Scand. J. clin. Lab. Invest. **43,** 127–131, 1983.

Effects of acute carbon tetrachloride intoxication on kinetics of galactose elimination by perfused rat livers

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Vilstrup, H. Effects of acute carbon tetrachloride intoxication on kinetics of galactose elimination by perfused rat livers. *Scand. J. clin. Lab. Invest.* **43,** 127–131, 1983.

Seventeen livers of 200 g rats, of which seven had received 435 μl of CCl_4 (LD_{15}) by gastric tube 36 h earlier, were isolated and perfused in a once-through system at 9 ml/min with a semi-synthetic medium to which galactose was added to concentrations from 0.1 to 3.3 mmol/l. The relative liver weight was increased by 13% by CCl_4. The portal pressure was 16 cmH_2O and the oxygen consumption of the livers 20 μmol/min, both unchanged by CCl_4. In each liver four to six sets of galactose elimination rate at different galactose concentrations were measured. The relation was examined by a model including modification of the simple Michaelis–Menten kinetics by allosterism. The resulting Vmax values were decreased by CCl_4 from 1.20±0.18 in controls to 0.78±0.19 μmol × min^{-1} × 100 g^{-1} body weight (mean±SEM, $P<0.001$). The affinity constant was decreased from 0.18±0.06 to 0.11±0.02 mmol/l (mean±SEM, $P<0.015$) in CCl_4-treated livers. The decrease in affinity constant may—if it also applies to other substances eliminated by the liver—have implications for the use of a clearance as a measure of functional capacity, since this presupposes that the affinity constant remains unchanged during liver disease.

Key-words: animals; galactose; kinetics; liver function tests; toxins

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The galactose elimination capacity (GEC) in man and rats is determined as the rate of disappearance from the blood of galactose at galactose concentrations sufficiently high to secure Vmax, or zero order kinetics, of the process [8, 17]. Decreased GEC during liver disease is taken to reflect reduced 'functional liver mass'.

0036–5513/83/0400–0127 $02.00

The molecular basis for the impaired galactose elimination is not always known, but the ability to eliminate galactose appears to be associated with unknown 'essential' liver functions.

GEC in rats subjected to experimental acute hepatitis by carbon tetrachloride (CCl_4) has been found to be decreased, down to 40% of control values depending on dose and time after intoxication [18]. It is not known, however, whether this injury also influences the

elimination rate at lower galactose concentrations, as reflected by changes in the affinity constant of the blood galactose disappearance kinetics as well as in Vmax.

This cannot be tested in the intact rat, because only one galactose concentration can be studied in each animal due to the time needed for equilibration, but in the isolated, perfused rat liver several elimination rate to concentration sets can be determined.

The purpose of this work was to investigate the effects of acute carbon tetrachloride intoxication on the kinetics of elimination of galactose from the blood by perfused rat livers.

MATERIAL AND METHODS

Experimental. The material consists of 17 female Wistar rats with an average body weight of 197 g (Table I). Seven of them were given 220 μl of CCl_4 per 100 g body weight, dissolved in 2 ml of corn oil, by gastric tube. Food was withheld for 12 h before and 1 h after gavage. The intoxicated rats were investigated 36 h after dosage.

The livers of the fed animals were isolated as described by Hems *et al.* [6]. The perfusion was performed at 37°C by a once-through system with a perfusate consisting of Krebs-Henseleit buffer [11], 3% bovine albumin (fraction V, Sigma), 3-days old washed bovine red cells to a haemoglobin concentration of 5.4 mmol/l (range 4.1–7.1), and galactose (Kabi) added to concentrations from 0.09 to 3.29 mmol/l. The perfusate was oxygenated with atmospheric air with 5% CO_2 and was titrated to pH 7.4. A roller pump maintained a perfusion flow rate of on the average 9.0 ml/min (range 7.5–11.0 ml/min). The perfusion (i.e. portal) pressure was measured in a side tubing immediately before the liver. The perfusions lasted for 75–105 min and were divided into five to seven periods of 15 min. The first period was for stabilization of the liver, during the remaining four periods the perfusate contained galactose at different concentrations, administered in random order. Samples were taken during the last 10 min of each period, one from the inlet reservoir, and three to six at the outlet from the liver. Galactose was measured enzymatically [12]. Oxygen consumption (Osm 1, Radiometer, Denmark) was measured in the middle of the sampling period. Following perfusion the livers were weighed after blotting an filterpaper.

Calculations. The velocity of the galactose elimination (v) was calculated for each period as $v = F \cdot (c_{in} - c_{out})$ (μmol$\times$min$^{-1}\times$100 g^{-1} body weight), where F = perfusion flow rate (ml/min), c_{in} = inlet perfusate galactose concentration (mmol), and c_{out} = the mean of measured outlet galactose concentrations (mmol/l). An earlier study (including the ten control experiments of this work) showed that the galactose elimination by perfused rat livers is describable by an allosteric kinetics with two active sites on the rate determining enzyme [19]. The sinusoidal galactose concentration was therefore calculated as

$$\hat{c} = \left[\sqrt{\left(\frac{c_{out}^{-1} - c_{in}^{-1}}{c_{in} - c_{out}} \right)} \right]^{-1} \quad [7],$$

which is a modification accommodating for allosterism of the sinusoidal concentration in simple Michaelis–Menten kinetics:

$$\hat{c} = \frac{c_{in} - c_{out}}{1n\, c_{in} - 1n\, c_{out}} \quad [3].$$

This expression compensates for the non-linear fall of galactose concentration in the perfusate

TABLE I. Physiological data and kinetic constants of galactose elimination by perfused livers of control rats and rats acutely intoxicated with CCl_4

	Total BW* (g)	Liver weight† (g)	Relative liver weight (%)	Portal pressure (cm H_2O)	Oxygen consumption‡ (μmol/min)	Vmax (μmol$\times$min^{-1} $\times$100 g^{-1}BW)	K (mmol/l)
Control (n=10)	202±15	7.6±1.0	3.8±0.4	14.3±3.9	20.1±2.8	1.20±0.18	0.18±0.06
CCl_4 (n=7)	190±7	8.2±0.6	4.3±0.3	17.4±3.1	19.5±2.7	0.78±0.19	0.11±0.02
P (t-test)	=0.05	=0.15 NS§	<0.05	=0.1 NS	=0.7 NS	<0.001	<0.015

*Body weight.
†After perfusion.
‡Chemically bound. Physically bound contributes with less than 10% [10].
§Not significant.

along the sinusoid. The relation between v and $\hat{c}$ is then given by the 'Hill equation' [15]:

$$v = \frac{Vmax \cdot \hat{c}^2}{K^2 + \hat{c}^2},$$

which is recognizable as a modification of the Michaelis–Menten kinetics. V_{max} is the maximum velocity of galactose elimination and K is the galactose concentration corresponding to half-maximal galactose elimination rate, i.e. the affinity constant. The kinetic constants were estimated in the double reciprocal Lineweaver-Burk plot:

$$1/v = \frac{1}{Vmax} + \left(\frac{K^2}{Vmax} \cdot \frac{1}{\hat{c}^2}\right),$$

where the y-intercept is $Vmax^{-1}$ and the slope is $k^2/Vmax$.

These constants were found by linear regression analyses by the least squares method of $^1/v$ on $^1/\hat{c}^2$. Differences between means were evaluated by *t*-tests [2]. *P*-values smaller than 0.05 were considered statistically significant.

RESULTS

Table I gives physiological data and kinetic results of the perfusions. The weight of livers of CCl_4-treated animals was slightly but not significantly higher than that of controls, but the liver/body weight ratio was significantly raised by 13%. The portal pressures increased slightly during perfusion, but the average values are not significantly different. The hepatic oxygen consumption did not change systematically with the rate of galactose elimination in different perfusion periods, and was not changed by CCl_4.

Figures 1 and 2 show v versus $\hat{c}$ plots of galactose elimination in control and CCl_4-treated livers, respectively, and Fig. 3 shows the double

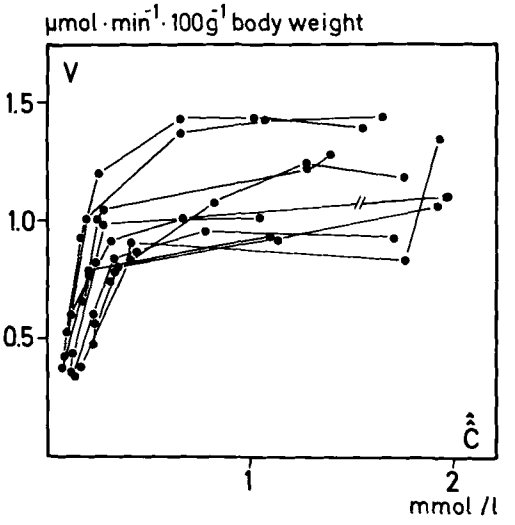

FIG. 1. Relation between galactose uptake rate (v) and sinusoidal galactose concentration ($\hat{c}$) in 10 perfused control rat livers. Points indicate experimental data, connected by straight lines for each liver. The interrupted line should continue to $\hat{c}$=3.18 mmol/l.

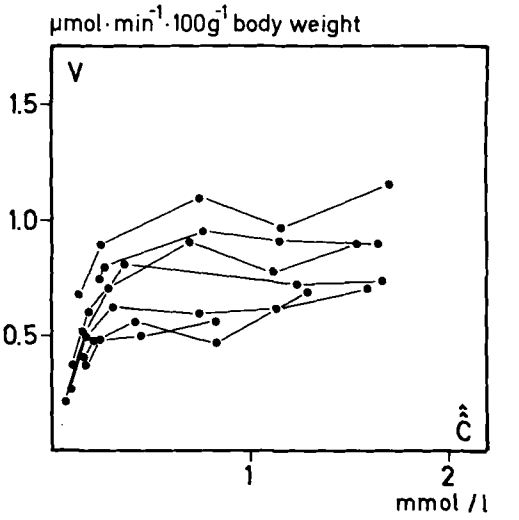

FIG. 2. Relation between galactose uptake rate (v) and sinusoidal galactose concentration ($\hat{c}$) in seven perfused livers of rats given CCl_4 36 h earlier. Points indicate experimental data, connected by straight lines for each liver.

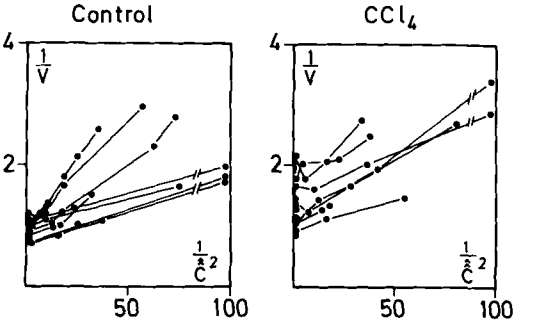

FIG. 3. Relation between 1/v and $1/\hat{c}^2$ of data shown in Fig. 1 to the left and in Fig. 2 to the right. Points indicate experimental data, connected by straight lines for each liver. The highest actual value on the abscissa is 230.

reciprocal plots. It is seen that a saturation pattern emerged in both control and CCl_4 livers, in the latter at a lower level. The double reciprocal plots show no systematic trend to curvilinearity. The resulting calculated average Vmax and K values of CCl_4-treated livers were both decreased by about one third as compared with controls (Table I).

DISCUSSION

The precise nature of the injurious effect of CCl_4 on the liver has not been established, and toxicity to other organs may contribute to mortality. The increase in liver weight is probably due to accumulation of fat and water. Possibly also regeneration, likely to have started after 36 h [4], may contribute although the increase was only half of that found after partial hepatectomy [20]. The insignificant increase in portal pressure during constant perfusion flow rate indicates unchanged flow resistance in the damaged livers.

The unchanged oxygen consumption after CCl_4 is in agreement with the normal hepatic contents of ATP found earlier [18]. Since the first and rate limiting step in the conversion of galactose is a bisubstrate ATP dependent kinase step [8], this finding indicates that the effect of CCl_4 on galactose metabolism is due to a general enzyme defect rather than a more specific effect.

Under normal conditions the transport of galactose across hepatocyte membranes is so fast that it does not interfere with elimination [5]. After CCl_4 membranes may be disorganized because of lipid peroxidation [16], and it is not known whether this will lead to increased passive influx of galactose or to compromised transport. In this work, however, the whole-organ elimination of galactose from the blood is measured. A distinction between different changes leading to decreased elimination rate is not possible, and also of minor importance for the purpose of the study, as long as a specific interaction between galactose and CCl_4 is not suspected.

In another model of liver injury, viz. 70% hepatectomy, the GEC has been found unchanged, probably due to a compensatory induction of galactose enzymes [20]. This is in contrast with the present and earlier findings in rats [18] and with findings in patients with severe hepatitis [14]. The reason may be that the regenerative response elicited by partial hepatectomy is less pronounced after CCl_4 intoxication and in fulminant hepatitis [13]. This means that acute CCl_4 intoxication may be a suitable experimental model of acute liver disease, at least as far as the galactose elimination is concerned.

The allosteric model used here for quantitation of the kinetic constants does not complicate interpretation of the results. Allosterism may explain convexity of the 1/v versus $1/\hat{c}$ plots, corresponding to a slightly sigmoid course of the v versus $\hat{c}$ plots. The inflection point, i.e. where the slope changes from increasing to decreasing in the latter plot, corresponds to $K/\sqrt{3}$ [15] on the abscissa, which in the present study is 0.1 mmol/l. This concentration is so low that the sigmoidity in Fig. 1 and 2 is undiscernable. The kinetic constants, when estimated in a simple Michaelis–Menten kinetics neglecting the sigmoidity, are about 25% larger than those reported here, and are both decreased by CCl_4. Thus the allosteric deviation from simple Michaelis–Menten in this situation may be regarded primarily as a mathematical model for calculation of the kinetic constants.

It is usually presumed that Vmax reflects the amount of active enzymes and changes accordingly, whereas the affinity constant is a protein characteristic of the enzyme, and as such is not subject to fluctuations.

This is the basis for use of the clearance measure for liver function tests: Vmax of the elimination of many test substances, e.g. antipyrine [1], can only be effectuated at concentrations above feasible levels. In such cases the capacity is quantitated indirectly by the clearance of the test substance, which is proportional with Vmax by the factor 1/(the affinity constant). Use of clearance as a measure of the capacity of the process thus presupposes that the affinity constant is the same in the normal and the diseased liver.

In the present work Vmax was decreased by CCl_4 as found earlier in intact rats [18]. Moreover, the affinity constant was decreased. As a result the Vmax/K ratio, which in a strict Michaelis–Menten kinetics would have been the (enzyme limited) clearance [9], was here increased from 6.7 to 7.1 ml/min by CCl_4. Thus, if this also applies to other test substances, clearance determinations yield misleading information as to the amount of active enzymes or 'the functional liver mass' after CCl_4.

The livers were investigated 36 h after CCl_4, at which time the effect on the capacity for galactose elimination is known to be at maximum [18]. However, the time course of changes in the affinity constant is not known, but it may not change in parallel with Vmax, so that larger variations in the calculated clearance may occur. This problem should be considered when dissociation between liver function tests is studied and when phenomena such as overshoot and rapid induction of functions are described. Therefore, direct measurement of Vmax is preferable for this purpose.

ACKNOWLEDGMENTS

I am grateful to P.B. Vendsborg for assistance with the liver perfusion technique.

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Received 9 August 1982
Accepted 3 November 1982