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METABOLISM OF [^{14}C]- and [^{36}Cl]-LABELED VINYL CHLORIDE *IN VIVO* AND *IN VITRO**

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Abstract—Label from [^{14}C]vinyl chloride was covalently bound to protein and nucleic acids *in vivo* and *in vitro* in the presence of rat liver microsomal fractions or highly purified cytochrome P-450 and NADPH-cytochrome P-450 reductase preparations. The ratio of bound to total non-volatile metabolites increased in going from the *in vivo* to the microsomal to the purified system. [^{36}Cl]vinyl chloride was metabolized by microsomes and highly purified systems: no label was bound and most could be accounted for as chloride ion. Phenobarbital pretreatment of rats did not induce total metabolism of vinyl chloride *in vivo* at either 10 or 250 ppm exposure levels; however, binding to protein and RNA was enhanced at the 10 ppm but not the 250 ppm level. Phenobarbital pretreatment increased the *in vitro* microsomal conversion of vinyl chloride to both total and bound metabolites. A sizeable fraction of the label of [^{14}C]vinyl chloride metabolized *in vivo* was recovered in the microsomal fraction of the liver, but sodium dodecyl sulfate polyacrylamide gel electrophoresis of *in vitro* incubations indicated that the metabolites were distributed among many microsomal proteins and not localized to cytochrome P-450. Evidence was obtained for the metabolism of the suspected vinyl chloride metabolite chloroethylene oxide by microsomal epoxide hydratase. However, the epoxide hydratase inhibitor 3,3,3-trichloropropylene oxide, which blocks the microsomal degradation of chloroethylene oxide, did not enhance the level of vinyl chloride bound to either protein or adenosine.

Vinyl chloride is an industrial chemical that has been demonstrated to be carcinogenic in laboratory animals [1] and humans [2, 3]. The concept that a metabolite(s) of vinyl chloride is responsible for the observed carcinogenicity [4, 5] is supported by the findings that NADPH-dependent microsomal activation is necessary for vinyl chloride-mediated mutagenesis in *Salmonella typhimurium* tester strains [6, 7] and for binding of vinyl chloride to protein and nucleic acids [8–10]. The vinyl chloride metabolite responsible for these activities has been postulated to be the potent electrophile chloroethylene oxide [11].

Liver microsomal cytochrome P-450 has been shown to activate vinyl chloride to a species that destroys the heme of that cytochrome [12]. Cytochrome P-450 has also been suggested, on the basis of CO-inhibition experiments [9], as the enzyme responsible for activating vinyl chloride to metabolites bound to tissue nucleophiles. Spectral evidence [13] for a role of cytochrome P-450 in vinyl chloride metabolism is rather weak when the limitations of such data are considered [14]. Previous workers had reported that induction with phenobarbital increases the ability of the liver to convert vinyl chloride to a P-450 destructive species *in vivo* [15] and *in vitro* [12], observed *in vivo* vinyl chloride hepatotoxicity [16], and the capacity to convert vinyl chloride to mutagenic products *in vitro* [6]; however, induction with phenobarbital had no effect on the levels of vinyl chloride metabolism by rats *in vivo* and *in vitro* [8]. Recently, Watanabe *et al.* [17] reported that, in rats exposed *in vivo* to 100 ppm vinyl chloride, phenobarbital increased binding approximately 2-fold but had no effect on the level of total vinyl chloride metabolism.

The studies described in this report were initiated in order to establish the roles of liver microsomal cytochrome P-450 and epoxide hydratase in the biotransformation of vinyl chloride to metabolites, particularly those bound to protein and nucleic acids.

MATERIALS AND METHODS

Materials. 1, N^6-ethenoadenosine was purchased from P-L Biochemicals (Milwaukee, WI), calf thymus DNA and *Escherichia coli* soluble RNA from Calbiochem (La Jolla, CA), research grade vinyl chloride from Matheson (E. Rutherford, NJ), and 3,3,3-trichloropropylene oxide. (TCPO) from Aldrich (Milwaukee, WI). Chloroethylene oxide was prepared, as described previously [12, 18], on the day of use; identity was confirmed by N.M.R. and mass spectra and by reaction with 4-(*p*-nitrobenzyl)-pyridine [11]. [1,2-^{14}C]vinyl chloride was prepared from [1,2-^{14}C]dichloroethane [19] and was diluted with carrier vinyl chloride; the purity was as described previously [17, 19]. [^{36}Cl]vinyl chloride (0.10 mCi/m-mole) was synthesized from H^{36}Cl (New England Nuclear) and acetylene [20]; the

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product was >99 per cent chemically and radiochemically pure as judged by gas chromatography (g.l.c.) and its identity was confirmed by mass spectrometry (M^+62,64).

Animals. Male Sprague–Dawley rats were fed commercial diets *ad lib.* and handled as described previously [4, 17]. For the *in vivo* experiments, rats weighing 200–250 g (Spartan Research Laboratories, Haslett, MI) were used and phenobarbital treatment consisted of daily intraperitoneal injections of 80 mg/kg for 3 days. For the preparation of microsomes and cytochrome P-450, 100–150 g rats (Harland, Cumberland, IN) drank a 0.1% phenobarbital solution for 5 days [21].

Preparation of microsomes and enzymes. Hepatic microsomal fractions were prepared as described previously [21]. Cytochrome P-450 was prepared from liver microsomes of phenobarbital-treated rats as described [21]; the enzyme eluted from the hydroxyapatite column with 90 mM potassium phosphate was treated with calcium phosphate to remove detergent and used as such. Such preparations contain predominantly the "B" fraction and traces of the "A" fraction [21]; the specific content of the preparation used here was 16.1 nmoles cytochrome P-450/mg of protein. NADPH-cytochrome P-450 reductase was purified from liver microsomes of phenobarbital-induced rats by octylamino-Sepharose 4B and 2′,5′-ADP agarose chromatography [21]; the preparation migrated as a single band upon electrophoresis in the presence of sodium dodecyl sulfate [22] and catalyzed the reduction of 39 μmoles cytochrome *c*/min/mg of protein [21].

Assays. Handling of vinyl chloride was done in fume hoods with flows of *ca.* 150 cubic feet/min; disposable gloves were used for safety. All vinyl chloride concentrations were experimentally deterined by g.l.c. (Porapak QS, 3 ft × $\frac{1}{8}$ in, stainless steel, 150°). EDTA and butylated hydroxytoluene (BHT) [23] were added to microsomal incubations to retard lipid peroxidation, which destroys cytochrome P-450 [24]; these compounds did not inhibit the metabolism of vinyl chloride or a number of other substrates.

Total non-volatile metabolites present in samples were estimated by sweeping aliquots under a strong stream of N_2 for 15 min (while heating the samples at 25° in a water bath) and counting in 5 ml of water and 10 ml of 3a70B mixture (Research Products International, Elk Grove Village, IL). Bound metabolites were determined by washing samples five times with 95% ethanol using the previously described homogenization/centrifugation technique [25]; in every case, the radioactivity found in the supernatant fluid at this point was less than 1.5 background. The residue was solubilized in 1.0 ml of 1 N NaOH for 60 min at 60° and centrifuged for 5 min at 1000 *g*; portions of the supernatant fluid were assayed for radioactivity as above and for protein, and the results are expressed as nmoles of bound metabolites/mg of total protein. Because this radioactivity could not be removed from the protein with ethanol, trichloroacetic acid, or several other solvents, the metabolites are assumed to be covalently bound.

Protein [26] and cytochrome P-450 [21] concentrations were estimated as described previously. Unless noted otherwise, all *in vitro* results are expressed as the means of triplicate experiments ± S. D. Statistical analyses used Student's *t*-test.

RESULTS

In vivo *metabolism of* [*1,2-*^{14}*C*]*vinyl chloride.* At the high vinyl chloride dose (250 ppm), phenobarbital pretreatment had no effect on any of the observed indices of metabolism (Table 1). However, at the 10 ppm dose, binding to protein and RNA, but not binding to DNA or lipid or total metabolism, was enhanced significantly (about 2- to 3-fold).

The distribution of bound vinyl chloride metabolites among subcellular fractions was investigated after *in vivo* exposure (Table 2). After phenobarbital pretreatment, the highest concentration of bound metabolites was found in the microsomal fraction at both levels of vinyl chloride exposure.

In vitro *microsomal metabolism of* [^{14}*C*]- *and* [^{36}*Cl*]*vinyl chloride.* The metabolism of [^{14}C]vinyl chloride to non-volatile and bound metabolites was fairly linear with respect to time up to at least 45 min of incubation (Fig. 1A) and linear with respect to protein concentration up to a microsomal protein concentra-

Table 1. *In vivo* metabolism of [^{14}C]vinyl chloride*

Pretreatment	Nominal vinyl chloride concn (ppm)	Total non-volatile metabolites (nmoles/g wet liver)	Total bound metabolites: Protein (nmoles/g wet liver)	DNA (pmoles/g wet liver)	RNA (pmoles/g wet liver)	Lipid (nmoles/g wet liver)
None	10	24 ± 2	0.229 ± 0.029	0.040 ± 0.008	0.059 ± 0.014	0.024 ± 0.007
Phenobarbital	10	23 ± 2	0.558 ± 0.067†	0.055 ± 0.021	0.162 ± 0.011†	0.075 ± 0.056
None	250	720 ± 80	8.46 ± 2.93	0.90 ± 0.02	2.61 ± 0.44	0.40 ± 0.25
Phenobarbital	250	740 ± 100	6.99 ± 2.07	1.01 ± 0.52	1.49 ± 0.63	0.65 ± 0.40

* Animals (five per treatment group) were simultaneously exposed to [^{14}C]vinyl chloride for 6 hr using a previously described system [4, 17, 27]. The analytically determined mean concentrations (± S. D.) were 7.6 ± 1.6 ppm (nominally 10 ppm) and 232 ± 50 ppm (nominally 250 ppm) with respective specific activities of 0.32 and 0.028 μCi/μmole of vinyl chloride. Non-volatile metabolites were measured after sweeping liver homogenates with nitrogen as described under Materials and Methods. Bound metabolites were measured after fractionation of livers according to Shibko *et al.* [28] and binding is expressed on the basis of wet liver weight. Each experimental grouping used five animals; results are expressed as means ± S. D.

† Statistically different from untreated animals exposed to the same concentration of vinyl chloride ($P < 0.05$).

Table 2. *In vivo* hepatic subcellular distribution of bound [^{14}C]vinyl chloride metabolites*

Pretreatment	Nominal vinyl chloride concn (ppm)	Bound vinyl chloride metabolites (nmoles/g wet liver)			
		Nuclei	Mitochondria	Microsomes	Cytoplasm
None	10	0.044	0.080	0.062	0.031
Phenobarbital	10	0.049	0.048	0.103	0.061
None	250	1.33	1.09	1.42	1.25
Phenobarbital	250	1.10	0.86	3.12	1.23

* Pretreatment and vinyl chloride exposure were as under Table 1. Liver homogenates were fractionated as described previously [25] and an aliquot of each fraction (approximately 20 mg) was used to determine bound metabolites after extensive washing with 95% ethanol as described under Materials and Methods. In this single experiment, the livers of five animals were pooled in each group.

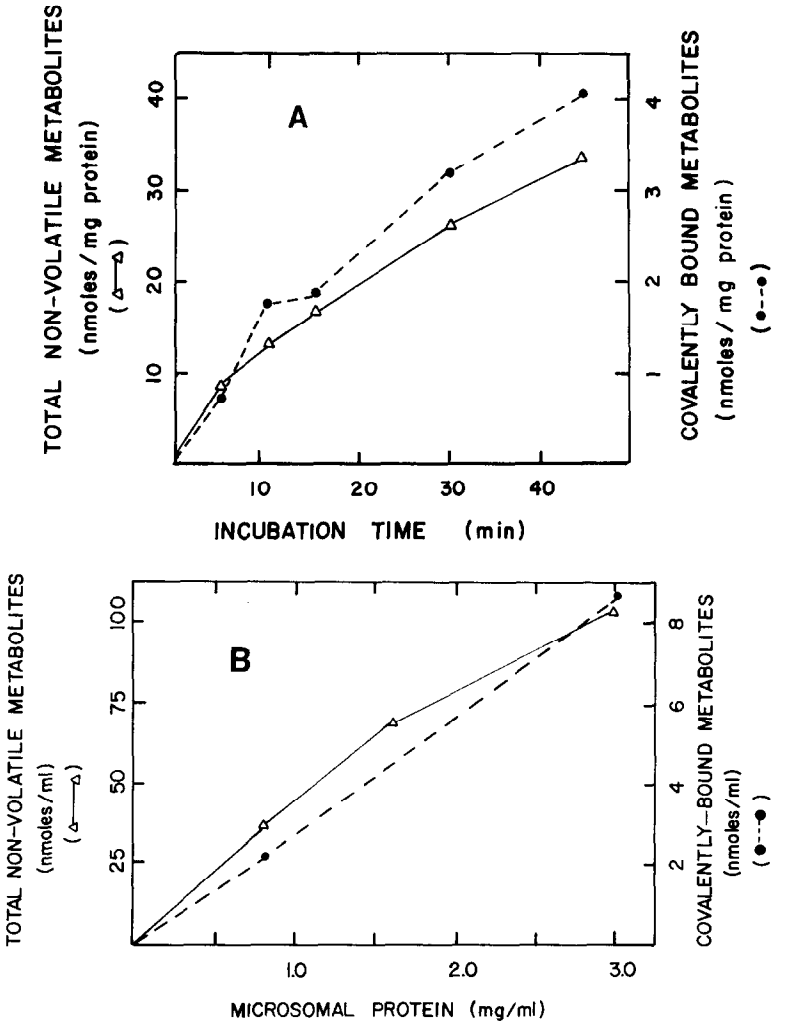

Fig. 1. Panel A: time course of metabolism of [1,2-^{14}C]vinyl chloride by liver microsomes. Incubations contained 3.0 mg/ml of microsomal protein from phenobarbital-treated rats, 50 mM potassium 4-(2-hydroxyethyl)-1-piperazineethanesulfonate (pH 7.7), 15 mM $MgCl_2$, 20 μM BHT, 1 mM EDTA, 0.5 mM NADP$^+$, 1.0 I.U./ml of glucose 6-phosphate dehydrogenase, and 10 mM glucose 6-phosphate in a final volume of 2.0 ml in vials capped with teflon stoppers; the gas phases (7.0 ml each) were equilibrated for 2 min with labeled vinyl chloride delivered from a Saran bag with a peristaltic pump through needles in the septa at a rate of 15 ml/min. Glucose 6-phosphate was omitted in the minus NADPH control experiments. Incubations were carried out in a shaking water bath at 37° and terminated by sweeping the samples with N_2 and freezing. Each data point represents a single determination.

Panel B: dependence of metabolism of [1,2-^{14}C]vinyl chloride upon microsomal protein concentration. Incubations were carried out as in panel A for 45 min. Each data point represents a single determination.

Table 3. *In vitro* metabolism of vinyl chloride by liver microsomes*

Substrate	Incubation system	Total non-volatile metabolites (nmoles/mg protein)	Bound metabolites (nmoles/mg protein)	Chloride recovered (nmoles/mg protein)
[1,2-^{14}C]vinyl chloride (5×10^4 ppm)	Phenobarbital microsomes, complete system	37.0 ± 2.0	3.92 ± 0.58	
	Phenobarbital microsomes, complete system − NADPH	0.72 ± 0.56	0.14 ± 0.09	
	Untreated microsomes, complete system	17.6 ± 2.0	1.71 ± 0.17	
	Untreated microsomes, complete system − NADPH	0.26 ± 0.08	0.10 ± 0.02	
	Phenobarbital microsomes, complete system + 10 μM TCPO	48.0 ± 8.6	3.94 ± 1.24	
	Phenobarbital microsomes, complete system + 5 mM glutathione	50.0 ± 9.4	2.77 ± 0.11	
[^{36}Cl]vinyl chloride (1.4×10^4 ppm)	Phenobarbital microsomes, complete system	11.95 ± 1.26	0.002 ± 0.005	8.46 ± 1.0
	Phenobarbital microsomes, complete system − NADPH	0.30 ± 0.02	0.001 ± 0.004	0.04 ± 0.02

* The ^{14}C and ^{36}Cl data were obtained in separate experiments. The complete system was used as described under Fig. 1A except that the concentration of microsomal protein was 2.5 mg/ml.

tion of 3 mg/ml (Fig. 1B); the remainder of the incubations were carried out under conditions where metabolism was maximized on the basis of these experiments. Results of experiments measuring metabolism and binding of [^{14}C]vinyl chloride under various conditions are shown in Table 3.

In a separate experiment, microsomes catalyzed the conversion of [^{36}Cl]vinyl chloride to non-volatile labeled metabolites. No bound chlorine label was detected, and almost all of the metabolized chlorine could be trapped with Ag^+ as AgCl, indicating that chloride ion is the fate of the chlorine atom of vinyl chloride under these conditions.

In vitro *microsomal metabolism of vinyl chloride to products bound to nucleic acids.* As shown in Table 4, liver microsomes catalyzed the NADPH-dependent

Table 4. Binding of vinyl chloride metabolites to nucleic acids catalyzed by liver microsomes*

Substrate	Incubation system	Vinyl chloride metabolites bound nucleic acid residue (nmoles/μmole)	Vinyl chloride metabolites bound to nucleic acid (nmoles/mg microsomal protein)
[1,2-^{14}C]vinyl chloride (5×10^4 ppm)	Microsomes, complete system + DNA	0.52 ± 0.06	1.37 ± 0.16
	Microsomes, complete system + RNA	0.19 ± 0.06	0.50 ± 0.16
	Microsomes, complete system − NADPH + DNA	0.036 ± 0.004	0.096 ± 0.010
[^{36}Cl]vinyl chloride (1.4×10^4 ppm)	Microsomes, complete system + DNA	0.0030 ± 0.0045	0.128 ± 0.048
	Microsomes, complete system + RNA	0.0041 ± 0.0023	0.106 ± 0.046
	Microsomes, complete system − NADPH + RNA	0.0048 ± 0.0061	0.15 ± 0.010

* The data with [^{14}C]- and [^{36}Cl]vinyl chloride were obtained in separate experiments. The complete systems were as under Table 3, except that 2 mg/ml of either DNA or RNA was added. All microsomes, present at 2.5 mg protein/ml, were prepared from phenobarbital-treated rats (levels of nucleic acids in these pyrophosphate-washed microsomes are negligible compared to the added DNA and RNA). Binding to nucleic acids was determined as described [21].

Table 5. Metabolism of vinyl chloride by reconstituted cytochrome P-450 systems*

Substrate	Incubation system	Total non-volatile metabolites (nmoles/nmole P-450)	Bound metabolites (nmoles/nmole P-450)	
			Protein	Nucleic acids
[1,2-^{14}C]vinyl chloride (5×10^4 ppm)	Complete + albumin	2.17 ± 0.38	1.23 ± 0.39	
	Complete + albumin, − NADPH	0.07 ± 0.02	0.08 ± 0.03	
	Complete + DNA			0.47 ± 0.08
	Complete + RNA			0.31 ± 0.15
	Complete + RNA, − NADPH			0.06 ± 0.04
[^{36}Cl]vinyl chloride (1.4×10^4 ppm)	Complete + albumin	4.43 ± 0.15	0.001 ± 0.001	
	Complete + albumin, − NADPH	1.17 ± 0.12	0.001 ± 0.002	

* The data with [^{14}C]- and [^{36}Cl]vinyl chlorides were obtained in separate experiments. The microsomal protein used with the systems described in Tables 3 and 4 was replaced with a mixture of 2.5 nmoles cytochrome P-450, 125 μg NADPH-cytochrome P-450 reductase, 100 nmoles L-α-dilauroylglyceryl-3-phosphorylcholine, and 0.5 μmole deoxycholate [20], with either 2 mg/ml of DNA or RNA (as under Table 4) or 2 mg/ml of bovine serum albumin in a total volume of 2.0 ml.

conversion of [^{14}C]vinyl chloride to metabolites bound to added DNA and RNA. As in the case of protein, no binding of the label from [^{36}Cl]vinyl chloride to nucleic acids was detectable.

In vitro *metabolism of vinyl chloride by highly purified cytochrome P-450 and NADPH-cytochrome P-450 reductase.* A highly purified cytochrome P-450 preparation catalyzed the metabolism of vinyl chloride to non-volatile products and to products tightly bound to added protein and nucleic acids. The level of total non-volatile metabolites formed was somewhat less than expected on the basis of the experiments carried out with the microsomes (which contained 2.0 nmoles cytochrome P-450/mg of protein) (Table 5). However, the ratios of bound to total metabolites were high and the binding catalyzed by microsomes can be attributed to cytochrome P-450 when the results are compared. As with microsomes, no chlorine from vinyl chloride became bound to added macromolecules.

Electrophoresis of solubilized radiolabeled proteins from vinyl chloride incubation systems. Radiochromatograpic profiles of electrophoretic separations of the proteins of the [^{14}C]vinyl chloride incubation systems are shown in Fig. 2. In the case of the microsomal system (Fig. 2A), bound label was distributed over a variety of proteins; the slightly higher binding of the label in the cytochrome P-450 region (50,000 daltons) is probably largely a reflection of the amount of protein (cytochromes P-450 and other proteins) in this region. In the reconstituted system (Fig. 2B) the incubation mixture contained albumin, cytochrome P-450, and NADPH-cytochrome P-450 reductase in a 27:1:1 ratio. Most of the binding was to the albumin and not to cytochrome P-450.

Studies on the role of epoxide hydratase in the metabolism of vinyl chloride. The epoxide hydratase inhibitor, TCPO, did not enhance binding of [^{14}C]vinyl chloride metabolites to microsomes (Table 3), in contrast to previous observations [9]. Another *in vitro* system [12], in which microsomes catalyze the conversion of vinyl chloride and adenosine to 1,N^6-ethenoadenosine (a known reaction product of chloroethylene oxide [11] or 2-chloroacetaldehyde [30] with adenosine), was set up in the presence of varying levels of TCPO. Concentrations of TCPO as high as 3 mM did not enhance the level of 1,N^6-ethenoadenosine recovered (Fig. 3).

The suitability of chloroethylene oxide as a substrate for epoxide hydratase could not be ascertained directly because of the instability of the compound in aqueous systems [11]. However, evidence for the destruction of chloroethylene oxide by epoxide hydratase was obtained in the experiment described in Fig. 4. The measured half-life of chloroethylene oxide was 4.35 min in phosphate buffer.† The half-life decreased to 2.5 min when rat liver microsomes were included with the buffer. When TCPO was mixed with the microsomes before the addition of the chloroethylene oxide, the half-life increased to 3.5 min, suggesting that microsomal epoxide hydratase enhances the destruction of the epoxide.

DISCUSSION

The levels of vinyl chloride metabolites bound to nucleic acids *in vitro* were significantly higher than those observed by Kappus *et al.* [9]. Moreover, the ratios of bound to total metabolites varied both *in vivo* and *in vitro* when compared to previous results [9,10]. In this work, the ratio increased with simplification of

† The half-lives reported are the means of two experiments and were computed from apparently first-order initial reaction phases, which account for at least two-thirds of each degradation.

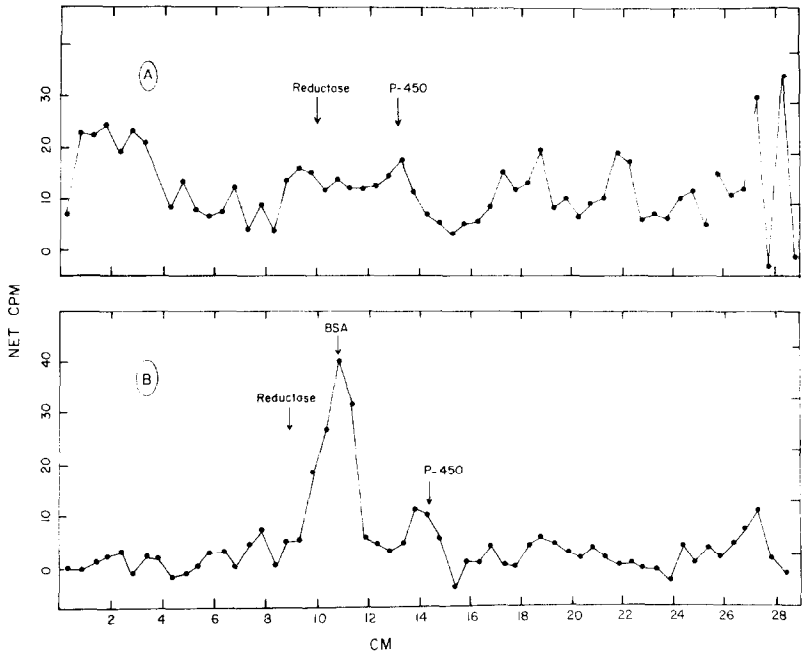

Fig. 2. Panel A: electrophoresis of microsomes incubated with [1,2-^{14}C]vinyl chloride and NADPH. Polyacrylamide gel electrophoresis of solubilized microsomal proteins was carried out in the presence of sodium dodecyl sulfate as described previously [22], except that a Hoefer slab gel system was used. Approximately 1 mg protein was solubilized [22] and applied to a 4 cm wide tract of a 3 mm × 30 cm gel. Standard proteins [21] were electrophoresed on a parallel gel. Samples moved through the stacking gel at a current limit of 10 mA/track and were electrophoresed in the separating gel at a current limit of 25 mA/track. Gels were strained and destained according to Fairbanks *et al.* [29] and dried onto paper sheets using a Hoefer slab gel drying apparatus. The dried gels were cut into 0.5 cm sections with a scissors; each section was digested with 2 ml of 15% H_2O_2 overnight at 50° in a closed scintillation counting vial (plastic-lined caps). The vials were cooled and to each was added 10 ml of a mixture consisting of 0.3% 2,5-diphenyloxazole (w/v), 0.01% 1,4-bis-[2-(5-phenyloxazolyl)benzene], and 33% Triton X-100 in toluene. The vials were then capped, shaken, and allowed to stand in darkness overnight before counting. The anode was at the right of the figure.
Panel B: electrophoresis (in the presence of sodium dodecyl sulfate) of a reconstituted cytochrome P-450 system incubated with [1,2-^{14}C]vinyl chloride, NADPH, and excess bovine serum albumin. Details of the incubation and electrophoresis are under Table 5 and Fig. 2A respectively. The anode was at the right of the figure.

the involved system; i.e. from *ca.* 0.01 *in vivo* to 0.1 with microsomes to as high as 0.6 with the purified *in vitro* system. Two other findings are in conflict with the results of Kappus *et al.* [9]: both total metabolism and binding were enhanced by phenobarbital pretreatment (Table 3) and the epoxide hydratase inhibitor, TCPO, did not enhance the binding of vinyl chloride metabolites.

In this work, phenobarbital induction had no effect on either metabolism or binding at the 250 ppm level of vinyl chloride, while at 10 ppm covalent binding to protein and RNA (but not DNA or lipid) was enhanced 3-fold (Table 1). In *in vitro* systems (5×10^4 ppm substrate) both total metabolism and binding were enhanced by pretreatment of animals with phenobarbital. The explanation for such a pattern is not straightforward. Previous studies by Hefner *et al.* [4] indicated than vinyl chloride may be metabolized by several different pathways which are dependent on the exposure concentration of vinyl chloride. Since phenobarbital induces a number of enzymes* involved in the activation and detoxification of vinyl chloride, different affinities of the various enzymes for their substrates (either vinyl chloride or its metabolites) might influence the distribution of bound and polar metabolites. Thus if phenobarbital shifts the pathways at low exposure levels without overt induction of overall metabolism of

* Two phenobarbital-induced enzymes, cytochrome P-450 and NADPH-cytochrome P-450 reductase, were shown here and elsewhere [12] to be involved in vinyl chloride metabolism. Two other enzymes induced by phenobarbital, epoxide hydratase [31] and glutathione-*S*-transferase [32], might be expected to metabolize an epoxide, if such a compound is a product. Of these enzymes, at least cytochrome P-450 [21, 33] and glutathione-*S*-transferase [32, 34] and possibly the other two enzymes [33, 35] exist in a number of forms.

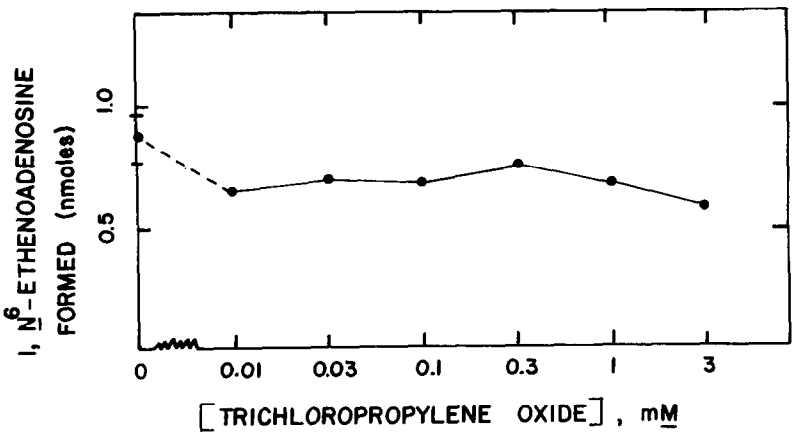

Fig. 3. Attempts to enhance microsomal conversion of vinyl chloride to 1, N^6-ethenoadenosine with varying concentrations of TCPO. The incubations contained microsomes from phenobarbital-treated rats in the usual system described under Table 3 with 5 × 10^{-4} ppm vinyl chloride in the gas phase and 3 mM adenosine in the aqueous phase. After 45 min at 37°, 0.15 ml of 10% (w/v) $ZnSO_4$–$7H_2O$ was added and the incubates were centrifuged. The supernatant fluids were applied to short columns (1 ml) of Dowex 1 × 8 (H^- form; previously batch-washed with 1 N NaOH, H_2O, 1 N HCl, and H_2O) and washed with 3.0 ml H_2O. The total eluents were assayed fluorimetrically (excitation 306 nm, emission 409 nm) for 1, N^6-ethenoadenosine [12, 30] using a standard curve.

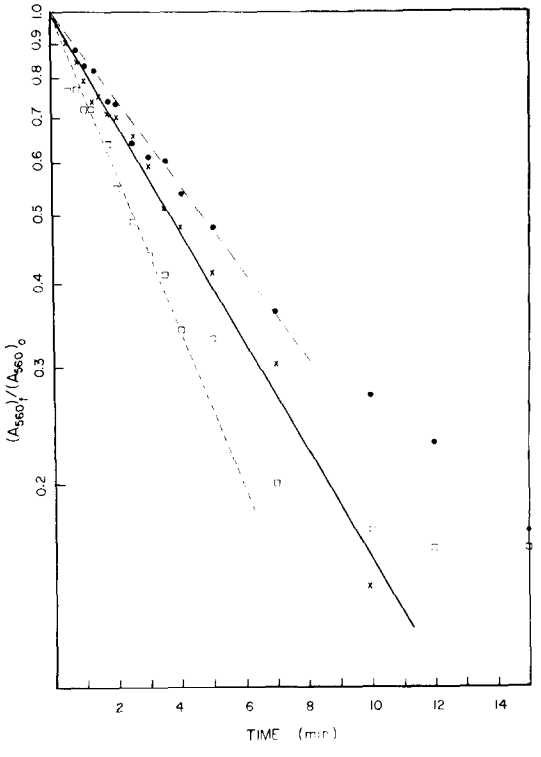

Fig. 4. Effect of rat liver microsomes on the breakdown of chloroethylene oxide. Chloroethylene oxide (1.6 μmoles) was added to 5.0 ml of a solution containing the indicated components in 50 mM potassium phosphate (pH 7.7) at 25°; aliquots were withdrawn at timed intervals and mixed with (4-(*p*-nitrobenzyl)-pyridine as described [11]: (●) buffer only; (buffer plus phenobarbital-treated rat microsomes, 2 mg/ml; (x) buffer plus microsomes (as above) plus 1 mM TCPO $(A_{560})_t$ and $(A_{560})_0$ refer to the absorbance due to chloroethylene oxide at each time point and at zero time. $(A_{560})_0$ was about 0.6 in all cases.

vinyl chloride, this might explain the increased binding observed following the 10 ppm exposure. At higher level exposure it appears that phenobarbital has little effect on either total metabolism or binding. This is consistent with other reports indicating that the metabolism of vinyl chloride is saturable both *in vivo* [17] and *in vitro* [9].

The subcellular distribution studies indicated that significant fractions of the ^{14}C label of vinyl chloride administered *in vivo* were bound to all of the subcellular fractions at both exposure levels, and that, after phenobarbital induction, the microsomal fraction contained the greatest fraction of bound metabolites (Table 2). Electrophoresis of solubilized microsomes previously incubated with [^{14}C]vinyl chloride *in vitro* (Fig. 3) indicated that the label was bound to numerous proteins. Since the amount bound to cytochrome P-450 was relatively small, the reactive metabolite(s) appears to be stable enough to migrate away from the site of formation (presumably cytochrome P-450). Assuming vinyl chloride-induced hemangiosarcoma (a tumor of endothelial origin) is caused by a reactive metabolite, the active species must either migrate from the hepatocyte to endothelial cells or be formed within the target cells. However, nonparenchymal cells (including endothelium) of the liver have a lower capacity to metabolize some foreign chemicals [36] and, therefore, these data tend to support the hypothesis that the active vinyl chloride metabolite can migrate from the hepatocyte to other sites.

The ^{36}Cl experiments indicate that any chemical mechanism for activation and binding of vinyl chloride includes provision for release of the chlorine atom as chloride ion, either in the actual activation mechanism or in rearrangement of the metabolite or adduct. Green and Hathaway suggested originally that chlorine might be retained in an *N*-acetylcysteine adduct isolated from

urine [37], but this chlorine may well be an artifact of HCl used in esterification [27, 38, 39].

The experiment depicted in Fig. 4 suggests that epoxide hydratase, which is inhibited by TPCO, can catalyze the degradation of chloroethylene oxide. However, TCPO enhanced neither the microsomal conversion of vinyl chloride to products covalently bound to protein (Table 3) nor the vinyl chloride-dependent conversion of adenosine to 1,N^6-ethenoadenosine (Fig. 3), suggesting that epoxide hydratase does not play a major role in the metabolism of vinyl chloride. Moreover, chloroethylene oxide would not appear to be an important alkylating agent, although a role as a precursor of 2-chloroacetaldehyde is plausible. 2-Chloroacetaldehyde is probably the best candidate to date as the actual alkylating agent, because (a) structures of isolated adducts of adenosine are consistent with such a reagent [6, 11, 30], (b) chloride ion would be released during nucleophilic attack, (c) 2-chloroacetaldehyde is apparently nearly as good, as a mutagen [6] and reactant with adenosine [11], as chloroethylene oxide, and (d) the stability of the compound in aqueous systems would allow for some migration from the endoplasmic reticulum to other cellular organelles and perhaps other cells. However, other mechanisms of activation, although less precedented, should still be considered; neither chloroethylene oxide nor 2-chloroacetalydehyde appear to be the vinyl chloride metabolite responsible for destruction of cytochrome P-450 heme [12].

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