Biochemical Pharmacology, Vol. 33, No. 24, pp. 3941–3946, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00

2,2-DIMETHYL-5-*t*-BUTYL-1,3-BENZODIOXOLE: AN UNUSUAL INDUCER OF MICROSOMAL ENZYMES

JON C. COOK and ERNEST HODGSON*
Interdepartmental Toxicology Program, North Carolina State University, Raleigh, NC 27695, U.S.A.

(*Received* 19 *December* 1983; *accepted* 12 *April* 1984)

Abstract—Our previous studies have shown that 2,2-dimethyl-5-*t*-butyl-1,3-benzodioxole (DBBD), a methylenedioxyphenyl (MDP) analog in which the methylene hydrogens have been replaced by methyl groups, does not form an inhibitory complex with cytochrome P-450 nor induce this cytochrome. However, in the present experiments, DBBD-treated male Dub:ICR mice showed an increase in NADPH-dependent cytochrome *c* (P-450) reductase and epoxide hydrolase activity. This separation of cytochrome P-450 induction from the induction of epoxide hydrolase and NADPH-dependent cytochrome *c* (P-450) reductase appears to be unique among inducers of xenobiotic metabolizing enzymes. In similar experiments, mice were treated with phenobarbital + DBBD or 3-methylcholanthrene + DBBD and the following parameters were measured: cytochrome P-450 content; NADPH-dependent reduction of cytochrome *c*; ethylmorphine and benzphetamine *N*-demethylase; 7-ethoxycoumarin *O*-deethylase; benzo[*a*]pyrene hydroxylase; and ethoxyresorufin *O*-deethylase. The microsomal proteins were examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Phenobarbital + DBBD treatment gave results which did not differ significantly from those obtained with phenobarbital alone. In contrast, cytochrome P-450 content and benzo[*a*]pyrene hydroxylase and ethoxyresorufin *O*-deethylase activities were less in mice treated with 3-methylcholanthrene + DBBD than in animals treated with 3-methylcholanthrene alone. SDS-PAGE confirmed that induction of cytochrome P-450 by 3-methylcholanthrene was reduced by DBBD, suggesting that the latter compound may be an antagonist to the Ah cytosolic receptor.

Methylenedioxyphenyl (MDP) compounds have a biphasic effect on cytochrome P-450 and monooxygenase activity, namely, inhibition followed by induction [1]. Inhibition is related to the formation of a stable MDP metabolite–cytochrome P-450 complex which is also responsible for the appearance of a Type III double soret optical difference spectrum [2, 3].

Fennell *et al.* [4] suggested that the formation of the MDP metabolite-cytochrome P-450 complex was the initiating event for cytochrome P-450 induction. To test this hypothesis, we [5] synthesized analogs of the MDP compound 5-tertiarybutyl-1,3-benzodioxole (BBD) and examined their effects on the microsomal cytochrome P-450-dependent monooxygenase system, following treatment *in vivo*. Substitution with methyl groups at the methylene carbon blocked both the formation of the MDP metabolite–cytochrome P-450 complex, *in vivo* and *in vitro*, and the induction of cytochrome P-450. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of microsomes from treated and control animals indicated that 2,2-dimethyl-5-tertiarybutyl-1,3-benzodioxole (DBBD) did, however, induce proteins other than cytochrome P-450.

Isosafrole, an MDP compound, can displace [^{3}H]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from its cytosolic receptor, suggesting that induction by MDP compounds may depend upon binding to the same cytosolic receptor [6]. Ohyama *et al.* [7] also described an isosafrole-inducible form of cytochrome P-450, which they named cytochrome P_2-450. They concluded that the induction of cytochrome P_2-450 is mediated by the cytosolic receptor for aromatic hydrocarbon hydroxylase (Ah). The mechanism for aromatic hydrocarbon induction has been the subject of numerous reviews (e.g. Refs. 8 and 9); the hypothesis enjoying the most support includes the following events. The aromatic hydrocarbon moves passively across the cell membrane [10] and, once inside, binds to a cytosolic Ah receptor [11, 12] coded for by the regulatory gene. Translocation of the inducer–receptor complex into the nucleus [13, 14] and its interaction with structural genes stimulate transcription of those structural genes [15, 16] which code for the different forms of cytochrome P-450. Several other inducible enzyme activities, including NADPH cytochrome P-450 reductase and epoxide hydrolase, are not associated with the Ah locus [17, 18]. We [5] suggested that substitution of the methylene hydrogens of MDP compounds with methyl groups may block MDP binding to the cytosolic receptor, thus preventing induction of cytochrome P-450.

To test this hypothesis, we have measured the levels of hepatic microsomal enzymes, including some other than cytochrome P-450, following treatment of mice with BBD and its analogs. We have also determined the effect of coadministration of DBBD with either phenobarbital or 3-methylcholanthrene.

MATERIALS AND METHODS

Experimental animals and treatment protocol. Male 25–30 g Dub:ICR mice were obtained from Dominion Laboratories, Dublin, VA. The animals

* Address all correspondence to: Dr. Ernest Hodgson, Interdepartmental Toxicology Program, Campus Box 7613, North Carolina State University, Raleigh, NC 27695.

were caged in groups of five, housed in a facility with a 12-hr light/dark cycle, and were given Wayne Feed Blox F-6 and tap water *ad lib*. The mice were held for 1 week before dosing. The following doses were administered i.p. daily for 3 days: sodium phenobarbital, 80 mg/kg in 0.9% sodium chloride or corn oil; 3-methylcholanthrene, 20 mg/kg in corn oil; and BBD, 2-methyl-5-tertiarybutyl-1,3-benzodioxole (MBBD), and DBBD, 150 mg/kg in corn oil. Control groups received either 0.9% sodium chloride or corn oil. The phenobarbital in oil and phenobarbital + DBBD in oil were stirred continuously during dosing.

Preparation of microsomes. The animals were killed on day 4 by decapitation, and microsomes were prepared as previously described [5].

Enzyme assays. Benzphetamine *N*-demethylase and ethylmorphine *N*-demethylase activities were determined spectrophotometrically by the method of Nash [19] as modified by Cochin and Axelrod [20]. 7-Ethoxycoumarin *O*-deethylase activity was determined by the method of Aitio [21]. Benzo[*a*]-pyrene hydroxylase activity was determined by the method of Yang and Kicha [22] as modified by Denison *et al.* [23]. Epoxide hydrolase activity was determined by the method of Jerina *et al.* [24] using [^{14}C]-styrene oxide as the substrate. Ethoxyresorufin *O*-deethylase activity was determined fluorometrically by the method of Pohl and Fouts [25]. NADPH cytochrome P-450 reductase activity was measured by determining the rate of reduction of cytochrome *c* [26]. NADPH was used for benzo[*a*]pyrene hydroxylase and NADPH-dependent cytochrome *c* reductase assays. The following NADPH-regenerating system was used in other assays: 25 mM $NADP^+$; 200 mM glucose-6-phosphate, and 40 units/ml glucose-6-phosphate dehydrogenase. Fifty microliters of the NADPH-regenerating system was used per ml reaction volume. Time course experiments were run for each assay to ensure first-order kinetics.

Spectral analysis. All spectra were recorded with an Aminco DW-2 UV/VIS spectrophotometer at room temperature. Cytochrome P-450 content was measured according to the method of Omura and Satu [27].

Protein was determined by the method of Lowry *et al.* [28] using bovine serum albumin fraction V as the standard.

Electrophoresis. SDS-PAGE [29] was performed as previously described except that a 3% stacking gel was included [5].

Materials. BBD was synthesized by the method of Clark *et al.* [30]. MBBD and DBBD were synthesized by the method of Cole *et al.* [31]. Identity and purity were confirmed by NMR spectroscopy, determination of boiling points, and thin-layer chromatography. NADPH, $NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, bovine serum albumin, porcine heart fumarase, bovine liver catalase, bovine liver L-glutamic dehydrogenase, ovalbumin, horse cytochrome *c*, and benzo[*a*]pyrene were obtained from the Sigma Chemical Co., St. Louis, MS. 7-Ethoxyresorufin was obtained from the Pierce Chemical Co., Rockford, IL. Other chemicals and sources follow: SDS, Coomassie Brillant Blue R-250, and ammonium persulfate, Bio-Rad Laboratories, Richmond, CA; ethylmorphine HCl and benzphetamine HCl, Applied Science Laboratories, State College, PA; corn oil, ICN Nutritional Biochemicals, Cleveland, OH; sodium phenobarbital, Gaines Chemical Inc., Pennsville, NJ; 3-methylcholanthrene, Eastman Kodak Co., Rochester, NY; and 1-phenyl-1,2-ethanediol (styrene glycol), 7-ethoxycoumarin, and 7-hydroxycoumarin, Aldrich Chemical Co., Milwaukee, WI. [^{14}C]Styrene oxide was a gift from Dr. J. R. Bend, National Institute of Environmental Health Sciences. All other chemicals were of at least reagent grade quality and were obtained from either the Sigma Chemical Co. or the Aldrich Chemical Co.

Statistical analysis. All data are expressed as the mean of three or four experiments. Statistical significance was determined using an analysis of variance to compute the least significant difference, with $P < 0.05$ used as a criterion for significance [32].

RESULTS

2,2-Dimethyl-5-tertiarybutyl-1,3-benzodioxole induction of NADPH-dependent cytochrome c reductase and epoxide hydrolase. Phenobarbital, 3-methylcholanthrene, and BBD treatment of mice increased the hepatic cytochrome P-450 content, while the cytochrome P-450 content of MBBD- and DBBD-treated mice was not significantly different from that of control animals (Table 1). The concentration of cytochrome P-450 following BBD treatment was different from that of the control, MBBD- and DBBD-treated mice.

The data in Table 1 also show that phenobarbital, 3-methylcholanthrene, BBD, MBBD, or DBBD treatment of mice increased the hepatic NADPH-dependent cytochrome *c* reductase activity compared to control values. In addition, it shows that the activity in DBBD-treated mice was higher than that in either BBD- or MBBD-treated mice.

Phenobarbital and DBBD treatment increased the hepatic microsomal epoxide hydrolase activity, while treatment of mice with 3-methylcholanthrene, BBD and MBBD did not (Table 1). The epoxide hydrolase activity in DBBD-treated mice was higher than that following BBD or MBBD treatment. The data shown in Tables 1 and 2 were derived from experiments utilizing commercial corn oil which we now believe to have contained antioxidants. Antioxidant compounds are known to induce epoxide hydrolase [33]. The epoxide hydrolase activity for the oil control was similar to the saline control when ICN corn oil, which lacks antioxidants, was used. Regardless of the corn oil used, the conclusions were not different.

The data in Table 2 demonstrate that treatment of mice with phenobarbital or 3-methylcholanthrene increased hepatic benzo[*a*]pyrene hydroxylase activity to the same level with each compound. However, BBD-, MBBD-, or DBBD-treated mice were not different from one another or from the controls in hepatic benzo[*a*]pyrene hydroxylase activity. Similarly, mice treated with either phenobarbital or 3-methylcholanthrene showed an increase in 7-ethoxycoumarin *O*-deethylase activity, while the activity of hepatic microsomes from BBD-, MBBD-, and DBBD-treated mice were unaffected (Table 2).

Table 1. Effects of treatment with various inducing agents on hepatic microsomal cytochrome P-450 content, NADPH-dependent cytochrome *c* reductase and epoxide hydrolase

Treatment	Cytochrome P-450* (nmoles/mg protein)	NADPH-dependent cytochrome *c* reductase* (nmoles/min·mg protein)	Epoxide hydrolase* (nmoles/min·mg protein)
0.9% Sodium chloride	1.09	222.6	1.78
Phenobarbital in 0.9% sodium chloride	1.85†	422.9†	5.55†
Oil	1.18	204.0	3.24
3-Methylcholanthrene	1.46†	264.7†	4.52
5-Tertiarybutyl-1,3-benzodioxole (BBD)	1.47†	262.9†	4.59
2-Methyl-5-tertiarybutyl-1,3-benzodioxole (MBBD)	1.05	274.1†	3.02‡
2,2-Dimethyl-5-tertiary butyl-1,3-benzodioxole (DBBD)	1.04	367.0†,§	6.20†,§

* Values are expressed as a mean of three experiments. The average S.E. per compound was 0.11, 24.0 and 1.01 for cytochrome P-450 content, NADPH-dependent cytochrome *c* reductase, and epoxide hydrolase, respectively.
† $P < 0.05$ compared to their respective control value.
‡ $P < 0.05$ compared to BBD.
§ $P < 0.05$ compared to BBD and MBBD.

However, the 7-ethoxycoumarin *O*-deethylase activity for phenobarbital-treated mice was higher than that for animals treated with 3-methylcholanthrene.

2,2-Dimethyl-5-tertiarybutyl-1,3-benzodioxole antagonism of 3-methylcholanthrene induction of cytochrome P-450. Phenobarbital, phenobarbital + DBBD, 3-methylcholanthrene, and 3-methylcholanthrene + DBBD treatment of mice increased the hepatic cytochrome P-450 content, but DBBD alone did not when compared to the control (Table 3). The cytochrome P-450 contents for phenobarbital-treated, as compared to phenobarbital + DBBD-treated, mice were not different from one another; however, the 3-methylcholanthrene-treated mice had a higher cytochrome P-450 content than those treated with 3-methylcholanthrene + DBBD.

The data in Table 3 indicate that all the treatments except 3-methylcholanthrene elevated hepatic NADPH-dependent cytochrome *c* reductase activity. Phenobarbital + DBBD-treated and 3-methylcholanthrene + DBBD-treated mice had higher NADPH-dependent cytochrome *c* reductase activities than the corresponding phenobarbital- or 3-methylcholanthrene-treated mice.

Phenobarbital-treated mice had increased ethylmorphine- and benzphetamine-*N*-demethylase activities, but the activity of hepatic microsomes from DBBD-, 3-methylcholanthrene-, and 3-methylcholanthrene + DBBD-treated mice was not different from the control (Table 3). Ethylmorphine- and benzphetamine *N*-demethylase activities were the same for phenobarbital compared to phenobarbital + DBBD treatments or 3-methylcholanthrene compared to 3-methylcholanthrene + DBBD treatments.

Mice treated with phenobarbital, phenobarbital + DBBD, 3-methylcholanthrene, or 3-methylchol-

Table 2. Effects of treatment with various inducing agents on hepatic microsomal monooxygenase activities

Treatment	Benzo[*a*]pyrene hydroxylase* (nmoles/min·mg protein)	7-Ethoxycoumarin *O*-deethylase* (nmoles/min·mg protein)
0.9% Sodium chloride	1.48	2.57
Phenobarbital in 0.9% sodium chloride	4.11†	9.46†,‡
Oil	1.40	2.63
3-Methylcholanthrene	4.51†	5.31†
5-Tertiarybutyl-1,3-benzodioxole (BBD)	1.13	2.23
2-Methyl-5-tertiarybutyl-1,3-benzodioxole (MBBD)	1.36	2.42
2,2-Dimethyl-5-tertiary-butyl-1,3-benzodioxole (DBBD)	1.57	2.60

* Values are expressed as a mean of three experiments. The average S.E. per compound was 0.24 for benzo[*a*]pyrene hydroxylase and 0.94 for 7-ethoxycoumarin *O*-deethylase.
† $P < 0.05$ compared to their respective control value.
‡ $P < 0.05$ compared to 3-methylcholanthrene.

Table 3. Effects of treatment with various inducing agents on hepatic microsomal monooxygenase parameters

Treatment	Cytochrome P-450* (nmoles/mg protein)	NADPH-dependent cytochrome *c* reductase* (nmoles/min·mg protein)	Ethylmorphine *N*-demethylase† (nmoles/min·mg protein)	Benzphetamine *N*-demethylase† (nmoles/min·mg protein)
Oil	0.90	257.8	10.3	11.5
2,2-Dimethyl-5-tertiarybutyl-1,3-benzo dioxole (DBBD)	0.88	433.3‡	10.4	11.7
Phenobarbital in oil	1.70‡	572.2‡,§	38.6‡	42.6‡
Phenobarbital + DBBD in oil	1.60‡	625.0‡	36.2‡	41.0‡
3-Methylcholanthrene	1.25‡,‖	295.8‖	12.4	14.6
3-Methylcholanthrene + DBBD	1.04‡	486.6‡	10.6	12.0

* Values are expressed as a mean of four experiments. The average S.E. per compound was 0.08 for cytochrome P-450 content and 29.0 for NADPH-dependent cytochrome *c* reductase.

† Values are expressed as a mean of three experiments. The average S.E. per compound was 2.3 for ethylmorphine *N*-demethylase and 2.7 for benzphetamine *N*-demethylase.

‡ $P < 0.05$ compared to control value.

§ $P < 0.05$ compared to phenobarbital + DBBD.

‖ $P < 0.05$ compared to 3-methylcholanthrene + DBBD.

anthrene + DBBD had elevated hepatic microsomal 7-ethoxycoumarin *O*-deethylase activity (Table 4). Both the phenobarbital-treated and phenobarbital + DBBD-treated mice had monooxygenase activities greater than the 3-methycholanthrene and 3-methylcholanthrene + DBBD values. In addition, the monooxygenase activities of the phenobarbital compared to phenobarbital + DBBD or 3-methylcholanthrene compared to 3-methylcholanthrane + DBBD treatments were not different. The DBBD-treated mice were not different from the control with respect to hepatic 7-ethoxycoumarin *O*-deethylase activity.

The data in Table 4 also demonstrate that all of the treatments, except DBBD alone, increased the hepatic microsomal benzo[*a*]pyrene hydroxylase activity and ethoxyresorufin *O*-deethylase activity compared to the controls. The responses of the two enzymes to phenobarbital or phenobarbital + DBBD treatment were similar. However, benzo[*a*]pyrene hydroxylase induction was greater with phenobarbital treatments while ethoxyresorufin *O*-deethylase induction was greater with 3-methylcholanthrene treatments. 3-Methylcholanthrene treatment enhanced activity of both enzymes more than 3-methylcholanthrene + DBBD treatment.

The gel in Fig. 1 shows that the various treatments induced the following molecular weight bands: phenobarbital - 50,000, 53,000, and 54,000; DBBD - 53,000; phenobarbital + DBBD - 50,000, 53,000 53,000, and 54,000; 3-methylcholanthrene - 53,000 and 54,000; and 3-methylcholanthrene + DBBD - 53,000, 53,000 and 54,000. DBBD induced a 53,000 molecular weight band which is just below the phenobarbital and 3-methylcholanthrene 53,000 molecular weight bands. Although not apparent in this gel, DBBD also induced molecular weight bands around 70,000 and 78,000. The arrow in Fig. 1 points to the 54,000 molecular weight band region. The 3-methylcholanthrene + DBBD 54,000 molecular weight band had a decreased staining intensity compared to the 3-methylcholanthrene 54,000 molecular weight band.

DISCUSSION

The methylenedioxyphenyl analogs, MBBD and DBBD, do not induce cytochrome P-450 nor do they form a metabolite–cytochrome P-450 complex [5]. However, DBBD, but not MBBD, induces proteins other than cytochrome P-450 at the following molecular weights; 53,000, 70,000 and 78,000. Hepatic NADPH cytochrome P-450 reductase, purified in our laboratory from the same strain of mice, has a molecular weight around 78,000 [34]. The failure to induce cytochrome P-450 is consistent with the hypothesis of Fennell *et al.* [4] that metabolite–complex formation is a necessary prerequisite for induction. Using a homologous series of 4-*n*-alkyl ethers of 1,2-methylenedioxybenzene, Wilkinson and coworkers [35] found that their results were consistent with this relationship but offered no mechanistic explanation. An alternate hypothesis has been suggested, namely, that MBBD and DBBD do not induce cytochrome P-450 because they are unable to interact with a cytosolic receptor [5] such as the Ah

Table 4. Effects of simultaneous treatment with various inducing agents on hepatic microsomal monooxygenase activities

Treatment	7-Ethoxycoumarin *O*-deethylase* (nmoles/min·mg protein)	Benzo[*a*]pyrene hydroxylase* (nmoles/min·mg protein)	Ethoxyresorufin *O*-deethylase* (nmoles/min·mg protein)
Oil	2.53	1.22	0.42
2,2-Dimethyl-5-tertiarybutyl-1,3-benzodioxole (DBBD)	2.52	1.12	0.26
Phenobarbital in oil	10.84†,‡	3.48†,‡	0.93†,‡
Phenobarbital + DBBD in oil	11.18†,‡	3.70†,‡	0.78†,‡
3-Methylcholanthrene	5.15†	2.81†,§	2.47†,§
3-Methylcholanthrene + DBBD	4.71†	1.83†	1.33†

* Values are expressed as a mean of four experiments. The average S.E. per compound was 0.77, 0.25, and 0.13 for 7-ethoxycoumarin *O*-deethylase, benzo[*a*]pyrene hydroxylase, and ethyoxyresorufin *O*-deethylase respectively.
† $P < 0.05$ compared to control value.
‡ $P < 0.05$ compared to 3-methylcholanthrene and 3-methylcholanthrene + DBBD.
§ $P < 0.05$ compared to 3-methylcholanthrene + DBBD.

receptor. NADPH cytochrome P-450 reductase and epoxide hydrolase, as well as many other inducible enzyme activities, are not associated with the Ah locus [17, 18]. If this later hypothesis were correct, it would not be inconsistent for DBBD to induce NADPH cytochrome P-450 reductase and epoxide hydrolase without inducing cytochrome P-450.

DBBD did induce NADPH-dependent cytochrome *c* reductase and epoxide hydrolase activities which were higher than those of BBD, MBBD, or the controls (Table 1). The cytochrome P-450 content and the benzo[*a*]pyrene hydroxylase and ethoxycoumarin *O*-deethylase activities for MBBD- and DBBD-treated mice were not different from the control (Tables 1 and 2). These data and earlier work [5] demonstrate that DBBD is able to induce epoxide hydrolase and NADPH-dependent cytochrome *c* reductase without inducing cytochrome P-450.

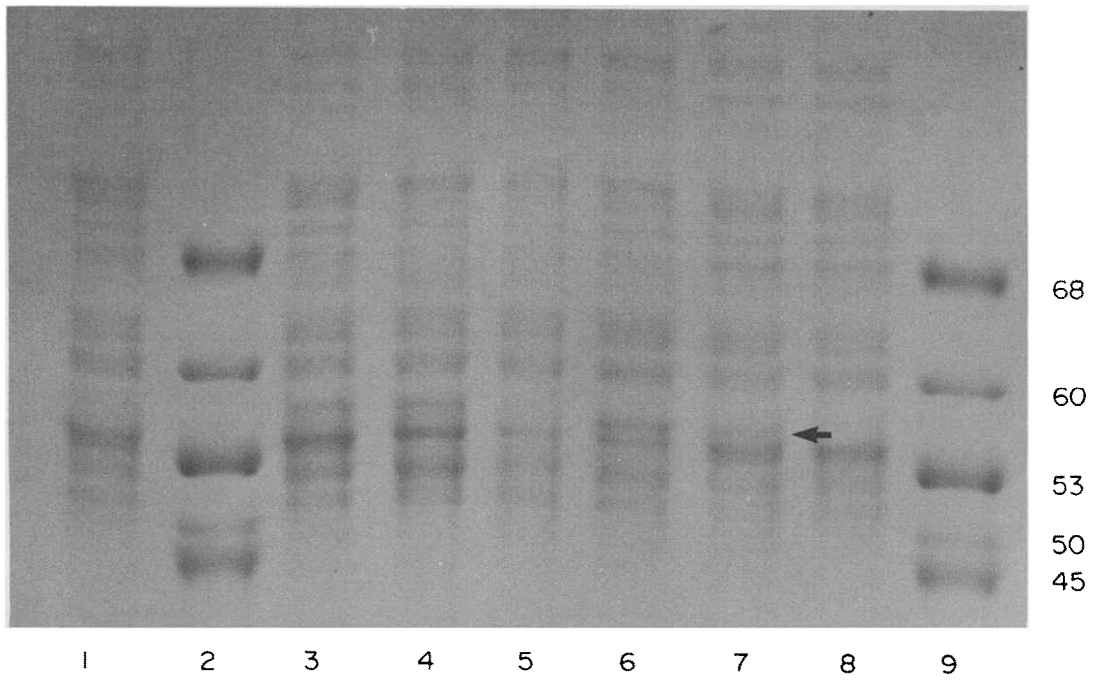

Fig. 1. SDS-PAGE of hepatic microsomes from Dub:ICR mice treated with various inducing agents. A mixture of molecular weight standards was applied to wells 2 and 9. Microsomes (20 μg protein) from treated mice were applied as indicated: (1) 2,2-dimethyl-5-tertiarybutyl-1,3-benzodioxole, (3) phenobarbital + 2,2-dimethyl-5-tertiarybutyl-1,3-benzodioxole, (4) phenobarbital, (5) oil, (6) 3-methylcholanthrene, (7) 3-methylcholanthrane + 2,2-dimethyl-5-tertiarybutyl-1,3-benzodioxole, and (8) 2,2-dimethyl-5-tertiarybutyl-1,3-benzodioxole. Migration is from the top of the gel to the bottom. Figures on the right represent molecular weight × 10^3. The arrow points to the 54,000 molecular weight band region. Note the decreased intensity of the 3-methylcholanthrene + DBBD 54,000 molecular weight band compared to 3-methylcholanthrene.

Phenobarbital is known not to interact with the Ah cytosolic receptor [11, 12], and attempts to establish the existence of a phenobarbital-type receptor have been inconclusive [36, 37]. However, there is evidence that isosafrole, an MDP compound, induces cytochrome P_2-450 via the Ah cytosolic receptor [6, 7]. If the BBD analogs are unable to induce cytochrome P-450 due to their inability to interact with the Ah cytosolic receptor [5], one would expect that the effect of coadministration of DBBD with either 3-methylcholanthrene or phenobarbital would not be different from either 3-methylcholanthrene or phenobarbital alone. Although this was the case with phenobarbital and all of the enzyme activities normally associated with phenobarbital, it was not the case for 3-methylcholanthrene (Tables 3 and 4). The cytochrome P-450, content and the benzo[*a*]pyrene hydroxylase and ethoxyresorufin *O*-deethylase activities for the 3-methylcholanthrene + DBBD-treated mice were all lower than those for mice treated with 3-methylcholanthrene alone. SDS-PAGE confirmed that 3-methylcholanthrene induction of cytochrome P-450 was reduced by DBBD. The 54,000 molecular weight band following 3-methylcholanthrene + DBBD treatment showed a decreased staining intensity compared to the same band following 3-methylcholanthrene alone (Fig. 1). No such reduction was seen for the phenobarbital and phenobarbital + DBBD-treated mice.

The ability of DBBD to attenuate 3-methylcholanthrene induction of cytochrome P-450 may be due to competition between 3-methylcholanthrene and DBBD for binding to the Ah cytosolic receptor, which would reduce the concentration of the 3-methylcholanthrene–Ah receptor complex reaching the nucleus. Tukey *et al.* [16] have shown a dose–response relationship between the amount of [^{3}H] TCDD–Ah receptor complex appearing in the nucleus and the amount of P_1-450 mRNA induced. No compound has been shown as yet to be an antagonist of the Ah cytosolic receptor [8].

If this is the case, the hypothesis that methylene carbon substitution blocks binding to the Ah receptor [5] must be modified to state that, although DBBD does bind to the receptor and can compete with 3-methylcholanthrene, it does not induce cytochrome P-450. The DBBD–Ah receptor complex may be unable to induce cytochrome P-450, either because it cannot be translocated into the nucleus or because it cannot be recognized by the Ah locus, possibly due to an unfavorable conformational change. Studies in progress using direct measurements of binding to the Ah receptor and other affinity binding proteins appear to confirm competition between DBBD and 3-methylcholanthrene.

Acknowledgements—Paper No. 9075 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC. Work was supported, in part, by PHS Grants ES-07046 and ES-00044 from the National Institute of Environmental Health Sciences. We are indebted to Dr. J. R. Bend, NIEHS, for the donation of [^{14}C]styrene oxide and the use of his laboratory.

REFERENCES

1. R. M. Philpot and E. Hodgson, *Chem. Biol. Interact.* **4**, 185 (1971/72).
2. R. M. Philpot and E. Hodgson, *Life Sci.* **10**, 503 (1971).
3. M. R. Franklin, *Xenobiotica* **1**, 581 (1971).
4. T. R. Fennell, B. C. Sweatman and J. W. Bridges, *Chem. Biol. Interact.* **31**, 189 (1980).
5. J. C. Cook and E. Hodgson, *Toxic. appl. Pharmac.* **68**, 131 (1983).
6. S. W. Bigelow and D. W. Nebert, *Toxic. Lett.* **10**, 109 (1982).
7. T. Ohyama, D. W. Nebert and M. Negishi, *J. biol. Chem.* **259**, 2675 (1984).
8. H. J. Eisen, R. R. Hannah, C. Legraverend, A. B. Okey and D. W. Nebert, in *Biochemical Actions of Hormones* (Ed. G. Litwock), p. 227. Academic Press, New York (1983).
9. D. W. Nebert, M. Negishi, M. A. Lang, L. M. Hjelmeland and H. J. Eisen, *Adv. Genet.* **21**, 1 (1982).
10. D. W. Nebert and L. L. Bausserman, *Ann. N.Y. Acad. Sci.* **179**, 561 (1971).
11. A. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
12. A. B. Okey, G. P. Bondy, M. E. Mason, G. F. Kahl, H. J. Eisen, T. M. Guenther and D. W. Nebert, *J. biol. Chem.* **254**, 11636 (1979).
13. W. F. Greenlee and A. Poland, *J. biol. Chem.* **254**, 9814 (1979).
14. A. B. Okey, G. P. Bondy, M. E. Mason, D. W. Nebert, C. J. Forester-Gibson, J. Muncan and M. J. Dulfresne, *J. biol. Chem.* **255**, 11415 (1980).
15. J. M. B. Carlstedt-Duke, U. B. Harnemo, B. Hogberg and J-A. Gustafsson, *Biochim. biophys. Acta* **672**, 131 (1981).
16. R. H. Tukey, R. R. Hannah, M. Negishi, D. W. Nebert and H. J. Eisen, *Cell* **31**, 275 (1982).
17. D. W. Nebert and N. M. Jensen, *CRC Crit. Rev. Biochem.* **6**, 383 (1979).
18. J. S. Felton, J. N. Ketley, W. B. Jakoby, A. Aitio, J. R. Bend and D. W. Nebert, *Molec. Pharmac.* **18**, 559 (1980).
19. T. Nash, *Biochem. J.* **55**, 416 (1953).
20. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
21. A. Aitio, *Analyt. Biochem.* **85**, 488 (1978).
22. C. S. Yang and L. P. Kicha, *Analyt. Biochem.* **84**, 154 (1978).
23. M. S. Denison, M. Murray and C. F. Wilkinson, *Analyt. Lett.* **16**, 381 (1983).
24. D. M. Jerina, P. M. Dansette, A. Y. H. Lu and W. Levin, *Molec. Pharmac.* **13**, 342 (1977).
25. R. J. Pohl and J. R. Fouts, *Analyt. Biochem.* **107**, 150 (1980).
26. J. L. Vermilion and M. J. Coon, *Biochem. biophys. Res. Commun.* **60**, 1315 (1974).
27. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
28. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
29. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
30. J. H. Clark, H. L. Holland and J. A. Miller, *Tetrahedron Lett.*, 3361 (1976).
31. E. R. Cole, G. Crank and H. T. Hai Minh, *Aust. J. Chem.* **33**, 675 (1980).
32. R. G. Steel and J. H. Torrie, *Principles and Procedures of Statistics. A Biometrical Approach.* McGraw-Hill, New York (1980).
33. Y. N. Cha, H. S. Heine and P. Moldeus, *Drug Metab. Dispos.* **10**, 434 (1982).
34. R. E. Tynes and E. Hodgson, *Biochem. Pharmac.* **32**, 3419 (1983).
35. M. Murray, C. F. Wilkinson, C. Marcus and C. E. Dube, *Molec. Pharmac.* **24**, 129 (1983).
36. A. Poland, I. Mak, E. Glover, R. J. Boatman, F. H. Ebetino and A. S. Kende, *Molec. Pharmac.* **18**, 571 (1980).
37. A. Poland, I. Mak and E. Glover, *Molec. Pharmac.* **20**, 442 (1981).