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EFFECT OF *o*-BENZYL-*p*-CHLOROPHENOL ON DRUG-METABOLIZING ENZYMES IN RATS

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Abstract—*o*-Benzyl-*p*-chlorophenol (BCP) is widely used as a broad spectrum disinfectant. Treatment of male Fischer 344 rats with BCP resulted in an increase in cytochrome P-450 content and an accompanying decrease in aryl hydrocarbon hydroxylase (AHH) activity in both liver and kidney microsomes. Several other drug-metabolizing enzymes were not affected by BCP treatment. However, in kidney, BCP induced NADPH-cytochrome *c* reductase and uridine diphosphate glucuronyl transferase activities and caused a small increase in total cytochrome P-450 content and glutathione concentration. The cytochrome P-450 isozymes induced by BCP were fractionated by high pressure liquid chromatography (HPLC). The HPLC profile following BCP treatment most closely resembled that seen after phenobarbital. Using an immunoblotting procedure and a radioimmunoassay, it was shown that the increase in cytochrome P-450 content in the liver after BCP treatment was, in part, due to an increase in the phenobarbital-inducible isozymes, P-450b + e. In the kidney, the increase in total cytochrome P-450 content after BCP exposure was not due to an increase in P-450b + e. The decrease in AHH activity appeared to be caused by noncompetitive inhibition of constitutive AHH activity by BCP. BCP also inhibited benzphetamine demethylation, although to a lesser extent. The failure to observe an increase in benzphetamine demethylase activity *in vivo*, despite the induction of P-450b, was probably due to the concomitant induction and inhibition of drug-metabolizing enzymes by BCP.

o-Benzyl-*p*-chlorophenol (BCP‡) is widely used in hospitals and households as a broad spectrum disinfectant. Due to its relatively nontoxic properties, it was introduced to replace another halogenated phenolic disinfectant, hexachlorophene. However, several incidents of neonatal hyperbilirubinemia occurred in hospitals subsequent to heavy usage of BCP [1]. This may have been due to inhibition of UDP-glucuronyl transferase [2]. BCP is also nephrotoxic, resulting in proximal tubule lesions in both mice and rats after repeated exposure [3]. The mechanism of BCP-induced nephrotoxicity is not understood.

Metabolic activation of xenobiotics plays an important role in tissue toxicity [4], and the cytochrome P-450 enzymes responsible for this activation can be inhibited or induced by prior administration of xenobiotics. Many previous studies indicate that inducers of cytochrome P-450 isozymes can be divided into at least three broad classes which are commonly represented by (i) phenobarbital (PB), (ii) 3-methylcholanthrene (3-MC) and (iii) pregnenolone-16α-carbonitrile (PCN) [5, 6]. The isozymes induced by these three classes of inducers differentially metabolize certain substrates. For example, aryl hydrocarbon hydroxylase (AHH) activity is induced by 3-MC in rat liver, while benzphetamine *N*-demethylase is induced preferentially by PB [7]. This preferential increase in the metabolism of certain substrates is the result of induction of specific cytochrome P-450 isozymes by each of the three classes of inducers (P-450b by phenobarbital, P-450$_{pcn}$ by PCN, and P-450c, P-450d and to a lesser extent P-450a by 3-MC) [5, 8].

Many xenobiotics such as SKF 525-A, piperonyl butoxide, metyrapone, and hexachlorophene inhibit drug-metabolizing enzymes both *in vivo* and *in vitro* [9–13]. However, the *in vivo* effects of these compounds are complicated by the fact that many of them (metyrapone, piperonyl butoxide and SKF 525-A) are also inducers of cytochrome P-450.

Induction of metabolic enzymes by xenobiotics in the kidney with 3-MC-type inducers has been reported [14], and existence of multiple forms of cytochrome P-450 was reported in rabbit kidney of untreated rabbits [15] and after 3-MC administration [16]. Due to increasing evidence of *in situ* metabolic activation of nephrotoxins such as chloroform and 4-ipomenol [17, 18], the modulation of metabolic enzymes by xenobiotics in kidney as well as in liver is of increasing concern.

It has been observed that BCP increases liver and kidney weight in both rats and mice after repeated

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‡ Abbreviations: BCP, *o*-benzyl-*p*-chlorophenol; AHH, aryl hydrocarbon hydroxylase; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; 3-MC, 3-methylcholanthrene, RIA, radioimmunoassay; UDPGA, uridine diphosphoglucuronic acid; and CDNB, 1-chloro-2,4-dinitrobenzene.

oral doses.* However, the biological significance of this observation has not been investigated. In this report, we describe the effects of BCP on drug-metabolizing enzymes in both liver and kidney in the male rat, which is more sensitive to BCP-induced toxicity than female rats or mice [3].

MATERIALS AND METHODS

Materials. *o*-Benzyl-*p*-chlorophenol (BCP) (chemical purity >99%) was provided by the Radian Corp., Austin, TX. [^{14}C]BCP was synthesized by the Midwest Research Institute, Kansas City, MO (specific activity 22.9 mCi/mmoles, radioactive purity >99%).

The chemical and radioactive purity was confirmed by HPLC using a reverse phase Radio Pak C_{18} column (Waters, Milford, MA) at a flow rate of 1 ml/min. A 5-min linear gradient from 0 to 85% acetonitrile in H_2O was followed by a 20-min linear gradient to 95% acetonitrile in H_2O. The u.v. absorbance was monitored at 254 nm using a Waters variable wavelength detector model 450. Radioactivity was monitored continuously using a Packard Tri-Carb RAM 7500 (Downer Grove, IL) radioactive detector with a solid crystal cell.

1-Chloro-2,4-dinitrobenzene (CDNB), benzo[*a*] pyrene, *p*-nitroanisole, NADPH, uridine diphosphoglucuronic acid (UDPGA), cytochrome *c* and *p*-nitrophenol were purchased from the Sigma Chemical Co., St. Louis, MO. Benzphetamine was a gift of the Upjohn Co., Kalamazoo, MI. All other reagents were of the highest grade commercially available.

Animals. Male Fischer 344 rats weighing between 175 and 200 g were obtained from the Charles River Breeding Laboratories (Portage, MI) and held 5 days prior to treatment. Rats were fed NIH 31 rat food and water *ad lib.* and maintained at a temperature of 22 ± 1°, 50 ± 10% humidity and with a 12/12 light/dark cycle.

Rats were treated orally with 500 mg/kg of BCP in 2.5 ml/kg of corn oil, 1 mg/ml of sodium PB in drinking water, 25 mg/kg i.p. 3-MC or 50 mg/kg i.p. PCN in corn oil for 3 consecutive days. Control rats also received 5 ml/kg corn oil. Animals were killed 24 hr after the last treatment, and the diet was removed 18 hr before sacrifice. Livers and kidneys from individual rats were homogenized with 4 vol. of 100 mM potassium phosphate buffer, pH 7.4, at 0°, and the homogenates were centrifuged at 10,000 *g* for 15 min. The supernatant fraction was then centrifuged at 105,000 *g* for 60 min. The microsomes were suspended in ice-cold 100 mM K^+-phosphate buffer, pH 7.4, and the 105,000 *g* supernatant fractions were analyzed for glutathione transferase activity. The microsomes and 105,000 *g* supernatant fractions were bubbled with N_2 and were stored at −80° before use.

Enzyme assays. AHH activity was measured by the method of Nebert and Gelboin [19] with some modifications. In brief, 0.1 mg microsomal protein, 100 μM benzo[*a*]pyrene and 0.5 mM NADPH were incubated in 50 mM Tris buffer containing 1 mg/ml bovine serum albumin and 3 mM $MgCl_2$, pH 7.5, in a total volume of 1 ml for 10 min at 37°. The apparent kinetic constants, K_m and V_{max}, were determined by the method of Lineweaver and Burk [20]. O-Demethylation of *p*-nitroanisole and N-demethylation of benzphetamine were assayed according to Hansen and Hodgson [21] and Cochin and Axelrod [22] respectively. NADPH-cytochrome *c* reductase was assayed by the method of Masters *et al.* [23]. Glutathione transferase activity was determined using CDNB as the substrate as described by Habig *et al.* [24]. UDP-glucuronyl transferase was assayed using *p*-nitrophenol as the substrate [25]. The ability of BCP to inhibit enzyme activity was assayed by incubating 10 μM BCP with microsomes in the absence of NADPH for 10 min followed by addition of substrates and cofactors. All enzyme assays were carried out in the linear range with respect to time and substrate concentration.

HPLC of solubilized microsomes. Fractionation of cytochrome P-450 isozymes was carried out by HPLC according to Kotake and Funae [26] with some modifications. Microsomes were solubilized at 4° for 30 min in 10 mM K^+-phosphate buffer, pH 7.4, containing 0.2% Emulgen 911, 0.5% (w/v) sodium cholate, 0.1 mM EDTA and 20% (v/v) glycerol [27] and then centrifuged at 105,000 *g* for 60 min. Greater than 95% of the cytochrome P-450 was recovered after solubilization. One hundred microliters of solubilized microsomes was applied to an Anpac anion exchange column (Anspec, Ann Arbor, MI) at a flow rate of 1.0 ml/min, and the proteins were monitored at 405 nm. The chromatogram was developed at room temperature using a 20-min linear gradient of 0 to 0.4 M sodium acetate in 20 mM Tris-acetate buffer, pH 7.4, containing 0.2% Emulgen 911 and 20% (v/v) glycerol according to Bansal *et al.* [28]. One-minute fractions were collected and monitored for cytochrome P-450 content as described below.

Electrophoresis and immunostaining (Western blots). Electrophoresis of microsomes, HPLC fractions, and pure cytochrome P-450b was performed as described by Laemmli [29]. Transfer of the proteins to nitrocellulose sheets (Western blots) and immunostaining with antibody to pure cytochrome P-450b were carried out with slight modifications [30] of the method of Towbin *et al.* [31].

Radioimmunoassay. Cytochrome P-450b purified from Sprague–Dawley rats [32] was radioionated by ICN (Irvine, CA) using the Bolton–Hunter Reagent to a specific activity of 4.0 μCi/μg. The cytochrome P-450b content of microsomes was determined by radioimmunoassay (RIA) as described for P-448 MC [30] except that the antibody was prepared and the RIA was modified accordingly for the use of goat antisera. Standard curves for microsomes from phenobarbital-treated animals produced lines with slopes parallel to that of the standard curve for P-450b.

In vitro *binding.* Solubilized microsomes (1 ml) were incubated with 0.1 μCi of [^{14}C]BCP at 25° for 30 min in 10 mM K^+-phosphate buffer, pH 7.4. The mixture was then chromatographed on a Sephadex G-25 column (20.0 × 1.0 cm) in the same buffer used for HPLC. The protein fractions were collected and analyzed for radioactivity using a Beckman (Fuller-

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Table 1. Effects of BCP on drug-metabolizing enzymes in rat liver and kidney

	Liver		Kidney	
	Control	Treated	Control	Treated
Cytochrome P-450 (nmoles/mg)	1.16 ± 0.14	1.51 ± 0.14*	0.20 ± 0.01	0.32 ± 0.03†
AHH (nmoles/min/mg)	1.25 ± 0.07	0.63 ± 0.15†	0.039 ± 0.006	0.024 ± 0.006*
N-Demethylation (nmoles/min/mg)	6.59 ± 0.50	7.73 ± 1.26	ND‡	ND
O-Demethylation (nmoles/min/mg)	0.05 ± 0.01	0.06 ± 0.02	ND	ND
NADPH-Cytochrome *c* reductase (nmoles/min/mg)	304.8 ± 50.2	368.1 ± 73.5	25.9 ± 3.5	45.4 ± 10.8*
UDP-glucuronyl transferase (nmoles/min/mg)	2.69 ± 0.51	3.02 ± 0.36	2.53 ± 0.12	4.33 ± 0.85*
GSH-*S*-transferase (μmoles/min/mg)	0.30 ± 0.04	0.38 ± 0.05	0.11 ± 0.02	0.12 ± 0.02
GSH content (μmoles/g tissue)	6.74 ± 0.06	6.74 ± 0.06	5.04 ± 0.10	6.30 ± 0.25†

Results are means of three animals ± S.D.
* Significantly different from control, $P < 0.05$.
† Significantly different from control, $P < 0.01$.
‡ ND indicates that the activity was not detectable.

ton, CA) LS 9000 liquid scintillation spectrometer. The fraction containing more than 90% of the radioactivity, which was eluted in the void volume, was concentrated by Amicon (Lexington, MA) ultrafiltration (YM10 filter) to a volume of 1 ml. One hundred microliters of this concentrate was then analyzed by HPLC with the radioactivity being monitored continuously as described above. The 1-min fractions were collected and analyzed for cytochrome P-450 content.

Other assays. Cytochrome P-450 content of liver microsomes and HPLC fractions was measured by the method of Omura and Sato [33] while that of kidney microsomes was measured by the method of Matsubara *et al.* [34]. In all cases, maximum reduction was assured by thorough mixing of the cytochrome preparation with dithionite for 3–5 min, and then waiting 15 min to allow the reduced-CO-P-450 to reach its maximum absorbance before determining the difference spectrum. Glutathione concentration was determined according to Mitchell *et al.* [35]. Protein concentration was determined as described by Lowry *et al.* [36] using bovine serum albumin as standard. Paired Student's *t*-test was used to determine statistical significance.

RESULTS

The effects of BCP treatment on hepatic and renal drug-metabolizing enzymes are shown in Table 1. Cytochrome P-450 content was increased in the liver microsomes by treatment with BCP, but none of the cytochrome P-450-dependent enzymatic activities were increased significantly. Cytochrome P-450 content, glutathione concentration, glucuronyl transferase activity and cytochrome *c* reductase activity were increased slightly in the kidney. However, AHH activity was decreased to 50% of the control levels in both liver and kidney. The effects of BCP on hepatic cytochrome P-450 content and AHH activity were compared to those observed after treatment with PB, 3-MC and PCN (Table 2). BCP was the least effective inducer of total cytochrome P-450 content but caused the greatest decrease in AHH activity. PB and PCN had little effect on AHH activity, whereas 3-MC increased AHH activity more than 3-fold.

We examined the effects of BCP on the apparent V_{max} and K_m of AHH enzyme (Table 3 and Fig. 1). BCP treatment significantly decreased the V_{max} for AHH, whereas an increase was observed in 3-MC-treated rat liver microsomes. Phenobarbital produced a smaller decrease in the V_{max} which was not statistically significant. However, both BCP and PB decreased the K_m. When BCP was incubated *in vitro* with control microsomes, the V_{max} was decreased even more dramatically; however, the K_m was not affected. Table 4 shows the effect of BCP when

Table 2. Effects of PB, 3-MC and PCN on cytochrome P-450 content and AHH activity in rat liver microsomes

Treatment	Cytochrome P-450 (nmoles/mg)	AHH (nmoles/min/mg)
Control	1.17 ± 0.14	1.25 ± 0.07
BCP	1.51 ± 0.14*	0.63 ± 0.15†
PB	3.20 ± 0.01*	1.07 ± 0.02*
3-MC	2.37 ± 0.20*	3.95 ± 0.01†
PCN	2.46 ± 0.02†	1.29 ± 0.04

Results are means of more than three animals ± S.D.
* Significantly different from control, $P < 0.05$.
† Significantly different from control, $P < 0.01$.

Table 3. Effects of inducers and inhibitors on the kinetics of AHH in rat liver microsomes

Treatment	K_m (μM)	V_{max} (nmoles/min/mg)
Control	68.66 ± 1.05	2.86 ± 1.43
In vitro inhibition*	83.57 ± 29.20	0.30 ± 0.10†
BCP	12.43 ± 4.81‡	0.97 ± 0.17†
PB	7.67 ± 0.03‡	1.25 ± 0.16
3-MC	15.08 ± 1.57‡	10.20 ± 0.82‡

Results are means of more than three animals ± S.D.
* Inhibition of AAH activity using 10 μM BCP.
† Significantly different from control, $P < 0.05$.
‡ Significantly different from control, $P < 0.01$.

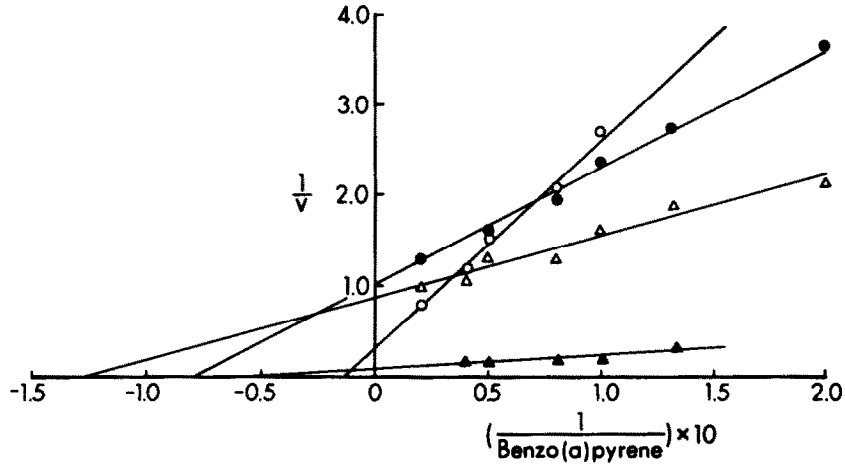

Fig. 1. Lineweaver–Burk plot of AHH activity from control and induced rat liver microsomes. The substrate concentration ranged from 5 to 50 μM. Key: (○) control, (●) BCP, (△) PB, and (▲) 3-MC.

added *in vitro* to microsomes from control, PB-, 3-MC- or BCP-pretreated rats. There was no difference in the results with or without NADPH. Addition of BCP to microsomes from control and BCP-treated rats resulted in a dose-dependent inhibition of AHH activity, with an EC_{50} of approximately 10 μM. AHH activity in microsomes from PB-induced rats was about half as sensitive to inhibition by BCP as the activity in microsomes from control or BCP-treated rats, while the activity in microsomes from 3-MC-induced rats was insensitive to inhibition by BCP. BCP also inhibited the demethylation of benzphetamine (54% in control and 39% in BCP microsomes), but a higher concentration (1 mM) was required.

To understand better the role of cytochrome P-450 isozymes in the induction and inhibition by BCP, we separated forms of cytochrome P-450 into four major fractions by HPLC as shown in Fig. 2. The large peak absorbing at 405 nm and eluting between 20 and 30 min was due to non-cytochrome P-450 heme. At least 70% of that cytochrome P-450 applied to the column was recovered. An increase in peak III was observed in microsomes from both BCP-treated (Fig. 2B) and PB-treated (Fig. 2C) rats compared to that of control rats (Fig. 2A). The chromatographic profile for the 3-MC-treated microsomes was clearly distinct from the others, resulting in an increase in peak IV. The CO-reduced difference spectrum had a maximum absorbance at 448 nm (data not shown). Although the cytochrome P-450 content in liver microsomes was increased after treatment with PCN, the chromatographic profile for PCN-treated liver microsomes was not distinct from that of control liver microsomes (Fig. 2E). In the kidney, only one cytochrome P-450 fraction was observed on HPLC. This fraction had a retention time similar to that of peak III from liver microsomes (Fig. 3) and increased approximately 2-fold after BCP treatment.

Electrophoretic and immunostaining procedures were performed on microsomes from control, BCP-, PB-, PCN-, and 3-MC-treated rats and on the major HPLC fractions from BCP-, PB-, and 3-MC-induced

Table 4. Inhibition of AHH and benzphetamine N-demethylation by various concentrations of BCP in control and induced rat liver microsomes

	% Inhibition of AHH activity					
Treatment	0 μM	5 μM	10 μM	15 μM	20 μM	100 μM
Control	0	25.9 ± 3.5	47.0 ± 3.5	61.6 ± 0.2	67.5 ± 0.1	95.0 ± 0.1
BCP	0	29.4 ± 4.0	48.2 ± 8.0	62.0 ± 0.1*	64.0 ± 0.1†	95.0 ± 0.1
PB	0		0		45.0 ± 0.5†	89.8 ± 0.5†
3-MC	0		0		0	0
	% Inhibition of benzphetamine N-demethylation					
	10 μM				1 mM	
Control	16.9 ± 8.6				54.2 ± 7.6	
BCP	14.2 ± 9.0				38.6 ± 16.1	

Results are means of three animals ± S.D.
* Significantly different from control, $P < 0.05$.
† Significantly different from control, $P < 0.01$.

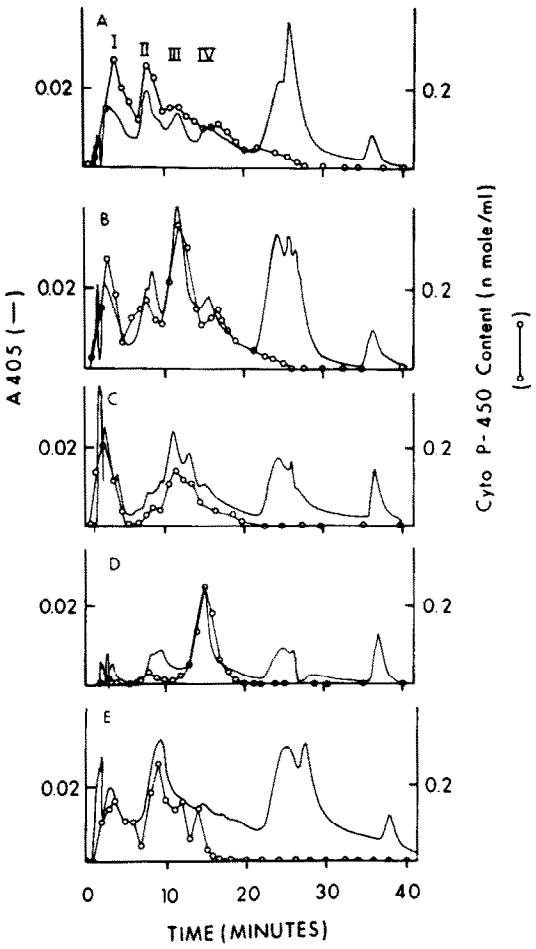

Fig. 2. HPLC separation of cytochromes P-450 from solubilized rat liver microsomes. Key: (A) 3.56 nmole control cytochrome P-450, (B) 3.54 nmole BCP-induced cytochrome P-450, (C) 2.53 nmole PB-induced cytochrome P-450, (D) 1.41 nmole 3-MC-induced cytochrome P-450, and (E) 2.83 nmole PCN-induced cytochrome P-450.

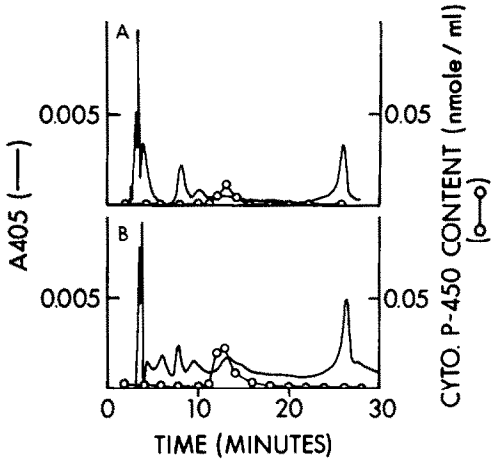

Fig. 3. HPLC separation of cytochromes P-450 from solubilized rat kidney microsomes. Key: (A) 0.053 nmole control cytochrome P-450, and (B) 0.11 nmole BCP-induced cytochrome P-450.

rats using an antibody to cytochrome P-450b (the major PB inducible form). Liver microsomes from BCP-treated Fischer rats contained increased amounts of two polypeptides which react with anti-P-450b isolated from PB-treated Sprague–Dawley rats (Fig. 4). One of these polypeptides had an electrophoretic mobility identical to pure P-450b, while the other had a mobility identical to a second immunochemically similar form of P-450 (P-450e) [37] found in both control and PB-treated rats. The mobilities of these proteins were identical in Fischer and Sprague–Dawley rats (not shown). Fraction III from PB-induced rats contained P-450b. Fraction III from BCP-induced rats also contains P-450b but to a lesser extent. We could not detect P-450b in kidney microsomes from control or BCP-treated rats.

A radioimmunoassay method was then employed to quantitate the microsomal content of cytochrome P-450b + P-450e, and the results are shown in Table 5. Cytochrome P-450b and P-450e constituted approximately 2% of the total cytochrome P-450 in control liver microsomes. BCP increased cytochrome P-450b + e by approximately 5-fold, while PB elicited a 45-fold increase in these isozymes. In kidney, $\leq 0.1\%$ of the total cytochrome P-450 was cytochrome P-450b + e in control rats. BCP had no effect on this enzyme in kidney, although PB may have produced a slight increase (2.8×).

It would be of interest to know which cytochrome P-450 isozymes account for the AHH activity which is inhibited by BCP. Solubilized microsomes were incubated with [^{14}C]BCP *in vitro*. The unreacted [^{14}C]BCP was then removed by chromatography on

Table 5. Cytochrome P-450b and cytochrome P-450e content in control, BCP- and PB-treated rat liver and kidney microsomes

Treatment	Liver		Kidney	
	Total P-450 (pmoles/mg)	P-450b + e (pmoles/mg)	Total P-450 (pmoles/mg)	P-450b + e (pmoles/mg)
Control	1170 ± 140	21.7 ± 1.5	200 ± 10	0.099 ± 0.030
BCP	1510 ± 140*	109.4 ± 16.5†	320 ± 36†	0.087 ± 0.031
PB	3200 ± 10†	959.5 ± 158.0†	430 ± 10†	0.276 ± 0.014†

Results are means of three animals ± S.D.
* Significantly different from control, $P < 0.05$.
† Significantly different from control, $P < 0.01$.

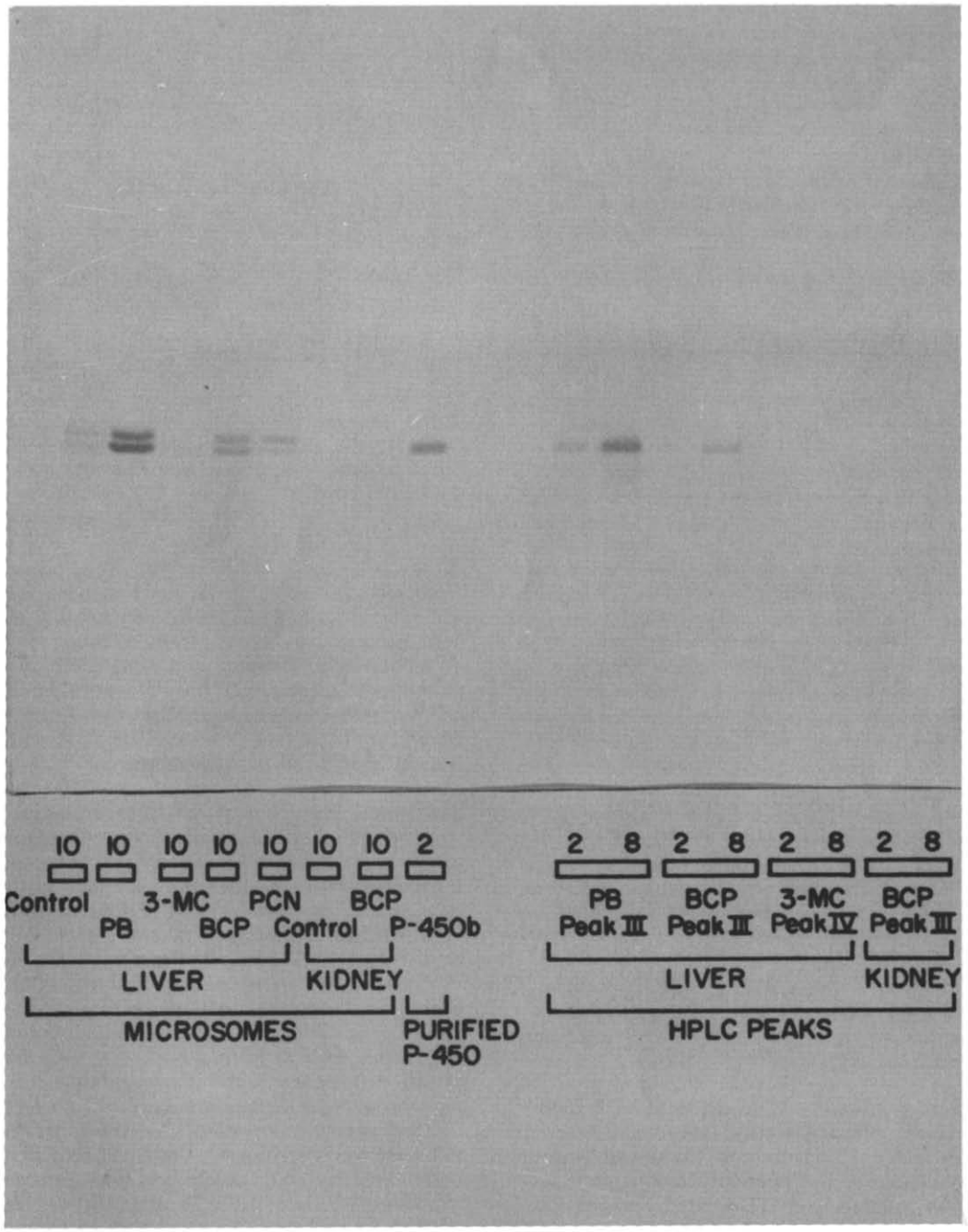

Fig. 4. Identification of cytochrome P-450b in control and induced rat liver and kidney microsomes by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by transfer of the protein to nitrocellulose and immunostaining of the proteins. Numbers indicate pmoles content of cytochrome P-450.

Sephadex G-25. The [^{14}C]BCP bound to microsomal proteins was then analyzed by HPLC. Any unbound [^{14}C]BCP elutes 3 min before the protein bound radioactivity under these conditions. Radioactivity was concentrated in peak II, presumably containing constitutive isozymes, instead of peak III which contains the inducible P-450b isozyme (Fig. 5). Using microsomes from control, PB-, or 3-MC-treated animals, [^{14}C]BCP was always found to elute with peak II. These results are consistent with *in vitro* inhibition studies, suggesting that BCP inhibits AHH and N-demethylation activities by binding preferentially to cytochrome P-450 isozyme(s) present in untreated microsomes. We also found that 10^{-6} M BCP produced a type I difference spectrum (peak, 390 nm; trough, 427 nm) in control rat liver microsomes, indicating that BCP binds to at least one form of cytochrome P-450 (data not shown).

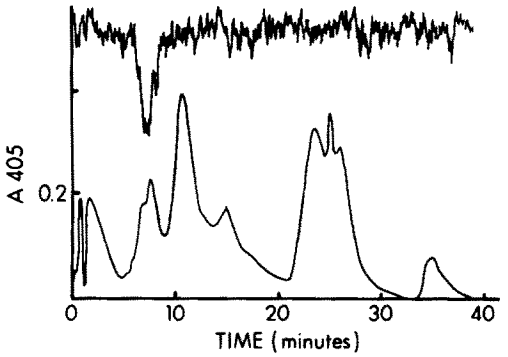

Fig. 5. Binding of [^{14}C]BCP (9 nCi) to BCP-treated liver microsomes. HPLC separation. Lower trace, absorbance at 405 nm; upper trace, radioactivity.

DISCUSSION

o-Benzyl-*p*-chlorophenol enjoys widespread use as a broad-spectrum germicide. It is moderately toxic to rodents after acute oral exposure and causes nephrotoxicity after repeated oral treatment [3]. Since BCP affects liver weight, produces renal lesions and is structurally similar to other halogenated phenolic compounds which affect drug metabolism, we examined its effects on both liver and renal drug-metabolizing enzymes. The dose used to treat the rats, 500 mg/kg, does not have acutely toxic effects, but repeated treatment with this dose does affect the kidney. In this study, involving 3 days of BCP treatment, no gross lesions were observed.

The effect of BCP on cytochrome P-450 in liver microsomes is a complex one involving both an increase in cytochrome P-450 content and a decrease in AHH activity. BCP increased cytochrome P-450b and P-450e, the major PB-inducible isozymes, indicating that it is a weak phenobarbital-type inducer. The content of cytochrome P-450b and P-450e increased 5- and 45-fold by BCP and PB respectively. Ryan *et al.* [37] have observed that phenobarbital induces two immunologically similar forms of cytochrome P-450, P-450b and P-450e, in Long–Evans rats. Cytochromes P-450b and P-450e from PB- or BCP-treated Fischer rats were immunologically and electrophoretically similar to cytochrome P-450b from Sprague–Dawley rats. Western blots of liver microsomes from control male Fischer rats indicated that P-450e was present in easily detectible amounts, while P-450b was much lower. However, in PB- and BCP-treated rats the two were present in roughly equivalent amounts, suggesting that cytochrome P-450b is induced preferentially over P-450e by both PB and BCP. The increase in P-450b and P-450e only accounted for approximately 25% of the increase in total P-450 after BCP administration. However, PB has been reported to increase at least two other P-450 isozymes, P-450$_{pcn}$ and P-450 PB-1 in rat liver [38, 39].

It has been reported that kidney cytochrome P-450 is induced by 3-MC type of inducers [32] but not by PB [40, 41]. However, in this study we observed a small increase in renal cytochrome P-450 content after both BCP and PB pretreatment (Table 5 and Fig. 3). This may simply be a strain difference since previous work on PB induction used Wistar rats and was based only on enzyme activity. Although the RIA indicated that renal cytochrome P-450b and P-450e were increased slightly by PB (Table 5), the increase in these isozymes was minimal (<0.1% of the total P-450). Moreover, neither cytochrome P-450b nor P-450e was detected on Western blots of kidney microsomes. The identity of the P-450 isozymes increased by Pb or BCP in kidney is not known, but does not appear to be P-450b or P-450e.

In vitro, BCP decreased the V_{max} but had no effect on the K_m value of AHH (Table 3), suggesting that BCP inhibited AHH noncompetitively [42]. Since BCP also decreased the V_{max} of AHH *in vivo*, we believe that the decrease in AHH activity *in vivo* may be due to noncompetitive inhibition of AHH by BCP (Table 3). Phenobarbital also produced a decrease in the V_{max} of AHH, although this decrease was smaller than that produced by BCP. This decrease after PB administration may reflect a decrease in a constitutive isozyme. The decrease in K_m after both BCP and PB probably indicates that both compounds change the pattern of P-450 isozymes in liver microsomes. However, BCP produces a larger decrease in AHH activity than PB, despite the fact that it is a much weaker inducer. Therefore, we believe that at least a portion of the decrease in AHH activity is due to direct inhibition of one or more P-450 isozymes. In fact, approximately 2 μmoles of BCP would have been present in the liver of rats administered BCP in this study.* However, we cannot rule out a decrease in the amount of a constitutive isozyme similar to the decrease observed in P-450-2c after administration of β-naphthoflavone or Aroclor 1254 [39].

Inhibition of cytochrome P-450 activities by other phenolic compounds has been reported. Miller *et al.* [43] demonstrated that hexachlorophene binds covalently to cytochrome P-450 in rats. Binding to cytochrome P-450 species was also observed by Yang *et al.* [44], who found that butyl hydroxyanisole (BHA), a phenolic antioxidant, was able to bind cytochrome P-448 (P-450c), which is the major cytochrome P-450 isozyme responsible for AHH activity, and reduce the mutagenicity of xenobiotics.

Radioactive BCP, after incubation with solubilized microsomes, chromatographed with a cytochrome P-450 fraction which was present in control and treated rats. This may suggest that BCP inhibits AHH activity by binding tightly to a constitutive cytochrome P-450 isozyme found in this fraction (fraction II). The interaction of BCP with a constitutive cytochrome P-450 species is also supported by the production of a type I spectrum [45] with control microsomes and by the preferential inhibition *in vitro* of AHH activity in control microsomes compared to liver microsomes from PB- or MC-treated animals.

Daum *et al.* [2] reported that BCP inhibits UDP-glucuronyl transferase activity *in vitro* but did not detect any inhibition after 3 days in an *in vivo* study. They hypothesized that the dose for the *in vivo* study was not enough to inhibit UDP-glucuronyl

* L. R. Kao and L. S. Birnbaum, manuscript submitted for publication.

transferase activity. We also failed to observe any change in hepatic UDP-glucuronyl transferase activity after *in vivo* treatment with BCP.

Similarly, inhibition of benzphetamine N-demethylation could explain the lack of significant induction of benzphetamine *N*-demethylase activity despite the 5-fold induction of cytochrome P-450b + e seen after pretreatment with BCP. However, this relatively weak induction of cytochrome P-450b + e resulting from BCP treatment may just be too low to result in the detection of an increase in the demethylation of benzphetamine.

The decrease in apparent K_m value for AHH after pretreatment with PB is not consistent with the K_m value reported by Alvares *et al.* [46]. However, the difference in apparent K_m value may be due to differences in protein concentration used in the different studies as reported by Hansen and Fouts [47] and Cumps *et al.* [48].

Increases in glutathione transferase and glutathione concentration can serve as protective mechanisms against liver and kidney toxicity induced by xenobiotics [36, 49]. In this study no change of glutathione concentration or glutathione transferase activity was found in the liver after treatment with BCP. In addition, BCP was shown to be a weak phenobarbital-type inducer, inducing P-450b, P-450e, and cytochrome *c* reductase. However, it decreased certain P-450 enzymatic activities preferentially, in particular, constitutive AHH activity. A decrease could also be demonstrated *in vitro*. In kidney, BCP increased total cytochrome P-450 content, cytochrome *c* reductase and glucuronyl transferase concomitant with a decrease in AHH activity. The increase in P-450 content could not be attributed to P-450b or P-450e but may reflect induction of a different isozyme. Whether these factors play a role in the nephrotoxicity observed after repeated exposure [3] requires further investigation.

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