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The galactose elimination capacity as a quantitative measure of liver function in acute carbon tetrachloride intoxication of rats

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Abstract. In rats given rising single doses of carbon tetrachloride (CCl_4) intragastrically the relation between dose and mortality, between time after injection and the quantitative liver function measured by the galactose elimination capacity (GEC), and between the dose and the GEC, was examined. The change in hepatic contents of galactose metabolites after CCl_4 was measured. There was a linear relation between the dose and mortality. No rat died later than 36 h after injection. Following injection of a dose lethal to 15% of rats the GEC fell to 40% of control after 36 h and was normalized after 72 h. There was a dose dependent decrease in the GEC with rising doses given 36 h earlier up to a dose lethal to 15%. Galactose metabolites other than UDP-galactose, which was decreased, were not affected by CCl_4, suggesting a general enzyme depression. The results are compatible with the concept of proportionality between the GEC and 'the functioning liver mass' and indicate that the GEC presents prognostically valuable information during acute hepatic insufficiency.

Key words. Liver function, carbon tetrachloride, galactose.

Introduction

The galactose elimination capacity (GEC) has been claimed to be a measure of the functional liver mass [1], and has as such been used as a prognostic indicator in acute liver failure [2]. The clinical usefulness of the GEC presupposes that it is reduced in parallel with the liver damage. The purpose of the present work is to test this hypothesis on the assumption that carbon tetrachloride (CCl_4) as a direct hepatotoxin produces dose and time dependent liver damage.

Material and Methods

Female Wistar rats (body weight 170–200 g) were given CCl_4 dissolved in 2 ml of corn oil by gastric tube. Food was withheld for 12 h before and 1 h after the administration. The GEC was measured [3] during thiopental anaesthesia (100 mg/kg body weight) after tracheostomy and nephrectomy. Galactose was given intravenously as a priming dose (200 μmol/100 g body weight) followed by a continuous infusion of 0.5–2.5 μmol/min for 1 h. Blood samples were drawn every 10 min during infusion for enzymatic determination of galactose concentration [4]. The mortality of the procedure was about 5%.

The GEC was calculated as

$$\text{GEC} = I - (dc/dt \times 0.4 \times \text{body weight})$$

where I is the infusion rate, dc/dt is the linear slope of the galactose blood concentration–time curve, and 0.4 x body weight is the estimated volume of distribution of galactose. Liver tissue was analysed after freeze-clamping. The following components were measured by the methods indicated: galactose [4], galactose-1-phosphate [5], UDP-galactose [6], UDPG [7], ATP [8], and ADP and AMP [9].

Results

The CCl_4 dose–mortality relation was determined by giving 100, 250, 400, 630, 890 and 1100 μl per 100 g body weight to groups of fifteen to twenty-seven rats. The lowest dose killed no animal, and the highest all animals. The mortality was linearly related to dose, and LD_{50} was about 600 μl CCl_4 per 100 g body weight. No animal died later than 36 h after the administration

Fig. 1 shows the change in GEC from 6 to 36 h after administration of 220 μl of CCl_4 per 100 g body weight (resulting in a mortality of about 15%). After 6 h it was reduced to 73% ($P < 0.01$), the lowest values (41%) were reached after 36 h, and after 72 h the values were similar to the control values. The liver weight decreased slightly initially and after 96 h it was increased by 29%. The GEC per g liver weight therefore remained below the control value after 96 h ($P < 0.02$). The relation between the dose of CCl_4 and the GEC was determined 36 h after CCl_4 administration (Fig. 2). At doses producing no mortality (55 and 110 μl 100 g body weight) the reduction in GEC was linearly related to the dose (to 76% and 57% of the control value, respectively). At higher doses with increasing mortality the reduction observed was relatively smaller, and at 220 μl/100 g body weight it was reduced to 41%. When 330 μl/100 g body

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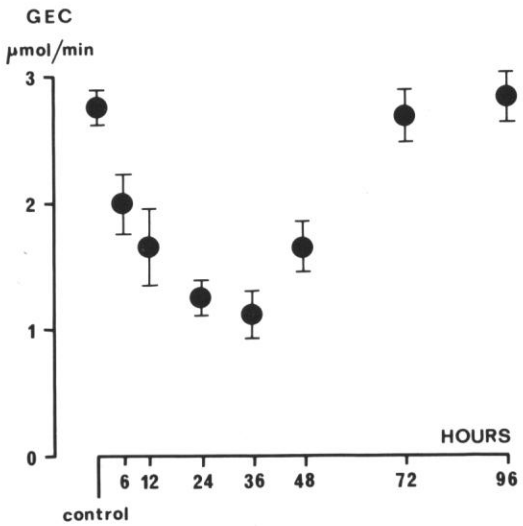

Figure 1. The time course of the galactose elimination capacity after intragastric injection of 220 μl of CCl_4 per 100 g rat. Dots and bars indicate mean and standard error of the mean, respectively ($N = 5–9$).

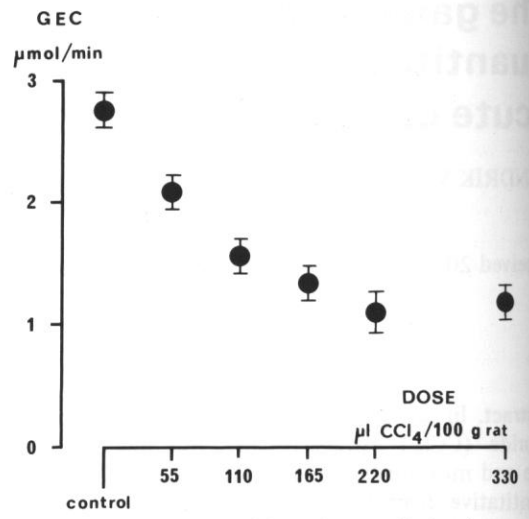

Figure 2. Dose dependence of the galactose elimination capacity of CCl_4 given intragastrically 36 h earlier. Dots and bars indicate mean and standard error of the mean, respectively ($N = 4–9$).

Table 1. Effect of 220 μl of CCl_4 given 12–36 h earlier on total hepatic contents of galactose metabolites in rats

	Metabolite (μmol)						
	Galactose	Gal-1-P	UDP-gal.	UDP-glucose	ATP	ADP	AMP
Control (11)*	19 ± 1†	6.5 ± 1.5	1.1 ± 0.1	0.9 ± 0.1	14 ± 1	9.2 ± 0.6	2.8 ± 0.6
CCl_4 (9)	20 ± 2‡	7.0 ± 1.3	0.7 ± 0.1	1.0 ± 0.2	11 ± 2	10.0 ± 1.4	6.0 ± 0.8

*Number of rats in parentheses.
†Mean ± SEM.
‡Corrected for increase in liver weight.

weight was given no further reduction of the GEC was seen. The liver weight was significantly increased (to 127%) after a dose of 100 μl/100 g body weight, but at higher doses no further increase was observed.

In liver tissue the concentration of UDP-galactose was decreased and that of AMP increased ($P < 0.01$), after 220 μl of CCl_4/100 g body weight, whereas galactose and other metabolites studied (Table 1) showed no consistent changes.

Discussion

Dose dependence of CCl_4 liver damage has been demonstrated morphologically [10] and by a correlation between the rise in serum enzyme levels and dose [11]. The impairment of (microsomal) drug metabolism also parallels the dose of CCl_4 given [12]. The linear dose–mortality curve supports this view, even if toxicity to other organs may contribute to mortality.

The GEC was progressively reduced by doses of CCl_4 up to 220 μl/100 g body weight. About 85% of animals survived this dose. Higher doses caused no further decrease in GEC. It should be noted, however, that only animals capable of surviving were examined, since all mortality occurred less than 36 h after CCl_4 administration, and the examination was made after 36 h where the reduction of GEC was found to be greatest.

These findings are subject to several different interpretations. One is that the mortality is not related to liver failure but is associated with damage to organs such as brain or kidney. It is equally possible, however, that liver damage, equivalent to a reduction of the GEC to less than 40% of normal, is incompatible with survival. Validation of the latter possibility will require a technical procedure which permits repeated determination of the GEC, showing either that the GEC is reduced to below 40% of control values, or that it falls more rapidly in animals dying from the intoxication. In that case the reduction to about 40% will represent the 'survival limit' of liver function. This is in good agreement with observations in patients with fulminant liver failure [4].

In the present study the GEC was most severely reduced 36 h after CCl_4. The rise in serum enzyme activities has been found to be at the maximum after a similar period [13] whereas necrosis is most extensive in histological preparations after 48 h [10]. The metabolism of amidopyrine and barbiturates is most severely impaired after 8 h [12] and the activity of hepatic glucuronyl transferase after 24 h [14].

This time sequence may reflect early (primary?) injury to the microsomal system and later (secondary?) disintegration of enzyme systems of the cytosol and mitochondria (e.g. galactokinase and ATP formation) along with loss of membrane structure (escape of cell constituents, morphological changes). In a patient with paracetamol intoxication, which may have a mechanism similar to that of CCl_4 [15], the maximum decrease in the prothrombin index – which depends on microsomal function [16] – was found to precede that of the GEC [17].

A reduction of the GEC to 40% does not necessarily imply that 40% of the liver function is preserved, since extrahepatic removal of galactose is likely to take place. In man the removal is estimated to be 20–30% of the normal elimination capacity [18]. As rats are known to be able to survive after removal of about 85% of the liver [19], the above findings are compatible with a similar extrahepatic elimination of galactose in the rat. In that case the 'survival limit' of liver function will be less accurately determined from measurements of GEC, unless the extrahepatic removal is rather precisely known.

These considerations leave out a dynamic aspect. Thus, for how long will the animal survive with a liver function below the 'survival limit'. Possible influences of CCl_4 and cell damage on regeneration are also unclear. Further studies are needed to evaluate the significance of GEC determinations for predicting the fatality of cases of toxic liver injury. Apparently measurements of serum enzyme activities and microsomal function (drug elimination) will not provide this information, since maximum changes are produced by non-fatal doses of CCl_4, i.e. about 100 μl/100 g body weight [11, 12].

The pattern of galactose metabolites identified in liver tissue indicates that the two first steps in the conversion of galactose to glucose metabolites were inhibited to approximately the same extent and more so than the third step. An impaired oxidative phosphorylation, as suggested by the increase in AMP, may contribute to the inhibition of the first – kinase – step. Thus the decrease in the GEC seems to depend primarily on a general metabolic depression, which means that the CCl_4 intoxication may be a relevant experimental model as to the effect of an acute liver injury on the GEC.

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References

1 Tygstrup N. (1966) Determination of the hepatic elimination capacity (Lm) of galactose by single injection. *Scand J Clin Lab Invest* **18**, 118–125.
2 Ranek L., Andreasen P.B. & Tygstrup N. (1976) Galactose elimination capacity as a prognostic index in patients with fulminant liver failure. *Gut* **17**, 959–964.
3 Keiding S. (1973) Galactose elimination capacity in the rat. *Scand J Clin Lab Invest* **31**, 319–325.
4 Kurz G. & Wallenfels K. (1970) D-Galactose, UV-test mit Galactose, UV-test mit Galactose-Dehydrogenase. *Methoden der Enzymatischen Analyse* (ed. by H.-U. Bergmeyer), p. 1241. Verlag Chemie, Weinheim.
5 Zöllner N. & Heuckenkamp P.-U. (1970) Alpha-D-Galactose-1-Phosphat, Bestimmung mit Uridyltransferase. *Methoden der Enzymatischen Analyse* (ed. by H.-U. Bergmeyer), p. 1250. Verlag Chemie, Weinheim.
6 Keppler D. & Decker K. (1970) Uridin-5'-Diphospho-Galactose. *Methoden der Enzymatischen Analyse* (ed. by H.-U. Bergmeyer), p. 2142. Verlag Chemie, Weinheim.
7 Keppler D. & Decker K. (1970) Uridin-5'-diphosphoglucose. *Methoden der Enzymatischen Analyse* (ed. by H.-U. Bergmeyer), p. 2141. Verlag Chemie, Weinheim.
8 Lamprecht W. & Trautschold I. (1970) ATP, Bestimmung mit Hexokinase und Glucose-6-Phosphat Dehydrogenase. *Methoden der Enzymatischen Analyse* (ed. by H.-U. Bergmeyer), p. 2024. Verlag Chemie, Weinheim.
9 Jaworek D., Gruber W. & Bergmeyer H.-U. (1970) Adenosin-5'-Diphosphat. *Methoden der Enzymatischen Analyse* (ed. by H.-U. Bergmeyer), p. 2051. Verlag Chemie, Weinheim.
10 Gardner G.H., Grove R.C., Gustafson R.K., Maire E.D., Thompson M.J., Wells H.S. & Lamson P.D. (1925) Studies on the pathological histology of experimental carbon tetrachloride poisoning. *Bull Johns Hopkins Hosp* **36**, 107–133.
11 Korsrud G.O., Grice H.O. & McLaughlan J.M. (1972) Sensitivity of several serum enzymes in detecting carbon tetrachloride-induced liver damage in rats. *Toxicol Appl Pharmacol* **22**, 474–483.
12 Dingell J.D. & Heimberg M. (1968) The effects of halogenated hydrocarbons on hepatic drug metabolism. *Biochem Pharmacol* **17**, 1269–1278.
13 Zimmerman H.J., Kodera Y. & West M. (1965) Rate of increase in plasma levels of cytoplasmic and mitochondrial enzymes in experimental carbon tetrachloride hepatotoxicity. *J Lab Clin Med* **66**, 315–323.
14 Chojecki A. & Kern F. (1961) The effect of dietary cirrhosis and CCl_4 poisoning on glucuronyl transferase activity of rat liver. *Gastroenterology* **40**, 521–531.
15 Mitchell J.R., Thorgeirsson S.S., Potter W.Z., Jollow D.J. & Keiser H. (1974) Acetaminophen-induced hepatic injury: Protective role of glutathione in man and rationale for therapy. *Clin Pharm Ther* **16**, 676–684.
16 Palade G. (1975) Intracellular aspects of the process of protein synthesis. *Science* **189**, 347–358.
17 Vilstrup H., Henningsen N.C. & Hansen L.F. (1977) Liver damage after paracetamol. *Ugeskr Laeg* **139**, 831–834.
18 Ranek L. (1977) Extrahepatic elimination of galactose in man. 12th Meeting of European Association for the Study of the Liver, September 1977, Greece. *Digestion* (in press).
19 Weinbren K. & Woodward E. (1964) The late incorporation of ^{32}P from orthophosphate into deoxyribonucleic acid of rat liver after subtotal hepatectomy. *Br J Exp Path* **45**, 442–449.