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INHIBITION OF LIVER-MICROSOME CALCIUM PUMP BY *IN VIVO* ADMINISTRATION OF CCl_4, $CHCl_3$ AND 1,1-DICHLOROETHYLENE (VINYLIDENE CHLORIDE)

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Abstract—CCl_4, $CHCl_3$ and 1,1-dichloroethylene (EDC) administered to rats resulted in a prompt dose-dependent inhibition of the ATP-dependent calcium pump isolated in the liver microsome fraction. EDC was slightly less potent than CCl_4 whereas $CHCl_3$ was approximately one-tenth as potent as CCl_4. Because neither EDC nor $CHCl_3$ increased lipid peroxidation *in vivo*, increased lipid peroxidation does not appear to be a prerequisite for inhibition of the liver-microsome calcium pump. Both CCl_4 and EDC have been shown to increase liver calcium early during intoxication, but $CHCl_3$ has been thought to be incapable of increasing liver calcium. Pretreatment with phenobarbital increased the $CHCl_3$ effect of inhibiting the liver-microsome calcium pump and resulted in a several-fold increase of liver calcium levels. $CDCl_3$, which is less hepatotoxic than $CHCl_3$, did not increase liver calcium or inhibit the liver-microsome calcium pump in phenobarbital-pretreated animals. These results suggest that chlorinated hydrocarbons may increase liver calcium as a result of inhibition of a microsomal calcium pump and that this inhibition is associated with the hepatotoxicity of these compounds.

An alteration of calcium metabolism has been shown to be an early consequence of intoxication with hepatotoxins and a potential mediator of cellular damage produced by hepatotoxins. Several workers have implicated this disruption of calcium metabolism in the early events of cellular necrosis produced by hepatotoxins including thioacetamide [1], carbon tetrachloride [2, 3] and galactosamine [4, 5]. It has been suggested that inhibition of the endoplasmic-reticulum calcium pump may be responsible for disruption of calcium metabolism in the CCl_4-intoxicated liver [6]. Certain hepatotoxins have been shown to induce lipid peroxidation in liver membranes. The classic example of this type of hepatotoxin is CCl_4 [7, 8]. It is not known if lipid peroxidation is a necessary prerequisite for inhibition of the endoplasmic-reticulum calcium pump in liver. To determine if lipid peroxidation is required for inhibition of this calcium pump and, hence, for disruption of liver calcium metabolism, two hepatotoxic compounds that have been reported not to induce lipid peroxidation were examined for their effects on a microsomal calcium pump *in vitro* and on calcium content of intact liver *in vivo*. $CHCl_3$ is less potent than CCl_4 as a hepatotoxin but does not increase lipid peroxides *in vivo* or *in vitro* [9, 10]. 1,1-Dichloroethylene (vinylidene chloride) is a potent hepatotoxin that does not increase lipid peroxides *in vivo* or *in vitro* [11].

METHODS

Animals and hepatotoxin administration. Male Sprague–Dawley (Taconic Farms) rats with body weights of 250–400 g were used for this study. Animals were allowed free access to food and water throughout the experiments. Chlorinated hydrocarbons were administered i.p. in corn oil; control animals received equivalent volumes of corn oil i.p. In certain experiments, rats were pretreated i.p. with phenobarbital (80 mg/kg) 72, 48 and 24 hr before administration of the chlorinated hydrocarbon.

Determination of liver calcium. Samples of liver were rinsed, weighed, dried overnight at 95°, and finally ashed for 72 hr at 600°. The ashed samples were collected in 0.1 N HCl containing 0.1% $LaCl_3$. Calcium in the samples was determined with a Perkin–Elmer 603 atomic absorption spectrophotometer using an acetylene–air flame. Results were expressed as μg calcium/g tissue (wet wt). Calcium associated with the microsomal fraction of liver was determined as described by Schmidt and Way [12]. Results were expressed as μg calcium/mg protein.

Calcium pump determination. Calcium pump activity was determined with the microsomal fraction as described previously [6, 13]. Briefly, a liver sample was excised and quickly cooled in 0.25 M sucrose. Homogenates were prepared with a Potter–Elvehjem homogenizer (Thomas type C) driven at about 1000 rpm. Homogenates were centrifuged at 1500 *g* for 10 min, and then at 12,500 *g* for 20 min in a SA 600 rotor (Dupont–Sorvall). The microsomal fraction was isolated from the 12,500 *g* supernatant fraction by centrifugation at 105,000 *g* for 60 min in a 50 Ti rotor (Beckman). 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 with imidazole to 6.8), 5 mM ammonium oxalate, 5 mM sodium azide, 20μM $CaCl_2$ ([$^{45}Ca^{2+}$], 0.1 μCi/ml) and 20–50 μg microsomal protein/ml. The assay was initiated by the addition of microsomes to the prewarmed (37°) assay medium. At timed intervals, 0.5-ml samples were removed and filtered through 0.45 μm nitrocellulose filters, and [$^{45}Ca^{2+}$] was determined by liquid scintillation spectrophotometry. Protein con-

centrations of microsomal suspensions were determined by the Lowry method as described by Shatkin [14].

Lipid peroxide and glucose-6-phosphatase determinations. Lipids were extracted from microsomes after *in vivo* administration of a chlorinated hydrocarbon, and lipid peroxidation was determined as conjugated dienes at 243 nm as described by Klaassen and Plaa [9]. Glucose-6-phosphatase (G-6-Pase) activity was determined in microsomal suspensions as described by Aronson and Touster [15].

RESULTS

Inhibition of the calcium pump by chlorinated hydrocarbons. As illustrated in Fig. 1, all three chlorinated hydrocarbons inhibited the liver endoplasmic-reticulum calcium pump after *in vivo* administration of the hydrocarbon. In these experiments, rats were treated with a uniform chlorinated-hydrocarbon dose of 1 ml/kg body wt. Animals were killed 2 or 24 hr after treatment, and microsomes were isolated for the calcium pump assay. Administration of CCl_4 i.p. resulted in a prompt inhibition of the calcium pump, similar to the effect previously reported after oral administration [6]. Within 2 hr after CCl_4 administration (1 ml/kg), calcium pump activity was inhibited 88 ± 1.5 per cent and remained inhibited for more than 24 hr. 1,1-Dichloroethylene (vinylidene chloride, EDC) was somewhat less inhibitory, and calcium pump activity was inhibited 69 ± 2.8 per cent 2 hr after 1 ml/kg. However, inhibition of calcium pump activity was virtually unchanged when animals were killed 24 hr after EDC administration. $CHCl_3$ was less inhibitory than either CCl_4 or EDC. Two hours after a $CHCl_3$ dose of 1 ml/kg, calcium pump activity was inhibited 31.8 ± 3.4 per cent. $CHCl_3$ has been observed to be consistently less potent than CCl_4 as a hepatotoxin [9, 10]. The yield of microsomal protein per gram liver was unaffected by chlorinated hydrocarbon treatment except that from CCl_4-treated animals at 24 hr after treatment. Twenty-four hours after CCl_4 (1 ml/kg) microsomes yielded 21.0 ± 1.1 mg protein/g liver, which can be compared to 30.4 ± 1.3 mg protein for control animals.

The dose–response curves for inhibition of the liver-microsome calcium pump are illustrated in Fig. 2. In these experiments, animals were killed 24 hr after administration of the chlorinated hydrocarbon. The dose–response curves are linear with log dose over the range tested, and in all cases the greatest inhibition of the endoplasmic-reticulum calcium pump was observed after administration of CCl_4. EDC was less potent than CCl_4, and $CHCl_3$ was approximately 10-fold less potent than CCl_4. Other measures of the hepatotoxicity of $CHCl_3$ and CCl_4 have demonstrated $CHCl_3$ to be approximately 10-fold less toxic than CCl_4 [9, 10]. It was not possible to test the effect of a 3 ml/kg dose of either EDC or $CHCl_3$ because at this dose both compounds were acutely toxic. Similar results were obtained when calcium pump activity was determined in crude homogenates from another group of animals. In these experiments, only non-mitochondrial calcium pump activity was determined. Twenty-four hours after a 1 ml/kg dose, the calcium pump activity of CCl_4-treated animals was inhibited an average of 45 ± 6 per cent, while with EDC- and $CHCl_3$-treated animals, inhibition averaged 34 ± 5 and 14 ± 7 per cent respectively.

It has been reported that stimulation of the mixed function oxidase system of liver endoplasmic reticulum with phenobarbital increased hepatotoxicity of both CCl_4 [16, 17] and $CHCl_3$ [18–20]. The effect of phenobarbital induction on $CHCl_3$ inhibition of the

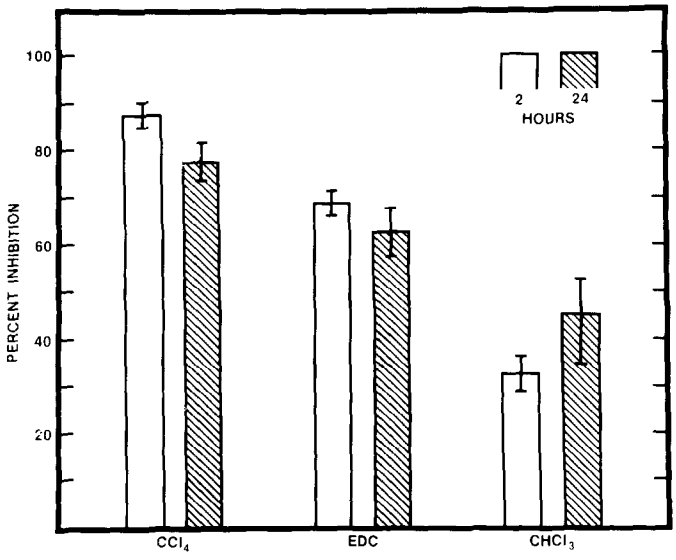

Fig. 1. Inhibition of liver-microsome calcium pump by chlorinated hydrocarbons administered *in vivo*. Two or twenty-four hours after chlorinated hydrocarbon administration at 1 ml/kg, liver microsomes were prepared and calcium pump activity was determined as described in Methods. Each point represents the mean ± S.E.M. of six animals. Control microsomal preparations had calcium uptake activities averaging 180 ± 22 nmoles calcium/mg protein/30 min (2 hr) and 202 ± 26 nmoles calcium/mg protein/30 min (24 hr).

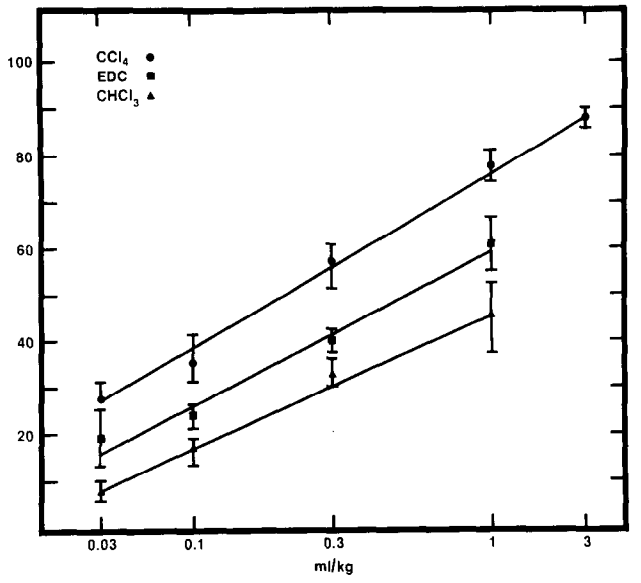

Fig. 2. Inhibition of liver-microsome calcium pump by various doses of chlorinated hydrocarbons. Twenty-four hours after administration of the test compound at the indicated dose, liver-microsome calcium pump activity was determined. Each point is the mean ± S.E.M. of five or six animals. Control microsome preparations had calcium uptake activities averaging 202 ± 26 nmoles calcium/mg protein/30 min. Ordinate is per cent inhibition.

endoplasmic-reticulum calcium pump has been examined. Pretreatment with phenobarbital significantly increased inhibition of the endoplasmic-reticulum calcium pump by $CHCl_3$ (Table 1). In these experiments, a $CHCl_3$ dose of 0.3 ml/kg inhibited the calcium pump 23.6 ± 6.2 per cent, but after phenobarbital pretreatment the same dose of $CHCl_3$ inhibited pump activity 81.6 ± 4.2 per cent. Comparable inhibition of calcium pump activity was observed when crude liver homogenates from another group of animals were examined.

Pohl and Krishna [21] have demonstrated that the deuterated analog of $CHCl_3$ was less hepatotoxic than the parent $CHCl_3$ in phenobarbital-pretreated rats. In the present study, $CDCl_3$ was a less potent inhibitor of the endoplasmic-reticulum calcium pump in rats that had been pretreated with phenobarbital (Table 2). In these experiments, $CHCl_3$ inhibited the calcium pump almost 70 per cent, while $CDCl_3$ inhibited the pump less than 5 per cent. Similar experiments have been performed in animals that have not been pretreated with phenobarbital at a chlorinated hydrocarbon dose of 1 ml/kg. Again, $CHCl_3$ produced significantly more inhibition (about 2-fold in this case) of the calcium pump than did $CDCl_3$ (data not shown).

Effect of chlorinated hydrocarbons on G-6-Pase *and lipid peroxidation in liver microsomes.* CCl_4 has been reported to inhibit a number of endoplasmic reticulum functions and enzymatic activities (for review, see Ref. 8). In parallel with the study of the effect of these three chlorinated hydrocarbons on

Table 1. Effect of phenobarbital pretreatment on $CHCl_3$ inhibition of liver endoplasmic-reticulum calcium pump and liver calcium*

Treatment group	Calcium pump inhibition (%)	Liver calcium ($\mu g\ Ca^{2+}$/g liver)
Control		44.7 ± 1.3
Phenobarbital	1.8 ± 3.9	43.5 ± 2.8
$CHCl_3$	23.6 ± 6.2	47.7 ± 3.7
Phenobarbital + $CHCl_3$	81.5 ± 4.2	253 ± 35

* Animals were pretreated with phenobarbital (80 mg/kg) 72, 48 and 24 hr before $CHCl_3$ administration. Twenty-four hours before the animals were killed, $CHCl_3$ was administered at 0.3 ml/kg diluted in corn oil. Control and phenobarbital groups received corn oil. Twenty-four hours after $CHCl_3$ administration, animals were killed, a sample of liver was removed for calcium determination, and another sample was removed for microsomal preparation and calcium pump activity determination as described in Methods. Each result is the mean ± S.E.M. for the determination in five to seven animals. Calcium pump activity averaged 224 ± 5.4 nmoles calcium/mg protein/30 min in microsomes prepared from the control group.

Table 2. Effects of $CDCl_3$ and $CHCl_3$ on liver calcium levels and liver endoplasmic-reticulum calcium pump*

Treatment group	Calcium pump inhibition (%)	Liver calcium ($\mu g\ Ca^{2+}$/g liver)
Control		42.4 ± 2.0
$CDCl_3$	3.43 ± 3.1	46.6 ± 4.59
$CHCl_3$	68.9 ± 4.5	87.9 ± 5.3

* All animals were pretreated with phenobarbital (80 mg/kg) 72, 48 and 24 hr before chlorinated hydrocarbon administration. Animals were killed 24 hr after administration of $CDCl_3$ or $CHCl_3$ at a dose of 0.15 ml/kg and liver samples were prepared as described in the legend for Table 1 and Methods. Each result is the mean ± S.E.M. for the determination in six or seven animals. Calcium pump activity averaged 215 ± 12 nmoles calcium/mg protein/30 min in microsomes prepared from the control group.

calcium pump activity, the effect of another microsomal enzyme, G-6-Pase, has been examined. As shown in Fig. 3, CCl_4 inhibited G-6-Pase activity in a dose-dependent manner at doses above 0.1 ml/kg. Similar results have been observed by Klaassen and Plaa [9]. Both EDC and $CHCl_3$ inhibited G-6-Pase only marginally (less than 20 per cent), and inhibition did not appear to be related to dose over the range tested. Similar results were observed when G-6-Pase activity was determined in crude liver homogenates. Twenty-four hours after a single 1 ml/kg dose, homogenate G-6-Pase activity was inhibited 37 ± 9 per cent by CCl_4, 7 ± 6 per cent by EDC and 4 ± 2 per cent by $CHCl_3$. In one group of animals pretreated with phenobarbital (80 mg/kg for 3 days), homogenate G-6-Pase activity was inhibited 19 ± 6 per cent 24 hr after a $CHCl_3$ dose of 0.3 ml/kg. In the same animals, homogenate calcium pump activity was inhibited 62 ± 5 per cent. Klaassen and Plaa [9] were also unable to demonstrate significant inhibition of G-6-Pase by $CHCl_3$. However, Jaeger *et al.* [11] demonstrated inhibition of G-6-Pase by EDC after oral administration. This discrepancy has not been explained but might be related to the different routes of administration employed (intraperitoneal vs oral).

The ability of these three compounds to produce lipid peroxidation in liver endoplasmic reticulum has been examined. As a quantitative estimate of the amount of lipid peroxidation occurring in the endoplasmic reticulum *in vivo*, conjugated dienes in lipids extracted from microsomes have been determined [9]. The results reported in Fig. 4 confirm the results of others and indicate that neither $CHCl_3$ [9, 10] nor EDC [11] increased lipid peroxides in the microsome fraction. In these experiments animals were treated with the indicated hepatotoxin 2 or 24 hr before the experiment. Calcium pump activity and G-6-Pase activity were determined in microsomes isolated from livers 24 hr after the hydrocarbon was administered. Lipid peroxidation is maximal shortly after hydrocarbon administration [9, 22]. Therefore, conjugated dienes were determined in lipids extracted from liver microsomes prepared from another group of animals killed 2 hr after hydrocarbon administration. In these experiments there appeared to be a correlation between the appearance of increased amounts of conjugated dienes and inhibition of G-6-Pase. There was no correlation between increased conjugated dienes and inhibition of the endoplasmic-reticulum calcium pump.

Liver calcium levels after hepatotoxin treatment. Calcium in the liver has been shown to increase to massive levels after administration of CCl_4 [2, 3, 6].

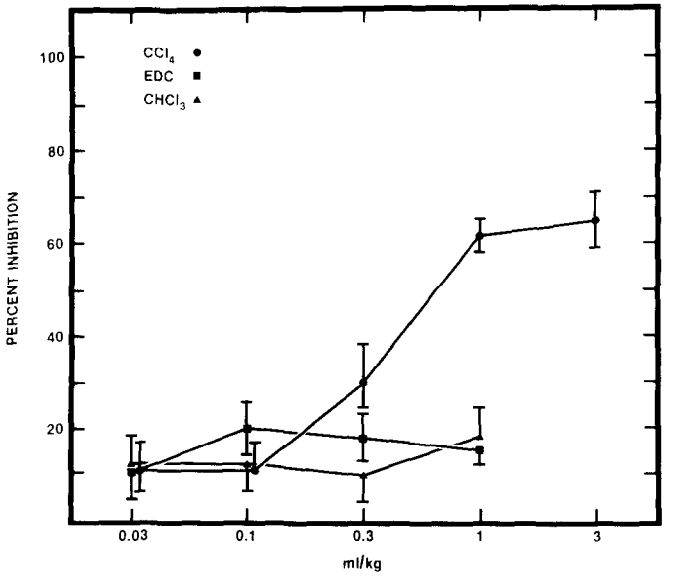

Fig. 3. Inhibition of liver microsome glucose-6-phosphatase activity by various doses of chlorinated hydrocarbons. Twenty-four hours after administration of the test compound at the indicated dose, glucose-6-phosphatase activity was determined in liver microsome preparations. Each point is the mean ± S.E.M. of five or six animals. Control microsomal preparations had glucose-6-phosphatase activity averaging 3.9 ± 0.3 μmoles phosphate/mg protein/15 min.

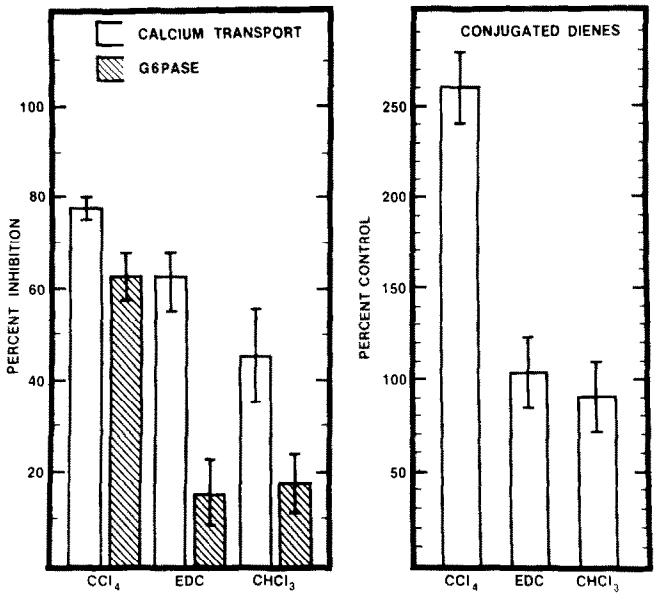

Fig. 4. Effect of chlorinated hydrocarbons administered *in vivo* on liver-microsome calcium pump activity, glucose-6-phosphatase activity and lipid peroxides. Lipid peroxidation was determined as conjugated dienes 2 hr after administration of the test compound (1 ml/kg). Control values averaged 0.140 ± 0.02 A_{243}. Calcium pump and glucose-6-phosphatase activities were determined 24 hr after administration of the test compound. Control values averaged 195 ± 28 nmoles calcium/mg protein/30 min and 3.8 ± 0.4 μmoles phophate/mg protein/15 min, respectively. Each point is the mean ± S.E.M. of five or six animals.

More recently the chlorinated hydrocarbon EDC has also been shown to result in calcium accumulation by the damaged liver [23]. However, Reynolds and Yee [24] have reported that no evidence of calcium accumulation could be found in livers of $CHCl_3$-intoxicated rats. Results reported in Table 1 confirm this observation but also demonstrate that, when the hepatotoxicity of $CHCl_3$ was increased by phenobarbital pretreatment, calcium levels in the liver increased more than 5-fold 1 day after $CHCl_3$ administration.

Data in Table 3 extend these observations and demonstrate that the time course of calcium accumulation in the liver was quite similar for CCl_4 and EDC administered to normal rats, and for $CHCl_3$ in phenobarbital-pretreated rats. In all three cases, significant calcium accumulation occurred between hours 2 and 4 after hydrocarbon administration *in vivo*. Even though this work does not demonstrate significant calcium accumulation until more than 2 hr after hydrocarbon administration, the effect of hydrocarbon administration on the microsomal calcium pump would suggest that calcium metabolism is disrupted much earlier. The data in Table 4 suggest that an effect of these hydrocarbons in the liver results in disruption of the calcium pool associated with membranes isolated in the microsomal fraction much earlier after hydrocarbon administration. Microsomal fractions were isolated from animals treated with CCl_4 or EDC (both 1 ml/kg) or from phenobarbital-pretreated animals treated with $CHCl_3$ (0.3 ml/kg), 2 hr after hydrocarbon treatment. Calcium associated with these membranes was decreased 50 per cent. Previously, Rey-

Table 3. Effects of chlorinated hydrocarbons on liver calcium*

Time after hydrocarbon administration (hr)	Calcium (μg/g liver)				
	Control	CCl_4	EDC	PB-induced control	PB-induced $CHCl_3$
1	41.6 ± 1.8	43.2 ± 2.9	40.2 ± 2.4	40.0 ± 1.7	43.8 ± 6.3
2	40.9 ± 1.4	44.0 ± 6.9	45.5 ± 3.9	41.1 ± 1.0	44.0 ± 3.0
4	39.5 ± 2.1	51.7 ± 3.0†	46.5 ± 3.9†	41.6 ± 1.0	55.7 ± 4.8†
12	43.2 ± 3.0	82.9 ± 10†	115 ± 31†	41.2 ± 2.4	105 ± 6.0†
24	41.1 ± 1.0	137 ± 25†	122 ± 34†	39.7 ± 1.7	216 ± 30†

* Control and phenobarbital (PB)-induced control rats were treated with corn oil. CCl_4 and EDC rats were treated with the hydrocarbon at a dose of 1 ml/kg. PB-induced animals were pretreated with daily injections of phenobarbital (80 mg/kg for three days before $CHCl_3$ treatment. $CHCl_3$ was administered at a dose of 0.3 ml/kg. Liver samples were prepared and calcium was determined as described in Methods. Data are expressed as the mean ± S.E.M. for the determination in five liver samples.

† $P < 0.01$ (Student's *t*-test).

Table 4. Effect of chlorinated hydrocarbons on liver microsome calcium levels*

Treatment group	Calcium in liver microsomes (μg Ca^{2+}/mg protein)
Control	0.22 ± 0.05
CCl_4	0.13 ± 0.03†
EDC	0.14 ± 0.03†
Phenobarbital-induced control	0.19 ± 0.03
Phenobarbital-induced + $CHCl_3$	0.10 ± 0.02‡

* Control and phenobarbital-induced control rats were treated with corn oil. CCl_4 and EDC (1 ml/kg) rats were treated with the hydrocarbon 2 hr before the animals were killed. Phenobarbital-induced animals were pretreated with daily injections of phenobarbital (80 mg/kg) for 3 days before $CHCl_3$ treatment. $CHCl_3$ was administered at a dose of 0.3 ml/kg 2 hr before the animals were killed. Liver microsomes were isolated as described in Methods. Calcium in the microsomal fraction was determined as described by Schmidt and Way [12]. Data are expressed as the mean ± S.E.M. for the determination in six microsomal preparations.

† $P < 0.05$ (Student's t-test).

‡ $P < 0.01$ (Student's t-test).

nolds *et al.* [25] demonstrated that this effect occurs within 15 min after CCl_4 treatment.

DISCUSSION

Disruption of calcium metabolism has been implicated in the production of hepatocyte death in response to a number of chemical agents [1–6] and to experimentally induced liver ischemia [26, 27]. Previous studies have demonstrated that the endoplasmic-reticulum (microsome) calcium pump is inhibited after CCl_4 [6] and hepatic ischemia [26]. It has been suggested that inhibition of this calcium pump may interfere with one of the normal pathways of calcium metabolism in the liver. Judah *et al.* [28] suggested that hepatotoxin action on cellular membranes may result in increased cytoplasmic calcium and, further, suggested that an intracellular store was the source of calcium important at early times after intoxication. Inhibition of the endoplasmic-reticulum calcium pump could result in the release of calcium from one of the intracellular sequestration sites. Calcium is important for regulation of membrane permeability, and it has been suggested that an increase of membrane calcium permeability could result from calcium activation of membrane-bound phospholipases and subsequent phospholipid degradation [27]. This cascade may be important in the massive calcium accumulation which reaches a maximum 24–48 hr after chlorinated hydrocarbon administration [2, 3, 6, 11].

All three chlorinated hydrocarbons reduced calcium pump activity in the microsome fraction. This represents an inhibition of the pump and does not seem to be due to a chlorinated hydrocarbon-induced alteration of the purity of the microsomal fraction, for the following reasons. Calcium pump activity was clearly reduced within 2 hr after hydrocarbon administration and, thus, at a time when the yield of microsomal protein was unaltered. In addition, if the purity of the microsomal fraction had been altered by chlorinated hydrocarbon treatment of the animals, one would expect calcium pump activity and G-6-Pase activity to decrease at a constant ratio. Clearly this is not the case for EDC- and $CHCl_3$-treated animals (Fig. 4). Finally, when microsome-like calcium pump activity in crude homogenates was examined, it was found to be inhibited by chlorinated hydrocarbon treatment. This would suggest that the isolation procedure was not responsible for the chlorinated hydrocarbon inhibition of calcium pump activity.

It is well known that CCl_4 produces lipid peroxidation in the liver (for review see Refs. 8 and 29). Because lipids are required for calcium pump function [27, 30, 31], it seemed possible that lipid peroxidation could be responsible for inhibition of the calcium pump in liver microsomes isolated from animals treated with chlorinated hydrocarbons. However, results from the experiments with $CHCl_3$ and EDC suggest that lipid peroxidation is not absolutely required for chlorinated hydrocarbon inhibition of this calcium pump. Other investigators have shown that neither $CHCl_3$ [9, 10] nor EDC [11] increases lipid peroxidation *in vitro* or *in vivo*, and this has been confirmed *in vivo* in the present study. Although lipid peroxidation may be responsible for CCl_4 inhibition of the calcium pump, other mechanisms may be responsible for inhibition of the calcium pump by $CHCl_3$ and EDC. Although the experiments presented in this work show that EDC and $CHCl_3$ do not increase total conjugated dienes in lipids extracted from the microsomal fraction, they do not rule out the possibility that peroxidation of a specific lipid may occur but not be detected in total lipid extracts.

Recent evidence suggests that phosgene may be the reactive metabolite responsible for $CHCl_3$ toxicity [21, 32, 33], but there is no indication that phosgene is produced by oxidative dehalogenation of EDC. Both $CHCl_3$ [34] and EDC [35] deplete hepatic glutathione, and depletion of hepatic glutathione increases the sensitivity of animals to both agents. Because the liver-microsome calcium pump is

inhibited by *p*-chloromecuribenzoic acid (PCMB) [13] and other compounds that are sulfhydryl inhibitors, it is possible that glutathione depletion by $CHCl_3$ and EDC contributes to inhibition of the liver-microsome calcium pump by $CHCl_3$ and EDC. Some credence is added to this argument because in phenobarbital-pretreated animals the deuterated analog of $CHCl_3$ is less potent as a hepatotoxin [21], is less potent as a depletor of hepatic glutathione [34], and is also less potent as an inhibitor of the microsome calcium pump (Table 2). Alternatively, it is possible that interactions of other reactive metabolites of both $CHCl_3$ and EDC with the endoplasmic reticulum are responsible for inhibition of the microsome calcium pump.

Finally, potent hepatotoxins are well known for their ability to substantially increase liver calcium levels [1–6], but it has been reported that $CHCl_3$ does not increase liver calcium levels [24]. Results presented here demonstrate for the first time that $CHCl_3$ has the potential to inhibit the liver-microsome calcium pump and to increase liver calcium levels. Previous work [6] has shown that $CHCl_3$ did not inhibit the liver-microsome calcium pump after oral administration. In the present study, however, intraperitoneal administration of $CHCL_3$ produced inhibition of the calcium pump. Although not examined, specifically this difference presumably reflects differences associated with the route of administration. When hepatotoxicity of $CHCl_3$ is increased by phenobarbital pretreatment [18–20], the compound is more potent as an inhibitor of the liver-microsome calcium pump. In phenobarbital-pretreated animals, massive levels of calcium accumulate in the livers of animals treated with $CHCl_3$. It is possible that substantial inhibition of this pump and, thus, substantial disruption of calcium metabolism in the liver are required to perpetuate the cascade of events responsible for increasing cytoplasmic calcium to a point which results in massive calcium accumulation. In the uninduced animals it is possible that $CHCl_3$ inhibits the liver-microsome calcium pump sufficiently to increase cytoplasmic calcium moderately but that, after phenobarbital induction, inhibition of the microsome calcium pump by $CHCl_3$ and disruption of liver calcium metabolism are sufficient to allow massive accumulation of calcium in the liver.

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