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PRESERVATION OF GLUCURONIDATION IN CARBON TETRACHLORIDE-INDUCED ACUTE LIVER INJURY IN THE RAT*

PAUL V. DESMOND, ROBERT JAMES, STEVEN SCHENKER, JOHN F. GERKENS and ROBERT A. BRANCH†

Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine and the Veterans Administration Medical Center, Nashville, TN 37203, U.S.A.

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Abstract—The influence of carbon tetrachloride on several routes of drug metabolism has been investigated in the rat *in vivo*, in isolated perfused livers, and in hepatic microsomal preparations to evaluate the hypothesis that hepatic glucuronyl transferase activity is preserved when mixed-function oxidase activity is impaired by this hepatotoxin. Impaired oxidative metabolism after acute administration of carbon tetrachloride was confirmed *in vivo* by a 65 per cent reduction in the rate of elimination of $^{14}CO_2$ in breath after [^{14}C]aminopyrine administration and a 58 per cent reduction in the clearance of lorazepam in the whole animal. In the isolated perfused livers of control rats, glucuronide metabolism of lorazepam accounted for 26 per cent of its overall elimination; thus, by inference, oxidative metabolism accounted for the remainder. Carbon tetrachloride pretreatment resulted in a 63 per cent reduction in total lorazepam clearance. In microsomal preparations, cytochrome P-450 concentration, cytochrome P-450 reductase activity, and activities of *o*-demethylation of *p*-nitroanisole and hydroxylation of aniline were reduced by 63, 32, 85 and 95 per cent, respectively, after exposure to carbon tetrachloride. In contrast, in the carbon tetrachloride-damage isolated perfused liver the proportion of lorazepam conjugated to lorazepam glucuronide increased from 26 to 43 per cent of the dose administered. Furthermore, *in vitro* microsomal glucuronidation of *o*-aminophenol was not decreased by carbon tetrachloride pretreatment, while glucuronidation of *p*-nitrophenol actually increased significantly by 49 per cent. Solubilization of microsomes with Triton X-100 resulted in a 10.4-fold increase in glucuronyl transferase activity in control microsomes, but in only a 5.7-fold increase in microsomes from carbon tetrachloride-treated rats. This suggests that carbon tetrachloride enhanced basal glucuronidating activity but decreased the total amount of enzymes present. These overall results suggest that microsomal glucuronidation activity is spared in an experimental liver injury in which oxidative metabolism is impaired.

Patients with parenchymal liver disease have impaired elimination of many drugs. Individuals with cirrhosis and acute viral hepatitis have reduced metabolic clearances of diazepam [1] and chlordiazepoxide [2], while the clearances of the closely related benzodiazepines, oxazepam [3] and lorazepam [4], are unaffected by liver disease. We have suggested previously that a possible explanation of these discordant findings lies in the different routes of elemination of these drugs. In man, diazepam and chlordiazepoxide are initially metabolized by a number of oxidative processes, whereas oxazepam and lorazepam are metabolized only to their respective glucuronides [5]. These observations could be accounted for by two explanations that are not mutually exclusive: (1) there could be a relative sparing of hepatic glucuronyl transferase enzymes in comparison to mixed-function oxidase enzymes in patients with parenchymal liver disease, and (2) there could be extrahepatic glucuronidation of these drugs.

To examine further the former of these two hypotheses, we have studied various oxidative and glucuronidative pathways of drug metabolism in an experimental model of carbon tetrachloride-induced liver injury in the rat. Lorazepam has been used as our drug model for a benzodiazepine since in the rat it has the advantage of being metabolized by both oxidative metabolism and by glucuronidation [6], allowing us to investigate simultaneously both routes of metabolism. In addition, we have investigated other substrates that undergo oxidation or glucuronidation in hepatic microsomal preparations to determine the generality of these findings.

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† Author to whom all correspondence should be addressed: R. A. Branch, M.D., Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, U.S.A.

METHODS

Experimental model of liver injury

Two groups of male Sprague–Dawley rats constituted control (30) and treatment (29) groups. Animals were fed Purina rat chow and water *ad lib*. One

group of rats (200–250 g) received carbon tetrachloride [0.8 ml/kg of a 1:3 (v/v) solution of carbon tetrachloride in corn oil] by i.p. injection; studies were performed 24 hr later, after a 12-hr fast. This dose of carbon tetrachloride produced histological damage in 50–60 per cent of hepatocytes as assessed by light microscopy; the serum aspartate aminotransferase at the time of study was 1114 ± 555 mU/ml. The rats in the control group were given 3.2 ml/kg of corn oil by i.p. injection and had serum aspartate aminotransferase levels of 65 ± 15 mU/ml. At the time of the study, the liver weights of the control and carbon tetrachloride-treated rats were 7.2 ± 0.9 and 10.0 ± 2.3 g respectively ($P < 0.50$).

Experimental protocol

In each group of rats the following studies were performed: (1) an aminopyrine breath test ($N_c = 6, N_c = 6$); (2) lorazepam kinetics in the whole animal ($N_c = 6, N_c = 6$); (3) lorazepam kinetics in the isolated perfused liver system ($N_c = 6$, Ne = 6); and (4) measurement of mixed-function oxidase and glucuronidation activities in microsomal preparations ($N_c = 12, N_c = 11$).

Pharmacokinetic studies

Aminopyrine breath test [7]. One-half ml containing 0.5 μCi of [^{14}C]aminopyrine (82 mCi/mmole, New England Nuclear Corp. Boston, MA) was injected i.p. into unanesthetized rats. Rats were housed in individual air-tight restraining cages; exhaled $^{14}CO_2$ was drawn through concentrated sulfuric acid to remove water and then through a scintillation vial containing 10 ml of 2:1 (v/v) methanol–ethanolamine mixture as a trapping agent to collect all CO_2 expired during consecutive 15-min periods starting 60 min after drug administration. Trapped radioactivity was determined after adding 10 ml A.C.S. (Amersham Searle, Arlington Heights, IL). A plot of the logarithm of the amount of radioactivity recovered in breath against the mid-point of each timed collection yielded a mono-exponential curve from which the half-life could be calculated by a least squares regression analysis.

Lorazepam clearance in whole animal. Cannulae were placed in a femoral artery and vein under light ether anesthesia. These cannulae were buried subcutaneously and brought out on the lower back of the animal. Animals were studied after they had recovered fully from the anesthesia. Lorazepam (2 mg/kg) was injected intravenously, and 0.5 ml of arterial blood was collected at 30-min intervals over 3 hr for lorazepam determinations.

Lorazepam clearance in isolated perfused liver. Livers from rats were removed surgically and perfused via the portal vein on an Ambec-Perfusion 210 apparatus as described by Evans *et al.* [8]. The bile duct was cannulated, and bile was collected throughout the experiment. The perfusate was 20% rat blood and 80% Krebs bicarbonate buffer solution equilibrated with 95% O_2–5% CO_2 to maintain a pH 7.4 at 37°. The volume of the perfusate was 100 ml, and a constant flow rate of 20 ml/min was maintained. After 20 min of equilibration, lorazepam (0.3 mg) was added as a single dose to the reservoir. Perfusate samples were collected at intervals over 150 min for lorazepam estimation. After the sample at 180 min, the liver was removed, weighed, and homogenized. Then the concentrations of larazepam in plasma, perfusate, bile, and liver homogenates were measured using g.l.c. with an electron capture detector as described previously [9]. Oxazepam was added as an internal standard to each sample of bile, homogenate, and perfusate prior to extraction. Lorazepam glucuronide was calculated by measuring lorazepam in the sample before and after Glusulase incubation [4].

The clearance of lorazepam (Cl) was calculated by dividing the dose by the area under the blood or perfusate concentration/time curve, using the trapezoidal method, and extrapolating to infinity, using linear regression analysis to determine the terminal rate constant of elimination from the log concentration/time relationship. Half-life ($T_{\frac{1}{2}}$) was calculated by dividing 0.693 by this rate constant, and the apparent volume of distribution (V_d) was estimated from:

$$V_d = \frac{Cl \times T_{\frac{1}{2}}}{0693} \quad (1)$$

Preparation of microsomes

Microsomal preparations were made according to the method of Franklin and Estabrook [10]. After removal of the liver, the portal vein was flushed with cold saline and the tissue was homogenized in 0.25 M sucrose. After an initial centrifugation at 19,000 *g*, the supernatent fraction was centrifuged at 100,000 *g*. The final microsomal pellet was suspended in Tris–sucrose.

Protein concentration was determined by the method of Lowry *et al.* [11]. Microsomes were frozen at −20° overnight and used immediately the next day.

Spectral and enzymatic determinations.

All spectral enzyme studies were performed at 25° on an Aminco DW-2 UV-Vis spectrophotometer. Cytochrome P-450 was measured by the method of Omura and Sato [12] using an extinction coefficient of 91 mM^{-1} cm^{-1}. NADPH–cytochrome *c* reductase was determined by the method of Masters *et al.* [13]. Nitroanisole *o*-demethylase activity in microsomes was measured using the method described by Buening and Franklin [14] and modified by Netter and Seidel [15]. Aniline hydroxylase activity was determined by the procedure of Imai *et al.* [16] based on the measurement of formaldehyde by Nash [17]. The assay for the glucuronidation of *o*-aminophenol and *p*-nitrophenol utilized the protein and drug concentrations of Litterst *et al.* [18], but the values for *o*-aminophenol are given as the change in optical density at 550 nm according to the method of Levvy and Storey [19]. All *in vitro* drug metabolism assay results are expressed in nanomoles per minute except for *o*-aminophenol glucuronidation. For the experiments measuring the rate of glucuronidation in microsomes solubilized by 0.05% Triton X-100, the concentration of *p*-nitrophenol was five times higher than in the "native" microsomes, and the assay was run 10, and not 15, min.

Statistics

Statistical evaluations were performed with the two-tailed unpaired Student's *t*-test. A P value of < 0.05 was considered to be the minimal level of statistical significance.

RESULTS

Treatment with carbon tetrachloride 24 hr before administration of [^{14}C]aminopyrine resulted in a marked change in the rate of elimination of $^{14}CO_2$ in breath (Fig. 1). The normal half-life of 52 ± 3 min increased to 151 ± 12 min ($P < 0.001$) after carbon tetrachloride treatment.

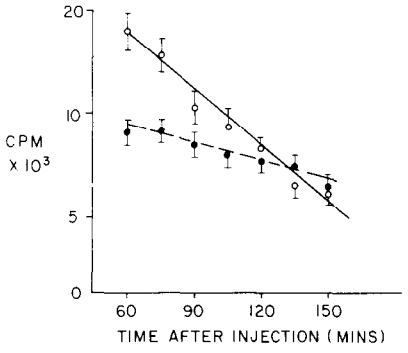

Fig. 1. Rate of $^{14}CO_2$ breath elimination after intravenous [^{14}C]aminopyrine administration (0.5 μCi of ^{14}C) in control rats (○) and rats pretreated with carbon tetrachloride (0.8 ml/kg, i.p.) 24 hr before the experiments (●) (mean ± S.E.; six rats in each group)

Carbon tetrachloride pretreatment caused a reduced rate of elimination of lorazepam in both the intact rat (*in vivo*) and in the isolated perfused rat liver studies (Fig. 2), such that plasma concentrations were higher in animals with liver injury compared to controls in all samples collected after 60 min. In the whole animal, clearance was significantly reduced by 58 per cent and this change in clearance was reflected by an increase in half-life of 126 per cent (Table 1). In the isolated perfused liver studies, the mean clearance in control animals was the same as the mean clearance in the whole control animal. The smaller apparent volume of distribution in the perfused liver experiments resulted in a shorter half-life compared to the whole animal. Carbon tetrachloride caused similar reductions in clearance in the isolated perfused liver studies (63 per cent) compared to that observed in the whole animal (58 per cent) and did not significantly change the apparent volume of distribution. The 3-fold increase in half-life in the perfused carbon tetrachloride-damaged liver experiments reflects the decrease in clearance. It is quantitatively greater than the change in half-life for the whole animal only because of the differences between the distribution volumes of the two models.

Having established that carbon tetrachloride-induced liver injury in the rat reduced the clearance of unchanged lorazepam, we investigated the extent

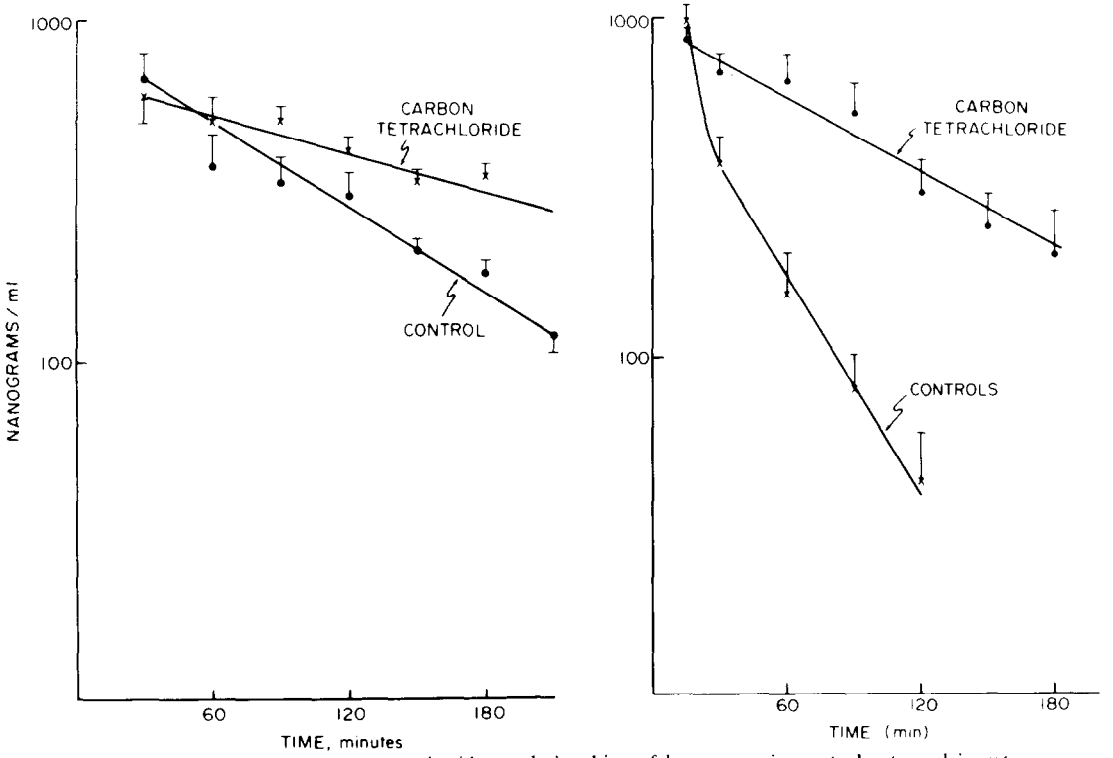

Fig. 2. Blood or perfusate concentration/time relationships of lorazepam in control rats and in rats pretreated with carbon tetrachloride (0.8 ml/kg) 24 h before the experiment. Left panel: following intravenous administration of 2 mg/kg lorazepam to the intact rat. Right panel: following addition of 0.3 mg lorazepam to a 100 ml reservoir in the isolated perfused rat liver experiment (mean ± S.E.; six rat in each group)

Table 1. Lorazepam disposition in rats pretreated with CCl_4*

	Clearance (ml/min)	Volume of distribution (ml)	Half-life (min)
Whole animal			
Control	9.1 ± 6.1	1358 ± 923	93 ± 31.8
CCl_4	3.8 ± 2.0†	1262 ± 862	244 ± 147
% Difference	−42	−7	+162
Isolated perfused liver			
Control	9.7 ± 4.9	216 ± 90	17 ± 8
CCl_4	3.6 ± 1.9†	306 ± 80	70 ± 27
% Difference	−63	+42	+312

* Rats were pretreated with 0.8 ml/kg (i.p.) of a 25% solution of carbon tetrachloride 24 hr before the study. The intact animals received 2 mg/kg of lorazepam by intravenous injection, while 0.3 mg of lorazepam was added to a 100 ml reservoir in isolated perfused rat liver experiments. Mean ± S.D. of six rats in each group.
† $P < 0.05$.

to which lorazepam was metabolized by conjugative and non-conjugative routes of elimination by measuring the total accumulation of lorazepam glucuronide and unchanged lorazepam at the end of each isolated perfused liver experiment (Table 2). From this we could infer the amount eliminated by oxidative pathways. In control rats there was essentially complete metabolism of the parent drug after 180 min with only 1.1 μg of the unchanged drug recovered from perfusate, liver, and bile combined. Approximately 26 per cent of the parent drug (80 μg) had been converted to lorazepam glucuronide, and of this 76 per cent was present in bile, 15 per cent in perfusate, and 9 per cent in the liver. Following carbon tetrachloride, bile volume was reduced from 1.99 ± 0.32 ml/180 min to 1.31 ± 0.2 ml/180 min ($P < 0.05$). In carbon tetrachloride-treated rats, most (89 per cent) of the parent drug was also metabolized by 180 min so that only 32.8 μg of the unchanged lorazepam was recovered at the end of the perfusion. Conjugation then accounted for 43 per cent (130 μg) of total metabolism. The major proportion of additional glucuronide was present in perfusate and liver.

In microsomal enzyme preparations from carbon tetrachloride-pretreated rats, there was a reduction in cytochrome P-450 concentration and evidence of impaired mixed-function oxidation activity for both *p*-nitroanisole *o*-demethylation and aniline hydroxylation (Table 3). In contrast, glucuronidation of *p*-nitrophenol significantly increased by 49 per cent and glucuronidation of *o*-aminophenol was unaltered. This rate of glucuronidation represents the "native activity" of microsomes per mg of microsomal protein without any specific procedures to enhance glucuronyl transferase activity. In a further series of experiments (Table 4), maximum glucuronidating activity was measured after solubilization of microsomes in Triton X-100 (0.05%). In the "native" microsomes, a small (29 per cent) but significantly higher glucuronidation activity was observed after carbon tetrachloride pretreatment. After solubilization, glucuronidation activity in control microsomes increased by 10.4-fold. In contrast, the glucuronidation activity in microsomes from carbon tetrachloride-treated rats increased by only 5.7-fold. Thus, in comparison to controls maximum glucuronidating activity was significantly reduced by 30 per cent. This represents a smaller decrease than the 63 per cent reduction in cytochrome P-450 concentration from the same microsomes (Table 3).

Table 2. Recovery of lorazepam and its glucuronide at the end of the isolated perfused liver experiments in control rats and CCl_4-pretreated rats*

	Lorazepam glucuronide (μg)		Unchanged lorazepam (μg)	
	Control	CCl_4	Control	CCl_4
Perfusate	12.4 ± 7.7	44.8 ± 18.6†	0	22.9 ± 20.4†
Bile	61.3 ± 12.6	73.3 ± 25.8	0.2 ± 0.1	0.3 ± 0.1
Liver	7.0 ± 7.9	12.2 ± 10.3†	0.8 ± 0.5	9.6 ± 6.6
TOTAL	79.2 ± 15.3	130.2 ± 26.0†	1.1 ± 0.5	32.8 ± 23.4†

* Rats were pretreated with carbon tetrachloride (0.8 ml/kg, i.p., of a 25% solution) 24 hr before the experiment; 0.3 mg of lorazepam was added to a 100 ml reservoir. Mean ± S.D. of six rats in each group.
† $P < 0.05$.

Table 3. Microsomal enzyme concentrations and drug-metabolizing activities in microsomes prepared from control rats and rats pretreated with carbon tetrachloride

	Control	CCl_4	% Difference
Cytochrome P-450 (nmoles/mg protein)	0.92 ± 0.11	0.34 ± 0.05†	− 63
NADPH–cytochrome *c* reductase [nmoles·(mg protein)$^{-1}$·min^{-1}]	124 ± 31	85 ± 22†	− 32
p-Nitroanisole *o*-demethylation [nmoles·(mg protein)$^{-1}$·min^{-1}]	0.72 ± 0.15	0.11 ± 0.04†	− 85
Aniline hydroxylation [nmoles·(mg protein)$^{-1}$·min^{-1}]	0.83 ± 0.17	0.06 ± 0.05†	− 93
p-Nitrophenol glucuronidation [nmoles·(mg protein)$^{-1}$·min^{-1}]	4.5 ± 1.3	6.7 ± 1.4†	+ 49
o-Aminophenol glucuronidation [O.D. × 10^3·(mg protein)$^{-1}$·min^{-1}]	4.3 ± 0.4	5.1 ± 0.2	+ 19

* Rats were pretreated with carbon tetrachloride (0.8 ml/kg, i.p., of a 25% solution) 24 hr before the experiment. Mean ± S.D. of six rats in each group.
† $P < 0.05$

Table 4. Influence of carbon tetrachloride pretreatment on *p*-nitrophenol glucuronidation before and after solubilization with Triton X-100 (0.05%)*

	p-Nitrophenol glucuronidation [nmoles·(mg protein)$^{-1}$·min^{-1}]	
	Before Triton X-100	After Triton X-100
Controls (N = 6)	5.1 ± 0.7	53.2 ± 3.4
CCl_4 (N = 5)	6.6 ± 0.9†	37.7 ± 6.3†

* Rats were pretreated with carbon tetrachloride (0.8 ml/kg, i.p., of a 25% solution) 24 hr before the experiments. Values are means ± S.D.
† $P < 0.05$, compared to control.

DISCUSSION

The results of the present study demonstrate that, 24 h after carbon tetrachloride administration to the rat, oxidative metabolism of lorazepam was impaired while glucuronidation was relatively preserved. These observations are similar to our findings in man where patients with both acute and chronic liver disease had impaired elimination of benzodiazepines metabolized by mixed-function oxidation [1, 2] but showed essentially no abnormality in the elimination of benzodiazepines metabolized by glucuronidation [3, 4]. The same divergent response has been observed by us with cimetidine, which inhibits the oxidative metabolism of chloridazepoxide but does not alter the glucuronidation of lorazepam or oxazepam in man [20].

It is well recognized that administration of carbon tetrachloride to rats causes extensive hepatocellular damage. Within 15 min of its administration, changes in membrane 5′-nucleotidase activity have been observed [21] and by 60 min there is evidence of an increase in diene conjugate content of microsomal lipids suggesting lipid peroxidation of the microsomal membrane [22], an increase in RNA cell sap indicating degranulation of the endoplasmic reticulum, reduced oxidative demethylation, and reduced glycine incorporation into protein [22]. These early changes indicate primary membrane damage, thought to be induced by homolytic cleavage of a carbon–chlorine bond with subsequent free radical effects on the tissue [23]. By 24 h, evidence of cell deaths is visible by light microscopy, and additional evidence of extensive cell damage, such as changes in mitochondrial function [24], is present. We chose to investigate drug metabolism 24 h after carbon tetrachloride administration as a model of extensive cell damage that might be found in severe acute viral hepatitis, rather than as a model of a particular type of hepatotoxin.

In preliminary experiments, we confirmed that oxidative metabolism *in vivo* was influenced by a dose of 0.8 ml/kg of a 25% solution of carbon tetrachloride in corn oil given 24 hr prior to testing. The approach adopted was to administer aminopyrine with a ^{14}C-label in each methyl position and to follow the elimination of $^{14}CO_2$ in the breath using an adaptation of the approach of Bircher *et al.* [25]. The aminopyrine is initially demethylated and the ^{14}C undergoes a series of metabolic changes before a proportion is excreted in the breath; the rate of this elimination has been shown to parallel the reduction in plasma level of parent durg [25]. This approach has been used previously in the rat as a measure of acetaminophen hepatotoxicity [26]. In the present study, carbon tetrachloride pretreatment was shown to increase the half-life of aminopyrine elimination from 52 to 151 hr (Fig. 1). This represents a 65 per

cent reduction in the rate of elimination of aminopyrine.

To investigate oxidative and glucuronidative metabolism in the same *in vivo* experiments, we chose lorazepam, a benzodiazepine, because in the rat it is metabolized by both routes of elimination, with oxidative metabolism being predominant [6]. In control animals, lorazepam kinetics in the whole animal indicated a high clearance with respect to expected liver blood flow, supporting the observation that it is a high intrinsic clearance drug in this species [6]. Furthermore, the similarity of the clearances in the whole animal and that in the isolated perfused liver (Table 1) suggests a predominant hepatic elimination. In both the whole animal and in the isolated perfused liver, pretreatment with carbon tetrachloride resulted in equivalent reductions in total clearance of unchanged drug.

To ascertain the relative contributions of oxidative and conjugative metabolism to lorazepam elimination, recovery of the glucuronide in perfusate, bile, and liver homogenate was measured. The difference between the sum of parent drug and conjugate then provided an indirect measure of oxidative metabolism. At a time of major reduction in total drug clearance, recovery of lorazepam glucuronide increased from 26 per cent of the dose of parent drug administered in controls to 43 per cent after carbon tetrachloride (Table 2), suggesting that glucuronidation had been either relatively spared or even increased. This concept must be interpreted with caution as reduced oxidative clearance would result in higher perfusate concentrations of lorazepam throughout the experiment. If glucuronidation was decreased, but to a lesser extent than oxidation, then a greater proportion of lorazepam should be conjugated when the drug is completely metabolized. This is more clearly illustrated by conceiving of total clearance as the sum of oxidative and glucuronidative clearances. In an analogous approach to measuring renal clearance, each component can be estimated by dividing the total recovery of that route of metabolism from bile, homogenate, and perfusate by the area under the perfusate parent drug concentration versus time curve. Using this approach, the control animals had oxidative and conjugative clearances of 7.4 ml/min and 2.56 ml/min respectively. Rats pretreated with carbon tetrachloride had clearances of 2.04 ml/min and 1.56 ml/min respectively. Thus, oxidative clearance had been reduced by 71 per cent and conjugative clearance by 39 per cent, confirming a relative sparing of conjugation rather than an actual increase.

Even though carbon tetrachloride pretreatment of rats is known to influence cytochrome P-450 at doses lower than those required to induce hepatotoxicity [27], the findings of preservation of conjugation, with a reduction in oxidation in livers with marked histological evidence of cellular damage, are consistent with the observations of Bock *et al.* using 1-napthol in the isolated perfused liver [28] and with observations in patients with hepatitis and cirrhosis of impaired clearance of diazepam and chlordiazepoxide, two drugs that are initially metabolized by oxidative processes, whereas the clearances of lorazepam and oxazepam, two drugs that in man are only conjugated, remain normal [29]. Further evidence that this difference in response is not confined to the benzodiazepine group of drugs is the observations of essentially normal conjugation of chloramphenicol [30] and salicylamide [31] in patients with cirrhosis and of normal hepatic bilirubin glucuronyl transferase activity in patients with hepatitis and cirrhosis [32].

Several possible explanations could account for the preservation of glucuronidation with hepatic damage. It is possible that differences in enzymes or cofactors could result in the mixed-function oxidation system being more susceptible than the microsomal glucuronidative system to hepatic injury. To investigate this further and to assess whether the metabolism of other substrates is affected similarly, we isolated microsomes from rats pretreated with carbon tetrachloride and from their appropriate controls and investigated mixed-function oxidase and glucuronidase activities. The reductions in cytochrome P-450 and mixed-function oxidase activities for the metabolism of *p*-nitroanisole and aniline were quantitatively similar to that observed in the [^{14}C]aminopyrine breath test in the whole animal, namely a reduction of approximately 60–90 per cent in animals with acute hepatotoxicity (Table 3). Thus, even in the presence of excess cofactors (i.e. NADPH, etc) there was evidence of reduced rates of metabolism that were quantitatively similar to the decrease in cytochrome P-450 concentration. In contrast, the rate of glucuronidation of *o*-aminophenol was not decreased, whereas that of *p*-nitrophenol apparently increased by 49 per cent. The relative preservation of glucuronidation in freshly prepared microsomes, at a time when oxidative drug metabolism is impaired, is similar to observations made with microsomal glucuronidation of 1-napthol and concentration of cytochrome P-450 [28]. These results suggest that perturbation influences the activity of these two species of microsomal enzymes to different extents. It has been suggested that mixed-function oxidase enzymes are located on the surface of the membrane, while glucuronyl transferase enzymes are distributed deeper within the membrane [33, 34]. As a consequence, under normal conditions, only a relatively small proportion of glucuronyl transferase enzymes would interact with substrate. Any technique that tends to disrupt or solubilize the membrane, however, would result in increased activity of these enzymes. In the present study, *in vitro* solubilization of microsomes with Triton X-100 resulted in a 10.4-fold increase in glucuronidating activity in control rats. In contrast, in the carbon tetrachloride-pretreated rats (even though initial native activity which most closely represents *in vivo* activity [26] was higher than in controls) the increase in activity with solubilization was only 5.7-fold, and the total maximum activity was less than in controls. Bock and coworkers have demonstrated similar trends with 1-napthol glucuronidation [28]. In their studies, native microsomal glucuronyl transferase activity was increased 24 hr after carbon tetrachloride pretreatment, and solubilization of microsomes further increased control activity by approximately 15-fold. Following carbon tetrachloride pretreatment the effects of solubilization

were decreased to a 7-fold increment such that the absolute maximum rate of glucuronidation was decreased by approximately 55 per cent. These two studies suggest that (1) the total amount of glucuronyl transferase enzyme was reduced by carbon tetrachloride pretreatment, (2) the native activity had been increased by the hepatotoxin, possibly by a process analogous to Triton X-100, and (3) even with severe hepatic damage there was a considerable reserve of enzyme not expressing any activity. It is possible that lipid peroxidation due to carbon tetrachloride could cause this microsomal membrane perturbation [22]. However, studies in mice and hamsters with acetaminophen and furosemide, hepatotoxins that do not cause lipid peroxidation, have demonstrated a similar sparing of microsomal glucuronidating activity [35]. This suggests that sparing of glucuronidation is a more generalized phenomenon associated with various causes of microsomal membrane damage. It is possible that a similar process of activation is sufficient to maintain the selective glucuronidation of drugs that has been observed in patients with hepatocellular disease. The deeper, "protected" location of glucuronyl transferases within the microsomes is another possible explanation for its preservation in the presence of liver injury. The possible contribution of extrahepatic glucuronidation of drugs in this setting also remains to be investigated.

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