

Hepatic galactose elimination kinetics in the intact pig

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The hepatic galactose elimination kinetics was studied in eight anaesthetized pigs by hepatic vein catheterization. Galactose was given as successive constant intravenous infusions so that steady-state concentrations, ranging from 50 $\mu\text{mol/l}$ to 8 mmol/l , were obtained. Concentrations were measured in a peripheral artery and in the hepatic veins. Hepatic blood flow was determined by constant infusion of indocyanine green. The elimination kinetics is described by a mathematical model which assumes that the liver sinusoids are perfused with unidirectional blood flow and that the elimination in the hepatocytes takes place according to Michaelis-Menten kinetics. This creates a decreasing sinusoidal blood concentration from the inlet to the outlet of the liver. The estimated maximal elimination rate, V_{max} , was on average $0.67 \text{ mmol min}^{-1} \text{ kg}^{-1} \text{ liver}$ (range 0.55-0.95) and the half saturation concentration K_m 0.25 mmol/l (0.12-0.58). The estimate of K_m is similar to the value found in the isolated perfused pig liver (0.23 mmol/l), whereas the estimate of V_{max} is about 50% higher ($0.43 \text{ mmol min}^{-1} \text{ kg}^{-1} \text{ liver}$), probably due to both extrahepatic elimination in the splanchnic area and a better function of the liver *in situ* than in the isolated preparation.

Key-words: enzyme kinetics; liver circulation; liver metabolism

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The galactose elimination by the isolated perfused pig liver has previously been described by a model of the sinusoidal perfusion [11] which describes the elimination of substances carried to the liver by the blood [1]. It is assumed that all sinusoids are identical and that the elimination takes place according to Michaelis-Menten saturation kinetics at the

blood concentration at each location along unidirectionally perfused sinusoids. It is a wall removal model in the sense that it describes the kinetics of the rate-determining process during steady state. It is assumed that in each cross-section of a sinusoid, diffusion maintains a uniform substrate concentration. The rate determining process can be the membrane transport or the intracellular conversion of the substrate, for example; the model does not

depict the specific type of process involved. The elimination kinetics is described in terms of the sinusoidal blood concentration. Due to elimination the concentration decreases in the direction of the blood stream. At steady state when the inlet and outlet concentrations are constant

$$v = F \cdot (c_i - c_o), \quad (1)$$

and according to the model [1]

$$(c_i - c_o) + K_m \cdot (\ln c_i - \ln c_o) = V_{\max}/F, \quad (2)$$

where c_i is the hepatic inlet concentration (mmol/l), c_o the hepatic outlet concentration (mmol/l), v the elimination rate (mmol/min), and F the hepatic blood flow rate (l/min); V_{\max} is the maximal elimination rate (mmol/min) and K_m the half saturation concentration (mmol/l).

In the present investigation the galactose elimination kinetics in the *in situ* pig liver is studied. It is examined if the sinusoidal perfusion model fits the data adequately. Furthermore, the results are compared with data obtained from the isolated perfused pig liver and from man.

MATERIALS AND METHODS

Hepatic vein catheterization

Eight female Danish land-race pigs, weighing from 29 to 45 kg, were fasted for 48 h but had free access to water. After premedication with 2 mg/kg of phenylcyclohexyl hydrochloride, the pigs were intubated and anaesthetized with N_2O and oxygen (75 : 25) by a respirator; they received 50 mg succinyl chloride every 60 min. Catheters were placed in the superior caval vein through the left jugular vein (for infusion of galactose and indocyanine green), in the right common carotid artery (for arterial blood sampling), and in one of the larger hepatic veins through the right internal jugular vein (for hepatic venous blood sampling). The position of the hepatic vein catheter was checked by means of X-ray two to four times during the experiment.

The intravascular pressures were recorded by electromanometers (Siemens), the mean arterial pressure was 143 ± 10 mmHg (mean \pm SD,

$n = 8$), and the pressure in the free hepatic vein 6.0 ± 2.1 mmHg. The hepatic blood flow rate was on average 1.15 ± 0.19 litre $\text{min}^{-1} \text{kg}^{-1}$ liver, the splanchnic oxygen uptake 3.31 ± 0.97 mmol $\text{min}^{-1} \text{kg}^{-1}$ liver, and the haematocrit 0.32 ± 0.02 . These values correspond to those found previously [10].

Experimental procedure

Each experiment consisted of six to seven steady-state periods with different concentrations of galactose. Galactose was given as successive continuous infusions, increasing from 0.17 to 1.3 mmol/min, following a priming dose. Each period consisted of 9–22 min for equilibration and 15 min for measurement during which five to six arterial and hepatic venous blood samples were drawn. Figure 1 gives an example.

Indocyanine green was given as a continuous infusion, 0.1 $\mu\text{mol}/\text{min}$ in 3% human albumin, following a priming dose of 0.65 μmol .

Analytical procedures

Galactose blood concentration was determined enzymatically [12]. Indocyanine green plasma concentration was determined by spectrophotometry [15].

Oxygen saturation, pO_2 and pH (Radiometer), haematocrit and haemoglobin concentrations were measured once or twice during each period. The splanchnic oxygen uptake rate was calculated as the blood flow rate multiplied by the difference between the blood concentrations of oxygen (chemically and physically absorbed) in the artery and the hepatic veins.

Estimation of parameters

Galactose concentration. The model presumes steady-state concentrations and complete distribution in the pre-hepatic splanchnic volume of distribution during each study period. As to the first condition the relative change in concentration was always less than 2% per min, and not systematic. No correction was made for this, and the mean concentration in each period was used for the calculations. The experimental

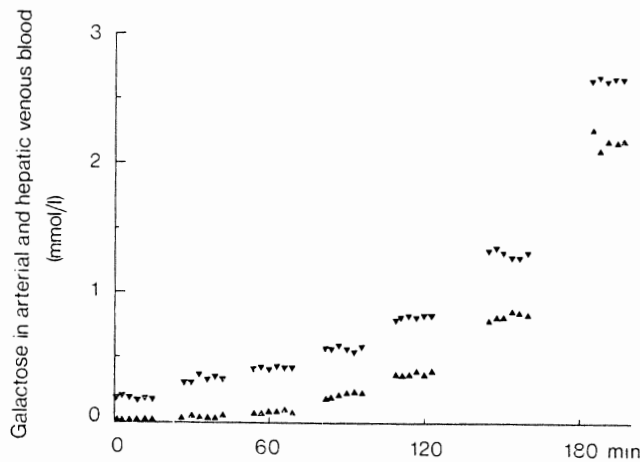


FIG. 1. Hepatic vein catheterization in pig 2. Galactose concentrations in plasma water during seven quasi steady-state periods used for estimating kinetic constants. Measurements were performed in samples from an artery (▼) and hepatic veins (▲). Infusion rates of galactose during successive periods were 0.30, 0.48, 0.61, 0.69, 0.78, 0.94, and 1.04 mmol/min. Each period was initiated by a single injection of galactose (0, 0, 0.8, 2.1, 2.1, 2.8, and 22 mmol respectively). Body weight was 42.5 kg, liver weight 0.84 kg and measured splanchnic blood flow rate on average 0.91 l/min (corresponding to 0.66 litres plasma water/min).

measurement error was estimated from the residual variation of the regression line of the concentrations on time. The galactose concentration in pig erythrocytes is negligible [11] and the mean blood concentration c_B (and the error) was converted to the concentration in erythrocyte-free plasma water, c_P , by

$$c_P = \frac{c_B}{(1 - \text{Hct}) \cdot f_P} \quad (3)$$

where f_P is the water content in plasma (0.93 [10]) and Hct the haematocrit.

Galactose elimination rate. This was calculated as a mean value in each period by $v = F(c_i - c_o)$, where F is the mean blood flow rate and c_i and c_o the mean galactose blood concentrations in the inlet and the outlet of the liver respectively. In this paper the term *elimination rate* represents splanchnic elimination, since portal blood concentrations and arterial and portal blood flow rates were not measured.

Flow rate. The splanchnic plasma flow rate was determined by means of infusion of indocyanine green (ICG). The mean concentration of ICG in each period and the error was calculated

as for galactose, and the elimination rate was calculated from the infusion rate corrected for accumulation of ICG, assuming the volume of distribution to be 5% of the body weight. The flow rate was calculated from an equation equivalent to equation (1) [10].

Statistical analysis

The purpose of the statistical analysis is to check if the model equation (2) is a reasonable fit to the measured data, as compared with the measurement error, and to estimate the parameters V_{\max} and K_m together with their relative errors. The values of c_i , c_o and F were calculated together with their measurement error $s(c_i)$, $s(c_o)$, and $s(F)$ as described above.

For each period one thus has three variables— c_i , c_o and F —which satisfy the functional relation (equation 2), but which are all measured with some error $s(c_i)$, $s(c_o)$ and $s(F)$.

We let c_i^{est} , c_o^{est} and F^{est} denote the values that fit equation (2), and we let c_i^{meas} , c_o^{meas} and F^{meas} denote the values of the three variables that were actually measured. We will measure the difference between the measured and estimated values by the weighted sum of squares

$$Q = \sum_{i=1}^n \frac{(c_o^{\text{meas}} - c_o^{\text{est}})^2}{s^2(c_o^{\text{meas}})} + \frac{(c_i^{\text{meas}} - c_i^{\text{est}})^2}{s^2(c_i^{\text{meas}})} + \frac{(F^{\text{meas}} - F^{\text{est}})^2}{s^2(F^{\text{meas}})}, \quad (4)$$

where n denotes the number of periods.

To find the best estimate of V_{max} and K_m we minimize Q with respect to all the estimated values and V_{max} and K_m subject to the non-linear equation (2). We thus have $3n+2$ parameters subject to n non-linear conditions (equation 2), corresponding to the $3n$ measured values. The distribution of the minimum value of Q (Q_{min}) will be approximately that of a χ^2 distribution with $[3n - (3n + 2 - n)] = n - 2$ degrees of freedom. This will be used to test the model (equation 2).

The technique used for minimizing Q is the classical Gauss-Newton method as described in Cleland [5]. It consists of replacing the non-linear regression problem by a succession of linear regression problems. Initial values for V_{max} and K_m were obtained from the

Lineweaver-Burk plot of $1/v$ against $1/c$, where $c = (c_i - c_o)/(\ln c_i - \ln c_o)$, which according to equations (1) and (2) will be a straight line—see equation (6) in the Discussion.

We tested whether the values of V_{max} and K_m from different animals were significantly different by calculating a variance ratio

$$\frac{1/(n-1) \sum_{i=1}^{i=n} (\ln V_{\text{max}} - \overline{\ln V_{\text{max}}})^2}{1/n \sum_{i=1}^{i=n} s^2(\ln V_{\text{max}})} \quad (5)$$

and a similar expression for K_m . These values measure the difference between the individual values of the parameters as compared to the precision with which they are determined. The values can be evaluated in a $\chi^2(f)/f$ distribution with $f = 8 - 1 = 7$ degrees of freedom.

RESULTS

The measured values of the galactose

TABLE I. Galactose elimination in pig liver *in vivo*. Mean plasma water flow (F , ml min⁻¹) and mean plasma water concentration of galactose in the carotid artery (c_i , $\mu\text{mol/l}$) and hepatic vein (c_o , $\mu\text{mol/l}$) \pm SEM*

Expt. No. (Liver, kg)	Parameter	Period						
		A	B	C	D	E	F	G
1 (0.79)	F	709 \pm 136	621 \pm 57	655 \pm 121	625 \pm 35	616 \pm 91	575 \pm 74	577 \pm 38
	c_i	147 \pm 1.4	298 \pm 7.9	483 \pm 12	878 \pm 14	1596 \pm 31	3404 \pm 24	7527 \pm 40
	c_o	24 \pm 9.1	30 \pm 8.1	62 \pm 9.0	313 \pm 7.9	995 \pm 25	2691 \pm 16	6602 \pm 31
2 (0.84)	F	623 \pm 45	675 \pm 31	640 \pm 47	651 \pm 96	646 \pm 72	722 \pm 70	1128 \pm 581
	c_i	301 \pm 8.7	535 \pm 15	660 \pm 4.3	901 \pm 13	1302 \pm 6.8	1999 \pm 23	3938 \pm 12
	c_o	29 \pm 4.2	62 \pm 9.6	125 \pm 6.4	335 \pm 4.4	608 \pm 8.1	1264 \pm 12	3208 \pm 54
3 (0.96)	F	734 \pm 41	709 \pm 90	807 \pm 79	756 \pm 57	936 \pm 56	738 \pm 17	
	c_i	146 \pm 5.0	252 \pm 6.4	405 \pm 1.7	601 \pm 11	879 \pm 11	4436 \pm 25	
	c_o	50 \pm 9.6	75 \pm 21	97 \pm 15	151 \pm 20	262 \pm 17	3391 \pm 28	
4 (0.97)	F	665 \pm 27	748 \pm 126	758 \pm 22	641 \pm 27	803 \pm 65	697 \pm 29	622 \pm 37
	c_i	174 \pm 2.3	428 \pm 4.8	566 \pm 8.2	1091 \pm 16	1822 \pm 15	3459 \pm 20	6422 \pm 51
	c_o	20 \pm 3.3	78 \pm 11	126 \pm 14	435 \pm 20	1199 \pm 11	2656 \pm 23	5533 \pm 27
5 (1.11)	F	985 \pm 39	987 \pm 45	923 \pm 57	866 \pm 45	898 \pm 231	597 \pm 92	309 \pm 14
	c_i	197 \pm 13	384 \pm 15	542 \pm 13	919 \pm 18	1600 \pm 14	3012 \pm 53	6229 \pm 43
	c_o	-11 \pm 4.9	25 \pm 3.3	63 \pm 3.4	228 \pm 5.3	683 \pm 23	1917 \pm 19	5533 \pm 90
6 (1.15)	F	583 \pm 35	641 \pm 60	626 \pm 74	566 \pm 39	780 \pm 60	937 \pm 28	906 \pm 200
	c_i	226 \pm 7.9	494 \pm 18	768 \pm 26	1121 \pm 14	2092 \pm 20	3234 \pm 11	6912 \pm 65
	c_o	0.55 \pm 1.6	37 \pm 11	133 \pm 11	315 \pm 15	1328 \pm 20	2587 \pm 20	6437 \pm 63
7 (1.19)	F	1002 \pm 45	864 \pm 24	860 \pm 37	908 \pm 43	904 \pm 24	950 \pm 23	
	c_i	90 \pm 5.2	175 \pm 6.9	356 \pm 4.1	435 \pm 8.9	675 \pm 21	2847 \pm 24	
	c_o	-5 \pm 3.6	2 \pm 3.6	15 \pm 5.4	38 \pm 5.6	89 \pm 7.0	2163 \pm 14	
8 (1.20)	F	612 \pm 58	541 \pm 23	591 \pm 51	601 \pm 50	580 \pm 33	454 \pm 40	429 \pm 19
	c_i	95 \pm 6.1	168 \pm 6.2	240 \pm 4.8	281 \pm 5.1	390 \pm 5.5	662 \pm 3.5	2141 \pm 28
	c_o	31 \pm 1.6	25 \pm 4.3	40 \pm 4.9	39 \pm 2.5	42 \pm 4.2	42 \pm 13	978 \pm 28

* SEM (standard error of the mean) is calculated from the residual variation of the regression of concentration on time. The data have been given with as many decimals as is needed for our analysis to be reproducible.

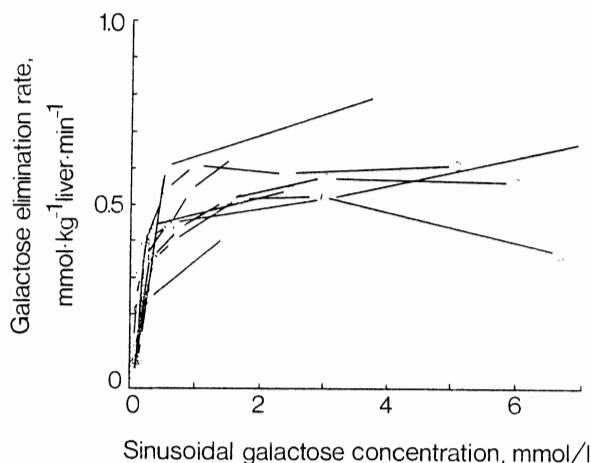


FIG. 2. Galactose elimination kinetics in eight pigs. Relation of elimination rate $v = F(c_i - c_o)$ to sinusoidal galactose concentration $\ell = (c_i - c_o)/\ln(c_i/c_o)$. Points give measured data, connected by straight lines for each experiment.

concentrations in the artery and in the hepatic veins (c_i and c_o) and the flow rate (F) are given in Table I together with their statistical errors.

Figure 2 shows plots of the elimination rates against the sinusoidal blood concentrations. It is seen that the elimination follows saturation kinetics in each of the experiments.

Table II gives the estimated values of V_{\max} and K_m and their relative errors.

The variation between the estimates from different experiments was evaluated by the

TABLE II. Kinetic parameters of galactose elimination in pig liver *in vivo*

Expt No.	V_{\max}^* (mmol min ⁻¹ kg ⁻¹ liver)	K_m^* (mmol litre ⁻¹ plasma water)	Q_{\min}^\dagger	$(n-2)^\ddagger$
1	0.72 (6)	0.21 (20)	21.5	5
2	0.73 (9)	0.26 (20)	6.6	5
3	0.95 (5)	0.55 (14)	25.4	4
4	0.64 (5)	0.31 (12)	6.2	5
5	0.64 (5)	0.12 (17)	10.2	5
6	0.55 (4)	0.18 (17)	4.3	5
7	0.60 (5)	0.13 (15)	9.2	4
8	0.61 (8)	0.58 (12)	48.9	5
Mean§	0.67 (5)	0.25 (21)		

* Numbers in parentheses denote percentage error of the estimates.

† Q_{\min} is the weighed residual sum of squares after having fitted all parameters. The weights are the reciprocal variances of the measurements (see equation 4).

‡ Degrees of freedom equal number of periods minus 2.
§ Mean values of V_{\max} and K_m are calculated from the mean of the logarithmic estimates.

variance ratio given in equation (5). The values were 7.67 for the estimates of V_{\max} and 3.13 for K_m , corresponding to P values <0.0001 and <0.001 respectively. Thus there was significant inter-individual difference between the animals for both V_{\max} and K_m .

To test the fit of the model to the data, we compared the calculated values of Q_{\min} with a 95% fractile in the χ^2 distribution with $n-2$ degrees of freedom (for $n-2=5$, $\chi^2_{0.95}=11.1$, and for $n-2=4$, $\chi^2_{0.95}=9.5$; n is the number of periods). For five of the experiments the model gives a satisfactory fit to the experimental data with the values of Q_{\min} being less than the values of $\chi^2_{0.95}$ (see Table II).

DISCUSSION

The study shows that the galactose elimination kinetics by the pig liver *in situ* can be described adequately by the model of the sinusoidal perfusion. In the model it is assumed that the local removal process in the hepatocytes can be described by saturation kinetics; this elimination creates a decreasing sinusoidal blood concentration in the direction of the blood stream.

The experimental design with successively increasing infusion doses could introduce systematic errors into the results. The functional integrity of the livers was maintained, however,

throughout the experimental period as evaluated by means of the oxygen uptake and vascular resistances. In another study using an equivalent pig preparation, we found that the galactose elimination rate (at 5 mmol/l) was unchanged throughout the experimental period, being of the same duration as in the present study [Vilstrup, H., to be published]. On the other hand, we cannot exclude systematic errors in the interpretation of the observed data due to distribution phenomena in the pre-hepatic splanchnic area in the present study.

The variation in flow between periods is relatively large, but of the same order of magnitude as in similar studies [10] and with no systematic changes. The variations probably reflect physiological variations during the experimental period, e.g. caused by variations in the depths of the anaesthesia.

Equations (1) and (2) of the model of the single sinusoid can be reformulated by eliminating the flow rate F [11] to

$$\begin{aligned} v &= \frac{V_{\max} \cdot \bar{c}}{K_m + \bar{c}}, \\ \bar{c} &= \frac{c_i - c_o}{\ln(c_i/c_o)}. \end{aligned} \quad (6)$$

This is the Michaelis–Menten relation with the concentration being the logarithmic average sinusoidal concentration \bar{c} . The \bar{c} concentration can be regarded as an ‘operative’ sinusoidal blood concentration [9]. If we assume all sinusoids to be identical, this equation for the single sinusoid is directly applicable for the whole liver with c_o being measured in the hepatic venous blood. A plot of $1/v$ against $1/\bar{c}$ therefore should give a straight line if the model fits the data.

Since there is probably complete mixing of the portal and arterial blood, all sinusoids receive blood with the same concentration. Variation of the enzyme activity and flow of the sinusoids [4] and of transit times [6] gives rise to a distribution of the outlet concentrations during physiological conditions. The hepatic venous blood consists of mixed blood coming from the different sinusoids. The concentration measured in the mixed hepatic venous blood will be higher from a liver with functional distribution of the sinusoids than from a liver without distribution due to less efficient

utilization of the enzymes. In this case the calculated value of the \bar{c} concentration will be ‘too high’—and the plot of $1/v$ against $1/\bar{c}$ will have a convex curvature. The larger the variability, the more pronounced the curvature will be [8]. In three of the experiments (Experiments 3, 4 and 8) the plots showed significant convex curvatures.

Bass & Robinson [2] have accounted for this by including an estimate of the variation of the functions between the sinusoids in the model. This so-called ‘functional variability’ is an estimate of the variation coefficient of the sinusoidal functions.* The estimated values of Q_{\min} calculated according to this distributed model show that this model gives satisfactory fits to the experimental data for five of the experiments; the fits are, however, not significantly better than for the undistributed model. For three experiments (Expts 1, 3 and 8), the values of Q_{\min} are large for both models. Thus in no case is the distributed model significantly better than the undistributed model.

The estimated values of the functional variability of the sinusoids (ϵ) range from 0 to 50%, being not significantly different from 0. A rough evaluation of the confidence intervals gives values from 0 to about 60–70% for each experiment. Thus any value of ϵ in this interval gives a good description of the data. This means that even large, perhaps the largest physiologically imaginable, variations of the sinusoidal functions cannot be detected by the present experimental method. A different analysis has been performed by Bass & Robinson [3]. A maximal limit of the functional variability of about 40% has been calculated for perfused rat livers [2].

Intrahepatic arteriovenous vascular shunts will also increase the concentration in the mixed hepatic venous blood, but the amount of shunts in normal livers is probably very small [10]. Furthermore, enzyme kinetics involving allosteric mechanisms or two parallel pathways will cause convex curvatures of the plots of $1/v$ against $1/\bar{c}$ [8], but these phenomena are not regarded as important in the present context since the rate-determining process in the hepatic galactose elimination is the irreversible

* We estimated the values of ϵ from the present data using equation (A1) from the paper of Bass, Robinson & Bracken [4]. We used a log-normal distribution to describe the variation of the values of V_{\max} between different sinusoids.

intracellular phosphorylation of galactose to galactose-1-phosphate [11].

We have previously shown that the galactose elimination in isolated perfused pig livers is adequately described by the undistributed model [11]. The estimates of V_{\max} ranged from 0.34 to 0.57 mmol min⁻¹ kg⁻¹ liver (mean 0.43, $n = 9$) and the estimates of K_m ranged from 0.17 to 0.30 mmol/l (mean 0.23). The estimates of K_m are in good agreement in both studies, whereas V_{\max} is significantly higher in the pig liver *in situ* than in the isolated perfused liver. The difference is probably due to a better function of the *in situ* liver compared to the isolated perfused preparation [13] and due to a small extrahepatic elimination in the splanchnic area.

In patients with no liver diseases [7], the splanchnic galactose elimination ranged from 1.18 to 1.76 mmol min⁻¹ kg⁻¹ liver (mean 1.43 mmol min⁻¹ kg⁻¹ liver, $n = 5$, liver weight assumed to be 2% of body weight). This is about twice as much as in the pig. The clearance of ICG in the pigs, as calculated in the present study, is 157 ± 64 ml plasma min⁻¹ kg⁻¹ liver (\pm SD, $n = 8$); this is about one-third of the values in man, being 492 ± 125 ml min⁻¹ kg⁻¹ liver ($n = 14$), liver weight assumed to be 2% of body weight, recalculated from the data of Winkler *et al.* [14]. The reason for this species difference is not clear, and it may be noticed that no difference between pig and man was found for ethanol elimination [10].

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