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MODULATION OF BENZENE-INDUCED LYMPHOCYTOPENIA IN THE RAT BY 2,4,5,2′,4′,5′-HEXACHLOROBIPHENYL AND 3,4,3′,4′-TETRACHLOROBIPHENYL

W.F. GREENLEE* and R.D. IRONS

Chemical Industry Institute of Toxicology. Department of Pathology, 6 Davis Drive, Research Triangle Park, NC 27709 (U.S.A.)

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SUMMARY

Repeated administration of benzene (440 mg/kg/day, s.c.) to 6-week-old male Fischer-344 rats resulted in a progressive decline in the number of circulating lymphocytes. Pretreatment of these animals with 2,4,5,2′,4′,5′-hexachlorobiphenyl (HCB) or 3,4,3′,4′-tetrachlorobiphenyl (TCB) protected against benzene toxicity for as long as 7 days, but not after 10 days of repeated dosing. Representative phase I (mixed-function oxidase) and phase II (conjugating) enzyme activities were measured to determine whether the altered susceptibility to benzene toxicity in TCB- and HCB-pretreated rats could be correlated with changes in the profile of hepatic oxidative and detoxification pathways. Measurement of 7-ethoxycoumarin *O*-deethylase and benzphetamine *N*-demethylase activities indicated that the loss of protection by HCB or TCB against benzene toxicity after 7 days was not associated with changes in the activities of hepatic mixed-function oxidases inducible by 3-methylcholanthrene or phenobarbital. The time course for the stimulation by TCB and return to control values, of UDP-glucuronosyl transferase activity, a potential route for the elimination of benzene metabolites, mirrored the time course for the protection against toxicity. Epoxide hydratase activity was induced 2- to 3-fold by HCB. Although stimulation of this pathway could result in a decreased concentration of phenol, this activity did not decline with the loss of protection. Hepatic 10 000 × g supernatant fractions, prepared from livers of rats given TCB, were incubated with a non-saturating concentration of [^{14}C] benzene (equivalent to 19 nmol/mg wet wt. tissue). Under these conditions the metabolism of benzene was depressed (40% of control) 2 days after pretreatment; after 14 days, the

*CIIT Postdoctoral Fellow.

Abbreviations: HCB, 2,4,5,2′,4′,5′-hexachlorobiphenyl; MC, 3-methylcholanthrene; PB, phenobarbital; TCB, 3,4,3′,4′-tetrachlorobiphenyl.

metabolism of benzene returned to control values. This pattern correlate: temporarily with the protection against lymphocytopenia. The data indicate that the protection against benzene toxicity in rats pretreated with HCB or TCB is not necessarily related to the capacity of these compounds to induce phase I activities. In rats pretreated with TCB, the data suggest that decreasing the concentration of primary benzene metabolites, either by inhibiting the hepatic metabolism of benzene or increasing hepatic conjugation activity is an important factor modulating toxicity.

INTRODUCTION

Chronic exposure to benzene results in a progressive degeneration of bone marrow structure and function [1,2]. In the peripheral blood benzene toxicity is expressed as leucopenia, thrombocytopenia, or anemia [3]. The metabolism of benzene has been extensively studied and the findings indicate that the toxicity of benzene to the hematopoietic system is associated with metabolism of the parent compound to one or more toxic species [4–6]. In the liver, benzene is a substrate for the cytochrome P-450-dependen: rnonooxygenases [7]. Several primary metabolites including phenol, catechol, hydroquinone, 1,2,4-benzenetriol and benzene dihydrociiol, and sulfate or glucuronic acid conjugates have been detected in the urine of rats given [^{14}C]benzene [8–10].

Evidence supporting the role of metabolism in the expression of benzene toxicity has resulted from the use of various inhibitors of inducers of mixed-function oxidase activities. Pretreatment of rats with 3-amino-1,2,4-triazole [11] or piperonyl butoxide [12], inhibitors of the hepatic microsomal monooxygenases, protected against benzene toxicity. Benzene-associated leucopenia was also alleviated by pretreating animals with phenobarbital (PB), an inducer [13–14]; however, pretreatment with 3-methylcholanthrene (MC), a prototype polycyclic aromatic hydrocarbon inducing a profile of hepatic microsomal monooxygenase activities different from that induced by PB [15], did not prevent the leucopenia resulting from a single large dose (equivalent to 5 ml/kg) of benzene [16]. Both PB and MC stimulated benzene metabolism as measured by the urinary excretion of phenol [16]. Attempting to resolve the paradox of the protective effect of both inhibitors and inducers of benzene metabolism, Snyder et al. [3] postulated that these agents elicit different responses in the liver (the major site of detoxification) and the bone marrow (the major site of toxicity): inducers stimulate hepatic detoxification, whereas inhibitors prevent the formation of reactive metabolites in the bone marrow.

Benzene associated radioactivity binds covalently to DNA in liver [17] and to macromolecules in both the liver and bone marrow [18]. It has been suggested that the chronic production of small amounts of benzene oxide in the bone marrow may result in reactions with critical cellular macromolecules, ultimately leading to bone marrow depression or leukemia [3]; however, studies o
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ever, studies on the covalent binding of benzene to liver microsomal protein have indicated that a metabolite of phenol and not benzene oxide was responsible for the observed binding [19].

We have observed that pretreatment of rats with a single dose of Aroclor 1254 (250 mg/kg) resulted in a decreased concentration of radioactivity associated with polyphenolic benzene metabolites in the bone marrow and protected against benzene toxicity [20]. Aroclor 1254 is a commercial mixture of chlorinated biphenyl isomers eliciting a complex pattern of induction of microsomal mixed-function oxidase activity comparable to that produced by the combined administration of PB and MC [21,22]. In this report we present findings on the effect of pretreatment with individual polychlorinated biphenyl isomers, HCB* and TCB, on benzene-mediated lymphocytopenia. Potential toxification and detoxification pathways in the liver were studied using various model substrates and compared with the in vitro metabolism of [^{14}C]benzene. The data indicated that both HCB and TCB protected against benzene toxicity and that protection is not necessarily associated with the capacity of these compounds to induce mixed-function oxidase activity.

METHODS

Animals

Male Fischer-344 rats were obtained from Charles River Breeding Labs (Wilmington, MA). The rats were housed in plastic cages with hardwood bedding: exposed to a day-night light cycle of 1 2 h, and given food (Wayne Lab Blox; Allied Mills, Chicago, IL) and water ad libitum.

Treatment

Rats pretreated with HCB (75 mg/kg, i.p.) or TCB (50 mg/kg, i.p.) for 2 consecutive days before the treatment period and naive animals were each divided into 2 groups: one group received 0.5 ml/kg/day corn oil (s.c.); the second group was given 1.0 ml/kg/day of a 50% solution of benzene in corn oil (s.c.). All rats weighed approx. 150 g at the beginning of dosing regimen. Benzene (thiophene-free) was obtained from Fisher Scientific Co. (Pittsburgh, PA) and HCB and TCB were purchased from RFR Corporation (Hope, RI).

Preparation of tissues

At various times during a 2-week treatment period, 5 animals from each group were anesthetized using methoxyflurane. Blood was collected by cardiac puncture into Vacutainer® tubes (Becton-Dickinson, Rutherford, NJ) containing potassium EDTA. Livers were dissected out, minced, washed in cold isotonic KCl, and homogenized in 4 vols. of SET (250 mM sucrose; 5 mM EDTA; 20 mM Tris, pH 7.4) buffer. The homogenate was centrifuged

*HCB and TCB are prototype inducers of PB- and MC-inducible mixed-function oxidase.

at 10 000 X g for 20 min and the supernatant fraction was carefully removed. Three 1-ml portions of this fraction were taken and stored at −80°C. The remainder was centrifuged at 105 000 X g for 60 min. The resulting supernatant fraction was decanted and the microsomal pellet was resuspended in SET buffer (concentration equivalent to 2 g wet wt. liver/ml) and stored at −80°C.

Hematology

All determinations were made on blood samples within 1 h of collection. Peripheral blood counts, hemoglobin, mean corpuscular volume and hematocrit were measured using a Coulter Counter (Model ZF, Coulter Electronics, Inc., Hialeah, FL). For the determination of differential white blood cell counts, blood smears were prepared and stained using a modified Wright stain containing added eosin.

Enzyme assays

7-Ethoxycoumarin O-deethylase [23], benzphetamine N-demethylase [24], epoxide hydratase [25] and UDP-glucuronosyl transferase [26] activities were assayed on the stored hepatic microsomal samples essentially as described in the references cited. 7-Ethoxycoumarin and 7-hydroxycoumarin were purchased from Aldrich Chemical Co. (Milwaukee, WI), benzphetamine from Applied Science Labs, Inc. (State College, PA) and benzo[*a*]pyrene-4,5-oxide from Midwest Research Institute (Kansas City, MO).

Assay of in vitro benzene metabolism

The assay was performed in 10-ml conical flasks fitted with screw cap Mininert® valves (Supelco, Inc, Bellefonte, PA). The reaction mixture, in a total volume of 0.9 ml, contained 100 pmol Tris-buffer (pH 7.5), 10 pmol $MgCl_2$, 0.5 pmol NADPH, 0.5 pmol NADP, 15 pmol glucose 6-phosphate, 0.5 U glucose-6-phosphate dehydrogenase and 0.10 ml 10 000 X g hepatic supernatant. Each flask was purged with O_2 before adding the substrate. The reaction was started by the addition of 560 nmol (0.5 μCi) of [^{14}C]benzene. The substrate was added in a total volume of 1 μl and the diluted benzene was prepared by adding the appropriate volume of methanol. The reaction mixture was incubated for 30 min at 37°C with vigorous shaking and the reaction was stopped by the addition of 0.125 ml of 15% (w/v) trichloroacetic acid. The samples were transferred to 15 ml conical centrifuge tubes and centrifuged at 2000 X g for 5 min.

Portions (100-μl or 200-μl) of the supernatants were analyzed for benzene metabolites using reverse phase high pressure liquid chromatography. Metabolites were separated on a Radial Pak A column containing octadecylsilane using a 12-min linear gradient (flow rate: 2.4 ml/min) from water to methanol. Both solvents were acidified with 100 μl/l formic acid. The metabolites eluted from the column within 10 min. Fractions (0.5-min) were collected into 20 ml glass scintillation vials and 10 ml of ACS scintillation cocktail

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For the systematic hydrolysis of conjugates, various incubation mixtures were divided into 0.3-ml portions and incubated for 12 h at 37°C with (a) 0.1 ml 0.2 M sodium acetate (pH 4.5), (b) 0.1 ml buffer containing 3000 U/ml sulfatase – 100 000 U/ml p-glucuronidase or (c) same as (b) with 15 mM saccharolactone. Both p-glucuronidase and saccharolactone were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical analysis

Statistical significance was assessed at the 5% level using a one-way ANOVA in combination with a least significant difference test.

RESULTS

Effect of pretreatment with HCB or TCB on benzene-induced lymphocytopenia

As shown in Figs. 1 and 2, daily benzene administration (440 mg/kg) resulted in a steady decline in the number of circulating lymphocytes. After 4 days, the lymphocyte count in the benzene-treated group was significantly depressed ($P < 0.05$) compared to control animals (Fig. 1). Pretreatment with TCB (Fig. 1) or HCB (Fig. 2) protected against benzene toxicity. After 7 days of benzene dosing, the values for the lymphocyte count in rats pretreated with TCB or HCB were significantly greater ($P < 0.05$) than the values for non-pretreated animals, but after 10 days, protection against benzene toxicity was not observed in rats in either pretreatment group (Figs. 1 and 2). One factor contributing to the loss of protection in the TCB pretreated rats may have been a delayed toxicity of TCB to lymphocytes as indicated by a significant ($P < 0.05$) depression in the lymphocyte count at days 10 and 14 in the TCB-pretreatment group which did not receive benzene (Fig. 1). No significant changes in the total red cell count, hemoglobin, mean corpuscular volume and hematocrit were observed in any of the treatment groups (data not shown).

Depressed leucocyte counts have been reported in rats on a restricted food intake [27]. In order to assess this factor in our studies, we monitored the body weights of the rats in the various treatment groups. As shown in Table I, initially, the body weight gains in the benzene-treated rats were less than in control animals. However, toward the end of the treatment period, we observed a recovery in the body weights of the benzene-exposed rats,

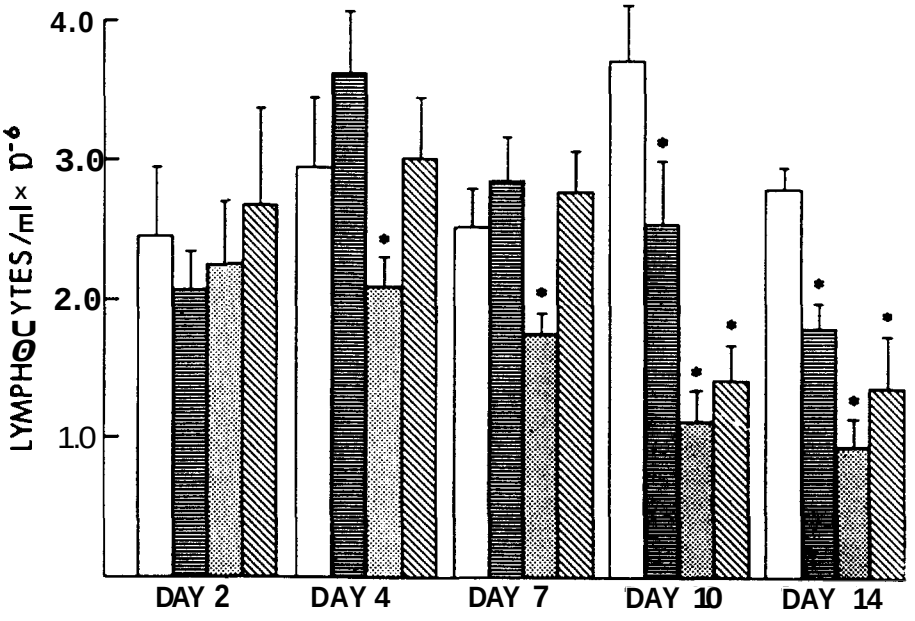


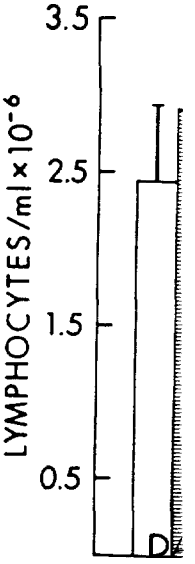
Fig. 1. Effect of repeated benzene administration on peripheral lymphocytes in rats pretreated with TCB. □, corn oil controls; ▨, benzene treated; ▩, TCB pretreated; ▧, pretreatment with TCB before receiving benzene. All values represent the mean ± S.E.M. of duplicate determinations on 5 animals. Asterisks indicate values significantly different ($P < 0.05$) from corn oil controls for a given group.

indicating that the hematologic changes in these animals were associated with the benzene treatment and were not the result of general debility.

Characterization of hepatic xenobiotic metabolism

Since benzene has been shown to be metabolized by cytochrome *P*-450-dependent monooxygenases in the liver [7], we measured representative phase I (mixed-function oxidase) and phase II (conjugating) enzyme activities to determine if the altered susceptibility to benzene toxicity in pretreated rats could be correlated with changes in the profile of hepatic oxidative and detoxification pathways. As shown in Fig. 3, pretreatment with HCB resulted in a sustained induction (3-fold) of benzphetamine Ndemethylase activity. 7-Ethoxycoumarin Odeethylase activity was induced (4- to 5-fold) by either TCB or HCB throughout the treatment period. These data indicate that the loss of protection against benzene toxicity in HCB- or TCB-pretreated animals (Figs. 1 and 2) cannot be associated with changes in representative MC- or PB-inducible mixed-function oxidase pathways.

Epoxide hydratase and UDP-glucuronosyl transferase activities were measured in livers from the same rats using benzo[*a*]pyrene-4,5-oxide and 7-hydroxycoumarin, respectively, as model substrates. Epoxide hydratase activity was induced 2- to 3-fold by HCB. The activity of this enzyme did not decline after 2 weeks (Fig. 4). UDP-glucuronosyl transferase activity



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Time (days)	Treatn
	Corn o (0.5 m
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0	159 ±
4	173 ±
7	192 ±
10	197 ±
14	215 ±

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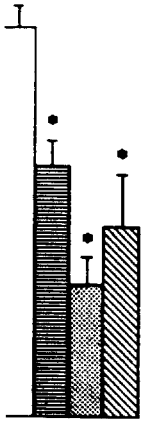


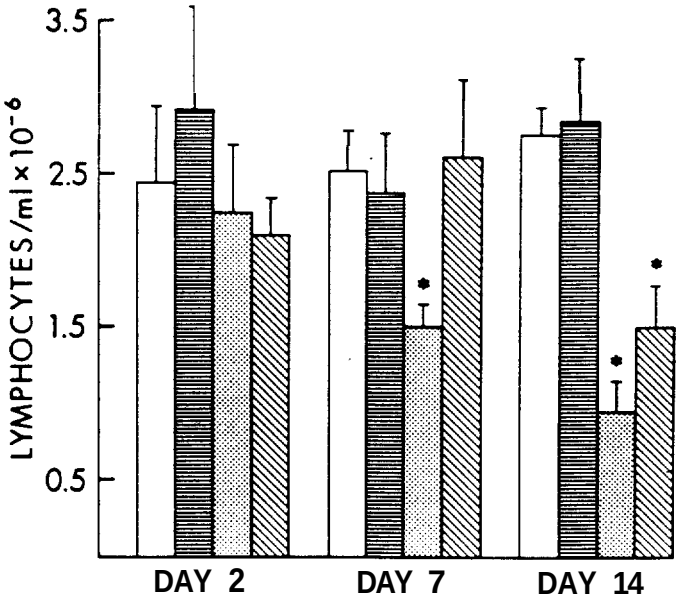


Fig. 2. Effect of repeated benzene administration on peripheral lymphocytes in rats pretreated with HCB. □, corn oil controls; ▨, benzene treated; ▤, HCB pretreated; ▩, pretreatment with HCB before receiving benzene. All values represent the mean ± S.E.M. of duplicate determinations on 5 animals. Asterisks indicate values significantly different ($P < 0.05$) from corn oil controls for a given group.

TABLE I

EFFECT OF CHRONIC BENZENE ADMINISTRATION ON BODY WEIGHTS OF RATS PRETREATED WITH HCB OR TCB

Male Fischer-344 rats were given HCB (Group 111) or TCB (Group IV) for two consecutive days before treating with benzene (Methods). Groups I and II received no pretreatment. Each value represents the mean ± S.E.M. The number of animals in each group ranged from 5 to 25.

Time (days)	Treatment			
	Corn oil (0.5 ml/kg/day)	Benzene[1] (0.5 ml/kg/day)		
	Group I	Group II	Group III	Group IV
0	159 ± 8 (100)[b]	156 ± 7 (100)	155 ± 10 (100)	142 ± 11 (100)
4	173 ± 13 (109)	158 ± 10 (101)	150 ± 13 (97)	147 ± 18 (104)
7	192 ± 15 (121)	162 ± 12 (104)	158 ± 14 (102)	155 ± 12 (109)
10	197 ± 16 (124)	172 ± 9 (110)	168 ± 22 (108)	160 ± 12 (113)
14	215 ± 18 (135)	191 ± 11 (122)	185 ± 22 (119)	181 ± 10 (127)

[1] Benzene was administered as a 50% solution in corn oil.
[b] Numbers in parentheses represent percent of body weight at day 0 for each group.

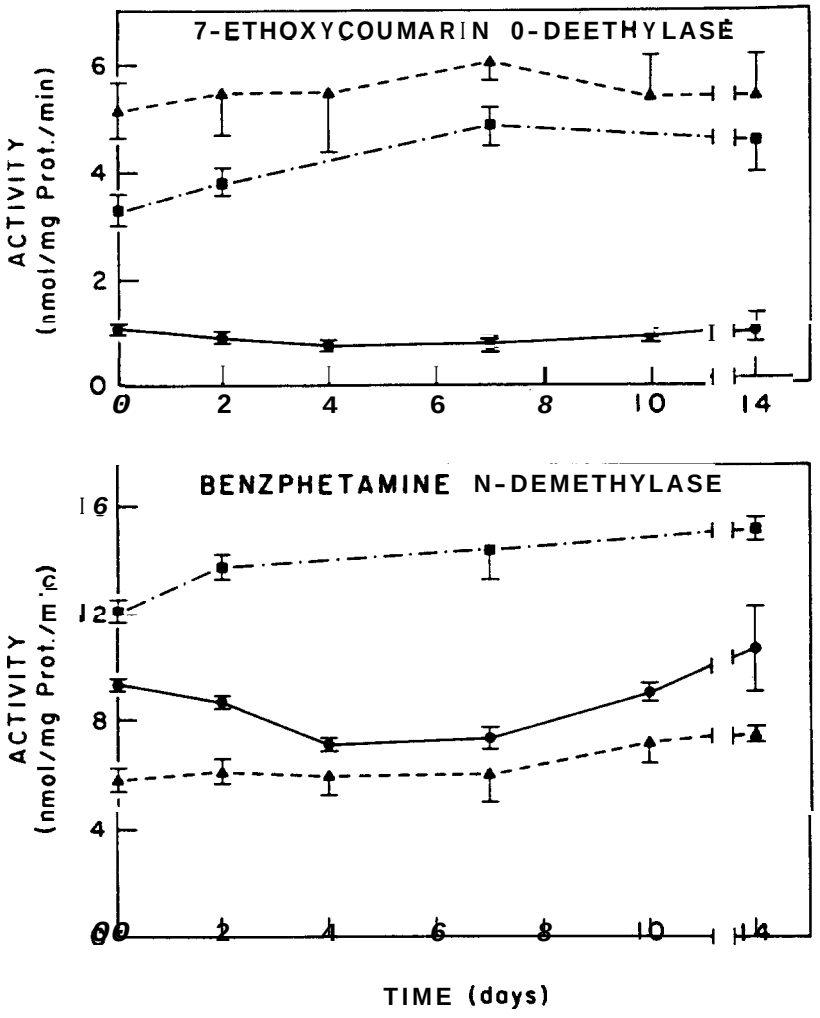
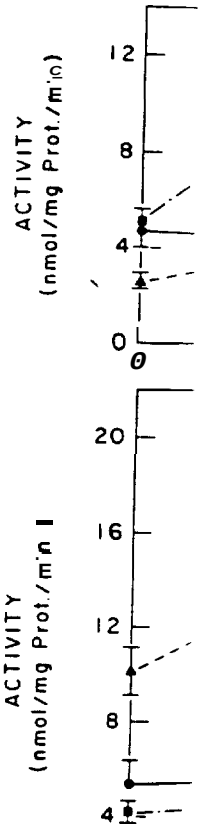


Fig. 3. Time course for the induction of 7-ethoxycoumarin *O*-deethylase and benzphetamine *N*-demethylase activities by TCB or HCB. ▲, TCB pretreated; ■, HCB pretreated; ●, no pretreatment. Each point represents the mean ± S.E.M. of duplicate determinations on 4 animals.

was induced by TCB. The time course for the stimulation (4-fold increase in activity) and return to control values of glucuronosyl transferase activity precisely paralleled the time course for the protection against benzene toxicity (Figs. 1 and 4), suggesting a possible relationship between glucuronidation and the modulation of benzene toxicity.

Hepatic metabolism of benzene

The metabolism of benzene by hepatic 10 000 × g supernatant fractions



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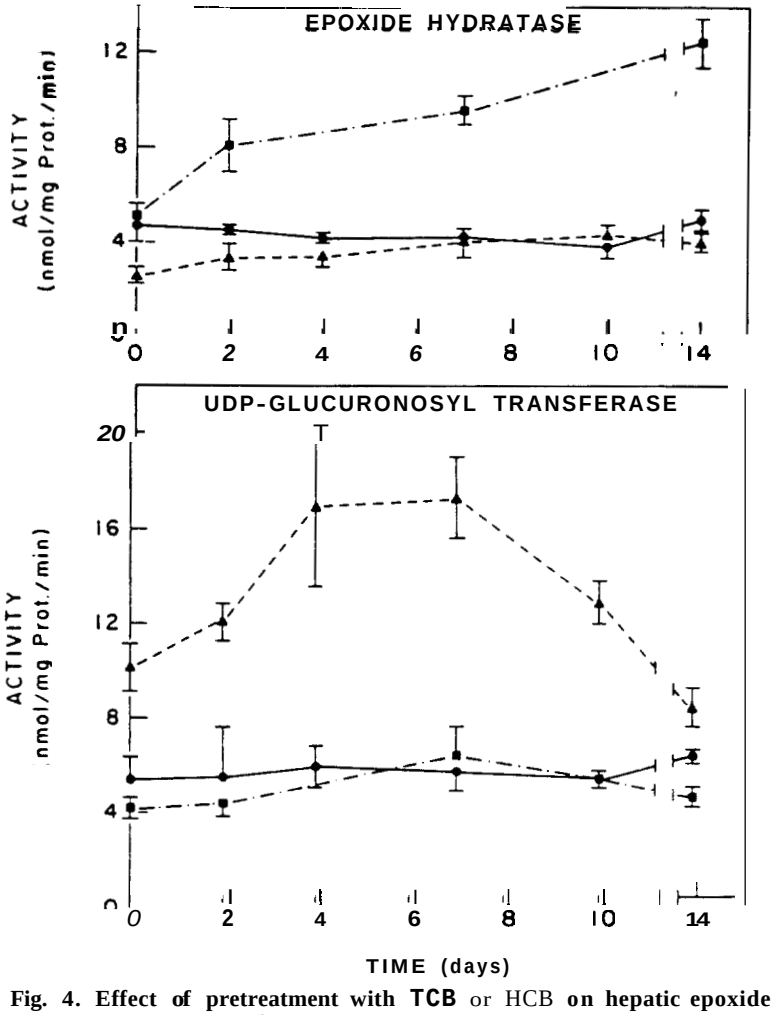

Fig. 4. Effect of pretreatment with TCB or HCB **on hepatic epoxide hydratase and UDP-glucuronosyl transferase. Symbols** *are* **the same as in Fig. 4. Each point represents the mean ± S.E.M. of duplicate determinations on 4 animals.**

was measured using reverse-phase high pressure liquid chromatography. The results **are** shown in Fig. 5 and Tables II *and* 111. Peaks coeluting with authentic samples of hydroquinone and phenol were consistently detected (Fig. **5A).** Under these incubation conditions we did not detect hydroquinone and only **a** small peak coeluting with phenol was observed (Table 111). Incubation of this sample with sulfatase in the presence of saccharolactone (a β-glucuronidase inhibitor), indicated that the conjugate formed in vitro was the sulfate ester of phenol. Hydroquinone was not detected after hydrolysis of the conjugate fraction.

In measuring the in vitro metabolism of benzene, a concentration equiva-

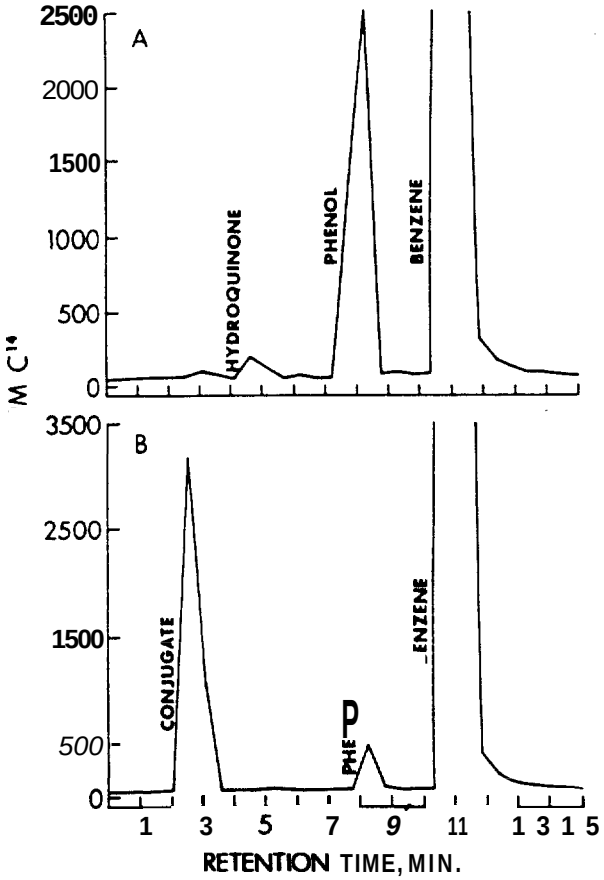

Fig. 5. HPLC profile of benzene metabolites after incubation of [^{14}C]benzene with hepatic 10 000 x g supernatants from naive rats. Metabolites were analyzed by reverse phase high pressure liquid chromatography using a Radial Pak A column containing octadecylsilane. A: incubation buffer containing NADPH and NADPH regenerating system. B: same as (A) plus ATP, sodium sulfate, and UDP-glucuronic acid.

lent to 19 nmol/mg wet wt. tissue was used. This is a nonsaturating substrate concentration, but more accurately reflects the hepatic concentrations of benzene reported in vivo [28,29]. In the presence of conjugating cofactors, the metabolism of benzene (assessed by total metabolite formation) was significantly depressed ($P < 0.05$) 2 days after pretreatment with TCB (day 0 of the start of the treatment period) and then returned to control values within 14 days (Table III). Under these same conditions a moderate, but statistically insignificant, increase in benzene metabolism was observed in livers from HCB-pretreated animals. In the absence of conjugating cofactors HCB-pretreatment stimulated the formation of hydroquinone at all time points examined (Table II); however, in the presence of these cofactors (Table III), hydroquinone was not detected in either free or conjugated form

TABLE II

METABOLISM OF
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Day	Pretreatn
0	Control
	HCB
	TCB
7	Control
	HCB
	TCB
14	Control
	HCB
	TCB

[a]Numbers in paren

TABLE III

METABOLISM OF
TCB OR HCB

All conditions were
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for 3 animals.

Day	Pretreatr
0	Control
	HCB
	TCB
7	Control
	HCB
	TCB
14	Control
	HCB
	TCB

[a]Values shown incl
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TABLE II

METABOLISM OF [^{14}C]BENZENE IN LIVERS FROM RATS PRETREATED WITH TCB OR HCB

Male Fischer-344 rats were pretreated with TCB or HCB (Methods). Animals were killed at the times indicated and 560 nmol (equilvalent to 9 nmol/mg wet wt. tissue) of [^{14}C]-benzene (0.89 μCi/μmol) was incubated with hepatic 10 000 x g supernatant fractions (Methods). All values represent the mean ± S.E.M. for 3 animals.

Day	Pretreatment	Hydroquinone (nmol/assay)	Phenol (nmol/assay)	Total metabolites[a] (nmol/assay)
0	Control	0.114 ± 0.003	2.76 ± 0.23	2.93 ± 0.32 (100)
	HCB	0.240 ± 0.014	4.18 ± 0.43	4.45 ± 0.49 (151)
	TCB	0.094 ± 0.002	1.60 ± 0.11	1.70 ± 0.24 (58)
7	Control	0.126 ± 0.002	3.55 ± 0.19	3.68 ± 0.23 (100)
	HCB	0.313 ± 0.025	4.98 ± 0.33	5.36 ± 0.57 (146)
	TCB	0.154 ± 0.005	3.03 ± 0.15	3.19 ± 0.21 (87)
14	Control	0.170 ± 0.007	3.12 ± 0.08	3.31 ± 0.12 (100)
	HCB	0.285 ± 0.025	5.01 ± 0.13	5.31 ± 0.24 (160)
	TCB	0.197 ± 0.010	3.58 ± 0.44	3.78 ± 0.54 (114)

'Numbers in parentheses represent percent of control value for each group.

TABLE III

METABOLISM OF [^{14}C]BENZENE IN LIVERS FROM RATS PRETREATED WITH TCB OR HCB

All conditions were the same as in Table II except that the incubation buffer contained ATP, sodium sulfate, and UDP-glucuronic acid. All values represent the mean ± S.E.M. for 3 animals.

Day	Pretreatment	Phenol (nmol/assay)	Conjugate (nmol/assay)	Total Metabolites[a,b] (nmol/assay)
0	Control	0.466 ± 0.022	3.32 ± 0.59	4.34 ± 1.31 (100)
	HCB	1.03 ± 0.08	5.16 ± 0.17	6.21 ± 0.37 (143)
	TCB	0.103 ± 0.001	1.76 ± 0.45	1.91 ± 0.41[c] (44)
7	Control	0.607 ± 0.132	5.16 ± 0.18	5.76 ± 0.61 (100)
	HCB	0.741[d]	4.54[d]	5.28[d] (92)
	TCB	0.434 ± 0.020	3.58 ± 0.39	4.01 ± 0.41 (70)
14	Control	0.437 ± 0.001	4.69 ± 0.48	5.12 ± 0.51 (100)
	HCB	0.06 ± 0.001	7.56 ± 6.1	8.62 ± 6.0 (170)
	TCB	0.293 ± 0.001	5.46 ± 3.8	5.76 ± 3.8 (112)

[a] Values shown includes contribution from a third metabolite (presumably hydroquinone).
[b] Numbers in parentheses represent percent of control value for each group.
[c] Significantly different ($P < 0.05$) from the control value as assessed by one-way ANOVA and least significant difference test.
[d] Average value for two animals.

in control or pretreated rats, suggesting that the conjugation of phenol effectively competed with the further metabolism of this compound to hydroquinone. The decline and subsequent recovery to control values for benzene metabolism in livers from TCB-pretreated rats correlated temporally with the protection against lymphocytopenia (Fig. 1; Table 111).

DISCUSSION

In this report we have shown that pretreatment of rats with HCB or TCB protects against benzene toxicity. Measurement of MC- and PB-inducible enzyme activities using the well-characterized prototype substrates, 7-ethoxycoumarin and benzphetamine, indicated that the protection against benzene toxicity elicited by TCB or HCB was not associated with changes in the profile of phase I activities. However, the data suggest that pathways purported to govern the concentration of free phenol (and thus metabolites of phenol such as hydroquinone) in the liver mediate the expression of benzene toxicity. This is indicated by the temporal correlation between protection against lymphocytopenia and (a) depressed benzene metabolism (40% of control) by hepatic 10 000 X g supernatant fractions (Fig. 1 and Table 111) or (b) the stimulation and return of control values of UDP-glucuronosyl transferase activity (Figs. 1 and 4) in rats pretreated with TCB. The depressed hepatic metabolism of benzene in vitro at a non-saturating substrate concentration (Tables II and 111) suggests that TCB may compete with benzene for specific enzyme sites. At a 20-fold greater substrate concentration than reported in Table II, benzene metabolism in livers from rats pretreated with either TCB or HCB was approx. 2 times greater than in livers from control animals (W.F. Greenlee and R.D. Irons, unpublished data).

In the broken cell preparation used in this study only the sulfate conjugate was detected (Table 111); however, in vivo both the sulfate and glucuronide conjugates of phenol have been identified [8,30] and it has been reported that at higher concentrations, the percentage of phenol excreted as the glucuronide increases in the absence of significant depletion of inorganic sulfate [31]. In a recent series of experiments characterizing the urinary metabolites of benzene (W.F. Greenlee et al., in prep.), we have found that hydroquinone is eliminated as the glucuronide conjugate, whereas phenol is excreted as both the sulfate and glucuronide conjugate. Further, the amount of hydroquinone found in the urine from control rats was greater than in urine from rats pretreated with Aroclor 1254, a regimen which protects against benzene toxicity [20].

No obvious relationship between protection against benzene toxicity and metabolism was seen in rats pretreated with HCB. The stimulation of epoxide hydratase activity by HCB (Fig. 4) would result in a decreased concentration of phenol and subsequent metabolites such as hydroquinone, but this activity did not decline with the loss of protection against lymphocytopenia (Figs. 2 and 4). Since catechol is a putative product from the oxidation of benzene dihydrodiol [3], catechol may have achieved a toxic concentration

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after 7 days of repeated benzene dosing in the HCB-pretreated animals. Catechol was not detected in the in vitro metabolism of benzene by hepatic 10 000 × *g* supernatants; however, this species is detected in the urine of rats after administration of benzene [8–10].

In reviewing the apparent discrepancies between in vivo and in vitro studies of benzene metabolism, Gut [32] suggested that either low concentrations of benzene in the liver or product inhibition could account for the low expression of induced hepatic rmcrosomal monooxygenase activity in benzene metabolism in vivo. Thus, it would appear that the non-saturating concentration of benzene used in our investigation approximates the in vivo situation and provides a reasonable index of the role of hepatic metabolism in the expression of benzene toxicity.

High concentrations of benzene metabolites have been demonstrated in the bone marrow of mice given 880 mg/kg [^{3}H] benzene [29]. The maximum concentration of these metabolites in the bone marrow occurred approx. 2 h after maximum concentrations were attained in the liver. Rickert et al. [28] reported that hydroquinone and catechol were retained in the bone marrow of rats exposed to 500 ppm benzene for 6 h and Sammett et al. [33] reported that the removal of 70–80% of the liver reduced both the metabolism and the toxicity of benzene in rats. We showed that in rats given ^{14}C-labelled benzene metabolites, radioactivity associated with hydroquinone or catechol, but not phenol, concentrated in the bone marrow and lymphoid organs [20]. Further, the amount of radioactivity in these tissues was reduced in rats pretreated with Aroclor 1254. Andrews et al. [34] have demonstrated benzene metabolism by rabbit bone marrow in vitro; however, the amount of phenol formed, the major metabolite, was less than 0.24% of the benzene added. After perfusion of an isolated femur in the rat with blood containing [^{14}C]benzene at a concentration 200- to 800-fold greater than the reported concentrations of toxic doses of benzene to mice or rats [28,29], it was found that the amount of metabolites formed was 3% of the administered dose of benzene [35]. These data suggest; (a) that the metabolism of benzene by bone marrow cannot account for the concentration of metabolites reported in that tissue in vivo [28,29] and (b) that the metabolism of benzene in the liver is important for the expression of toxicity with polyphenolic metabolites such as hydroquinone and catechol being taken up and concentrated by target tissues for benzene toxicity such as the bone marrow.

Hydroquinone is an inherently reactive species capable of being oxidized to semiquinone or benzoquinone [36]. The formation of free radical intermediates or the potential generation of superoxide anion may be causative factors leading to cytotoxicity. If toxicity is mediated by the superoxide anion, the low levels of superoxide dismutase reported in bone marrow [37] could be a determinant of the selective toxicity of benzene metabolites to that tissue.

In summary, we have shown that pretreatment of rats with HCB or TCB protects against benzene toxicity. This protection is a complex process

involving metabolic events other than the induction of phase I activities. In rats pretreated with TCB, the data suggest that decreasing the concentration of primary benzene metabolites, either by inhibiting the hepatic metabolism of benzene or increasing hepatic conjugating activity, is an important factor regulating the expression of toxicity.

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L.M. ALLEN, MARI

Department of Onc
Miami, FL 33101 (V.

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Introduction

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