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Metabolism of Carbon Tetrachloride in Hepatic Microsomes and Reconstituted Monooxygenase Systems and Its Relationship to Lipid Peroxidation

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SUMMARY

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The metabolism of carbon tetrachloride has been investigated in liver microsomes and in reconstituted cytochrome *P*-450-dependent monooxygenase systems. In both cases cytochrome *P*-450 appears to be the sole site of carbon tetrachloride reduction. In aerobic incubations of microsomal preparations from phenobarbital-treated rats or rabbits, carbon tetrachloride induces large increases in NADPH oxidation and oxygen uptake. This cofactor utilization is not directly related to monooxygenase-mediated metabolism. Under aerobic conditions carbon tetrachloride induces lipid peroxidation, a reaction which is not inhibited by carbon monoxide. Under anaerobic conditions, carbon monoxide is a potent inhibitor of the cytochrome *P*-450-mediated reduction of carbon tetrachloride. These facts are inconsistent with a role for carbon tetrachloride metabolism in the potentiation of lipid peroxidation.

INTRODUCTION

The mechanism of haloalkane-induced hepatotoxicity, particularly that of carbon tetrachloride (CCl_4), has received a great deal of attention over the last 15 years (for reviews, see Refs. 1–4). Several mechanisms have been proposed for the toxicity of CCl_4 and it is generally accepted that the trichloromethyl radical, resulting from a one-electron reduction, is the reactive intermediate involved (1–6), $CCl_4 + e^- \rightarrow \cdot CCl_3 + Cl^-$. It is still in dispute whether the resulting cell necrosis is due to the covalent binding of the radical to microsomal proteins and lipids or to trichloromethyl radical-induced peroxidation of membrane-bound unsaturated lipids (1, 2, 7, 8).

The metabolism of CCl_4 is catalyzed by the cytochrome *P*-450-dependent monooxygenase system. Slater has proposed that reduction is mediated by the flavoprotein, NADPH–cytochrome *P*-450 reductase (see Ref. 1), although it has been shown that cytochrome *P*-450 can reduce halomethanes (9, 10). Whether both of these enzymes can reduce CCl_4 is still unclear. In order to clarify this point, we have investigated the metabolism of CCl_4 in reconstituted cytochrome *P*-450-dependent monooxygenase systems.

Indirect techniques, such as the covalent binding of $^{14}CCl_4$ to macromolecules (1, 2, 4, 9, 11, 12) or CCl_4-induced lipid peroxidation (see Refs. 1, 2, and 4), have generally been used as criteria for metabolism. Few investigators have monitored CCl_4 metabolism directly, and the requirement for CCl_4 metabolism in such reactions as lipid peroxidation has not been clearly demonstrated. We have investigated the metabolism of CCl_4 in aerobic and anaerobic microsomal systems by determining the formation of chloroform ($CHCl_3$) and carbon monoxide (CO) (10, 13), and by measuring CCl_4 disappearance. The effects of CCl_4 on microsomal oxygen uptake and NADPH oxidation, reactions normally associated with the metabolism of monooxygenase substrates, have also been investigated. The relationships between cofactor utilization, metabolite formation, and lipid peroxidation are discussed.

MATERIALS AND METHODS

Male New Zealand white rabbits (2 kg) and Sprague–Dawley rats (200–250 g) were used. Rabbits were treated with phenobarbital (1 mg/ml of drinking water) for 1

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week prior to sacrifice. Rats were treated with phenobarbital as above or received 100 mg/kg (i.p.) for 3 consecutive days before the preparation of hepatic microsomal fractions (14). Cytochrome *P*-450 determinations were made according to the method of Omura and Sato (15) and protein determinations according to the method of Lowry *et al.* (16). Microsomal incubations used in the determination of lipid peroxidation, oxygen consumption, $CHCl_3$ formation, or CCl_4 disappearance contained 2 mg microsomal protein/ml, NADPH (1 mM), and CCl_4 (0.025–20 mM). Incubations were carried out in 0.1 M Tris–HCl buffer, pH 7.6, at 37°C for periods of up to 10 min, in stoppered glass tubes; CCl_4 was added to the incubations directly or in 1 μl of methanol. NADPH oxidation was determined in essentially the same incubation system; however, the microsomal protein concentration was 0.5 to 2.0 mg/ml and the NADPH concentration was 150 μM. The rate of loss of the NADPH absorption peak at 340 nm was monitored with time. An extinction coefficient of 6.02 mM^{-1} was used. Anaerobiosis was achieved in some incubations by gassing the samples gently for 5 min with nitrogen or by using an oxygen scavenging system (17). Lipid peroxidation was determined by measuring malonaldehyde formation as described by Slater and Sawyer (18). The metabolism of CCl_4 to carbon monoxide was determined as previously reported (10). Spectral measurements were made in an Aminco DW-2A spectrophotometer fitted with a temperature-controlled cuvette holder. Oxygen concentration was monitored with a Yellow Springs oxygen monitor (Model 53; Yellow Springs Instrument Co., Yellow Springs, Ohio). Chloroform or CCL_4 concentrations were determined by gas–liquid chromatography following extraction from the incubation medium with *n*-heptane (1 ml/ml incubation). A Chromasorb 101 column (80–100 mesh, 2-m × 4-mm glass) was used with conditions as follows: N_2 flow rate, 100 ml/min; injection temperature, 250°C; oven temperature, 185°C; detector temperature, 250°C. A [^{3}H]scandium electron capture detector was used.

Components of the hepatic cytochrome *P*-450-dependent monooxygenase system were isolated from phenobarbital-treated rabbits. The major cytochrome induced by this compound was purified to 17 to 20 nmol/mg protein using a method reported previously (19). NADPH–cytochrome *P*-450 reductase was separated from the other monooxygenase components (19) and then purified to homogeneity by affinity chromatography on 2′,5′-ADP Sepharose. The experimental details for the recombination of these components to give enzymatically active monooxygenase systems have been described previously (20).

Materials. Phenyl-*t*-butylnitrone (PBN) was purchased from Eastman-Kodak Co. (Rochester, N.Y.), and 2-methyl-2-nitrosopropane (MNP) from Aldrich (Milwaukee, Wis.). All other reagents were obtained from commercial sources and were of the highest purity available.

RESULTS

Effect of CCl_4 on lipid peroxidation, NADPH oxidation, and O_2 uptake. In agreement with findings of other investigators (1, 2), CCl_4 increased the rate of NADPH-dependent lipid peroxidation in hepatic microsomal preparations from phenobarbital-treated or untreated rats (Table 1). Similar results were obtained with hepatic microsomal preparations from phenobarbital-treated rabbits (CCl_4-induced rate = 0.26 nmol malonaldehyde formed/min/mg protein). The rate of lipid peroxidation was dependent on the CCl_4 concentration [maximum rates were obtained at approximately 10 mM CCl_4 (Fig. 1)], proportional to the microsomal protein concentration (Fig. 2), and linear for 10–15 min.

TABLE 1

CCl_4-induced lipid peroxidation and cofactor utilization in rat liver microsomal samples

The concentration of CCl_4 was 5 mM. Microsomal protein concentration was 2 mg/ml for the determination of malonaldehyde formation and oxygen uptake, and 1 mg/ml for NADPH oxidation. Other experimental details are described in Materials and Methods.

Determination	Rate (nmol/min/mg protein)					
	Untreated rats			Phenobarbital-treated rats		
	Endogenous	+CCl_4	CCl_4-induced rate	Endogenous	+CCl_4	CCl_4-induced rate
Malonaldehyde	0.078	0.261	0.183	0.071	0.371	0.30
NADPH oxidized	6.0	6.7	0.7	8.5	21.0	12.5
Oxygen uptake	9.4	17.0	7.6	20.1	55.5	35.4

The addition of CCl_4 to hepatic microsomal preparations from phenobarbital-treated rats resulted in large increases in both NADPH oxidation and O_2 uptake (Table 1). Maximum rates of NADPH oxidation occurred at CCl_4 concentrations of 0.5 to 1.0 mM (K_m = 2.0 × 10^{-4} M, V_{max} = 16 nmol/min/mg protein). O_2 uptake was maximum at CCl_4 concentrations of 5 to 10 mM and did not conform to Michaelis–Menten kinetics. In microsomal preparations from untreated rats, CCl_4 induced NADPH oxidation only very slightly (Table 1).

Inhibition of lipid peroxidation, O_2 uptake, and NADPH oxidation by free radical scavengers and carbon monoxide. Possible relationships between CCl_4-induced lipid peroxidation (a free radical reaction), O_2 uptake, and NADPH oxidation were investigated (Fig.

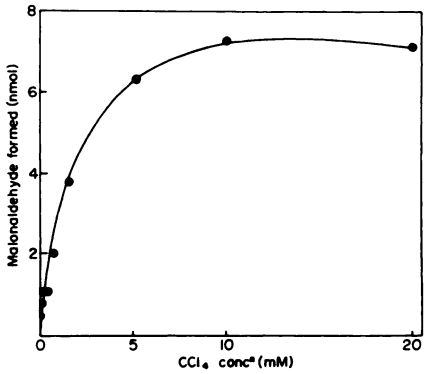

FIG. 1. *The effect of carbon tetrachloride concentration on the rate of malonaldehyde formation in liver microsomal samples from phenobarbital-treated rats*

Incubation conditions: microsomal protein concentration, 2 mg/ml; and NADPH concentration, 1 mM. Incubations were carried out at 37°C for 10 min. Other details are described in Materials and Methods.

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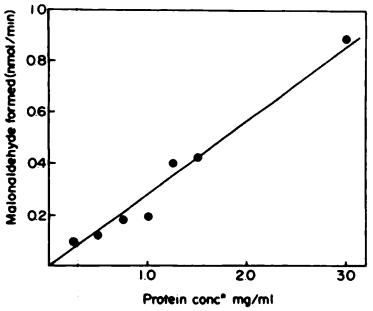

FIG. 2. *The effect of microsomal protein concentration on the CCl_4-induced rate of malonaldehyde formation*

In the experiment shown microsomal samples from phenobarbital-treated rats were used. Incubations were at 37°C for 10 min. CCl_4 concentration was 5 mM. Other details are described in Materials and Methods.

3). MNP and PBN were used because they are known radical scavengers and carbon monoxide (CO) was used to inhibit cytochrome *P*-450-mediated reactions.

Endogenous and CCl_4-induced lipid peroxidations were inhibited approximately 75% by PBN (Fig. 3) and 100% by MNP (10 mM). PBN did not affect the CCl_4-induced rate of NADPH oxidation but inhibited the induced oxygen uptake by 62% (Fig. 3). In addition, the concentration of CCl_4 required for maximal oxygen uptake was lowered by PBN from 10 to 1 mM, a concentration similar to that required for maximal CCl_4-induced NADPH oxidation. In fact, in the presence of PBN, CCl_4-induced oxygen uptake followed similar kinetics to the induced NADPH oxidation and gave a K_m value of approximately 1.0×10^{4} M. Although inhibition of CCl_4-induced oxygen uptake by PBN appears related to inhibition of lipid peroxidation, PBN may also be a substrate for cytochrome *P*-450 as suggested by its effect on the endogenous cofactor utilization (Fig. 3).

The presence of $CO:O_2$ (4:1), which inhibited the cytochrome *P*-450-dependent metabolism of *p*-nitroanisole beyond the limits of detection, did not alter either the endogenous or the CCl_4-induced rates of lipid peroxidation (Fig. 3). In fact, the rates were slightly increased. The same results were obtained using microsomal samples from untreated animals. The endogenous oxygen uptake was partially inhibited (38%) by the gas mixture, as was the CCl_4-induced rate (78%). Endogenous NADPH oxidation was inhibited 18% by $CO:O_2$, whereas the CCl_4-induced rate was completely inhibited (Fig. 3). The CCl_4-induced oxygen uptake could be completely inhibited by the concomitant use of PBN and $CO:O_2$.

Metabolism of CCl_4 in microsomal and purified systems. The metabolism of CCl_4 and its role in CCl_4-induced lipid peroxidation and cofactor utilization were determined by investigating the conditions required for the formation of $CHCl_3$ and CO and the disappearance of CCl_4. In hepatic microsomal preparations from phenobarbital-treated rats, the metabolism of CCl_4 to $CHCl_3$ (0.5 to 2.6 nmol formed/min/mg protein) required NADPH and was inhibited by air to an undectable level. (The detection limit for $CHCl_3$ was equivalent to a rate of 0.01 nmol formed/min/mg protein in these experiments.) The formation of $CHCl_3$ was completely inhibited by PBN (14 mM), MNP (10 mM), or CO and increased by FMN and FAD (two- to threefold). The rate of formation of $CHCl_3$ was dependent on the concentration of CCl_4, and a K_m of 1.5×10^{-4} M and a V_{max} of 2.63 nmol/min/mg protein were calculated from results obtained using hepatic microsomal preparations from phenobarbital-treated rats. Similar kinetic constants ($K_m = 1.2 \times 10^{-4}$ M, $V_{max} = 2.56$ nmol/min/mg protein) were calculated for CCl_4-induced NADPH oxidation in anaerobic incubations. Metabolism of $CHCl_3$ could have accounted for the lack of any detectable metabolite under aerobic conditions. However, the con-

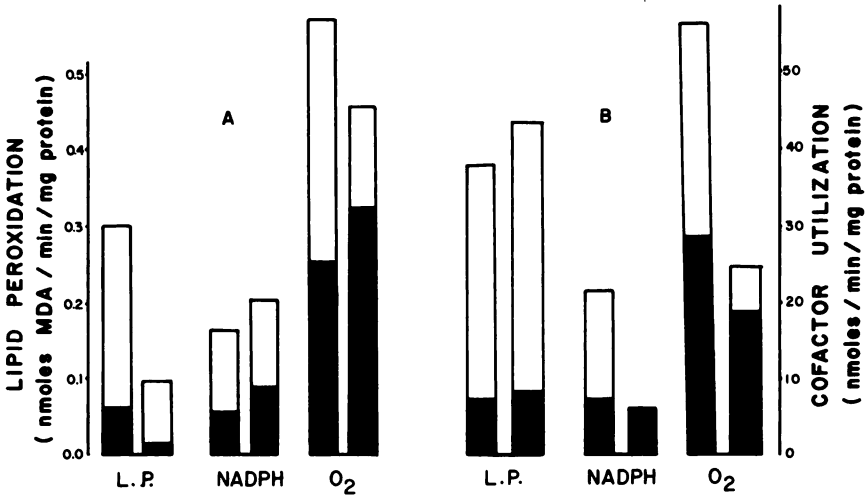

FIG. 3. *Effect of PBN (14 mM) (A) and carbon monoxide (B) on the endogenous and CCl_4-stimulated rates of lipid peroxidation and cofactor utilization in hepatic microsomal samples from phenobarbital-treated rats*

Lipid peroxidation was determined by measuring malonaldehyde formation, and cofactor utilization was determined by measuring oxygen uptake (O_2) or NADPH oxidation (NADPH). Incubations contained 2 mg microsomal protein/ml. The CCl_4 concentration was 5 mM. In experiments using CO, a gas mixture $CO:O_2$ (4:1) was bubbled gently through the incubation medium for 5 min before the addition of CCl_4 or NADPH. Other details are described in Materials and Methods. Solid bars represent endogenous rates; open bars represent rates measured in the presence of CCl_4.

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TABLE 2

Comparison of CCl_4-induced lipid peroxidation, cofactor utilization, $CHCl_3$ formation, and CCl_4 disappearance measured in microsomal samples from phenobarbital-treated rats

The CCl_4 concentration was 100 μM. Microsomal protein concentration was 2 mg/ml. The incubation for $CHCl_3$ and malonaldehyde formation and also CCl_4 disappearance was for 10 min at 37°C. Other details are described in Materials and Methods.

	Rate (nmol/min/mg protein)	
	Nitrogen atmosphere	Air atmosphere
$CHCl_3$ formed	0.60	<0.01
Malonaldehyde formed	0.0	0.14
NADPH oxidized	0.72	4.6
Oxygen uptake	—	6.2
CCl_4 disappearance	1.20	0.1

centration of added $CHCl_3$ (equivalent to the amount produced during an anaerobic incubation) did not change in aerobic incubations that contained microsomes, NADPH, and CCl_4. In addition, no disappearance of CCl_4 could be detected in aerobic incubations even though significant rates of CCl_4-induced lipid peroxidation and cofactor utilization could be measured (Table 2 and Fig. 4; see also Table 1). The value of 0.1 nmol/min/mg for CCl_4 disappearance was within the experimental error of the technique used. In control experiments (minus NADPH) recovery of CCl_4 by extraction with *n*-heptane was essentially 100%. Similar results were obtained with microsomal preparations from rat or rabbit. In anaerobic incubations, CCl_4 disappearance exceeded $CHCl_3$ formation (Table 2), a result that suggests the presence of additional routes of metabolism for CCl_4, for example, covalent binding of reactive metabolites to proteins and lipids.

A monooxygenase system reconstituted from NADPH–cytochrome *P*-450 reductase and cytochrome

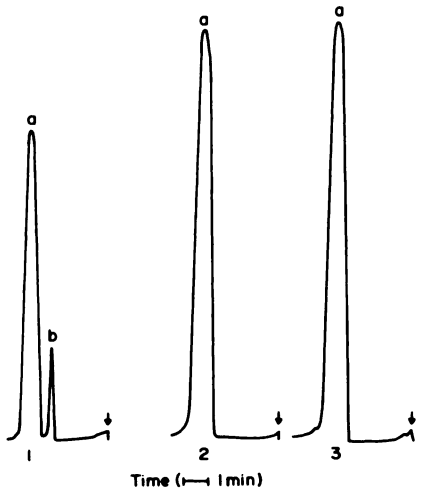

FIG. 4. *CCl_4 metabolism in microsomal samples from phenobarbital-treated rats determined by measuring substrate disappearance and chloroform formation*

(1) Anaerobic (N_2 atmosphere); (2) aerobic; (3) incubation in the absence of NADPH. (a) CCl_4 peak; (b) $CHCl_3$ peak; (↓) injection point. The CCl_4 concentration used in this experiment was 100 μM; the microsomal protein concentration was 2 mg/ml.

P-450, purified from the livers of phenobarbital-treated rabbits, was used to identify the enzyme responsible for catalyzing the reduction of CCl_4 to $CHCl_3$. The formation of $CHCl_3$ could be detected only in anaerobic incubations that contained both the reductase and the cytochrome (Table 3). The reaction was inhibited by air, CO, MNP, or PBN and was potentiated by FMN. No $CHCl_3$ was formed in the absence of the cytochrome, even at a reductase concentration 20 times that present in microsomal incubations. Hepatic microsomal phospholipids inhibited the formation of $CHCl_3$ by 66% in the reconstituted system. This could be explained by a competition between the covalent binding of reactive metabolites to the lipid fraction and the hydrogen abstraction reaction to give $CHCl_3$.

Carbon monoxide, another metabolite that results from the reduction of CCl_4, was detected in incubations that contained cytochrome *P*-450 and dithionite or in complete systems (cytochrome plus reductase) incubated anaerobically in the presence of NADPH (Fig. 5). No CO was formed in the absence of cytochrome *P*-450. A characteristic cytochrome *P*-450–CCl_4 ligand complex (10) formed concomitantly with the production of CO. The spectrum obtained, which may well be a composite of both of these ligands, is shown in Fig. 6. The spectral peak at 416 nm appears to be ferrous cytochrome *P*-450 that has not formed a ligand complex.

DISCUSSION

It has been thought for some time that cytochrome *P*-450 can metabolize haloalkanes (9, 10, 12, 21) to free radicals (see reviews, Refs. 1–4) or carbene (10, 13, 22) intermediates via one- and two-electron reductions, respectively. Some evidence has also indicated that NADPH–cytochrome *P*-450 reductase can catalyze the reduction of CCl_4 (1, 23). For example, carbon monoxide, a potent inhibitor of cytochrome *P*-450-catalyzed reactions, has been reported to incompletely inhibit the reduction of CCl_4 to $CHCl_3$ and the covalent binding of CCl_4 metabolites to microsomal proteins and lipids (9). Also, carbon monoxide has no effect on CCl_4-induced

TABLE 3

Metabolism of CCl_4 to $CHCl_3$ in reconstituted monooxygenase systems

Expt No.	Components present in the incubation				$CHCl_3$ formed
	Cytochrome *P*-450 (0.5 μM)	Cyt. *P*-450 reductase (8000 U/ml)	NADPH (0.5 mM)	Other	
					% control
1	+	+	+	—	100*
2	+	+	—	—	<1
3	—	+	+	—	<1
4	+	+	+	Air	<1
5	—	+	+	Air	<1
6	+	+	+	Phospholipid (50 μg/ml)	34
7	+	+	+	FMN (0.1 mM)	331
8	+	+	+	Riboflavin (1 mM)	225
9	+	+	+	Benzoquinone (0.1 mM)	25
10	+	+	+	Carbon monoxide	10

* Control rates of $CHCl_3$ formation ranged between 0.8 and 1.4 nmol/min/nmol *P*-450. Incubations (2 ml) were carried out for 10 min at 37°C in 0.05 M phosphate buffer, pH 7.6, in a nitrogen atmosphere. The CCl_4 concentration was 5 mM. The phospholipid used was extracted from hepatic microsomes.

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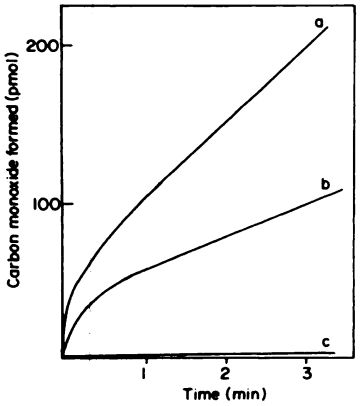

FIG. 5. *Rate of CCl_4 metabolism to carbon monoxide in reconstituted monooxygenase systems under anaerobic conditions*

(a) Cytochrome *P*-450 (0.5 μM) and sodium dithionite; (b) cytochrome *P*-450 (0.5 μM), cytochrome *P*-450 reductase (8000 U/ml), and NADPH (0.5 μM); (c) cytochrome *P*-450 reductase (8000 U/ml) and NADPH or sodium dithionite. Incubations (at 37°C) were in 0.05 M phosphate buffer, pH 7.6 CCl_4 (1 mM) was present in all incubations.

lipid peroxidation (1), a reaction which is thought to be initiated by the metabolism of CCl_4 to a free radical. In addition, NADPH–cytochrome *P*-450 reductase is known to catalyze the one-electron reduction of a number of compounds (3). Our results, obtained using purified enzymes, demonstrate that cytochrome *P*-450 can catalyze both the one- and the two-electron reductions of CCl_4 (10):

$$CCl_4 \xrightarrow{e^-} CCl_3 + Cl^- \xrightarrow{e^-} :CCl_2 + Cl^-.$$
$$\downarrow RH \qquad \downarrow H_2O$$
$$CHCl_3 \qquad CO$$

Furthermore, these results strongly suggest that NADPH–cytochrome *P*-450 reductase cannot reduce CCl_4. First, cytochrome *P*-450 is required for the reduction of CCl_4 in a reconstituted system; cytochrome *P*-450 reductase alone does not catalyze the reaction. Second, the formation of $CHCl_3$ in anaerobic microsomal incubations is completely inhibited by carbon monoxide.

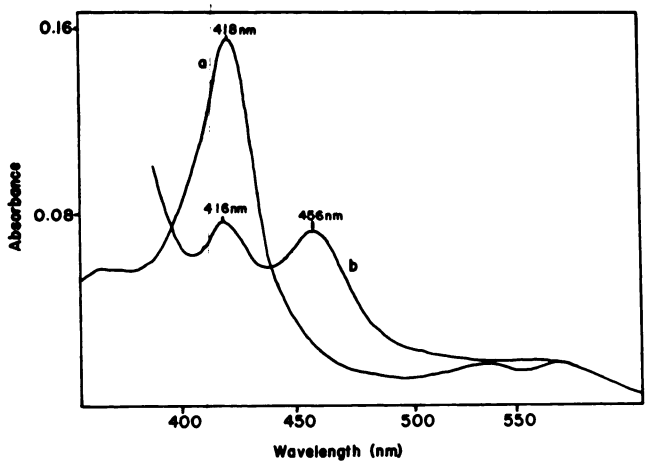

FIG. 6. *Formation of the CCl_4–cytochrome P-450 complex with cytochrome P-450 purified from phenobarbital-treated rabbits under anaerobic conditions*

(a) Ferric cytochrome *P*-450 spectrum; (b) spectrum obtained 5 min after the addition of CCl_4 (5 mM) and sodium dithionite. The cytochrome *P*-450 concentration was 1.5 nmol/ml.

Although metabolism of CCl_4 could be detected only in the absence of air, CCl_4 significantly increased microsomal rates of lipid peroxidation and cofactor utilization in aerobic incubations. The relationships between these reactions appear to be complex and a correlation between the metabolism of CCl_4 and CCl_4-induced lipid peroxidation is not evident. It has been proposed that the metabolism of CCl_4 to a trichloromethyl radical ($\cdot CCl_3$) initiates CCl_4-induced lipid peroxidation by the abstraction of a hydrogen atom from an unsaturated lipid to form $CHCl_3$ and a lipid radical (1, 2, 4). This hypothesis is supported by Slater and Sawyer (18), who concluded that a linear relationship (correlation coefficient, 0.82) exists between the induced rate of lipid peroxidation and the square root of the CCl_4 concentration. This argument is weakened by theoretical considerations that preclude the differentiation of a square root function from typical equilibrium kinetics when the substrate range is limited by experimental conditions. Data from a hypothetical double reciprocal plot (substrate concentrations from 1/20 to 20 K_m) are linear when plotted as a square root function (correlation coefficient, 0.91). The data from Fig. 1 are linear in both double reciprocal (correlation coefficient, 0.96) and square root (correlation coefficient, 0.90) plots. In addition, our observations, that $CHCl_3$ formation was completely inhibited by air and that CO does not inhibit lipid peroxidation, argue against hydrogen abstraction by $\cdot CCl_3$ as the initiating event in CCl_4-induced lipid peroxidation. It has been reported that air inhibits the covalent binding of CCl_4 metabolites to microsomal proteins (9, 12) and, more important, almost totally inhibits their binding to microsomal lipids (24). It is possible that lipid peroxidation is induced by extremely low levels of CCl_4 metabolism. Using electron spin trapping techniques, evidence for the formation of the trichloromethyl radical under aerobic conditions has been presented (25). This work was criticized on the basis that a similar signal could be observed on reaction of the spin trap (PBN) with a carbon-centered lipid radical in the absence of CCl_4 (26). However, the formation of the trichloromethyl radical has been shown conclusively using $^{13}CCl_4$ (27), and this finding has been confirmed by us. The PBN–$^{13}CCl_3$ adduct formed by uv photolysis gives an identical ^{13}C hyperfine coupling (a^c = 9.6 G) to that obtained in microsomal incubations (unpublished data). Indirect evidence for the formation of the trichloromethyl radical was presented by Shah *et al.* (28), who showed that CCl_4 is metabolized to give phosgene. It is possible that trace amounts of $\cdot CCl_3$ are formed aerobically and react with oxygen to yield CCl_3–O–O$\cdot$. This species could degrade to give phosgene or could abstract a proton and initiate lipid peroxidation. Chloroform would not result from such a reaction sequence. However our data suggest that cytochrome *P*-450 is not involved in CCl_4-induced lipid peroxidation. This is in conflict with the report that cytochrome *P*-450 is required in order to induce this reaction in a soluble system containing partially purified cytochrome *P*-450 and NADPH–cytochrome *P*-450 reductase (29), a result that we have been unable to obtain with highly purified enzymes in a reconstituted system that catalyzes CCl_4-induced lipid peroxidation (unpublished).

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CCl_4-induced NADPH oxidation in aerobic incubations containing microsomal preparations from phenobarbital-treated rats or rabbits appears to be unrelated to both the metabolism of CCl_4 and CCl_4-induced lipid peroxidation. Unlike lipid peroxidation, the induced rate of NADPH oxidation is mediated by cytochrome *P*-450 and can be totally inhibited by carbon monoxide. This effect of CCl_4 appears to be the same as that of CCl_3F, which increases NADPH oxidation and O_2 uptake without increasing lipid peroxidation (30; Wolf, Netter, Parke and King, submitted) and may be caused by an uncoupling reaction similar to that reported by Staudt *et al.* (31). A lack of a correlation between induced lipid peroxidation and NADPH oxidation is also apparent with microsomes from untreated rats, in that CCl_4 increases lipid peroxidation without significantly affecting NADPH oxidation in these preparations. Whether or not increased cofactor utilization plays a role in the potentiation of CCl_4 toxicity by phenobarbital remains to be determined.

In contrast to the metabolism of CCl_4 and CCl_4-induced NADPH oxidation, CCl_4-induced O_2 uptake is not completely inhibited by carbon monoxide. However, it is totally inhibited by carbon monoxide plus PBN, a compound that inhibits lipid peroxidation. Thus, CCl_4-induced O_2 uptake is not entirely a function of cytochrome *P*-450 and is partially related to CCl_4-induced lipid peroxidation.

CONCLUSIONS

It appears that CCl_4-induced lipid peroxidation is not initiated by the abstraction of a hydrogen atom by $\cdot CCl_3$. These investigations also indicate that the cytochrome *P*-450-mediated reduction of CCl_4 and CCl_4-induced lipid peroxidation are independent reactions. This is most clearly demonstrated by the difference in the sensitivity of these reactions to carbon monoxide.

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