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EFFECT OF HYPOXIA ON CARBON TETRACHLORIDE HEPATOTOXICITY*

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Abstract—The effect of hypoxia on carbon tetrachloride-induced hepatotoxicity was studied. Male rats were exposed to carbon tetrachloride for 2 hr in the presence of differing oxygen concentrations. Serum glutamate-pyruvate transaminase (SGPT) activities were measured 24 hr after the end of the exposure. Exposure of rats to 5000 ppm carbon tetrachloride in the presence of 100, 21, 12, or 6% oxygen resulted in SGPT activities of 489, 420, 3768, and 1788 I.U./l respectively. Exposure of rats to air and 0, 1250, 2500, 5000, or 7500 ppm carbon tetrachloride gave SGPT activities of 35, 32, 69, 420, and 2188 I.U./l respectively; when 12% oxygen was used, the corresponding SGPT activities were 32, 665, 691, 3768, and 4200 I.U./l respectively. Exposure of rats to hypoxia produced histopathologically detectable condensation of hepatic cytoplasmic material, and exposure to 5000 ppm carbon tetrachloride in the presence of air produced mild centrilobular necrosis, which was much more severe when rats were exposed to 5000 pm carbon tetrachloride in the presence of 12% oxygen. Hepatic microsomal conjugated diene concentrations were increased by hypoxia and by exposure to carbon tetrachloride, but no synergistic interaction was observed. Hepatic microsomal cytochrome P-450 concentrations were decreased after exposure to carbon tetrachloride, but were the same after exposure to carbon tetrachloride and 12 or 21% oxygen. Hepatic carbon tetrachloride concentrations were the same in rats exposed to carbon tetrachloride in the presence of 12 or 21% oxygen; hepatic chloroform concentrations were higher in rats exposed to carbon tetrachloride in the presence of air than in the presence of 12% oxygen. The covalent binding of [^{14}C]carbon tetrachloride metabolites to hepatic microsomal lipids and proteins was increased markedly by hypoxia as compared with normoxia. The covalent binding of metabolites of carbon tetrachloride to cellular macromolecules may play a role in the potentiation of carbon tetrachloride toxicity by hypoxia.

Carbon tetrachloride is a well-known hepatotoxin. Reductive metabolism of carbon tetrachloride is thought to play an important role in the initiation of lipid peroxidation by carbon tetrachloride [1, 2] and in the biotransformation of carbon tetrachloride to chloroform [3–5], hexachloroethane [6–8], and carbon monoxide [5, 9]. Recent evidence suggests that phosgene, an oxygenated metabolite of carbon tetrachloride, may play a role in the potentiation of carbon tetrachloride-induced liver damage by 2-propanol [10].

The hepatotoxicity of halothane is potentiated by hypoxia in animals treated with phenobarbital or polychlorinated biphenyls [11–13], and this potentiation is associated with an increased irreversible binding of [^{14}C]halothane metabolites to hepatic microsomal protein and lipids [13, 14], increased plasma fluoride concentrations [14, 15], and increased formation of 2-chloro-1,1,1-trifluoroethane and 2-chloro-1,l-difluoroethylene, reduced metabolites of halothane [15, 16].

Since reductive metabolism may play an important role in carbon tetrachloride bioactivation, hypoxic conditions may potentiate carbon tetrachloride hepatotoxicity. It has been reported previously that hypoxia has no effect [17] or increases [18] carbon tetrachloride hepatotoxicity. The objective of these studies was to investigate the effect of hypoxia on the hepatotoxicity of carbon tetrachloride.

MATERIALS AND METHODS

Male Long–Evans rats (Blue Spruce Farms, Altamont, NY) weighing 230–300 g were maintained on a light (6:00 a.m. to 6:00 p.m.)–dark cycle and provided with food and water *ad lib*. The rats were exposed by inhalation to 6, 12, 21 (filtered room air), or 100% oxygen:nitrogen mixtures for 3 hr and to carbon tetrachloride (Omnisolv, MCB Manufacturing Chemists, Inc., Cincinnati, OH) concentrations of approximately 1250, 2500, 5000, or 7500 ppm for the last 2 hr of the exposure period. The Leach-type exposure chambers were fabricated from 8.65 l glass dessicators and were fitted with a steel mesh grid floor. The chambers were closed with an aluminium lid, which was fitted with gas inlet and outlet ports and two sampling valves, and sealed with a rubber O-ring. No more than four rats were placed in a chamber. Chamber temperatures during the exposures ranged from 23° to 27°.

The oxygen concentration of the gas mixture entering the chamber was measured polarographi-

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cally with an Ohio model 201 oxygen monitor (Ohio Medical Products, Madison, WI), which was placed immediately downstream from the vaporizer.

Carbon tetrachloride was delivered to the vaporizer, which was heated to 72°, from a 30 cc glass syringe driven by a model 355 Sage Instruments infusion pump (Orion Research, Inc., Cambridge, MA). Carbon tetrachloride concentrations were measured by gas chromatography every 15 min during the exposure. The samples were analyzed with a Varian Series 1400 gas chromatograph (Varian Aerograph, Walnut Creek, CA) equipped with a flame ionization detector and a gas sampling valve with a 0.25 ml loop. A 6 ft by 2 min i.d. glass column containing 0.2% SP-1000 on 60/80 mesh Carbopack B (Supelco Inc., Bellefonte, PA) was used. Nitrogen (20 cc/min) was the carrier gas, and the hydrogen and air flow rates were 30 and 190 cc/min respectively. The oven temperature was 175°, and the injection port and detector temperatures were both 195°. The retention time of carbon tetrachloride was 2.9 to 3.1 min. The peak corresponding to carbon tetrachloride was quantified by integration with a Hewlett–Packard 3390A Integrator (Hewlett–Packard, Inc., Avondale, PA).

For the [^{14}C]carbon tetrachloride binding studies, 240 g of anhydrous calcium sulfate (Drierite, 8 mesh, W. A. Hammond Drierite Co., Xenia, OH) was mixed with 150 g of a barium hydroxide–lime absorbent (Baralyme, Chemetron Medical Products, St. Louis, MO) and placed in the exposure chamber to absorb water and carbon dioxide. Rats were placed in the chamber and exposed for 1 hr to air or a 12% oxygen:88% nitrogen mixture. At the end of this time, the gas flow was stopped, the chamber was connected to a spirometer filled with oxygen, and 100 μl of [^{14}C]carbon tetrachloride (1.1×10^6 dpm/ml) was injected into the chamber to achieve a carbon tetrachloride concentration of approximately 5000 ppm. Rats were exposed to [^{14}C]carbon tetrachloride for 1 hr.

Quantification of serum glutamate-pyruvate transaminase (SGPT) activity was used to estimate liver damage. For the time course studies, blood samples were taken at 0, 24, 36, 48, and 72 hr after the end of the exposure to 5000 ppm carbon tetrachloride. SGPT activities were measured at 30° with a Beckman System TR Enzyme Activity Analyzer and Beckman Liquid-STAT ALT-UV Liquid Enzyme Reagent (Beckman Instruments, Inc., Fullerton, CA). Enzyme activities are expressed in International Units per liter (I.U./l).

Hepatic microsomal fractions were prepared as described previously [19] from livers perfused *in situ* with ice-cold 0.15 M potassium chloride solution. The microsomal pellet was suspended in ice-cold 0.15 M potassium chloride solution. Microsomal protein concentrations were determined by the method of Lowry *et al* [20] with bovine serum albumin as the standard. Hepatic microsomal cytochrome P-450 concentrations were measured by the method of Omura and Sato [21]. Hepatic carbon tetrachloride and chloroform concentrations were measured by gas chromatography. Rats were removed from the inhalation chambers immediately after the termination of the carbon tetrachloride exposure, stunned by a blow on the head, and decapitated. The liver was perfused *in situ* with ice-cold 0.15 M potassium chloride solution, excised, blotted dry, and weighed. The liver was minced, and a 50% homogenate (w/v) was prepared in ice-cold 0.15 M potassium chloride solution with a Dounce homogenizer (15 strokes with a loose pestle). One hundred microliters of the homogenate was placed in a 1 ml Hypo-Vial (Pierce Chemical Co., Rockford, IL) containing 1 ml of heptane (Omnisolv). The vials were capped with Teflon-lined silicone disks and aluminium crimp-on seals. The contents were mixed well, and 1 μl of the organic phase was analyzed on a Hewlett–Packard 5730A gas chromatograph equipped with a ^{63}Ni electron capture detector. The carrier gas was 95% argon:5% methane (60 ml/min). The 6 ft × 2 mm i.d. glass column was packed with 0.2% Carbowax 1500 on 80/100 mesh Carbopack C (Supelco, Inc.). The oven temperature was 100°, the injection port temperature was 150°, and the detector temperature was 250°. The retention times for chloroform and carbon tetrachloride were 1.3 and 2.1 min respectively. Carbon tetrachloride and chloroform concentrations were quantified by measuring peak heights. Recovery of chloroform and carbon tetrachloride from liver homogenates ranged from 92 to 98%.

Lipid peroxidation was evaluated by measuring the formation of microsomal lipid conjugated dienes

Table 1. Serum glutamate-pyruvate transaminase (SGPT) activities after exposure of rats to carbon tetrachloride under normoxic or hypoxic conditions*

Carbon tetrachloride† (ppm)	Atmosphere	SGPT‡ (I.U./l) Time after exposure (hr) 0	24	36	48	72
4811 ± 154	Air	45 ± 4	420 ± 156§	385 ± 61§	482 ± 68§	134 ± 21§
4930 ± 45	12% O_2:88% N_2	47 ± 1	3768 ± 783§‖	3137 ± 460§‖	1462 ± 146§‖	232 ± 40§

* Rats were exposed for 3 hr to 12% O_2:88% N_2 or air as indicated. Carbon tetrachloride was administered for the last 2 hr of the exposure period. Blood samples were collected at 0, 24, 36, 48, and 72 hr after the end of the exposure. Serum samples were analyzed for SGPT activities as described in Materials and Methods.

† Chamber carbon tetrachloride concentrations were measured by gas chromatography and are shown as means ± S.E.

‡ Values are shown as means ± S.E. for four rats.

§ Significantly different ($P < 0.05$) from the 0 hr value (*t*-test).

‖ Significantly different ($P < 0.05$) from corresponding normoxic (air) value (*t*-test).

Table 2. Effect of carbon tetrachloride and oxygen concentrations on serum glutamate-pyruvate transaminase (SGPT) activities*

Oxygen† (%)	SGPT activities (I.U./l) Carbon tetrachloride‡ (ppm) 0	1243 ± 37	2523 ± 70	4842 ± 92	7565 ± 117
100	27 ± 3	ND§	ND	489 ± 246‖	ND
21	35 ± 4	32 ± 8	69 ± 9	420 ± 156‖	2188 ± 665
12	32 ± 4	665 ± 297¶	691 ± 265¶	3768 ± 783‖¶**	4200 ± 1007
6	46 ± 6	ND	ND	1788 ± 488‖	ND

* Rats were exposed to carbon tetrachloride as indicated during the last 2 hr of a 3-hr exposure to either air or an oxygen:nitrogen mixture as indicated. Blood samples were taken 24 hr after the end of the exposure. Serum samples were analyzed for SGPT activities as described in Materials and Methods. Each value is the mean ± S.E. for four rats.

† Oxygen concentrations were measured by polarography.

‡ Chamber carbon tetrachloride concentrations were measured by gas chromatography and are shown as means ± S.E.

§ Not done.

‖ The effect of carbon tetrachloride concentration (0 vs 5000 ppm) at differing oxygen concentrations is significant ($P < 0.05$; analysis of variance).

¶ The effect of oxygen concentration (12 vs 21%) is significant ($P < 0.05$; analysis of variance).

** The synergistic interaction between oxygen and carbon tetrachloride concentrations is significant ($P < 0.05$; analysis of variance).

according to the procedure of Klaassen and Plaa [22]; conjugated diene formation was determined immediately after termination of the exposure.

The covalent binding of [^{14}C]carbon tetrachloride metabolites to hepatic microsomal lipids and proteins was measured by the procedures of Castro *et al.* [23] and Uehleke *et al.* [7] respectively.

Liver samples taken 24 hr after treatment for histopathological examination were fixed in neutral buffered formalin, and the sections were stained with hematoxylin-eosin. The coded slides were examined by light microscopy.

Results were evaluated statistically with Student's *t*-test or two-way analysis of variance. A level of $P < 0.05$ was chosen for acceptance or rejection of the null hypothesis.

RESULTS

Exposure of rats to 5000 ppm of carbon tetrachloride in the presence of 12% oxygen increased SGPT activities measured 24, 36, and 48 hr after exposure as compared with rats exposed to the same concentration of carbon tetrachloride in the presence of air (Table 1).

The effect of differing oxygen and carbon tetrachloride concentrations on carbon tetrachloride hepatotoxicity was also studied (Table 2). When the oxygen concentration was varied, maximal SGPT activities were observed in rats exposed to 12% oxygen and 5000 ppm carbon tetrachloride. When the carbon tetrachloride concentration was varied and the oxygen concentration was 12%, maximal SGPT activities were seen in rats exposed to 5000 or 7500 ppm carbon tetrachloride; rats exposed to 21% oxygen showed maximal SGPT activities at 7500 ppm carbon tetrachloride.

Hepatic carbon tetrachloride concentrations were the same in rats exposed to 5000 ppm carbon tetrachloride in the presence of either 12 or 21% oxygen (Table 3), but hepatic chloroform concentrations in carbon tetrachloride-exposed rats were lower in rats exposed to 12% oxygen than in rats exposed to 21% oxygen.

Exposure of rats to 12 or 21% oxygen did not alter hepatic microsomal cytochrome P-450 concentrations (Table 4). Exposure of rats to 5000 ppm carbon tetrachloride in the presence of either 12 or 21% oxygen lowered microsomal cytochrome P-450 concentrations, but the values were the same at both oxygen concentrations.

Rats exposed to 12% oxygen showed an increase

Table 3. Effect of hypoxia on hepatic carbon tetrachloride and chloroform concentrations*

Carbon tetrachloride† (ppm)	Atmosphere	Chloroform‡ (nmoles/g liver)	Carbon tetrachloride‡ (nmoles/g liver)
4898 ± 192	Air	63 ± 6	144 ± 48
5292 ± 62	12% O_2:88% N_2	44 ± 2§	182 ± 50

* Rats were exposed to carbon tetrachloride during the last 2 hr of a 3-hr exposure to either air or a 12% oxygen:88% nitrogen mixture. Hepatic carbon tetrachloride and chloroform concentrations were measured immediately after the end of the exposure as described in Materials and Methods.

† Chamber carbon tetrachloride concentrations were measured by gas chromatography and are shown as means ± S.E.

‡ Values are shown as means ± S.E. for three rats.

§ Significantly different ($P < 0.05$) from normoxic values (*t*-test).

Table 4. Effect of carbon tetrachloride and hypoxia on hepatic microsomal cytochrome P-450 concentrations*

Carbon tetrachloride† (ppm)	Atmosphere	Cytochrome P-450‡ (nmoles/mg protein)
None	Air	0.93 ± 0.14
None	12% O_2:88% N_2	0.92 ± 0.14
5450 ± 149	Air	0.63 ± 0.11§
5370 ± 156	12% O_2:88% N_2	0.52 ± 0.09§

* Rats were exposed to carbon tetrachloride as indicated during the last 2 hr of a 3-hr exposure to either air or a 12% oxygen:88% nitrogen mixture. Hepatic microsomal fractions were isolated from rats killed immediately after the end of the exposure, and cytochrome P-450 concentrations were measured as described in Materials and Methods.

† Chamber carbon tetrachloride concentrations were measured by gas chromatography and are shown as means ± S.E.

‡ Values are shown as means ± S.D. for five to six rats.

§ Significantly different ($P < 0.05$), 0 vs 5000 ppm carbon tetrachloride (*t*-test).

Table 5. Effect of carbon tetrachloride and hypoxia on hepatic microsomal lipid peroxidation*

Carbon tetrachloride† (ppm)	Atmosphere	Conjugated dienes‡
None	Air	0.16 ± 0.02
None	12% O_2:88% N_2	0.23 ± 0.04§
5401 ± 163	Air	0.28 ± 0.06‖
5239 ± 97	12% O_2:88% N_2	0.36 ± 0.07§‖¶

* Rats were exposed to carbon tetrachloride as indicated during the last 2 hr of a 3-hr exposure to either air or a 12% oxygen:88% nitrogen mixture. Hepatic microsomal fractions were isolated from rats killed immediately after the end of the exposure, and conjugated diene formation was determined as described in Materials and Methods.

† Chamber carbon tetrachloride concentrations were measured by gas chromatography and are shown as means ± S.E.

‡ Values are expressed as absorbance at 243 nm of a 1 mg/ml solution of microsomal lipids and are shown as means ± S.D. for four to eight rats.

§ Oxygen effect is significant ($P < 0.05$) compared to respective control (*t*-test).

‖ Carbon tetrachloride effect is significant ($P < 0.05$) compared to respective control (*t*-test).

¶ The effect of oxygen and carbon tetrachloride is significant ($P < 0.05$) compared to respective control (*t*-test), but the synergistic interaction of oxygen and carbon tetrachloride is not significant ($P > 0.05$; analysis of variance).

Table 6. Effect of hypoxia on the covalent binding of [^{14}C]carbon tetrachloride metabolites to hepatic microsomal lipids and proteins*

Atmosphere	Covalent binding†	
	Lipid (nmoles/mg)	Protein (nmoles/mg)
Air	4.7 ± 2.0	0.39 ± 0.07
12% O_2:88% N_2	12.7 ± 1.4‡	0.79 ± 0.14‡

* Rats were exposed to approximately 5000 ppm of [^{14}C]carbon tetrachloride during the last hour of a 2-hr exposure to either air or a 12% oxygen:88% nitrogen mixture. Hepatic microsomal fractions were isolated from rats killed immediately after the end of the exposure, and the covalent binding of [^{14}C]carbon tetrachloride metabolites to lipids and proteins was measured as described in Materials and Methods.

† Values are expressed as nmoles [^{14}C]carbon tetrachloride equivalents bound per mg of microsomal lipid or protein and are shown as means ± S.D. for three rats.

‡ Significantly different ($P < 0.05$) from normoxic value (*t*-test).

in lipid peroxidation, as measured by conjugated diene formation, when compared with rats exposed to 21% oxygen (Table 5). Exposure of rats to 5000 ppm carbon tetrachloride and 12 or 21% oxygen increased lipid peroxidation and, in carbon tetrachloride-exposed rats, lipid peroxidation was greater in animals exposed to 12% oxygen than in animals exposed to 21% oxygen.

The covalent binding of [^{14}C]carbon tetrachloride metabolites to both hepatic microsomal lipids and proteins was increased by exposure to 12% oxygen as compared with 21% oxygen in rats exposed to 5000 ppm carbon tetrachloride (Table 6).

Histopathological examination of the livers of rats exposed to 12% oxygen alone showed an unusual condensation of cytoplasmic material (Fig. 1). Rats exposed to 5000 ppm carbon tetrachloride and 12% oxygen showed centrilobular necrosis which was more progressive and extensive than in rats exposed to 5000 ppm carbon tetrachloride in the presence of 21% oxygen.

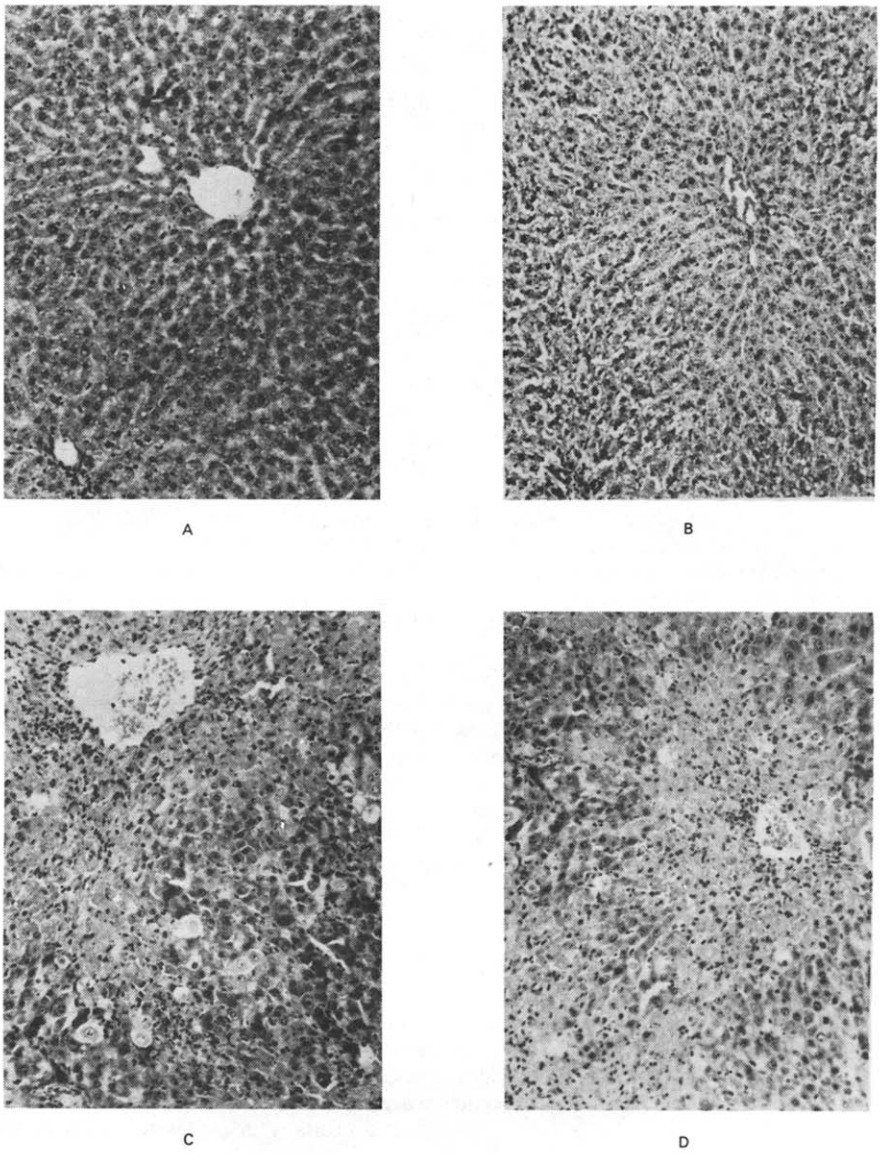

Fig. 1. Photomicrographs of liver tissue of rats exposed to room air (A), 12% oxygen (B), 5000 ppm carbon tetrachloride in the presence of air (C) or 12% oxygen (D). Magnification: 40×, H and E.

DISCUSSION

In the present study, rats exposed to hypoxia (12% oxygen:88% nitrogen) and 5000 ppm carbon tetrachloride showed a marked potentiation of carbon tetrachloride-induced hepatotoxicity as compared with rats exposed to 21% oxygen and 5000 ppm carbon tetrachloride. Rats exposed to 1250 ppm carbon tetrachloride and hypoxic (12% oxygen) conditions developed hepatotoxicity, but no effect was observed in rats exposed to 1250 ppm carbon tetrachloride under normoxic conditions. Strubelt and Breining [18] found that rats exposed to 6% oxygen for 6 hr immediately after the intraperitoneal administration of carbon tetrachloride (0.5 ml/kg) showed an increase in hepatotoxicity when compared with rats given carbon tetrachloride in the presence of air. In contrast, Suarez *et al.* [17] found that exposure of rats to 7.5% oxygen for 2 hr and carbon tetrachloride (3799 ppm for the last 30 min of exposure) did not alter carbon tetrachloride hepatotoxicity. The conflicting reports on the potentiating effect of hypoxia on carbon tetrachloride-induced hepatotoxicity may be due to differences in the oxygen and carbon tetrachloride concentrations used and in the exposure times employed. The relatively short carbon tetrachloride exposure time along with the low oxygen (7.5%) and carbon tetrachloride (3799 ppm) concentrations used by Suarez *et al.* [17] may have prevented the development of liver injury, since in the present investigation the observed potentiation of hepatotoxicity due to hypoxia was reduced markedly at 2500 ppm as compared with 5000 ppm carbon tetrachloride. Furthermore, rats exposed to

5000 ppm carbon tetrachloride and 6% oxygen exhibited less liver damage than rats exposed to 5000 ppm carbon tetrachloride and 12% oxygen.

The mechanisms involved in the potentiation of carbon tetrachloride-induced hepatotoxicity by hypoxia are not well-understood, but alterations in hepatic blood flow could contribute to the production of liver damage. The decrease in hepatic blood flow due to carbon tetrachloride administration [24–26] and hypoxia [27] could alter carbon tetrachloride delivery to, and extraction by, the liver and could be a contributory factor in the potentiation of carbon tetrachloride-induced hepatotoxicity by hypoxia. However, no difference in hepatic carbon tetrachloride concentrations was observed between normoxic and hypoxic animals exposed to carbon tetrachloride, indicating that any changes in liver blood flow did not affect carbon tetrachloride delivery to, or extraction by, the liver.

An alternative explanation for the effect of hypoxia on carbon tetrachloride-induced hepatotoxicity is that hypoxia may enhance the reductive metabolism or bioactivation of carbon tetrachloride. Since hypoxia was also found to increase the reductive metabolism of halothane, as measured by an increase in reduced halothane metabolites in liver and blood after hypoxia and halothane exposure [15], an increase in the reductive metabolism of carbon tetrachloride was also expected to occur under hypoxic conditions. Nevertheless, rats exposed to hypoxia and carbon tetrachloride had lower hepatic chloroform concentrations than rats exposed to carbon tetrachloride under normal oxygen tensions. The decreased hepatic chloroform concentrations seen in animals exposed to carbon tetrachloride under hypoxic conditions may be attributed to a decreased biotransformation of carbon tetrachloride to chloroform or to the diversion of the trichloromethyl radical from chloroform formation to other pathways of disposition. Although chloroform may undergo metabolism to phosgene [28, 29], increased chloroform metabolism under hypoxic conditions seems unlikely.

The decrease in hepatic chloroform concentrations under hypoxia was accompanied by a substantial increase in the covalent binding of [^{14}C]carbon tetrachloride metabolites to hepatic microsomal lipids and proteins. Under hypoxic conditions, the trichloromethyl radical, a precursor of chloroform and perhaps the covalently bound metabolites of carbon tetrachloride, may bind selectively to cellular macromolecules rather than form chloroform. Wolf *et al.* [30] found that, in a reconstituted monooxygenase system of purified NADPH–cytochrome P-450 reductase and cytochrome P-450, phospholipids inhibited the formation of chloroform from carbon tetrachloride; these authors suggested that competition may exist between the covalent binding of reactive intermediates of carbon tetrachloride to microsomal lipids and the hydrogen abstraction reaction, which is thought to produce chloroform. This competition could account for both the decrease in chloroform formation and the increase in covalent binding of [^{14}C]carbon tetrachloride metabolites to hepatic microsomal lipids under hypoxia as opposed to normoxia.

Exposure of rats to 12% oxygen increased lipid peroxidation, as measured by conjugated diene formation. The mechanism by which hypoxia increases lipid peroxidation is not clear, but hypoxic conditions are known to produce liver damage [31–33]. Hypoxia-stimulated lipid peroxidation may be involved in the condensation of cytoplasmic materials observed in liver sections from rats exposed to 12% oxygen alone. It is possible to speculate that the lipid peroxidation seen under hypoxic conditions may occur secondarily to iron release from necrotic cells. Maines [34] has provided evidence for a catalytic role of endogenous iron in the peroxidation of microsomal membranes, which is accelerated after the initial destruction of microsomal heme. Carbon tetrachloride exposure increased lipid peroxidation in rats exposed to either air or 12% oxygen, but no interaction between the effects of hypoxia and carbon tetrachloride exposure was detected. Thus, enhanced lipid peroxidation does not appear to explain completely the increase in carbon tetrachloride toxicity seen under hypoxic conditions.

The decrease in hepatic microsomal cytochrome P-450 concentrations observed after carbon tetrachloride exposure was the same under both normoxic and hypoxic conditions, and hypoxia alone did not alter hepatic cytochrome P-450 concentrations. Although lipid peroxidation has been implicated in carbon tetrachloride-induced cytochrome P-450 destruction *in vitro* [35], lipid peroxidation may not be responsible for the loss of hepatic cytochrome P-450, which may be due to an attack by reactive metabolites on the hemoprotein [36–38].

Hypoxia-potentiated carbon tetrachloride hepatotoxicity and the covalent binding of carbon tetrachloride metabolites to microsomal lipids and proteins appear to be correlated. The observation that the covalent binding of [^{14}C]carbon tetrachloride metabolites to microsomal lipids was greater than to microsomal proteins is in agreement with the results from other studies [39–42]. Changes in the covalent binding of reactive metabolites have been found in many instances to correlate with changes in the incidence and severity of toxicity [43]. Treatments, such as acetone, isopropanol, or dibenamine administration, that alter the severity of carbon tetrachloride-induced liver necrosis cause parallel changes in the covalent binding of carbon tetrachloride metabolites to cellular macromolecules [43–45]. The changes in the degree of covalent binding may reflect changes in the rate of bioactivation or in the disposition of reactive metabolites of carbon tetrachloride. It has also been reported that hypoxia increases the covalent binding of halothane metabolites *in vivo* [14]. In the present study, the hypoxic potentiation of carbon tetrachloride hepatotoxicity correlated more closely with the increase in covalent binding of carbon tetrachloride metabolites than with lipid peroxidation. Diaz Gomez *et al.* [46] also found that a better correlation exists between the degree of covalent binding of [^{14}C]carbon tetrachloride metabolites and necrosis in various animal species, although covalent binding may not be the primary event responsible for necrosis since Diaz Gomez *et al.* [40] observed that covalent binding does not consistently predict eventual necrosis. The present

investigation, however, indicates that the covalent binding of carbon tetrachloride metabolites to cellular macromolecules may be a major event during the potentiation of carbon tetrachloride-induced hepatotoxicity by hypoxia.

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