would be expected that 1,1-DCE could produce functional changes in both the ER calcium pump and ER contribution to calcium homeostasis without observable changes in ER morphology, MFOS activity or G6Pase activity.

In summary, this study shows that 1,1-DCE promptly inhibits a calcium homeostatic function of liver ER. The correlation with GSH depletion and the effect of MFOS induction on calcium pump inhibition suggest that this is a direct effect of a 1,1-DCE metabolite on the calcium pump. As a result of calcium pump inhibition, calcium released from the ER may serve to trigger changes that result in a massive influx of extracellular calcium and, ultimately, cytotoxicity.

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Carbon disulfide hepatoxicity and inhibition of liver microsome calcium pump

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Certain chlorinated hydrocarbon hepatotoxins inhibit the liver endoplasmic reticulum (ER,* microsome) calcium pump after exposure *in vivo* or *in vitro* [1–3]. Some investigators suggest that disruption of calcium homeostasis and alteration of intracellular calcium distribution by these toxins may initiate a cascade of events that terminates in hepatic necrosis [1–5]. Because of a possible mechanistic role of calcium pump inhibition in hepatotoxin action, it is of interest to examine other classes of hepatotoxins for the ability to inhibit the liver ER calcium pump. CS_2 is a hepatotoxin that produces extensive centrilobular necrosis in phenobarbital-pretreated, starved rats, while in normal rats CS_2 produces fatty infiltration but little necrosis [6–8]. This compound is appropriate as a model of non-halogenated hepatotoxins because its toxic actions range from lipid accumulation in the liver to hepatocytic necrosis.

Male Sprague–Dawley rats (250–400 g) were used for this study. Animals were allowed free access to food and water throughout the experiments. CS_2 (Fisher Scientific Co., Fair Lawn, NJ) was diluted in corn oil and administered i.p. or orally. Rats were pretreated with phenobarbital (J. T. Baker Chemical Co., Phillipsburg, NJ) (80 mg/kg) 72, 48 and 24 hr before CS_2. Microsomal calcium pump activity was measured in the following medium: 100 mM KCl, 30 mM imidazole–histidine buffer (pH 6.8), 5 mM $MgCl_2$, 5 mM ATP (pH adjusted to 6.8 with imidazole), 5 mM ammonium oxalate, 5 mM sodium azide, 20 μM $CaCl_2$ ([$^{45}Ca^{2+}$], 0.2 μCi/ml) and 20–50 μg microsomal protein (or equivalent 12,500 *g* supernatant fluid)/ml [1, 2]. The assay was initiated by addition of the membrane fraction to prewarmed assay medium (37°). At timed inter-

* Abbreviations: ER, endoplasmic reticulum; G6Pase, glucose-6-phosphatase; SGPT, serum glutamic pyruvic transaminase; 1,1-DCE, 1,1-dichloroethylene; GSH, glutathione; and MFOS, mixed function oxidase system.

Table 1. Effect of phenobarbital pretreatment on CS_2 inhibition of liver ER calcium pump*

	Microsomal calcium pump activity† nmoles Ca^{2+}	Microsomal calcium μg Ca^{2+}	Microsomal G6Pase activity‡ μmoles PO_4
	mg protein	mg protein	mg protein
Normal animals			
Control	188 ± 13	0.34 ± 0.01	2.9 ± 0.2
CS_2	169 ± 22	0.31 ± 0.03	2.9 ± 0.1
	(90 ± 12)	(89 ± 7)	(102 ± 5)
Phenobarbital-pretreated			
Control	172 ± 17	0.32 ± 0.02	2.3 ± 0.2
CS_2	101 ± 10	0.21 ± 0.01	2.3 ± 0.2
	(60 ± 6)	(63 ± 5)	(106 ± 12)

* Normal and phenobarbital-pretreated animals received corn oil i.p. CS_2-treated rats received CS_2 (1 ml/kg) diluted in corn oil, 1 hr before being killed. Phenobarbital-pretreated animals received 80 mg/kg 72, 48 and 24 hr before CS_2. The microsomal fraction was isolated and used to determine calcium pump activity, G6Pase activity, and calcium as described in the text. Data are expressed as the means ± S.E.M. for the determination in samples from six or seven livers. Data in parentheses are percent control.

† Total nmoles Ca^{2+} accumulated during 30 min, per mg protein.

‡ Total μmoles PO_4 accumulated during 15 min, per mg protein.

vals, samples were removed and filtered through 0.45 μm nitrocellulose cellulose filters, and [$^{45}Ca^{2+}$] was determined by liquid scintillation spectrophotometry. Microsomal calcium was determined by atomic asborption spectrophotometry, as previously described [2]. Glucose-6-phosphatase activity was determined as described by Aronson and Touster [9]. Glutathione (GSH) was determined as described by Jaeger *et al.* [10]. Lipids were extracted from microsomes after *in vivo* administration of CS_2, and the extent of lipid peroxidation was determined as conjugated dienes at 243 nm as described by Klaassen and Plaa [11]. As a measure of hepatotoxicity, serum glutamic pyruvic transaminase (SGPT) activity (Sigma Reagent Kit, Sigma Chemical Co., St. Louis, MO) was determined 24 hr after CS_2 treatment. Protein was determined by the Lowry method as described by Shatkin [12].

ER calcium pump activity, G6Pase activity, or the amount of calcium isolated with the microsomal fraction was not altered 1 hr after i.p. CS_2 administration to normal rats (Table 1). CS_2 administration to phenobarbital-pretreated rats inhibited ER calcium pump activity 40% after 1 hr and reduced the amount of calcium associated with the microsomal fraction 40%. However, activity of an ER marker enzyme (G6Pase) was not altered after CS_2 administration to phenobarbital-pretreated rats (Table 1). These effects could have been anticipated in that chlorinated hydrocarbon hepatotoxins have been shown to produce a similar spectrum of effects. $CHCl_3$ has been shown to be a potent hepatotoxin in phenobarbital-pretreated animals [2, 13] and was a potent inhibitor of the ER calcium pump only in phenobarbital-pretreated animals [2]. CCl_4, $CHCl_3$ and 1,1-dichloroethylene (1,1-DCE) have been shown to reduce ER calcium pump activity and calcium associated with the microsomal fraction simultaneously [2, 14]. Both $CHCl_3$ and 1,1-DCE inhibited the ER calcium pump but did not inhibit the ER marker enzyme G6Pase [2, 10, 11, 14].

Similar effects on calcium pump activity, G6Pase activity and microsomal calcium were seen after oral administration of CS_2 to phenobarbital-pretreated rats (Table 2). In addition, CS_2 increased lipid peroxidation (conjugated dienes) in microsomes isolated from phenobarbital-pretreated animals 1 hr after toxin administration (Table 2). This effect of CS_2 resembled CCl_4 [11], yet was dissimilar to $CHCl_3$ [11] and 1,1-DCE [10]. In a separate experiment, conjugated dienes were not elevated 1 hr after CS_2 (1 ml/kg) in normal animals but were elevated significantly in phenobarbital-pretreated animals ($P < 0.05$, Student's *t*-test, $N = 6$, data not shown). This experiment also confirmed that calcium pump inhibition occurred only in the

Table 2. Effect of orally administered CS_2*

	Calcium pump activity†	Microsomal calcium	G6Pase activity‡	Conjugated dienes	Glutathione	SGPT activity
	nmoles Ca^{2+}	μg Ca^{2+}	μmoles PO_4		mg GSH	units
	mg protein	mg protein	mg protein	O.D. 243 nm	g liver	ml
Control	146 ± 8.5	0.32 ± 0.04	2.1 ± 0.1	0.117 ± 0.01	5.6 ± 0.2	70 ± 3
CS_2	109 ± 6.1	0.20 ± 0.01	2.0 ± 0.1	0.223 ± 0.01	4.2 ± 0.2	336 ± 40
	(76 ± 5)	(66 ± 6)	(95 ± 7)	(194 ± 18)	(74 ± 3.5)	(536 ± 61)

* All animals received phenobarbital (80 mg/kg) 72, 48 and 24 hr before orally administered CS_2 (1 ml/kg) diluted in corn oil. Control animals received corn oil, and 1 or 24 hr later both control and treated groups were killed. All determinations were performed as described in the text. All data except SGPT activity were collected from animals killed 1 hr after CS_2. SGPT activity was determined from animals killed 24 hr after CS_2. Data are expressed as the means ± S.E.M. for the determination on material from four to six rats. Data in parentheses are percent control.

† Total nmoles Ca^{2+} accumulated during 30 min, per mg protein.

‡ Total μmoles PO_4 accumulated during 15 min, per mg protein.

phenobarbital-pretreated group of rats (data not shown). Conversely, $CHCl_3$, [15], 1,1-DCE [10] and CS_2 (Table 2) depleted GSH while CCl_4 [15] did not. Finally, as a biochemical measure of hepatotoxicity, SGPT activity was determined 24 hr after CS_2. Following CS_2 treatment, SGPT activity was increased more than 5-fold (Table 2). Calcium pump inhibition 24 hr after CS_2 administration to phenobarbital-pretreated animals was $30 \pm 3\%$ (N = 5).

CS_2 would appear to be a useful compound to study the role of liver ER calcium pump inhibition in development of hepatic necrosis. In addition, CS_2 would appear to be the first hepatotoxin, other than chlorinated hydrocarbons, that has been shown to be an inhibitor of the ER calcium pump. This compound can produce moderate hepatotoxicity (lipid accumulation) in the normal rat or severe toxicity (cell death) in the phenobarbital-pretreated rat [6–8]. Calcium pump inhibition was seen 1 hr after CS_2 administration only in that group of rats (phenobarbital-pretreated) that would subsequently develop cellular necrosis. This difference of calcium pump inhibition is similar to that noted between $CHCl_3$ and the much less toxic $CDCl_3$ in phenobarbital-pretreated rats [2]. A possible mechanism of CS_2 activation has been proposed. CS_2 has been shown to be metabolized to an active metabolite by the liver mixed function oxidase system (MFOS) [16]. During metabolism of CS_2 to COS, sulfur was released and covalently bound to microsomal proteins [17]. Additional studies suggested that a portion of the sulfur released reacted with sulfhydryl groups of cysteine residues in microsomal proteins to form a hydrodisulfide [18]. A portion of this sulfur interacts with cytochrome P-450 and inhibits the MFOS. However, 9 moles of sulfur interacts with microsomal proteins per mole of cytochrome P-450 [18]. A portion of this sulfur may also interact with and inhibit the liver ER calcium pump. The liver ER calcium pump has been shown to be sensitive to sulfhydryl reagents [19]. One may speculate that the mechanism by which CS_2 administration produces calcium pump inhibition is via hydrodisulfide formation.

In summary, this work has shown that CS_2 promptly inhibits the liver ER calcium pump only in those animals that subsequently develop hepatic necrosis. In this respect, inhibition of the ER calcium pump by CS_2 resembles the actions of chlorinated hydrocarbon hepatotoxins. This lends further support to the suggestion that disruption of calcium homeostasis is an important early step in the action of at least some hepatotoxins [1–5]. CS_2 appears to be the first example of a hepatotoxin other than chlorinated hydrocarbons that inhibit the liver ER calcium pump early in the course of intoxication. Finally, studies by others [17, 18] suggest a mechanism by which CS_2 can interact with and inhibit the liver ER calcium pump.

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