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Galactose elimination kinetics in perfused rat liver after two thirds hepatectomy

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Galactose elimination kinetics was studied in isolated perfused livers (single pass) from rats subjected to two thirds partial hepatectomy 6 h earlier or immediately before investigation. No difference was found between the two groups in the maximum galactose elimination rate or in the half saturation galactose concentration. This is in contrast with *in vivo* observation of restored galactose elimination capacity 6 h after two thirds hepatectomy. The discrepancy indicates that extrahepatic factors may be of importance for the metabolic regulation in the liver during regeneration.

Key-words: liver physiology; liver regeneration; mathematical models

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The galactose elimination capacity (GEC) is taken to reflect the functional liver mass in man [11] and in animals [15, 12]. In accordance with this concept removal of two thirds of the rat liver immediately reduces the GEC corresponding to the liver mass reduction. However, within 6 h after the hepatectomy GEC is restored to control values [15] and the activity of galactose metabolizing enzymes is increased [1]. This indicates changes in the kinetics of galactose elimination during regeneration and may be a clue to metabolic regulation in that situation. The purpose of this work was to study this phenomenon by comparing the kinetics of galactose elimination of perfused rat livers from animals in which two thirds hepatectomy was performed immediately before and 6 h earlier.

MATERIAL AND METHODS

Experimental. Fed, female Wistar rats with a body weight of 180 to 200 g were anaesthetized with diethylether and sham-operated (i.e. laparotomy and exteriorization of the liver with subsequent replacement) or had two thirds of the liver resected according to Higgins & Anderson [3]. Six hours later a single pass perfusion of the liver of six sham-operated animals and five hepatectomized animals was established with a medium consisting of Krebs-Henseleit buffer with 3% albumin (Fraction V, sigma, distilled to remove residual ethyl alcohol), 1 to 3 days old washed bovine erythrocytes added to a haematocrit about 0.23 l/l, and oxygenated with atmospheric air and carbon dioxide (95:5). The temperature was 37°C and pH 7.40.

Immediately after establishment of the artificial hepatic perfusion two thirds of the liver of the sham operated control animals was removed. Each liver was perfused for 20 min without galactose in the medium followed by six periods of 15 min duration each, with different

galactose concentrations (0.05 to 4.0 mmol/l) in a randomized sequence.

During the last 5 min of each experimental period three duplicate samples of the medium were taken simultaneously from the hepatic inlet and outlet for enzymatic determination of galactose concentration [7]. Prior to each period oxygen saturation and pO_2 was determined (OSM 2, ABL, Radiometer, Denmark) in the hepatic inlet and outlet for determination of the oxygen consumption.

After the last period the livers were removed, blotted on filter paper and weighed. The perfusion flow rate was 1.7 ml/(min · g liver weight), the oxygen uptake rate was constant during all perfusions ($P>0.05$, two-way analysis of variance) but was different from perfusion to perfusion (mean ± SD: 2.48 ± 0.78 μmol/ (min · g liver), $P<0.05$, two-way analysis of variance). Thus galactose elimination was determined under conditions that are independent of flow and haematocrit [6].

Calculations. The galactose elimination rate (v) was calculated for each period as $v = F \cdot (c_{in} - c_{out})$ (μmol/min · g liver weight) where F = perfusion flow rate (ml/min), c_{in} = mean inlet perfusate galactose concentration (mmol/l), and c_{out} = the mean outlet galactose concentrations (mmol/l).

In an earlier study it was found that the galactose elimination by perfused rat livers was described better by allosteric kinetics assuming two active sites on the rate determinating enzyme than by conventional Michaelis–Menten kinetics [14]. The sinusoid galactose concentration in this model is calculated as the geometric mean of in- and outlet concentrations:

$$\hat{c} = \sqrt{c_{in} \cdot c_{out}} \quad [4]$$

The relation between v and c is then given by the 'Hill equation':

$$v = \frac{V_{max} \cdot \hat{c}^2}{(\hat{c}_{0.5})^2 + \hat{c}^2} \quad [10]$$

where V_{max} is the maximum velocity of galactose elimination and $\hat{c}_{0.5}$ is the sinusoid galactose concentration corresponding to half-maximum galactose elimination, i.e. an affinity constant.

Estimation of the kinetic constants was performed by a non-linear interative regression analysis of v on $\hat{c}$ (ROSFIT [2]). Data were weighted by $1/v^2$ where the weights were normalized to sum up to the number of periods, since the variation of the estimation of the velocity of galactose elimination was found to increase proportionally with the sinusoid galactose concentration.

RESULTS

The estimated kinetic constants for the 11 perfusions are given in Table I, and an example of the fitted curves of elimination rate versus the sinusoid galactose blood concentration is depicted in Fig. 1.

There was no significant difference between the maximum velocity of galactose elimination (V_{max}) in animals partially hepatectomized immediately before investigation and animals hepatectomized 6 h earlier ($P = 0.53$, two-tailed t-test). The mean difference was −0.038 μmol/ (min · g liver weight) (95% confidence limits: −0.369 to 0.294). Also there was no significant

TABLE I. Kinetic constants of galactose elimination by perfused livers

Immediately after 70% hepatectomy			6 h after 70% hepatectomy		
Expt. no.	V_{max}*	$\hat{c}_{0.5}$†	Expt. no.	V_{max}	$\hat{c}_{0.5}$
1	0.220	0.148	7	0.174	0.179
2	0.416	0.075	8	0.352	0.081
3	0.164	0.054	9	0.271	0.251
4	0.217	0.141	10	0.098	0.093
5	0.249	0.089	11	0.260	0.126
6	0.350	0.094			
	0.269±0.095‡	0.100±0.037		0.321±0.097	0.146±0.070

*μmol/(min · g liver).
†mmol/l.
‡mean ± SD.

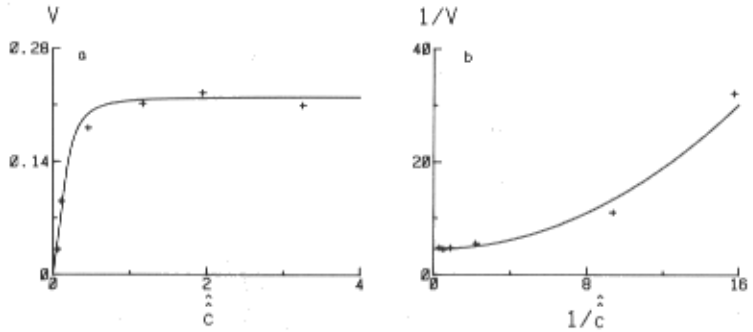

FIG. 1. Galactose elimination kinetics in perfused rat liver following two thirds hepatectomy immediately before perfusion. (a) The relation between elimination rate (V) and the sinusoid blood galactose concentration (c); (b) the double reciprocal plot. Points give experimental data of experiment no. 1, the curves are plotted from the estimated kinetic constants.

difference between the affinity constant ($\hat{c}_{0.5}$) in the two groups ($P = 0.23$). The mean difference was −0.046 mmol galactose/l (95% confidence limits: −0.239 to 0.147).

There was no difference between the liver weights after the perfusions (2.43 ± 0.28 versus 2.42 ± 0.71, mean ± SD).

DISCUSSION

By the partial hepatectomy two thirds of the liver mass is removed. The maximum rate of galactose elimination and the oxygen uptake per g liver weight was about the same as in intact livers [5]. Thus partial hepatectomy can be regarded as a simple reduction in the number of galactose-metabolizing liver cells. Likewise, the half saturation galactose concentration ($\hat{c}_{0.5}$) after partial hepatectomy was not changed 6 h after the resection. This is in contrast to the situation after toxic liver injury with carbon tetrachloride where $\hat{c}_{0.5}$ was decreased [13].

In vivo the galactose elimination capacity per g liver was increased threefold 6 h after partial hepatectomy [15], probably due to an increased activity of galactose-metabolizing enzymes [1]. The 95% confidence limits of the difference in V_{max} has an upper limit of 0.3 μmol/(min · g liver). Thus the present data may fail to detect a twofold increase in V_{max}, whereas the threefold increase of the galactose elimination capacity can safely be excluded.

The finding of increased galactose elimination after partial hepatectomy *in vivo* [15] but not in the present perfusion studies may raise the hypothesis of a circulating factor *in vivo* which stimulates galactose metabolism. Whether such a factor is identical to the factor that is supposed to regulate hepatic proliferation [8] cannot be determined since it has not yet been identified. The present study does not allow for testing the hypothesis of the specific nature of a humoral factor which would stimulate the galactose elimination. It may be virtually any circulating substance, for example one related to the increase in amino acids and glucagon or the decrease in insulin and thyroxin [9] seen *in vivo* after partial hepatectomy.

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