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ENHANCEMENT OF THE METABOLISM AND HEPATOTOXICITY OF TRICHLOROETHYLENE AND PERCHLOROETHYLENE

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Abstract—Trichloroethylene anesthesia (1% for 2 hr) caused acute hepatic injury in rats pretreated with five different inducers of the hepatic mixed function oxidase system, phenobarbital, Aroclor 1254, hexachlorobenzene, 3-methylcholanthrene and pregnenolone-16-α-carbonitrile. Injury did not occur after trichloroethylene in rats pretreated with spironalactone or controls given vehicle alone. Morphologic liver injury was most severe in the phenobarbital- and Aroclor 1254-pretreated animals and was accompanied by marked perturbations in liver electrolyte content and more than 20-fold elevations in serum transaminase. Extent of serum transaminase elevation appears to relate directly to prolongation of anesthesia recovery time and the enhanced urinary excretion of trichlorinated metabolites. Metabolism of perchloroethylene (7.5 m-moles/kg, p.o.) was increased 5- and 7-fold, respectively, in phenobarbital- and Aroclor 1254-pretreated animals, but liver injury after perchloroethylene appeared only in Aroclor 1254 animals. Differential induction of various components of the microsomal mixed function oxidase system was quantified in parallel experiments using animals similarly pretreated with isomolar doses of the six inducers and the vehicle control and sacrificed at times corresponding to onset of chloroethylene exposure. Magnitude of induction of cytochrome P-450 among these seven groups of animals correlates with the mean extent of trichloroethylene-induced liver injury as quantitated by serum transaminases level ($r = 0.95$), with prolongation of anesthesia recovery time ($r = 0.95$) and with enhanced urinary excretion of trichlorinated metabolites ($r = 0.88$).

Trichloroethylene (TRI) and perchloroethylene (PER) are versatile solvents with numerous commercial, industrial and household applications. TRI is especially valuable as a degreaser and PER as a dry cleaning solvent. In medicine, TRI is occasionally used for obstetric anesthesia [1] and one of its metabolites, chloralhydrate, is a valuable sedative [2]. Neither of these unsaturated chloroethylenes is considered a potent hepatotoxin [3–5]. However, preliminary evaluations of National Cancer Institute (U.S.A.) studies indicate that TRI exposure may have a tumorigenic potential analogous to that of vinyl chloride [6].

Although little is known of the biochemical effects of TRI and PER or their metabolites on the liver cells, the mechanism of their metabolism has been the focus of numerous investigations. In 1945, the demonstration by Powell [7] of oxidized metabolites of TRI in human urine led her to suggest that TRI was metabolized via an unstable epoxide intermediate, and to comment. "It would be remarkable if this channnel of metabolism could be followed with so little evidence of ill-effect." In 1961, Yllner [8] conducted metabolic studies with ^{14}C-labeled TRI which indicated the formation of an epoxide intermediate. In 1963, Daniel [9], after measuring the specific activity of ^{36}Cl-labeled TRI metabolites isolated from urine, proposed that intramolecular rearrangement of chlorines after epoxidation would account for both the lack of loss of ^{36}Cl to the chloride pool and the known metabolites, trichloroacetaldehyde, trichloroethanol and trichloroacetic acid. Daniel [9] conducted similar studies with ^{36}Cl-labeled PER, and concluded that epoxidation, followed by a chloride shift, would lead to an acid chloride which would be rapidly hydrolyzed to trichloroacetic acid, the major metabolite of PER.

Further metabolic investigations [10–12] have identified the liver mixed function oxidase system (MFOS) as the stystem primarily responsible for the oxidation of TRI and PER. Other enzyme systems, including those in the adjacent cytoplasm, participate in subsequent hydration, oxidation and/or reduction and conjugation of secondary metabolites [13].

Leibman and McAllister [14] found that pretreatment of animals with phenobarbital (PBT), classic MFOS inducer, caused alterations in the rate and route of TRI metabolism. Carlson [15] reported that pretreatment with PBT or 3-methylcholanthrene (3-MC) exacerbated the hepatotoxicity of TRI. We have found that potentiation of the acute hepatotoxicity of TRI or PER in the rat, produced by pretreating animals with chemicals that induce components of the MFOS, appears related to enhanced metabolism of these chloroethylenes.

EXPERIMENTAL

Treatment of animals. A series of 200 g male Sprague–Dawley rats from Charles River were given isomolar doses (400 μmoles/kg) of PBT, 3-MC, hexachlorobenzene (HCB), spironolactone (SNL), pregnenolone-16-α-carbonitrile (PCN) or 150 μmoles/kg

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or 300 μmoles/kg of Aroclor 1254 (A-1254) by gavage once daily for 7 days. All compounds were solubilized in water with traces of Tween 80. "Control" animals received this vehicle. Throughout the induction period animals were housed in wire floored cages over processed clay animal litter and allowed free access to food and drinking water. On the morning of day 8 after a 16-hr fast, animals were sacrificed for determination of microsomal enzymes, or exposed to TRI, or PER, or room air. Animals pretreated with 300 μmoles/kg of A-1254 failed to survive TRI anesthesia and this dose level of A-1254 was not used in further studies.

Chloroethylene administration. Four groups of eight animals (one animal from each pretreatment regimen) were exposed to 1% TRI in air for 2 hr in an inhalation chamber previously described [16]. Based on the data of Guyton [17] the total dose of TRI inhaled was approximately 20–30 m-moles. Immediately after cessation of the anesthetic, animals were placed in individual metabolism cages to collect urine for determination of trichlorinated excretion products. PER (0.75 ml/kg diluted with mineral oil) was given by gavage to vehicle, PBT- and A-1254-pretreated rats (four of each) and these animals were placed in metabolism cages. All animals were sacrificed 24 hr after administration of the chloroethylene.

Assessment of metabolism and liver injury. Anesthesia recovery time, the interval between removal from the inhalation chamber and return of the righting reflex, was noted for each animal. Serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase activities were assayed with Sigma reagent kits. Transaminase changes were consistently measured by both assays. Samples of each liver were rapidly frozen on dry ice for histochemical detection of increased calcium and to provide material for determination of metal contents [18], or fixed in formalin for histologic examination by standard techniques.

The alkali–pyridine–toluene method of Tanaka and Ikeda [19] was followed to quantitate urinary trichloroacetic acid and total trichlorinated products. This method was selected because its vigourous oxidizing conditions were reported to give good recoveries of total trichlorinated metabolites in rat urine. In preliminary studies, we found that added trichloroethanol gave minimal response without the oxidizing procedure and that neither 2,2-dichloroethanol nor 2,2-dichloroacetic acid was detected by this method. These observations suggest that this procedure detects only 2,2,2-trichlorinated metabolites of chlorinated ethylenes. Twenty-four-hr urine collections from vehicle–air, PBT-air, or A-1254-air animals gave virtually no color response even after the oxidation step.

Microsomal enzyme assays. Differential induction of MFOS components by the six inducers was verified in a series of parallel experiments each of which contained at least one animal from each pretreatment regimen. At times compatible with the onset of chloroethylene exposure, the animals were sacrificed, and the livers were perfused and homogenized with cold 0.25 M sucrose; the microsomal fraction was isolated by differential centrifugation essentially as previously described [16]. Activities of the microsomal enzymes were assayed on the day of sacrifice.

Protein contents were determined by the method of Lowry *et al.* [20]. Difference spectra of cytochrome P-450 and b_5 were charted after Dallner *et al.* [21] using a Gilford 2400-2 in the split beam mode. Rates of cytochrome *c* reduction by NADPH and β-NADH were recorded spectrophotometrically using the respective reaction systems of Wilson [22] and Hogeboom [23]. Oxidative *N*-demethylation of dimethylaminoantipyrine was assayed by the method of Orrenius [24]. Glucose 6-phosphatase activity was determined according to Sell and Reynolds [25]. Activities of zoxazolamine and 3,4-benzpyrene hydroxylase were measured by the methods of Nebert and colleagues [26, 27].

Statistical analysis. Student's *t*-test and regression correlations were calculated using a Wang 600 programmable calculator and assessed after Snedecor and Cochran [28, 29]. Chloroethylene-induced changes in SGOT and liver metal contents were analyzed for statistical significance by comparison with values obtained from similarly pretreated animals exposed to room air.

RESULTS

Trichloroethylene injury. Effects of the seven chemical pretreatment regimens on the quantitative parameters examined after TRI anesthesia are detailed in Tables 1 and 2. In vehicle-pretreated (control) animals, TRI exposure was without effect on SGOT activity, liver Na, K, Mg, Ca, Zn and Fe contents or the microscopic morphology of the liver (Fig. 1) as compared to similarly pretreated animals exposed to air. In striking contrast, animals pretreated with PBT or A-1254 had at least doubled anesthesia recovery times, marked increases in urinary excretion of trichlorinated metabolites of TRI, more than 20-fold increases in SGOT, marked perturbations of liver Na, K and Ca contents, and frank and extensive hepatocellular necrosis. In PBT animals, hepatic necrosis

Table 1. Effects of MFOS inducers on the metabolism of trichloroethylene*†

Pretreatment‡		Anesthesia recovery time (min)	Trichlorinated urinary metabolites (μmoles/24 hr/animal) Total	TCA
Vehicle	(4)	81 ± 10	314 ± 6	30.0 ± 3.0
PBT	(4)	196 ± 12§	823 ± 38§	62.1 ± 3.2
A-1254	(4)	244 ± 17§	829 ± 65§	71.8 ± 9.2
HCB	(4)	94 ± 6	371 ± 45	33.1 ± 7.0
3-MC	(4)	79 ± 5	401 ± 17‖	60.4 ± 7.7
SNL	(4)	71 ± 3	282 ± 22	22.5 ± 5.0
PCN	(4)	82 ± 14	268 ± 33	29.6 ± 4.1

* Mean ± S.E.M.

† Trichloroethylene: 1% × 2 hr in air.

‡ Chemicals were given by gavage once daily for 7 days; the number in parentheses indicates the number of animals.

§ Statistically different from vehicle-TRI group (line 1) $P < 0.001$.

‖ Statistically different from vehicle-TRI group (line 1) $P < 0.01$.

Table 2. Effects of MFOS inducers on chemical indicators of liver injury 24 hr after trichloroethylene*†

Pretreatment‡		SGOT (Karmen units)	Liver metal contents (mg metal/g liver)		
			Na	K	Ca
Vehicle	(4)	185 ± 16	0.43 ± 0.03	3.5 ± 0.1	0.032 ± 0.003
PBT	(4)	4418 ± 843§	1.33§ ± 0.10	1.9 ± 0.2§	0.213 ± 0.028§
A-1254	(4)	7497 ± 1066§	0.92 ± 0.06§	2.7 ± 0.1§	0.131 ± 0.017‖
HCB	(4)	739 ± 121§	0.72 ± 0.06‖	4.0 ± 3.0	0.084 ± 0.019‖
3-MC	(4)	253 ± 41§	0.46 ± 0.02	3.7 ± 0.3	0.026 ± 0.002
SNL	(4)	183 ± 26	0.47 ± 0.03	3.3 ± 0.3	0.034 ± 0.004
PCN	(4)	1015 ± 502‖	0.82 ± 0.18	3.0 ± 0.4	0.087 ± 0.046

* Mean ± S.E.M.

† Trichloroethylene 1% × 2 hr in air.

‡ Chemicals were given by gavage once daily for 7 days; the number in parentheses indicates the number of animals.

§ Statistically different from similarly pretreated animals exposed to air $P < 0.001$.

‖ Statistically different from similarly pretreated animals exposed to air $P < 0.01$.

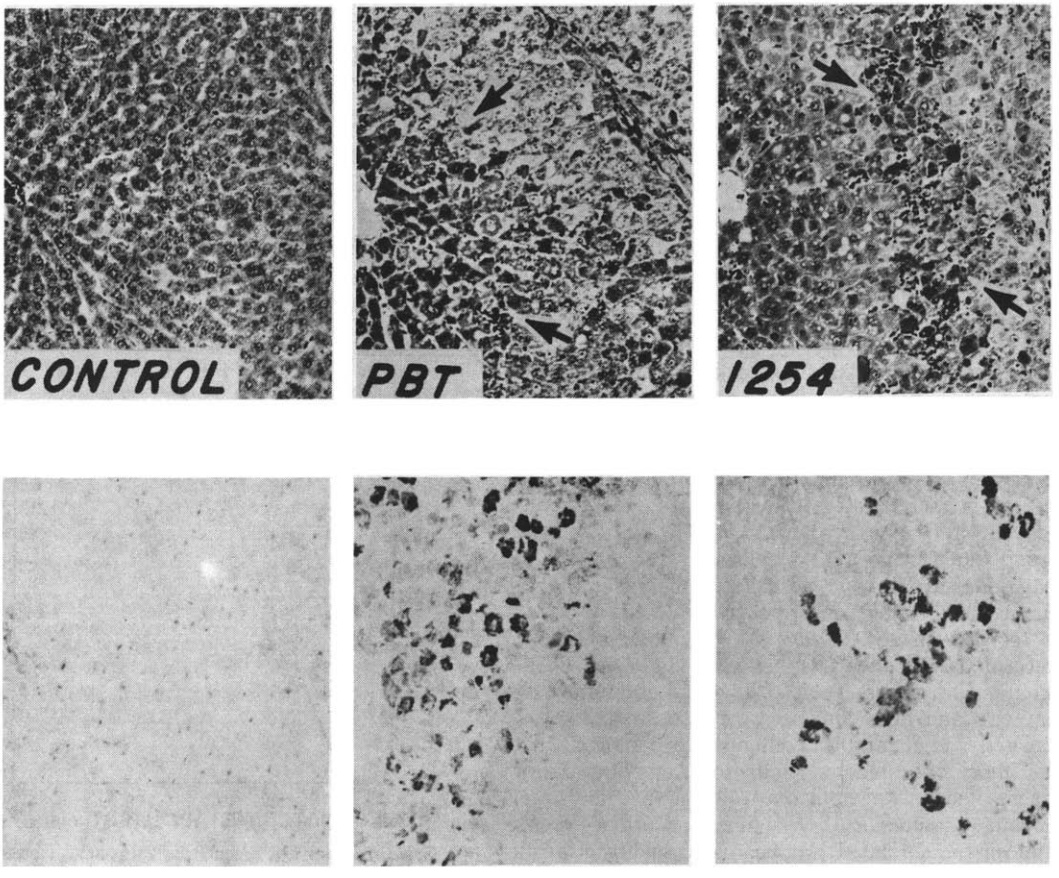

Fig. 1. Effect of PBT or A-1254 pretreatment on hepatocellular injury 24 hr after TRI exposure (1% for 2 hr). Top row shows hematoxylin and eosin stained paraffin sections, and bottom row the corresponding cryostat sections stained for calcium with Alizarin red S. In each panel the central vein is at the left with portal areas to the right. No necrosis or intracellular calcium deposits are seen in the TRI-exposed, vehicle-pretreated "control" at left. In the PBT (center) and A-1254 (right) livers, necrotic bands of pyknotic hepatocytes are present in the centrolobular and midzonal regions respectively (arrows). Alizarin red S stains calcium-rich necrotic cells in corresponding regions. (magnification: × 150).

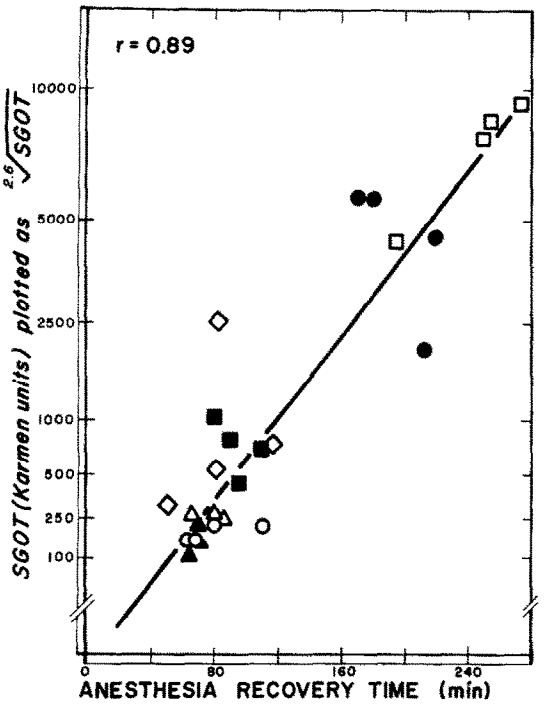

Fig. 2. Correlation between anesthesia recovery times and serum SGOT levels 24 hr after exposure to trichloroethylene (1% × 2 hr) in the seven pretreatment groups. Each point represents the data for an individual animal. Symbols represent the different pretreatments: (○) vehicle, (●) PBT, (□) A-1254, (■) HCB, (△) 3-MC, (▲) SNL and (◇) PCN. The power relationship is such that each 2-fold increase in anesthesia recovery time corresponds approximately to a 10-fold increase in SGOT (significance ≪ 1 per cent, $df = 26$, $r = 0.89$).

and histochemically stainable calcium were centrolobular, while in A-1254 animals stainable calcium was shifted periportally and necrotic zones appeared as prominent midzonal or periportal stripes (Fig. 1). In both groups, liver injury was extensive although TRI caused greater changes in liver Na, K and Ca contents of PBT animals, while the SGOT values at 24 hr were higher in the A-1254 animals. Liver contents of Mg, Zn and Fe were not altered in any group of animals 24 hr after TRI exposure.

Increases in SGOT of a lesser magnitude were also measured 24 hr after TRI in 3-MC-, HCB- and PCN-pretreated animals (Table 2). Elevated transaminases in HCB and PCN animals were associated with increased liver Na and Ca contents. Morphologic hepatic injury appeared mild with focal necrotic and/or vacuolated cells in both centrolobular and midzonal regions. Anesthesia recovery times and urinary excretion of trichlorinated products were slightly if at all increased in these animals (Table 1).

All parameters examined in the SNL-pretreated animals after TRI were unaltered (Tables 1 and 2).

Correlations between anesthesia recovery time, urinary excretion of trichlorinated metabolites and hepatic injury. Interpretation of anesthesia recovery times after TRI anesthesia is not straightforward. At least two metabolites of TRI, chloral hydrate and trichloroethanol, also have anesthetic properties [2]. Enhancement of the rate of metabolism of TRI should lead to a more rapid clearing of TRI from the blood after termination of anesthetic exposure. However, the anesthesia recovery time may not necessarily be shortened if enhanced TRI clearance is coupled to increased production of chloral hydrate and trichloroethanol. In this experiment, we did find a highly significant linear association (≪ 1 per cent) between elevated urinary excretion of total trichlorinated metabolites and prolongation of anesthesia recovery time ($df = 26$, $r = 0.92$). Therefore, prolongation of anesthesia recovery time appears a valid indicator of enhanced TRI metabolism.

The extent of hepatic injury 24 hr after TRI as quantitated by SGOT in individual animals appears directly related to enhancement of TRI metabolism in terms of both prolongation of anesthesia recovery times (Fig. 2) and increased urinary excretion of trichlorinated metabolites (Fig. 3). Both of these relationships are significant at the 1 per cent level by linear regression analysis as well as the power function analyses illustrated (Figs. 2 and 3). The ratio of trichloroacetic acid to total trichlorinated metabolites was similar in all the animals except for the animals pretreated with 3-MC.

Enzyme induction. The varied effects of the pretreatment regimens on components of the MFOS are detailed in Tables 3 and 4. PCN, PBT and A-1254,

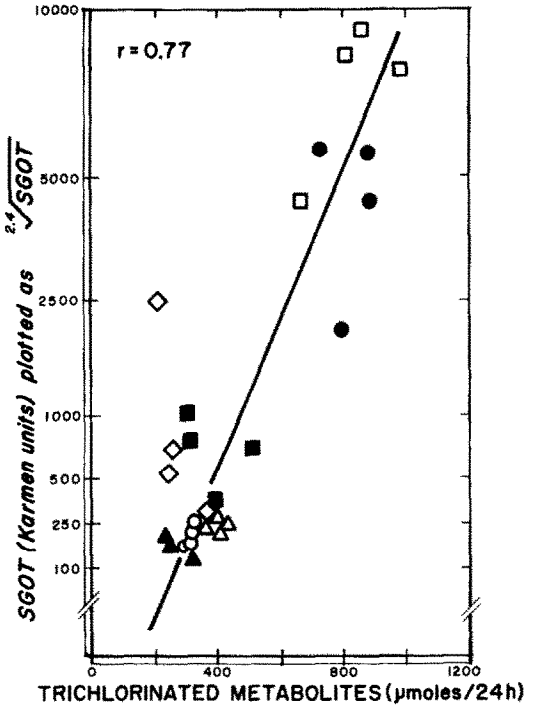

Fig. 3. Correlation between total urinary excretion of trichlorinated metabolites and serum SGOT levels 24 hr after exposure to trichloroethylene (1% × 2 hr) in the seven pretreatment groups. Each point represents the data for an individual animal. Symbols represent the different pretreatments: (○) vehicle, (●) PBT, (□) A-1254, (■) HCB, (△) 3-MC, (▲) SNL and (◇) PCN. The power relationship is such that each 2-fold increase in urinary trichlorinated metabolites corresponds approximately to a 10-fold increase in SGOT (significance < 1 per cent, $df = 26$, $r = 0.77$).

Table 3. Differential induction of electron transport components of the liver MFOS*

Pretreatment†		Cytochrome (nmoles/mg protein) P-450	Cytochrome (nmoles/mg protein) b_5	Cytochrome *c* reductase (nmoles product/mg protein·min) NADPH	Cytochrome *c* reductase (nmoles product/mg protein·min) NADH
Vehicle	(13)	0.77 ± 0.03	0.71 ± 0.04	48 ± 6	211 ± 16
PBT	(11)	1.83 ± 0.11‡	0.75 ± 0.03	89 ± 15§	130 ± 11‡
A-1254	(11)	2.52 ± 0.15‡	0.84 ± 0.04	83 ± 6‡	143 ± 8§
HCB	(9)	1.05 ± 0.09‡	0.76 ± 0.04	59 ± 11	160 ± 13§
3-MC	(9)	1.18 ± 0.10‡	0.84 ± 0.06	62 ± 11	169 ± 6
SNL	(9)	0.76 ± 0.05	0.73 ± 0.04	57 ± 8	144 ± 12§
PCN	(11)	1.38 ± 0.06‡	0.88 ± 0.04‡	78 ± 8§	98 ± 11‡

* Mean ± S.E.M.

† Chemicals were given by gavage once daily for 7 days; the number in parentheses indicates the number of animals.

‡ Statistically different from vehicle group (line 1) $P < 0.005$.

§ Statistically different from vehicle group (line 1) $P < 0.05$.

in that order, markedly increase cytochrome P-450 content. Peaks of the cytochromes induced by A-1254 or 3-MC show hyperchromic shifts ("P-448"). Cytochrome b_5 is increased significantly only by PCN. NADPH-cytochrome *c* reductase activity is enhanced by those agents which increase cytochrome P-450, while NADH-cytochrome *c* reductase is usually proportionally decreased. PBT, HCB, SNL and to a lesser extent PCN increase oxidative *N*-demethylation of dimethylaminoantipyrine, while glucose 6-phosphatase, an enzyme localized in the endoplasmic reticulum but not associated with the MFOS is decreased by PBT, A-1254 and PCN. Both A-1254 and 3-MC strikingly increase arene hydroxylase activities.

Correlations between differential induction of MFOS components and trichloroethylene toxicity. To determine if induction of specific MFOS components by chemical pretreatment regimens was associated with potentiation of TRI-induced liver injury, mean MFOS activities (assayed at times compatible with onset of TRI exposure) were compared with mean SGOT levels of similarly pretreated animals sacrificed 24 hr after TRI exposure. The correlation between cytochrome P-450 contents and SGOT levels was significant at the 1 per cent level by both linear and power (Fig. 4) analysis. The relationship between the rates of reduction of cytochrome P-450 by NADPH (measured as NADPH-cytochrome *c* reductase) and SGOT levels was less perfect, and no other relationship between the MFOS components and SGOT levels was apparent. When we looked for similar correlations between the corresponding induction of specific MFOS components and the enhancement of trichloroethylene metabolism, highly significant linear correlations were found between mean cytochrome P-450 content and both the mean anesthesia recovery time ($df = 5$, $r = 0.95$) and the mean urinary excretion of total trichlorinated metabolites ($df = 5$, $r = 0.88$).

Perchloroethylene injury. PER was metabolized primarily to TCA. Urinary recoveries of total trichlorinated products were approximately 5- and 7-fold greater in the PBT- and A-1254-pretreated animals (Table 5). Pretreatment with A-1254 enhanced the hepatotoxicity of PER as manifest both by doubling of SGOT and the appearance of focal areas of vacuolar degeneration and necrosis along the posterior aspect of the liver at 24 hr. Cornish *et al.* [30] also found slight elevations of SGOT in non-induced and PBT-induced rats after oral administration of PER.

Table 4. Differential induction of microsomal enzyme activities*

Pretreatment†		Oxidative *N*-demethylase (nmoles product/mg protein min)	Glucose 6-phosphatase (nmoles product/mg protein min)	Arene hydrocarbon hydroxylase: Zoxazolamine (nmoles product/mg protein min)	Arene hydrocarbon hydroxylase: Benzyprene ($O.D._{522}$/mg protein min)
Vehicle	(13)	10.5 ± 0.8	0.23 ± 0.01	0.50 ± 0.09	22 ± 2
PBT	(11)	16.4 ± 0.8‡	0.14 ± 0.01‡	0.61 ± 0.02	26 ± 3
A-1254	(11)	9.3 ± 0.6	0.11 ± 0.01‡	14.5 ± 1.30‡	66 ± 6‡
HCB	(9)	15.6 ± 0.5‡	0.20 ± 0.01	1.05 ± 0.07§	23 ± 5
3-MC	(9)	11.0 ± 1.4	0.23 ± 0.02	6.0 ± 0.60‡	38 ± 1§
SNL	(9)	15.1 ± 0.8‡	0.22 ± 0.02	0.60 ± 0.07	17 ± 2
PCN	(11)	13.8 ± 1.0§	0.19 ± 0.01‡	0.85 ± 0.20	15 ± 2

* Mean ± S.E.M.

† Chemicals were given by gavage once daily for 7 days; the number in parentheses indicates the number of animals.

‡ Statistically different from vehicle group (line 1) $P < 0.005$.

§ Statistically different from vehicle group (line 1) $P < 0.05$.

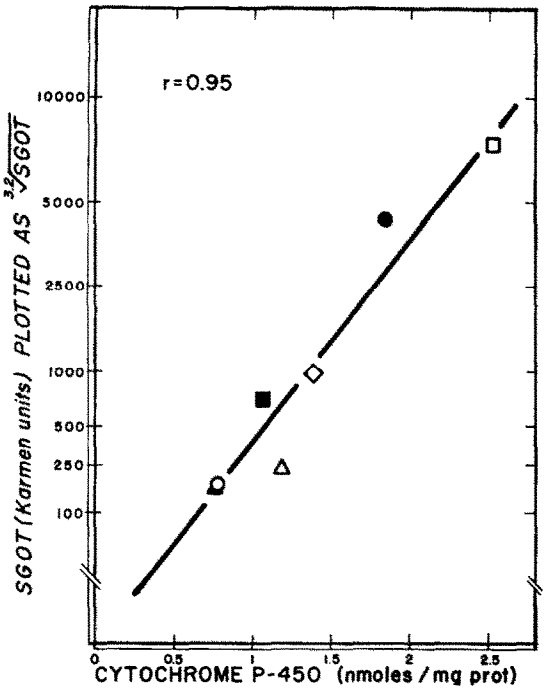

Fig. 4. Correlation between mean contents of microsomal cytochrome P-450 (nmoles/mg of protein) for the seven groups of chemically pretreated animals using data from Table 3 and the mean serum SGOT levels 24 hr after exposure to TRI using data from Table 2. Symbols represent the different pretreatments: (○) vehicle, (●) PBT, (□) A-1254, (■) HCB, (△) 3-MC, (▲) SNL and (◇) PCN. The power relationship is such that, within the experimental range, each 2-fold increase in cytochrome P-450 corresponds approximately to a 10-fold increase in SGOT (significance < 1 per cent, $df = 5$, $r = 0.95$).

DISCUSSION

Our working hypothesis is that the chlorinated ethylenes are activated to reactive molecular species by an enzyme system localized in the liver endoplasmic reticulum, and that induction of components of this system can alter the rates and/or routes of chloroethylene biotransformation. In this and prior studies [31, 32], we have demonstrated that pretreatment of rats with chemicals capable of inducing components of the liver mixed function oxidase system potentiates the acute hepatotoxicity of monochloroethylene (vinyl chloride), trichloroethylene and perchloroethylene.

Oxidized metabolites of di-(1,1- and 1,2-), tri-, and tetra-(per) chlorinated ethylenes have been identified in the perfusate of isolated perfused liver preparation [34]. Hepatic cytochrome P-450 appears to have a central function in the biotransformation of these chloroethylenes. Microsomal enzymes, presumably the mixed function oxidases, have been reported to oxidize vinyl chloride [34], 1,1- and 1,2-dichloroethylene [12], TRI [10, 11] and PER [12], and to transform vinyl chloride and 1,1-dichloroethylene into mutagens [35]. The metabolism of TRI by microsomes is competitively inhibited by carbon monoxide, hexobarbital or aniline, each of which binds to cytochrome P-450 [11]. Covalent binding of ^{14}C-labeled vinyl chloride is suppressed *in vitro* by 1-naphthyl-4(5)-imidazole, an inhibitor of microsomal cytochrome P-450 dependent oxidation [34]. Exposure *in vivo* to vinyl chloride (5% × 6 hr) resulted in a pattern of microsomal enzyme deactivation suggestive of a relatively toxic action or reaction centered about cytochrome P-450 [36]. Contents of cytochrome P-450 and the oxidative *N*-demethylation of dimethylaminoantipyrene and ethylmorphine were markedly decreased, while glucose 6-phosphatase, cytochrome b_5 and NADPH- and β-NADH-cytochrome *c* reductase were scarcely affected in both induced and non-induced animals sacrificed 24 hr after vinyl chloride.

In parallel *in vivo* studies using animals from seven pretreatment regimens, we found a direct correlation between mean cytochrome P-450 content (at the time of chloroethylene exposure) and the respective mean extent of liver injury (SGOT level) 24 hr after exposure to either TRI or vinyl chloride [32]. Paradoxically, the hepatotoxicity of 1,1-dichloroethylene (vinylidene chloride), a very reactive molecule [37], was reduced in animals pretreated with most effective inducers of cytochrome P-450 [32]. However, the hepatotoxicity of the relatively stable fully chlorinated ethylene, PER, was potentiated in animals pretreated with Aroclor 1254.

In this study, the metabolism of TRI and PER was enhanced in animals pretreated with inducers of cytochrome P-450. In fact, the trichloroethylene data indicate direct relationships between the mean magnitude of cytochrome P-450 induction and both the prolongation of anesthesia recovery time and the enhanced urinary excretion of trichlorinated metabolites. Additional support for a relationship between cytochrome P-450 content and extent of trichloroethylene metabolism can be inferred from the data of Bartoníček and Teisinger [38] and Stripp *et al.* [39]. Bartoníček and Teisinger [38] reported decreased urinary excretion of TRI metabolites coupled with increased recovery of TRI from the expired air of subjects previously given disulfuram. They attributed this shift to a disulfuram-induced suppression of TRI oxidation. Stripp *et al.* [39] have shown in the rat that disulfuram produces prolonged impairment of mixed

Table 5. Effects of PBT or Aroclor 1254 pretreatment on the metabolism and hepatotoxicity of perchloroethylene*†

Pretreatment‡		Urinary metabolites (μmoles/24 hr/animal) Total	TCA	SGOT (Karmen units)
Vehicle	(4)	4.1 ± 0.5	2.8 ± 0.4	211 ± 9
PBT	(4)	19.4 ± 1.1§	15.8 ± 0.9§	212 ± 36
A-1254	(4)	29.5 ± 2.5§	24.8 ± 2.5§	336 ± 31‖

* Mean ± S.E.M.

† Perchloroethylene: 0.75 ml/kg (7.5 m-moles/kg).

‡ Chemicals were given by gavage once daily for 7 days; the number in parentheses indicates the number of animals.

§ Statistically different from vehicle-PER group (line 1) $P < 0.001$.

‖ Statistically different from similarly pretreated animals exposed to air $P < 0.001$.

function oxidase components including decreased cytochrome P-450 content.

It is far from clear which of the metabolites of TRI is the ultimate toxin. Trichloroethylene is metabolized in a multi-stage process. The postulated epoxide intermediate rearranges to form an aldehyde (chloral); subsequent metabolism involves aldehyde and alcohol dehydrogenase and/or glucuronidases [13]. The components of this multimolecular process may vary in response to chemical induction. Non-uniform enzymatic induction could account for the changes Leibman and McAllister [14] described in the rate and route of TCE metabolism in PBT-induced animals.

Alternatively, factors other than enzyme induction could influence the hepatotoxicity of chloroethylenes, for example, changes in the redox state of the hepatocyte, or depletion of cofactors required for specific metabolic steps. Cornish and Adefuin [40] found striking elevations in SGOT levels in rats given ethanol 16 hr prior to TRI exposure (0.5% × 4 hr), while TRI-exposed rats not receiving ethanol had normal SGOT levels. Alcohol metabolism increases the NADH/NAD ratio in the liver and thus can slow reactions which require NAD or enhance reactions requiring NADH or NADPH [41]. Hepatic microsomal oxidation of TRI is NADPH dependent [10], and reducing equivalents for the reduction of chloral hydrate can be supplied by NADH or NADPH [42].

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