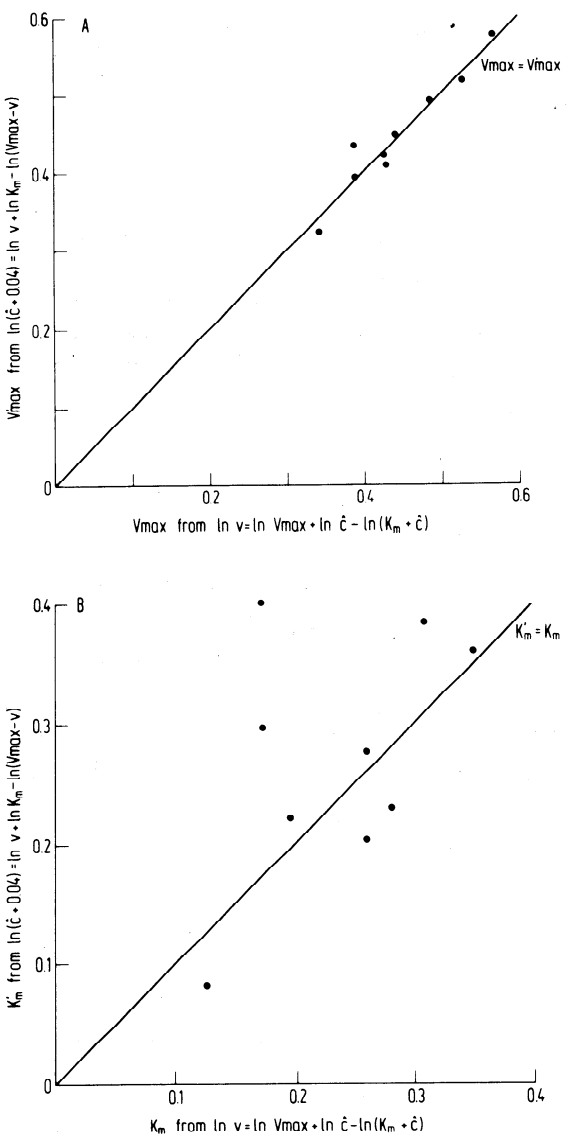

FIG. A2. Relation between galactose V_{max} estimated from regression of ln v on $\hat{c}$ against V_{max} estimated from regression of $\ln(\hat{c} + 0.04)$ on v *(A)*, and relation between corresponding estimates of K_m *(B)* from relation between elimination rate v and concentrations in inflow c_i and outflow c_o: $v = V_{max}\cdot\hat{c}/(K_m + \hat{c})$, where $\hat{c} = (c_i - c_o)/(\ln c_i - \ln c_o)$.

Standard deviation, s(v) of estimate v of galactose elimination rate. This deviation was estimated from double determinations of the measurements. Plots of $s(v)$ against v within each single experiment gave points scattered randomly around straight lines through zero, and accordingly $s(v)$ was assumed proportional to v or, in other words, the relative uncertainty within each experiment could be assumed independent of v. Since $s(\ln v)$ is approximately $s(v)/v$, logarithmic transformation of the data was performed yielding approximately constant variances; $s^2(\ln v)$ was estimated for each experiment by averaging $s^2(v)/v^2$. These results are reported in Table 2, showing relative uncertainties of v between 0.02 and 0.07.

The variability of the estimate of v is almost exclusively (more than 95%) because of uncertainties concerned with the galactose infusion flow rates and concentrations. For statistical comparison purposes the number of degrees of freedom of $s^2(\ln v)$ given in Table 2 was, therefore, based on the number of double determinations of these quantities.

Standard deviation, s(ĉ), of estimate ĉ of plasma water concentration of galactose. In each period $s(\hat{c})$ was estimated as the standard error of the mean of the estimate of $\hat{c}$.

Standard deviation of hepatocyte water concentration of galactose, c_H. This value, estimated from double determinations (18), was proportional to c_H and ranged from 16 to 24%.

Acceptability of model and estimation of V_{max} *and* K_m. The model *equation A9* was used in the form

$$\ln v = \ln V_{max} + \ln \hat{c} - \ln (K_m + \hat{c}) \qquad (A10)$$

and estimates of V_{max} and K_m were found by a nonlinear regression of $\ln v$ on $\hat{c}$, i.e., by minimizing

$$Q(V_{max}, K_m) = \sum_{k=1}^{n} (\ln v_k - \ln V_{max} - \ln \hat{c}_k + \ln(K_m + \hat{c}_k))^2 \qquad (A11)$$

Estimates of the standard deviations of the estimates of V_{max} and K_m were derived from $s^2(\ln v)$ by the usual inverse Fisher information values (or δ method); cf. e.g., Kendall and Stuart (25).

The fit of the data to the model was evaluated by comparison of the residual variance

$$s^2_{residual} = \min Q(V_{max}, K_m)/(n - 2) \qquad (A12)$$

with the estimates of s^2 $(\ln v)$ (see Table 2).

A check on the validity of this reasoning was obtained by the following argument: the above analysis studies a functional relationship between the two variables v and $\hat{c}$, both measured with uncertainty, by the regression of $\ln v$ on $\hat{c}$. This may give a biased estimate of the relationship in question, but it is known that a dual regression of $\hat{c}$, or some function of $\hat{c}$, on v will yield estimates which are biased in the opposite direction. Thus if these two regressions give identical results, the bias should be negligible. We found the best variance-stabilizing transformation of $\hat{c}$ as $\ln (\hat{c} + 0.04)$ and studied the regression

$$\ln(\hat{c} + 0.04) = \ln(V_{max} - v + 0.04 \cdot K_m \cdot v) - \ln v - \ln K_m \qquad (A13)$$

of $\ln(\hat{c} + 0.04)$ on v. The estimates of both V_{max} and K_m obtained from *equation A13* were in good agreement with the estimates from *equation A10* as shown in Fig. A2. The regression of $\ln v$ on $\hat{c}$ was used because it is numerically more stable to fit a horizontal asymptote. If reliable estimates of the uncertainties of v and $\hat{c}$ are available, one can estimate the parameters by minimizing a weighed sum of squares corresponding to the two regressions, *equations A10* and *A13*. It was felt, however, that the uncertainties obtained might underestimate the true variances, and it was decided not to use them in the analysis.

The two findings that the residual variance of $\ln v$ is of the same order of magnitude as the measurement uncertainty of $\ln v$ (Table 2) and that straight lines are obtained by plotting $1/v$ against $1/\hat{c}$ (Fig. 3) thus indicate that the model describes the data well, and that a reasonable estimate of the uncertainty of $\ln v$ has been obtained.

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