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Formation and Inactivation of a Chemically Reactive Metabolite of Vinyl Chloride[1]

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Formation and Inactivation of a Chemically Reactive Metabolite of Vinyl Chloride. PESSAYRE, D., WANDSCHEER, J. C., DESCATOIRE, V., ARTIGOU, J. Y., AND BENHAMOU, J. P. (1979). *Toxicol. Appl. Pharmacol.* 49, 505–515. The mechanisms responsible for, and protecting against, metabolic activation of vinyl chloride were investigated in the rat. When [^{14}C]vinyl chloride was incubated with hepatic microsomes, a ^{14}C-labeled material became irreversibly bound to microsomal proteins; binding required NADPH, was decreased by CO, SKF 525-A, or glutathione, and was increased by 1,1,1-trichloropropene-2,3-oxide (TCPO). Inhalation of a 5% vinyl chloride atmosphere decreased hepatic cytochrome *P*-450 and glutathione. Pretreatment of the animals with DDT or phenobarbital (1) increased *in vitro* irreversible binding to microsomal proteins measured in the presence, but not in the absence, of TCPO and increased the *in vivo* loss of cytochrome *P*-450 during inhalation of vinyl chloride, but (2) decreased the *in vitro* irreversible binding to 10,000*g* supernatant proteins and the *in vivo* loss of glutathione during inhalation of vinyl chloride. These results are consistent with the views that (1) vinyl chloride is activated by cytochrome *P*-450 into its chemically reactive epoxide, (2) the epoxide may be inactivated by epoxide hydrase or by binding to glutathione, (3) pretreatment with DDT or phenobarbital increases both the formation rate of the epoxide and its inactivation rate by epoxide hydrase, and (4) cytochrome *P*-450 destruction is related to the formation rate of the epoxide, whereas glutathione depletion seems related to only that fraction of the formed epoxide which has escaped inactivation by microsomal epoxide hydrase and has diffused in the cytosol.

Vinyl chloride (chloroethylene) is massively used in the manufacture of plastics: Polymerization of this monomer leads to polyvinyl chloride, the most widely used synthetic plastic (Berk *et al.*, 1976). In workers exposed to vinyl chloride, angiosarcomas of the liver and/or hepatic fibrosis have been observed (Berk *et al.*, 1976). Hepatic angiosarcomas and liver lesions have been reproduced in experimental animals chronically exposed to vinyl chloride (Torkelson *et al.*, 1961; Lester *et al.*, 1963; Viola *et al.*, 1971; Maltoni and Lefemine, 1975; Prodan *et al.*, 1975).

Several carcinogenic and/or hepatotoxic compounds are transformed by the hepatic microsomal mixed-function oxidase system (MFOS) into chemically reactive metabolites that (1) covalently bind *in vitro* to microsomal proteins, (2) covalently bind *in vivo*

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to tissue proteins, and (3) may destroy hepatic cytochrome *P*-450 and deplete hepatic glutathione (Mitchell and Jollow, 1975; Mazel and Pessayre, 1976). In several reports, it has been shown that vinyl chloride may produce one or the other of the above-mentioned effects (Barbin *et al.*, 1975; Reynolds *et al.*, 1975; Bolt *et al.*, 1976; Kappus *et al.*, 1976; Reynolds *et al.*, 1976; Guengerich and Strickland, 1977; Ivanetich *et al.*, 1977; Osterman-Golkar *et al.*, 1977; Watanabe *et al.*, 1978). However, a global understanding of the mechanisms for, and the interplay between, these various effects was hampered by the lack of a comprehensive work studying all these parameters both in non-pretreated and in variously pretreated animals.

METHODS

Animals and treatments. Male Sprague–Dawley rats, weighing 160–200 g, were obtained from Charles River France (Elbeuf, France). Rats were allowed water and food (Autoclave 113, UAR) *ad libitum*. Some animals received one of the following pretreatments: (1) cobaltous chloride ($CoCl_2 \cdot 6H_2O$), 35 mg/kg sc twice daily for 3 days; (2) 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A), 75 mg/kg, ip; (3) 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT); 200 mg/kg ip; or (4) phenobarbital, 100 mg/kg ip daily for 5 days.

Pretreated animals were exposed to vinyl chloride or sacrificed (1) 18 hr after the last dose of $CoCl_2$, (2) 15 min after the single dose of SKF 525-A, (3) 8 days after the single dose of DDT, or (4) 24 hr after the last dose of phenobarbital. Six rats at a time were placed in a 15-liter chamber that was continuously gassed with 2 liters/min of a 95% air–5% vinyl chloride atmosphere. Vinyl chloride (99.99%) was purchased from Merck Company, Darmstadt, Federal Republic of Germany. Animals were sacrificed after various lengths of exposure; it must be noted that the proportion of vinyl chloride in the atmosphere inhaled by the rats did not reach 5% immediately.

Preparation of tissue homogenates and tissue fractions. Rats were stunned; blood was drawn from the inferior vena cava; the liver, the kidneys, the spleen, and the sartorius muscle were removed. Blood samples and tissue fragments were homogenized with a glass-Teflon Potter-Elvehjem homogenizer in 3 vol of 0.154 M KCl, 0.01 M Na^+K^+-phosphate buffer, pH 7.4. The crude homogenates were centrifuged at 10,000*g*; microsomal pellets were prepared by centrifuging 3 ml of the 10,000*g* supernatant with 9.5 ml of 0.154 M KCl, 0.01 M Na^+K^+-phosphate buffer, pH 7.4, at 100,000*g*. The microsomal pellets were resuspended in the phosphate buffer and centrifuged again at 100,000*g*.

Transaminases, glutathione, cytochrome P-450. Serum glutamic-pyruvic transaminase (SGPT) activity was measured by the method of Reitman and Frankel (1957). Some liver fragments were placed in Bouin's solution, embedded in paraffin, and stained with hematoxylin and eosin. Hepatic glutathione was determined in the whole liver homogenate by the method of Ellman (1959) in animals sacrificed at 10–12 AM. Microsomal cytochrome *P*-450 was measured by the method of Omura and Sato (1964).

Irreversible binding in vitro. [^{14}C]Vinyl chloride (specific activity, 1 mCi/mmol), labeled on the two carbon atoms and dissolved in methanol (1 mmol/ml), was purchased from Commissariat à l'Energie Atomique, Saclay, France; its radiopurity, checked by gas–liquid chromatography, was higher than 99%. [^{14}C]Vinyl chloride (5 μmol, 5 μCi) in 5 μl of methanol was added, in 25-ml vials, to 1 ml of ice-cold 0.154 M KCl, 0.01 M Na^+ K^+-phosphate buffer, pH 7.4, containing microsomes or 10,000*g* supernatant from 125 mg of liver and the following reduced nicotinamide adenine dinucleotide phosphate (NADPH)-generating system: NADP (0.4 μmol), glucose 6-phosphate (6 μmol), glucose 6-phosphate dehydrogenase (3 enzyme units), and $MgCl_2$ (6 μmol). The vials were capped and the mixture was incubated at 37° for 15 min in a Gallenkamp shaking incubator. In some vials, the NADPH-generating system was omitted; in other vials, various substances were added; other vials were flushed with a 90% CO–10% O_2 atmosphere, capped, and incubated. Radioactivity irreversibly bound to microsomal or 10,000*g* supernatant proteins was measured exactly as previously described (Allemand *et al.*, 1978): Proteins were precipitated with trichloroacetic acid (TCA), dried, washed three times with TCA, and repeatedly extracted with various solvents (methanol, heptane, and ether); no detectable radioactivity was found in the three last solvent phases; ^{14}C material remaining on the proteins was then counted in an Intertechnique ABAC SL 40 scintillation counter.

Irreversible binding in vivo. [^{14}C]Vinyl chloride (6.25 mg, 100 μmol, 100 μCi) was administered ip in 250 μl of methanol; rats were sacrificed 4 hr later; tissue fragments were homogenized in 3 vol of 0.154 M KCl, 0.01 M Na^+K^+-phosphate buffer, pH 7.4. ^{14}C-Labeled material irreversibly bound to tissue proteins was measured as previously described (Allemand *et al.*, 1978) and summarized above.

Statistical analysis. Student's *t* test (two-tailed) for independent data was used as a test of significance

between means. The relationship between parameters was assessed by linear regression analysis and calculation of the coefficient of correlation (r).

RESULTS

Vinyl chloride inhalation. In animals sacrificed after an 18-hr exposure to a 5% vinyl chloride atmosphere, SGPT was unchanged and histological examination did not show hepatic necrosis in control rats or in rats treated with $CoCl_2$, SKF 525-A, DDT, or phenobarbital (data not shown).

The effects of inhalation of vinyl chloride on hepatic cytochrome *P*-450 and glutathione are shown in Figs. 1–3. Hepatic microsomal cytochrome *P*-450 decreased linearly with time during inhalation of vinyl chloride in control rats (Fig. 1); the decrease was negligible or lower in rats treated with $CoCl_2$ or SKF 525-A and was higher in rats treated with phenobarbital or DDT (Fig. 3). Hepatic glutathione rapidly decreased during the first hour of exposure, reaching a constant low value after 6 hr of exposure in control rats (Fig. 2); the decrease was negligible or lower in rats treated with $CoCl_2$, SKF 525-A, DDT, or phenobarbital (Fig. 3).

In vivo binding. After administration of [^{14}C]vinyl chloride, a ^{14}C-labeled material was irreversibly bound to whole tissue proteins (Table 1); binding to blood, kidney, and spleen proteins was about 33% of that to liver proteins; binding to muscle proteins was about 3% (Table 1). Binding in all organs tested was less in rats treated with $CoCl_2$ than in control rats (Table 1).

In vitro binding. When [^{14}C]vinyl chloride was incubated under air with hepatic microsomes and a NADPH-generating system, a radioactive material became irreversibly

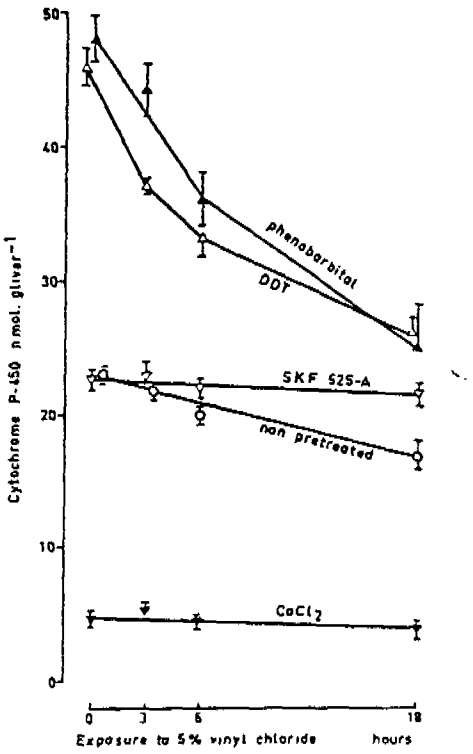

FIG. 1. Effects of vinyl chloride inhalation on hepatic microsomal cytochrome *P*-450. Cytochrome *P*-450 concentrations were measured in animals sacrificed after various lengths of exposure to a 5% vinyl chloride-95% air atmosphere. Results are means ± SD for at least six rats.

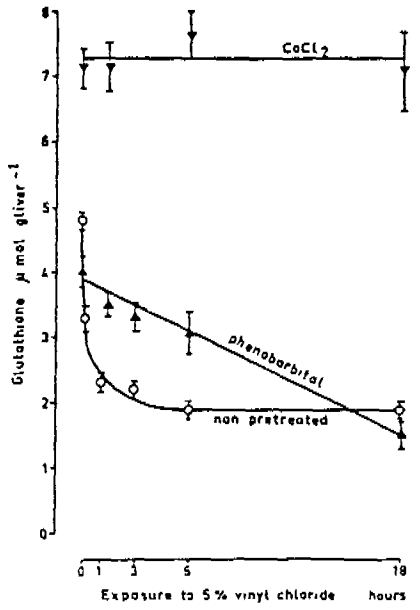

FIG. 2. Effects of vinyl chloride inhalation on hepatic glutathione. Glutathione concentrations were measured in animals sacrificed after various lengths of exposure to a 5% vinyl chloride-95% air atmosphere. Results are means ± SD for at least six rats.

bound to microsomal proteins (Fig. 4), whereas no detectable binding occurred under identical conditions with microsomes from blood, kidney, spleen, or muscle (data not shown). Binding to hepatic microsomal proteins was negligible when the NADPH-generating system was omitted (Fig. 4); binding was decreased when the incubation was made under a 90% CO–10% O_2 atmosphere or in the presence of SKF 525-A (Fig. 4). Similar findings were obtained when [^{14}C]vinyl chloride was incubated with hepatic 10,000*g* supernatant and the ^{14}C-labeled material irreversibly bound to 10,000*g* supernatant proteins was measured (Fig. 4).

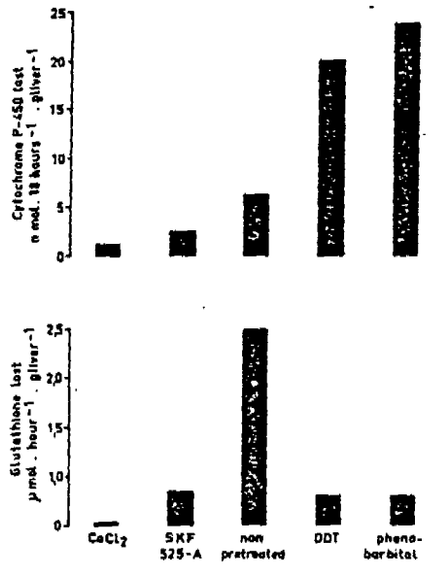

FIG. 3. Effects of various treatments on the loss of cytochrome *P*-450 and that of glutathione. Cytochrome *P*-450 lost is the difference between the mean for hepatic microsomal cytochrome *P*-450 concentration in rats not exposed, and in rats exposed, to a 5% vinyl chloride–95% air atmosphere for 18 hr, the former and the latter rats being simultaneously pretreated and sacrificed. Hepatic glutathione lost was similarly calculated after exposure to vinyl chloride for 1 hr.

In vitro binding to hepatic microsomal proteins was decreased by addition to the incubation mixture of glutathione or cysteine, but not *S*-methyl glutathione or glycine (Fig. 5); it was not increased by diethylmaleate (5 mM) (data not shown). Addition of diethylmaleate (5 mM) to the 10,000*g* supernatant incubation mixture decreased endogenous glutathione in the medium and increased *in vitro* binding to 10,000*g* supernatant proteins (Fig. 6).

Addition of 0.5 mM 1,1,1-trichloropropene 2,3-oxide (TCPO) to the incubation mixture increased *in vitro* covalent binding to microsomal proteins (Table 2); whereas binding was increased by only 37% in control rats, binding was increased by 134 and 200% in rats treated with DDT or phenobarbital, respectively (Table 2).

Treatment of the animals with $CoCl_2$ or SKF 525-A decreased (1) *in vitro* binding to 10,000*g* supernatant proteins, (2) *in vitro*

TABLE 1

VINYL CHLORIDE MATERIAL IRREVERSIBLY BOUND TO WHOLE TISSUE PROTEINS *in Vivo*[a]

	Vinyl chloride material irreversibly bound to proteins (nmol·4 hr^{-1}·g tissue^{-1})				
	Liver	Blood	Kidney	Spleen	Muscle
Control rats	69±10	24±4	22±10	22±5	2.0±0.9
$CoCl_2$-Treated rats	16±5[b]	5±3[b]	5±2[b]	4±1[b]	0.3±0.1[b]

[a] [^{14}C]Vinyl chloride (100 μmol; 100 μCi) in 250 μl of methanol was administered ip to control rats and to rats treated with $CoCl_2$; 4 hr later, the animals were sacrificed and the amount of ^{14}C-labeled material irreversibly bound to whole tissue proteins was measured; results are the means ± SD of four rats.

[b] Significantly different from that in control rats, $p < 0.001$.

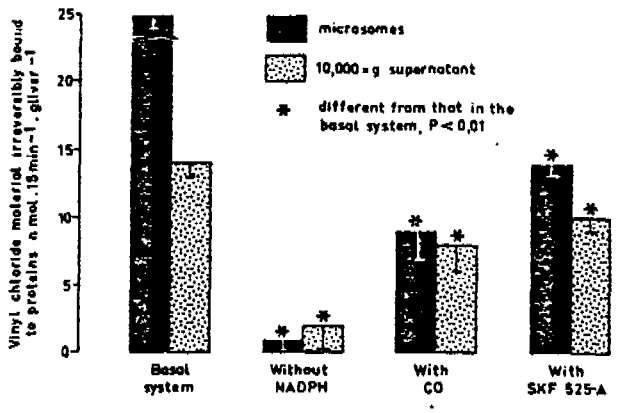

FIG. 4. Irreversible binding of a vinyl chloride material to proteins *in vitro*. In the basal system, [^{14}C]vinyl chloride (5 mM) was incubated under air with microsomes or 10,000*g* supernatant from 125 mg of liver from non-pretreated rats and with a NADPH-generating system; in some flasks, the NADPH-generating system was omitted; other flasks were incubated under a 90% CO–10% O_2 atmosphere; in other flasks, SKF 525-A (5 mM) was added to the incubation mixture. After 15 min of incubation, the amount of vinyl chloride material irreversibly bound to microsomal or 10,000*g* supernatant proteins was measured; columns and bars represent means and SD for three experiments.

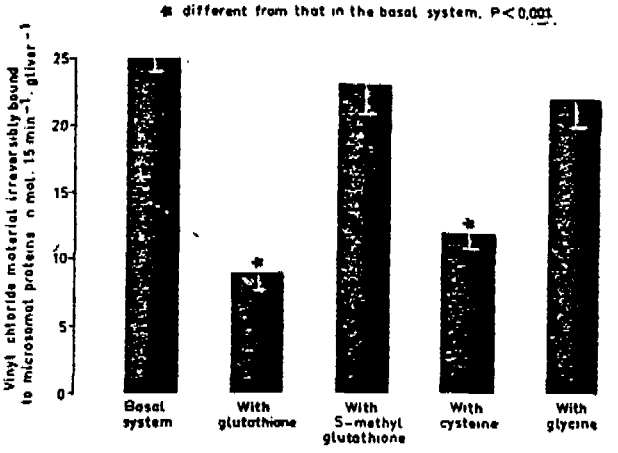

FIG. 5. Effects of SH compounds on the irreversible binding of a vinyl chloride material to microsomal proteins *in vitro*. In the basal system, [^{14}C]vinyl chloride (5 mM) was incubated under air with microsomes from 125 mg of liver from non-pretreated rats and with a NADPH-generating system; in other flasks, a 5 mM concentration of various compounds was added to the incubation mixture. Columns and bars represent means and SD for three experiments.

binding to microsomal proteins measured without added TCPO, and (3) *in vitro* binding to microsomal proteins measured in the presence of 0.5 mM TCPO (Table 2). Treatment of the animals with DDT or with phenobarbital (1) decreased *in vitro* binding to 10,000*g* supernatant proteins, (2) did not change binding to microsomal proteins measured without TCPO, and (3) increased binding to microsomal proteins measured in the presence of TCPO (Table 2).

The loss of hepatic microsomal cytochrome *P*-450 after inhalation of vinyl chloride for 18 hr was (1) not correlated ($r = 0.077$) with

TABLE 2

VINYL CHLORIDE MATERIAL IRREVERSIBLY BOUND TO PROTEINS *in Vitro*[a]

	Vinyl chloride material irreversibly bound to proteins (nmol·15 min^{-1}·g liver^{-1})		
	10,000 *g* supernatant	Microsomes without added TCPO[b]	Microsomes with added TCPO[b]
Control rats	14±1	24±7	33±3[c]
$CoCl_2$-Treated rats	2±2[d]	7±3[d]	9±2[d]
SKF 525-A-Treated rats	10±2[d]	13±4[d]	18±9[d]
DDT-Treated rats	8±1[d]	23±5	54±6[c,d]
Phenobarbital-Treated rats	8±2[d]	25±8	75±26[c,d]

[a] [^{14}C]Vinyl chloride (5 mM) was incubated under air with 10,000*g* supernatant or microsomes from 125 mg of liver and with a NADPH-generating system for 15 min; the amount of vinyl chloride material irreversibly bound to 10,000*g* supernatant or microsomal proteins was measured; results are means ± SD of at least six rats.

[b] 2 μl of acetone either pure or containing 1,1,1-trichloropropene-2,3-oxide (TCPO) (final concentration in the incubate, 0.5 mM) was added to the incubation mixture.

[c] Significantly different from that bound to microsomes without added TCPO, $p < 0.05$.

[d] Significantly different from that in control rats, $p < 0.05$.

in vitro binding to 10,000*g* supernatant proteins, (2) better correlated ($r = 0.771$) with *in vitro* binding to microsomal proteins measured without added TCPO, and (3) strikingly correlated ($r = 0.972$) with *in vitro* covalent binding to microsomal proteins measured in the presence of TCPO (Fig. 7).

The loss of hepatic glutathione after inhalation of vinyl chloride for 1 hr was (1) well correlated ($r = 0.891$) with *in vitro* binding to 10,000*g* supernatant proteins (Fig. 8), (2) poorly correlated ($r = 0.560$) with *in vitro* binding to microsomal proteins measured without added TCPO, and (3) not correlated ($r = 0.061$) with *in vitro* binding to microsomal proteins measured in the presence of TCPO.

DISCUSSION

Metabolic activation in the liver into a chemically reactive metabolite. In vitro, when [^{14}C]vinyl chloride was incubated under air with rat liver microsomes but without a NADPH-generating system, there was negligible radioactivity irreversibly bound to microsomal proteins (Fig. 4), which shows that vinyl chloride itself does not bind to proteins. *In vitro* binding to microsomal proteins required NADPH and was inhibited by CO or SKF 525-A (Fig. 4). Similar results were obtained by Kappus *et al.* (1976). These findings suggest that the radioactive material that bound to proteins *in vitro* (Fig. 4) was not vinyl chloride itself but a metabolite of it formed by the MFOS.

The bound metabolite could not be removed by repeated washings and repeated extractions with solvents of various polarities; this apparently irreversible binding suggests that the metabolite was attached to proteins by a covalent bond. A covalent bond between a drug molecule, or a metabolite of it, and a tissue macromolecule may result from two main reactions: (1) the spontaneous reaction between a chemically reactive compound and an already formed macromolecule, or (2) the

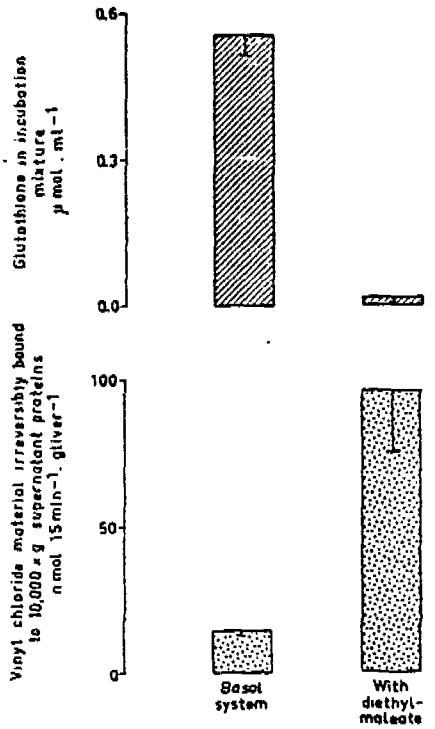

FIG. 6. Effects of diethylmaleate on the irreversible binding of a vinyl chloride material to 10,000*g* supernatant proteins *in vitro*. In the basal system, [^{14}C]vinyl chloride (5 mM) was incubated under air with 10,000*g* supernatant from 125 mg of liver from non-pretreated rats and with a NADPH-generating system; in other flasks 5 mM diethylmaleate was added. After 15 min of incubation, the amount of glutathione in the incubation mixture and the amount of vinyl chloride material irreversibly bound to 10,000*g* supernatant proteins were measured. Columns and bars represent means and SD for three experiments.

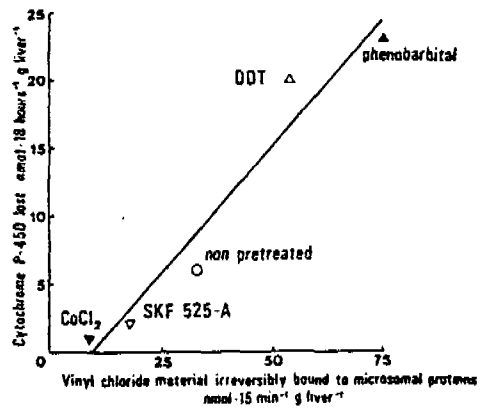

FIG. 7. Correlation between loss of cytochrome *P*-450 and irreversible binding to microsomal proteins *in vitro*. The loss of hepatic microsomal cytochrome *P*-450 after inhalation of a 5% vinyl chloride–95% air atmosphere for 18 hr (Fig. 3) is plotted against the amount of vinyl chloride material that became irreversibly bound to microsomal proteins when 5 mM 1,1,1-trichloropropene-2,3-oxide (TCPO) was added to the incubation mixture (Table 2). The drawn line is the least-squares regression line.

enzyme-mediated incorporation of a stable compound into a built-up macromolecule (Mazel and Pessayre, 1976). While the latter reaction might occur to some extent *in vivo*, it is highly unlikely to occur in an *in vitro* system restricted to microsomes and a NADPH-generating system. Thus, covalent binding to microsomal proteins was probably the consequence of the formation of a chemically reactive metabolite of vinyl chloride which reacted with, and covalently bound to, microsomal proteins.

In vivo, after ip administration of [^{14}C]vinyl

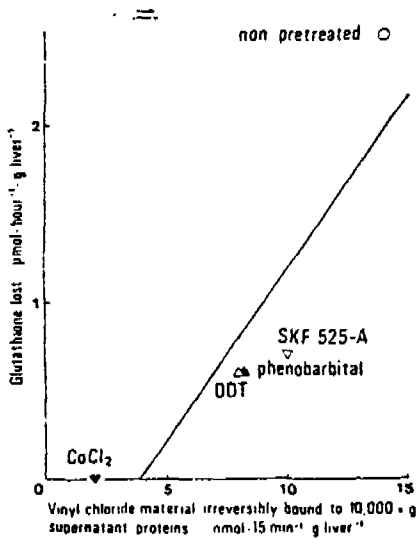

FIG. 8. Correlation between loss of glutathione and irreversible binding to 10,000*g* supernatant proteins *in vitro*. The loss of hepatic glutathione after inhalation of a 5% vinyl chloride–95% air atmosphere for 1 hr (Fig. 3) is plotted against the amount of vinyl chloride material that became irreversibly bound to 10,000*g* supernatant proteins (Table 2). The drawn line is the least-squares regression line.

chloride to non-pretreated rats, a radioactive material became irreversibly bound to hepatic proteins; binding was markedly decreased by $CoCl_2$ (Table 1). These findings suggest that a chemically reactive metabolite is also formed *in vivo*.

Covalent binding in extrahepatic organs. After ip administration of [^{14}C]vinyl chloride, substantial amounts of metabolite were covalently bound to whole blood, kidney, and spleen proteins (Table 1), whereas no detectable covalent binding occurred when microsomes from these organs were incubated with [^{14}C]vinyl chloride. These findings would suggest that the chemically reactive metabolite formed in the hepatocytes may partly leave the liver and bind to proteins in other tissues. However, it is not excluded that extrahepatic organs may also form some small amounts of the reactive metabolite that were undetected in our *in vitro* system.

Protective role of glutathione. Inhalation of vinyl chloride decreased hepatic glutathione in control rats but not in $CoCl_2$-treated rats (Fig. 2), which suggests that glutathione was depleted by a metabolite. It has been shown that vinyl chloride is metabolized to cysteine adducts and other sulfur-containing metabolites in non-pretreated rats (Green and Hathway, 1975; Watanabe *et al.*, 1976). Several chemically reactive metabolites have been shown to covalently bind to, and thus deplete, hepatic glutathione (Mazel and Pessayre, 1976). These observations suggest that glutathione was depleted (Fig. 2) because it was consumed by a reactive metabolite of vinyl chloride.

Addition of exogenous glutathione decreased (Fig. 5), and removal of endogenous glutathione by diethyl maleate increased (Fig. 6), the *in vitro* covalent binding to hepatic proteins. These findings are consistent with the view that the chemically reactive metabolite of vinyl chloride may bind either to glutathione or to proteins and that binding to glutathione decreases the amount of metabolite available for binding to proteins.

Proposed metabolism of vinyl chloride. It is generally hypothesized (Barbin *et al.*, 1975; Reynolds *et al.*, 1975; Bolt *et al.*, 1976; Kappus *et al.*, 1976; Reynolds *et al.*, 1976; Osterman-Golkar *et al.*, 1977) that vinyl chloride (chloroethylene) is oxidized by the MFOS into vinyl chloride epoxide (chloroethylene oxide); spontaneous rearrangement of this epoxide leads to chloroacetaldehyde which is oxidized into chloroacetic acid. Although both vinyl chloride epoxide and, to a lesser extent, chloroacetaldehyde are chemically reactive metabolites, evidence has been presented suggesting that *in vivo* alkylation of proteins in mice was mainly due to the epoxide rather than to the aldehyde (Osterman-Golkar *et al.*, 1977).

Protective role of epoxide hydrase. This inducible microsomal enzyme transforms reactive epoxides into inactive dihydrodiols (Oesch, 1972). Addition of TCPO (0.5 mM) to the incubation mixture increased *in vitro* covalent binding to microsomal proteins (Table 2). TCPO has two effects: (1) It decreases glutathione concentrations, and (2) it inhibits epoxide hydrase (Oesch and Daly, 1972). It is unlikely that the effects of TCPO (Table 2) were related to reduced glutathione concentration in the incubate because there is little glutathione left in washed microsomes and because addition of diethylmaleate (5 mM) to the incubation mixture did not increase *in vitro* covalent binding to microsomal proteins of normal or of phenobarbital-treated rats. Rather, it is likely that TCPO inhibited the inactivation of vinyl chloride epoxide by epoxide hydrase (Kappus *et al.*, 1976). This interpretation is further supported by the observation that addition of TCPO to the incubation mixture increased binding much more with induced microsomes, which have increased activities of epoxide hydrase (Oesch, 1972), than with microsomes from control rats (Table 2).

Effects of various treatments on covalent binding, cytochrome P-450, and glutathione. Treatment of the animals with $CoCl_2$ or SKF 525-A decreased *in vitro* covalent binding to hepatic proteins (Table 2) and the

loss of cytochrome *P*-450 and of glutathione (Fig. 3) after inhalation of vinyl chloride. These findings are consistent with the view that these treatments decreased the formation rate of the reactive epoxide and, thus, its binding to cytochrome *P*-450 and to hepatic glutathione.

More complex effects were observed after treatment with DDT or phenobarbital. (1) Whereas these treatments increased cytochrome *P*-450, they did not increase covalent binding to microsomal proteins measured in the absence of TCPO (Table 2). A similar observation was made by Kappus *et al.* (1976). This discrepancy might be understood if treatment with DDT or phenobarbital had induced both cytochrome *P*-450 and epoxide hydrase, resulting in both an increased formation rate and an increased inactivation rate of the reactive epoxide. Indeed, when the inactivation of the epoxide was inhibited by added TCPO, treatment of the animals with DDT or phenobarbital now markedly increased covalent binding to microsomal proteins as compared to that in control rats (Table 2). (2) Whereas treatments with DDT or phenobarbital increased the destruction of cytochrome *P*-450, they decreased the depletion of glutathione (Fig. 3). Similar observations have been made previously (Reynolds *et al.*, 1975). This finding could be explained if cytochrome *P*-450 were destroyed by the epoxide it just forms or has just formed and if glutathione were depleted by only that fraction of the formed epoxide that has escaped inactivation by epoxide hydrase and has diffused in the cytosol; according to this view, cytochrome *P*-450 destruction would be related to the formation rate of the epoxide, while glutathione depletion would be related to the release rate of the epoxide in the cytosol. *In vitro* covalent binding to microsomal proteins measured in the presence of the epoxide hydrase inhibitor may be the best *in vitro* estimate for the formation rate of the epoxide. A striking correlation was found between this *in vitro* covalent binding and the loss of cytochrome *P*-450 after inhalation of vinyl chloride, in variously treated animals (Fig. 7). *In vitro* covalent binding to 10,000*g* supernatant proteins may be a good *in vitro* estimate for the release rate of the epoxide in the incubation mixture. A good correlation was found between this binding and the loss of glutathione after inhalation of vinyl chloride (Fig. 8).

GENERAL COMMENTS

This work confirms and extends the previously held view that vinyl chloride is activated in the liver into a chemically reactive epoxide that covalently binds to hepatic proteins and might be responsible for the hepatotoxic and carcinogenic effects of chronic inhalation of vinyl chloride (Kappus *et al.*, 1976). In the past few years, it has been recognized that a vast array of chemicals, including a number of widely used drugs, is activated to unstable intermediates in rodents and probably in man (Mitchell and Jollow, 1975; Mazel and Pessayre, 1976; Pessayre *et al.*, 1977, 1978; Pessayre and Benhamou, 1978). Indeed the question now is not so much how these compounds may produce liver necrosis or cancer but rather why they do not do so more often. This study exemplifies several mechanisms that may protect the hepatocytes: (1) Destruction of cytochrome *P*-450 may prevent further metabolism and toxicity; in induced animals, higher initial concentrations of cytochrome *P*-450 are associated with a higher destruction rate of cytochrome *P*-450. (2) Microsomal epoxide hydrase is closely associated with cytochrome *P*-450 and may inactivate epoxides shortly after they are formed (Oesch, 1972); induction of cytochrome *P*-450, which increases the rate of formation of the epoxide, is associated with induction of epoxide hydrase (Oesch, 1972), which increases the inactivation rate of the epoxide. (3) Finally, the epoxide may bind to cytosolic glutathione which is offered as an alternative target. Eventually, only a tiny fraction of the formed epoxide does bind to macromolecules.

In induced animals, destruction of cytochrome *P*-450 during inhalation of vinyl chloride was increased, whereas depletion of glutathione was decreased. These observations suggest the need for a compartmentalized analysis of the effects of the chemically reactive metabolite. As a possible interpretation of these findings, it is suggested that cytochrome *P*-450 may be destroyed by the epoxide it just forms or has just formed and that, accordingly, cytochrome *P*-450 destruction is related to the formation rate of the epoxide, whereas glutathione would be depleted by only that fraction of the formed epoxide which has escaped inactivation by microsomal epoxide hydrase and has diffused in the cytosol.

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