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EFFECT OF SINUSOIDAL PERFUSION ON GALACTOSE ELIMINATION KINETICS IN PERFUSED RAT LIVER[1]

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ABSTRACT

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Elimination of substrates from the blood by the intact liver depends on the processes of metabolism, hepatic flow and anatomical arrangements of the sinusoids. In the present study a model which assumes the elimination to take place at a sinusoidal concentration decreasing along the flow (sinusoidal perfusion model) was evaluated and compared with an earlier approach assuming the elimination to take place at the outflow concentration. Ten livers from rats (200 g) were perfused in a recirculating system; a constant galactose elimination rate was ensured by constant galactose infusion. Each experiment consisted of three steady-state periods with a blood flow of 15, 9 and 15 ml/min, respectively. The sinusoidal perfusion model predicts that reduction of flow at a given elimination rate will reduce hepatic outflow concentration, in contrast to the other model which predicts the outflow concentration to be constant. According to the sinusoidal perfusion model the logarithmic average concentration ($\hat{c}$) is independent of flow at a given elimination rate; $\hat{c} = (c_i - c_o)/\ln (c_i/c_o)$, where c_i is the inflow concentration and c_o the outflow concentration. There was a significantly lower outflow concentration during the period with low flow than during periods with high flow, whereas the logarithmic average concentration was not changed. These observations are consistent with the sinusoidal perfusion model but do not comply with the earlier proposed use of the outflow concentration as approximation to the sinusoidal concentration.

The elimination of substrates from the blood by the liver at steady-state conditions depends on the processes of metabolism and biliary excretion, and on the rate of delivery of substrate to the liver by the hepatic blood flow. The anatomical arrangement of the sinusoids may be of importance for the elimination kinetics and the influence of hepatic blood flow, thus possibly influencing pharmacokinetics (Gillette, 1971) and quantitative evaluation of liver function (Brauer, 1963).

The purpose of the present study was to provide an experimental comparison of a mathematical model recently proposed to describe the elimination kinetics of the intact liver

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(Winkler *et al.*, 1974; Bass *et al.*, 1976) with an earlier approach (Rowland *et al.*, 1973; Bischoff and Dedrick, 1968).

At steady-state with no time-dependent change in blood concentration, $dc/dt = 0$, we have

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

where v is the elimination rate, F the flow rate, c_i the inflow concentration, and c_o the outflow concentration.

The first model, the "sinusoidal perfusion model" describes the effect of the directional sinusoidal perfusion in the intact liver on the kinetic of hepatic elimination of substances from the blood. The elimination is assumed to take place in hepatocytes lining sinusoidal tubes, perfused unidirectionally from the inlet to the outlet. This creates a sinusoidal concentration decreasing along the flow due to elimination at the local concentration at each site along the sinusoid; the elimination is assumed to be an irreversible process, following Michaelis-Menten kinetics with a maximal elimination rate V_{max} and half-saturation concentration K_m. The mathematical description of these phenomena gives (Bass *et al.*, 1976)

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

This is the Michaelis-Menten relation where the concentration $\hat{c}$ is the logarithmic average of c_i and c_o. This relation is independent of flow F, whereas c_o depends on F at a given elimination rate:

$$c_o = \frac{v}{F \cdot (e^{(V_{max}-v)/FK_m} - 1)} \quad (3)$$

Figure 1 illustrates the flow dependence of c_o at the elimination rate being two-thirds of V_{max}. It is seen that at large values of V_{max}/K_m relative to F, changes of flow will cause larger changes of c_o than at smaller values of V_{max}/K_m. For V_{max}/FK_m less than 0.1, c_o is practically independent of flow.

The earlier approach (Rowland *et al.*, 1973) approximated the concentration in the sinusoids (and hepatocytes) by the hepatic outflow concentration (or a concentration proportional to it). In the first-order range v is proportional to c_o, and a so-called "intrinsic clearance" v/c_o is used as a flow-independent measurement of the hepatic capacity to eliminate substrates

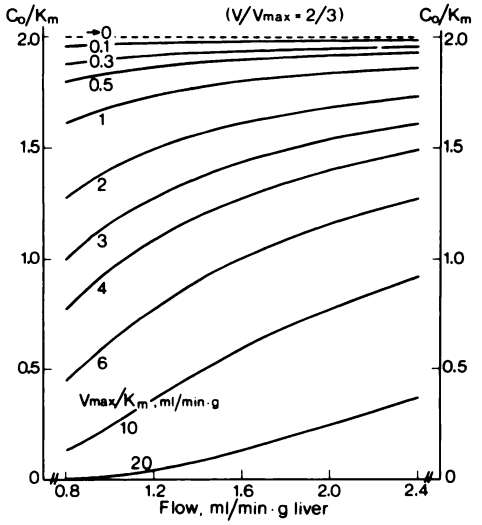

FIG. 1. Sinusoidal perfusion model. Effect of the hepatic perfusion F on the outflow concentration c_o (equation 3). The degree of saturation, *i.e.*, v/V_{max}, is 2/3. F and V_{max}/K_m are given in milliliters of plasma water per minute and gram of liver. The outflow concentration is given relative to K_m. The figure shows that c_o decreases for decreasing flow rate and that the reduction of c_o depends on V_{max}/K_m in relation to F; in the limiting case where $V_{max}/FK_m \rightarrow 0$, c_o becomes independent of F.

from the blood in the "perfusion limited model" by Rowland *et al.* (1973). If this model is extended to describe the whole concentration interval, assuming Michaelis-Menten kinetics, we get (Gillette, 1971; Rane *et al.*, 1977)

$$v = \frac{V_{max} \cdot c_o}{K_m + c_o} \quad (4)$$

At a given elimination rate, c_o thus is independent of flow, and as seen on figure 1 this equals the limiting case of the sinusoidal perfusion model for $V_{max}/FK_m \rightarrow 0$. In this case, $\hat{c}$ may be approximated by c_o. In the following we refer to the relation in equation 4 as the "venous equilibration model."

It has been shown that the galactose elimination kinetics in the isolated perfused pig liver can be adequately described by the sinusoidal perfusion model (Keiding *et al.*, 1976), and recently this has also been shown for ethanol elimination in isolated perfused pig liver and *in situ* pig liver (Keiding and Christiansen, 1976). The model with c_o as the operative concentration has been used to describe the elimination of lidocaine in perfused rat liver (Shand *et al.*, 1975), lidocaine in monkey and

man (Benowitz *et al.*, 1974), propranonol in perfused rat liver (Evans *et al.*, 1973; Branch *et al.*, 1973) and actinomycin-D in dogs (Lutz *et al.*, 1977). Experiments designed for direct comparison of the two models have not yet been reported.

The sinusoidal perfusion model predicts that at a given v, a reduction of F will reduce c_o (equation 3 and fig. 1) and increase c_i (from equations 1 and 3) in such a way that the logarithmic average concentration $\hat{c}$ remains constant (equation 2). According to the venous equilibration model reduction in F will not change c_o (equation 4) but will increase $\hat{c}$ (from equations 2 and 4).

In the present study these predictions of the two models were tested by examination of the effect of flow changes on the elimination of galactose in perfused rat livers. The livers were perfused in a recirculating system so that a constant elimination rate of galactose could be ensured by a constant galactose infusion. The blood flow rate was changed experimentally, and the changes in the inflow and outflow concentrations were observed.

Methods

Animals. Female Wistar rats weighing 179 to 211 g were starved for 18 hours before the liver perfusion.

Liver perfusion. The perfusion technique was that of Hems *et al.* (1966) with minor modifications. The medium was 1 to 3 days old washed bovine erythrocytes in Krebs-Henseleit buffer (Krebs and Henseleit, 1932) with a hematocrit of about 0.23 liter/liter. The albumin solution (bovine serum albumin powder, fraction V, Sigma Chemical Co., St. Louis, Mo.) was distilled *in vacuo* at 40°C for 5 hours. A total volume of about 92 ml of the medium was used for the perfusion. It was recirculated through the liver by means of a calibrated roller pump (O. Dick, Copenhagen, Denmark) at constant flow rates of 9 to 15 ml/min as described below. The blood was oxygenated by air + CO_2 (95:5) at an airflow of 5 liters/min; the blood flow through the oxygenator was kept at 60 ml/min by means of a digital pump; blood which was not perfused through the liver was returned to the reservoir. The inflow pressure was measured. The temperature was 37°C, and pH was kept at 7.4 by titration with 1 N NaOH.

Experimental design. The data consist of 13 experiments. Galactose was given as a priming dose so that a concentration in the medium of 0.1 to 0.3 mM was obtained and as a constant infusion of 1.3 to 1.9 μmol/min at a flow rate of 0.2 ml/min by means of a LKB ReCyChrom type 4912 A peristaltic pump given into the reservoir. The infusion was started when the liver was connected to the perfusion system.

Each of 10 experiments consisted of three periods of 50, 40 and 40 minutes, respectively. During the first and last period the blood flow was 15.0 ± 1.4 ml/min (mean ± S.D.), and during the intermediate period it was 9.0 ± 0.9 ml/min. During the last 25 minutes of each period, four to six samples of the inflow and outflow were taken at 5-minute intervals for galactose determinations. Three control experiments were performed with a flow rate of 17, 14 and 9 ml/min throughout the experimental period (Experiments 11, 12 and 13, respectively).

Analysis and calculations. The galactose concentration in blood was measured enzymatically (Kurz and Wallenfels, 1970) after precipitation by 0.3 N perchloric acid. Galactose was not detectable in the erythrocytes, and the mean plasma water concentration (Keiding *et al.*, 1976) in each period was estimated from the measured blood concentrations, the hematocrit and the mean concentration of solids in plasma [0.02 ml/ml (Keiding and Vinterby, 1976)].

The blood volume was 92 ± 6.6 ml (mean ± S.D., N = 10) at the start of the experimental period, and it decreased 14.9 ± 15.2 ml during the experiment. Blood sampling amounted to 10 ml, and in half of the experiments there was a bleeding of 4 to 10 ml. The hematocrit was 0.23 ± 0.01 liter/liter at the start of the experiment and it decreased 0.029 ± 0.014 liter/liter (the concentration of free hemoglobin in serum increased from zero to on the average 0.3 mM). The volume of galactose infusate given during the experiment was 22 ± 2 ml (mean ± S.E.M.). The estimated plasma water volume in the last period, corrected for loss of blood due to sampling and bleeding, was not significantly different from the measured volume in the first period, being 2.1 ± 11.1 ml larger (mean ± S.E.M., N = 10, $P > .50$). Evaporation of water in the oxygenator therefore probably was approximately equal to the volume of galactose infusate. Accordingly the measured concentrations in plasma water were not corrected for volume changes during the experimental period.

The oxygen saturation was measured twice in each period, and the oxygen uptake was calculated as blood flow multiplied with the inflow-outflow concentration difference.

Physiological control data. The liver weight was on the average 6.87 ± 0.88 g (mean ± S.D., N = 10).

There was no significant change in the oxygen uptake from the first to the last period ($P > .30$, paired t test), nor between the average of the values in the high flow periods and the value in the low flow period ($P > .10$); the mean oxygen uptake was 19.3 ± 4.3 μmol/min (N = 10).

The inflow pressure increased significantly from the first to the last period, the mean values being

9.3 ± 1.7 and 13.4 ± 3.0 cm of water (N = 10), respectively. In the low flow periods it was 7.2 ± 1.2 cm of water.

The experiments were performed with the flow in the last period being the same as in the first period, so that the functional anatomy of the liver preparation in the course of the experimental period could be evaluated. A total number of 17 experiments with blood flow rates 15-9-15 ml/min plus the three control experiments were performed. Seven experiments with a difference between the mean inflow plasma water concentration in the first and the last period larger than 2 times the standard deviation of the inflow concentration measurements, *i.e.*, >0.050 mM, were excluded from the data. The inflow concentration was larger in the first period than in the last period in eight experiments, and smaller in nine experiments.

Results

Figure 2 shows the galactose concentrations in one of the eperiments. In table 1 the mean plasma water concentrations in each of the three periods are given for all experiments. The average values of the concentrations in the first and last periods in each experiment were used for statistical analysis.

The comparison between outflow concentrations in the high flow and low flow periods is shown in figure 3. It is seen that the concentration was lower at the low flow than at the high flow in all of the experiments. The difference was significant as evaluated by a paired t test ($P < .01$, $N = 10$). In the three control experiments there was no significant difference (table 1). Reduction of flow thus caused a fall in the outflow concentration.

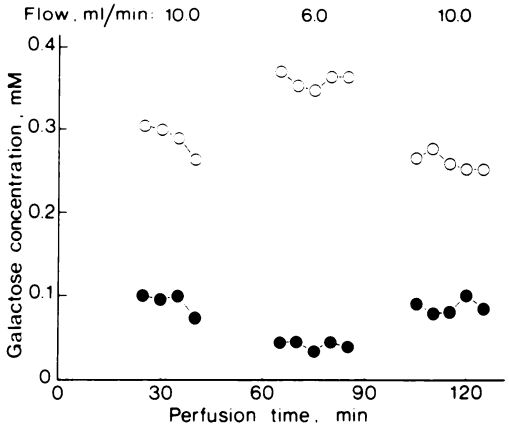

FIG. 2. Effect of flow changes on steady-state galactose plasma water concentrations in inflow (○) and outflow (●) of a rat liver perfused in a recirculating system (experiment 9). The plasma water flow rates are given on the figure, the galactose infusion was 1.83 μmol/min and the liver weight was 7.7 g.

TABLE 1

Galactose concentrations in rat livers from 200 g rats, perfused in a recirculating system with constant galactose infusions from 1.3 to 1.9 μmol/min

Expt. No.	Liver Weight	Inflow Concentration[a] at Flow[b] of			Outflow Concentration[a] at Flow[b] of		
		11 ml/min	7 ml/min	11 ml/min	11 ml/min	7 ml/min	11 ml/min
	g	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
1	5.51	0.259	0.305	0.283	0.122	0.060	0.168
2	5.65	0.363	0.410	0.349	0.240	0.150	0.197
3	6.31	0.105	0.173	0.134	0.060	0.046	0.066
4	6.77	0.353	0.449	0.367	0.152	0.137	0.172
5	6.84	0.087	0.158	0.102	0.062	0.071	0.082
6	6.88	0.255	0.297	0.257	0.130	0.118	0.155
7	7.18	0.277	0.365	0.305	0.138	0.142	0.159
8	7.37	0.211	0.318	0.258	0.097	0.118	0.139
9	7.71	0.289	0.361	0.263	0.093	0.043	0.089
10	8.39	0.312	0.362	0.347	0.185	0.141	0.205
		Control experiments with constant blood flow rates					
11	6.19	0.263	0.261	0.252	0.105	0.115	0.118
12	6.29	0.205	0.208	0.206	0.111	0.130	0.113
13	7.94	0.260	0.272	0.292	0.120	0.122	0.127

[a] Plasma water concentration c_P calculated from measured blood concentration c_B by $c_P = c_B/(1 - \text{Hct} - 0.02)$ where Hct is the hematocrit and 0.02 liter/liter the average concentration of solids in plasma.

[b] Plasma water flow in three experimental periods.

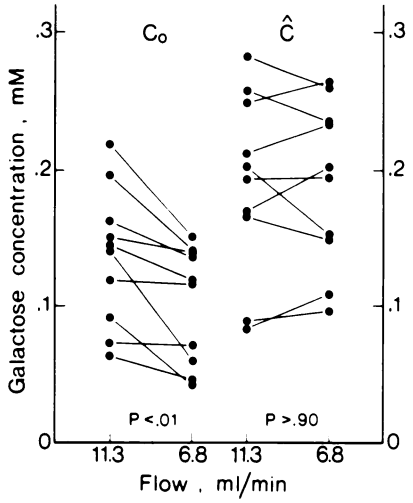

FIG. 3. Effect of flow changes on steady-state galactose concentrations in 10 rat livers, perfused in a recirculating system. Liver weight was, on the average, 6.8 g, and galactose was given as constant infusions of 1.3 to 2.0 μmol/min. Average plasma water flow rates are shown on the figure. The concentrations c_i and c_o are mean inflow and outflow plasma water concentrations of galactose, and $\hat{c}$ is the logarithmic average of c_i and c_o, $\hat{c} = (c_i - c_o)/(\ln c_i/c_o)$. Points from the same experiment are connected. Paired t tests are used for statistical analysis.

Figure 3 also shows the logarithmic average concentrations $\hat{c}$, and it is seen that in six experiments it was higher at the low flow than at the high flow, and *vice versa* in the remaining four experiments. The difference was not significant, $P > .90$. In the three control experiments the $\hat{c}$ concentrations were practically identical throughout the experiment. Reduction of flow thus caused no significant change in $\hat{c}$.

Discussion

In the present study the recirculating rat liver perfsion was chosen as the experimental model, since this preparation permits experiments with a constant elimination rate (ensured by a constant infusion) and observations of flow-dependent variations in the inflow and outflow concentrations. This design permits a qualitative test of the predictions of flow-independence, at a given elimination rate, of $\hat{c}$ or c_o according to the sinusoidal perfusion model or the venous equilibration model, respectively. The concentration which "determines" the hepatic elimination of substrates from the blood therefore can be estimated without knowledge of kinetic parameters as, *e.g.*, V_{max} and K_m. In the once-through perfusion technique, however, the inflow concentration is the controllable variable, and experimental changes in the flow rate will create changes in both elimination rate and outflow concentration according both to the sinusoidal perfusion model (equations 1 and 2) and to the venous equilibration model (equations 1 and 4). Evaluation of whether $\hat{c}$ or c_o may be best suited for approximation of the sinusoidal concentration, therefore, requires quantitative estimates of the variations in v, c_o and c_i and of V_{max} and K_m. The latter experimental model is therefore less suited for experimental comparison of the two models.

The analysis of the results requires that the metabolism in the hepatocytes and the number and geometry of the sinusoids are not influenced by the flow changes. In a previous study blood flow of less than about 0.9 ml/min and g of liver reduced the oxygen uptake and galactose elimination capacity, possibly due to collapse of sinusoids (Vilstrup and Keiding, 1976). In the present study the blood flow ranged from 1.00 to 2.84 ml/min and g of liver, and the oxygen uptake was not influenced by the low flow.

No systematic variation in the galactose elimination rate in the course of the experiment was seen. A change in the number of functioning hepatocytes (V_{max}), however, will change the blood concentrations. In order to eliminate the effect of functional changes on the results it was decided not to include seven experiments with a significant difference between the concentrations in the first and last periods (see "Methods"). However, if these seven experiments fulfilling the criteria for exclusion were nevertheless included in the analysis, the overall result remained the same: c_o was reduced ($P < .001$, $N = 17$) during low-flow period, in contrast to $\hat{c}$ ($P > .70$).

In the present rat liver perfusions, reduction of the hepatic blood flow at a constant galactose elimination rate decreased the outflow concentration and increased the inflow concentration, whereas there was no significant change in the logarithmic average concentration. These observations are consistent with the sinusoidal perfusion model and demonstrate that the decreasing concentration along the sinusoids has to be taken into account in evaluation of elimi-

nation kinetics in intact liver as first suggested by Goresky *et al.* (1973). The outflow concentration is not an appropriate approximation to the sinusoidal concentration for hepatic elimination of galactose.

For hepatic galactose elimination V_{max}/FK_m is about 2 to 4 with K_m about 0.1 to 0.2 mM (Vilstrup *et al.*, 1974; Keiding, 1973; Keiding *et al.*, 1976); the observed fall in c_o from on the average 0.13 to 0.10 mM in the present study is in agreement with these values (*cf.* fig. 1). For substances with smaller values of V_{max}/FK_m, however, the outflow concentration may be suitable as an approximation of the sinusoidal concentration (Keiding, 1976). Figure 1 shows the approximate flow independence of c_o at small values of V_{max}/K_m and high values of F, for v/V_{max} = 2/3. For smaller values of v/V_{max} the ratio V_{max}/FK_m has to be smaller to give approximate flow independence of c_o. For example, a reduction of c_o by 5% or less in the whole concentration range produced by a 2-fold reduction of F requires that V_{max}/FK_m is less than or equal to 0.1. Only at concentrations high enough nearly to saturate the elimination process may c_o or c_i be used equally in the Michaelis-Menten formula, irrespective of the values of V_{max}/FK_m.

As to future extension of the model it is seen on the basis of the present study that models of elimination kinetics in intact liver should include the effect of the directional hepatic perfusion, but it might, for example, take into account the variation, within the liver, of enzyme contents per sinusoid, of flow per sinusoid (L. Bass *et al.*, to be published) or of communications between the sinusoids.

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