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METABOLISM OF BENZO[*a*]PYRENE BY GUINEA PIG ADRENAL AND HEPATIC MICROSOMES

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Abstract—Studies were carried out to compare the metabolism of benzo[*a*]pyrene (BP) by adrenal and hepatic microsomes obtained from adult male guinea pigs. Adrenal microsomes produced fluorescent metabolites (primarily phenols) approximately three to four times more rapidly than hepatic microsomes, but the differences in the rates were considerably smaller when total BP metabolism was assessed using an isotopic assay. The apparent discrepancy between the two assays is attributable to differences in the profiles of BP metabolites produced by adrenal and liver. Separation of metabolites by high pressure liquid chromatography revealed that adrenal microsomes converted BP to primarily a phenolic metabolite with a retention time identical to that of 3-hydroxy-BP. Liver microsomes, by contrast, produced approximately equal amounts of compounds co-chromatographing with 3-hydroxy-BP and BP-4,5-dihydrodiol. Small amounts of other metabolites were also produced by adrenal and hepatic microsomes. Liver microsomes catalyzed the conversion of BP to metabolites that became covalently bound to exogenous DNA. The amount of binding was dependent upon the duration of incubation and concentration of microsomal protein. Adrenal microsomes, by contrast, did not promote BP binding to DNA. Inhibition of microsomal epoxide hydratase activity with trichloropropene oxide (TCPO) blocked the formation of dihydrodiol metabolites of BP by adrenal and liver microsomes. In the presence of TCPO, liver microsomes produced large amounts of a BP metabolite co-chromatographing with BP-4,5-oxide. TCPO also increased the rate of production of DNA-binding metabolites by liver microsomes but had no effect on the formation of DNA-binding metabolites by adrenal microsomes. The results demonstrate major differences in the pathways of BP metabolism by guinea pig adrenal and hepatic microsomes. Although adrenal microsomes metabolize BP more rapidly than hepatic microsomes, far greater amounts of reactive metabolites are produced by the liver. Thus, adrenal metabolism of BP may be of little toxicological significance.

The extrahepatic metabolism of xenobiotics has been studied extensively in recent years (see Ref. 1). Metabolism in organs which represent portals of entry to the body for foreign compounds has received the greatest attention, in part because high concentrations of xenobiotics might be expected at such sites. However, biotransformation of xenobiotics has also been investigated in a large number of other tissues. One of the reasons for the interest in extrahepatic xenobiotic metabolism is the potential for the formation of reactive metabolites, producing locally toxic effects. It is well known that a large number of chemicals require metabolic activation for their toxicity to be manifested. Among the compounds requiring activation by microsomal enzymes are the polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (BP) [2–4]. The cytotoxic, carcinogenic, and mutagenic actions of BP are dependent upon the formation of highly reactive epoxide derivatives. Since epoxides can be subsequently converted to a number of relatively innocuous metabolites, the balance between epoxide production and further metabolism appears to be a major determinant of BP actions in various tissues.

Among the extrahepatic organs in which xenobiotic metabolism has been demonstrated are several endocrine glands, including the adrenal cortex [5–22]. In fact, some foreign compounds are metabolized more rapidly by adrenal than by hepatic microsomal preparations. The rates of adrenal xenobiotic metabolism are particularly high in the human and monkey fetus and in the guinea pig [7–15]. BP is one of the compounds metabolized by adrenal microsomal enzymes, but relatively little is known about the nature of the adrenal metabolites. Because of the importance of microsomal metabolism in the cytotoxic, mutagenic, and carcinogenic effects of polycyclic aromatic hydrocarbons (PAH), the following studies were carried out to compare the metabolism of BP by guinea pig adrenal and hepatic microsomes. The identities and relative quantities of the various metabolites were studied using high pressure liquid chromatography and the potential toxicity was assessed by the covalent binding of metabolites to DNA.

MATERIALS AND METHODS

Adult (600–700 g) male guinea pigs of the Magnun strain were obtained from Biological Systems (Toms River, NJ) and maintained under standardized conditions of light (6:00 a.m.–6:00 p.m) and temperature (22°) on a diet of Wayne Guinea Pig Diet and water *ad lib*. All animals were allowed at least 10 days to become acclimated to the housing conditions before use in experiments. Animals were decapitated between 8:00 and 9:00 a.m. Adrenals and livers were

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quickly removed and placed in cold 1.15% KCl containing 0.05 M Tris–HCl (pH 7.4). Tissues were homogenized and microsomes were obtained by differential centrifugation, as previously described [15]. Microsomal pellets were washed once with KCl–Tris buffer (pH 7.4) and resuspended to a concentration of 2–3 mg microsomal protein/ml.

Benzo[*a*]pyrene (BP) hydroxylase activity in adrenal and hepatic microsomes was estimated using two different techniques. The fluorometric assay described by Nebert and Gelboin [23] measures primarily phenolic metabolites, especially 3-hydroxy-BP and 9-hydroxy-BP [24]. For that assay, the incubation medium contained 2.7 mM $MgCl_2$, 0.05 M Tris–HCl (pH 7.4), 480 μM NADPH (Sigma Chemical Co., St. Louis, MO), 0.5 mg of bovine serum albumin (Sigma Chemical Co.) and 0.1 ml of adrenal or hepatic microsomes (equivalent to 0.25 and 0.5 mg protein, respectively) in a total volume of 1.0 ml. The reaction was initiated by the addition of 100 nmoles BP (Eastman-Kodak Co., Rochester, NY) in 50 μl acetone following a preincubation period of 3 min at 37°. Samples were incubated for 8 min at 37° under air, and the reaction was terminated by the addition of 1.0 ml of cold acetone. Quinine sulfate was calibrated against authentic 3-hydroxy-BP (provided by the chemical repository of the National Cancer Institute) and routinely used as the fluorescence standard. Metabolism of BP was also determined by the isotopic method of DePierre *et al.* [25], as modified by Van Cantfort *et al.* [26]. The latter assay provides an estimate of total BP metabolism. In this assay, incubation conditions were similar to those described above. For the isotopic assay, [G-^{3}H]BP was obtained from the Amersham Corp. (Arlington Heights, IL), further purified as described by DePierre *et al.* [25], and diluted with unlabeled BP to a specific activity of 12.5 μCi/μmole. Samples for scintillation counting were added to 10 ml Aquasol (New England Nuclear Corp., Boston, MA), and radioactivity was measured using a Beckman LS-9000 liquid scintillation counter. The amount of total [^{3}H]BP metabolites formed was determined by multiplying the amount of substrate added (100 nmoles) by that fraction of the total radioactivity recovered in the aqueous phase after hexane extraction of the incubation medium [26]. All values were corrected for the amount of radioactivity found in unincubated samples (zero-time controls) or in samples incubated without NADPH or incubated with heat-inactivated (boiled) microsomes. Similar results were obtained regardless of the type of blank employed. Quenching of samples was determined by internal standardization and values were corrected accordingly. Epoxide hydratase activity in adrenal and liver microsomes was determined using [7-^{3}H]styrene oxide (Amersham Corp.) as the substrate as described by Oesch *et al.* [27] except that the substrate was added in tetrahydrofuran. Samples incubated without microsomes or with heat-inactivated (boiled) microsomes were carried through the entire procedure to correct for any non-enzymatic conversion of styrene oxide to styrene glycol. Where indicated, the epoxide hydratase inhibitors, trichloropropene oxide and cyclohexene oxide (Aldrich Chemical Co., Milwaukee, WI), were added to the incubation flasks in small volumes of tetrahydrofuran, and the control flasks received the vehicle only. For all enzyme assays, the rates of product formation were linear with respect to both incubation times and microsomal protein concentrations.

Analysis of BP metabolism by high pressure liquid chromatography was done essentially as described by Yang *et al.* [28]. Microsomal incubations with BP (^{3}H-labeled or unlabeled) were carried out as described above for the BP hydroxylase assays except that the incubation volume was increased to 5.0 ml. After incubation, the acetone–ethyl acetate (1:2) extracts were filtered, dried down under nitrogen, and the residues dissolved in 200 μl of HPLC grade methanol (Fisher Scientific Co., Pittsburgh, PA). Aliquots (50 μl) were injected into a Waters high pressure liquid chromatograph (model 204, Waters Associates, Milford, MA) equipped with a model 440 absorbance detector, a model 660 solvent flow programmer, a model 730 data module (electronic integrator and data analysis system), and a model 710B automatic sample injector. BP and its metabolites were separated with a 30 cm Waters μBondapak C18 column (i.d. 3.9 mm). The column was eluted with a concave (curve 8) gradient of 65% methanol in water to 90% methanol over 45 min followed by 15 min at 90% methanol. The solvent flow was 1.0 ml/min and the eluent was monitored at 254 nm. In addition, when [^{3}H]BP (sp. act. 12.5 μCi/μmole) was used as substrate, 0.5-min fractions were collected and radioactivity was determined as described above. Metabolite retention times, using standards provided by the chemical repository of the National Cancer Institute, were the following: BP-9,10-diol, 7.4 min; BP-4,5-diol, 14.5 min; BP-7,8-diol, 17.2 min; BP-1,6-quinone, 24.4 min; BP-3,6-quinone, 25.1 min; BP-6,12-quinone, 26.8 min; BP-4,5-oxide, 30.1 min; 9-OH-BP, 35.7 min; 3-OH-BP, 38.8 min; and BP, 49.4 min. All of the peaks were fully resolved except for the quinones which overlapped to varying degrees from experiment to experiment. Metabolites were quantitated from the amounts of radioactivity under those peaks corresponding to standards and the specific activity of the [^{3}H]BP substrate. In addition, by establishing a series of ratios between the u.v.-peak area of each metabolite and the amount of radioactivity in the peak as described by Fahl *et al.* [29], and by establishing standard curves for the u.v. absorbance of known amounts of the authentic metabolites, the metabolites could be quantitated by integration of u.v. peaks. Similar results were obtained regardless of whether calculations were based upon radioactivity or u.v. absorbance. Incubation of heat-inactivated (boiled) microsomes was used to correct for the amount of radioactivity or u.v. absorbance in the absence of BP metabolism. Microsomal metabolites were also characterized by retention times on a Partisil ODS microparticulate column (30–100% methanol–water gradient) and were found to cochromatograph with the same standards as on the μBondapak C18 column. In addition, the u.v. and visible absorption spectra of the major metabolites produced by hepatic (BP-4,5-diol, 3-OH-BP) and adrenal (3-OH-BP) microsomes were

Table 1. Benzo[a]pyrene hydroxylase and epoxide hydratase activities in guinea pig adrenal and hepatic microsomes*

	Adrenal	Liver
Microsomal protein (mg/g tissue)	43.8 ± 3.6	30.7 ± 2.8†
Cytochrome P-450 (nmoles/mg protein)	1.7 ± 0.2	1.1 ± 0.2†
Benzo[a]pyrene hydroxylase (nmoles/min × mg protein) (10^{-1})		
Fluorometric	5.2 ± 0.4	1.4 ± 0.1†
Isotopic	7.6 ± 0.5	3.7 ± 0.4†
Epoxide hydratase (nmoles/min × mg protein)	2.9 ± 0.4	7.3 ± 1.3†

* Values are means ± S.E; ten to twelve animals per value.
† $P < 0.05$ (vs corresponding adrenal value).

compared with those of the authentic standards with which they cochromatographed, and were found to be identical.

The microsome-mediated covalent binding of [^{3}H]BP to DNA was determined essentially as described by Gelboin [30]. Incubation conditions were similar to those described above for the BP hydroxylase assays except that each flask also contained 2.0 mg of calf thymus DNA (Sigma Chemical Co.) in a total volume of 3.0 ml. The specific activity of the [^{3}H]BP was 125 μCi/μmole. After incubation, the DNA was isolated and exhaustively extracted as described by Gelboin [30]. The DNA pellets were then hydrolyzed in 0.5 N perchloric acid and aliquots were taken for DNA measurement by the diphenylamine reaction and for determination of radioactivity as described above. Incubations with heat-inactivated (boiled) microsomes or without NADPH were done to correct for the apparent binding in the absence of BP metabolism.

Cytochrome P-450 concentrations in adrenal and hepatic microsomes were determined as described by Omura and Sato [31] and microsomal protein concentrations were measured by the method of Lowry *et al.* [32] using bovine serum albumin as the standard.

RESULTS

Microsomal protein and cytochrome P-450 concentrations were higher in guinea pig adrenals than in livers (Table 1). As reported previously [15, 22],

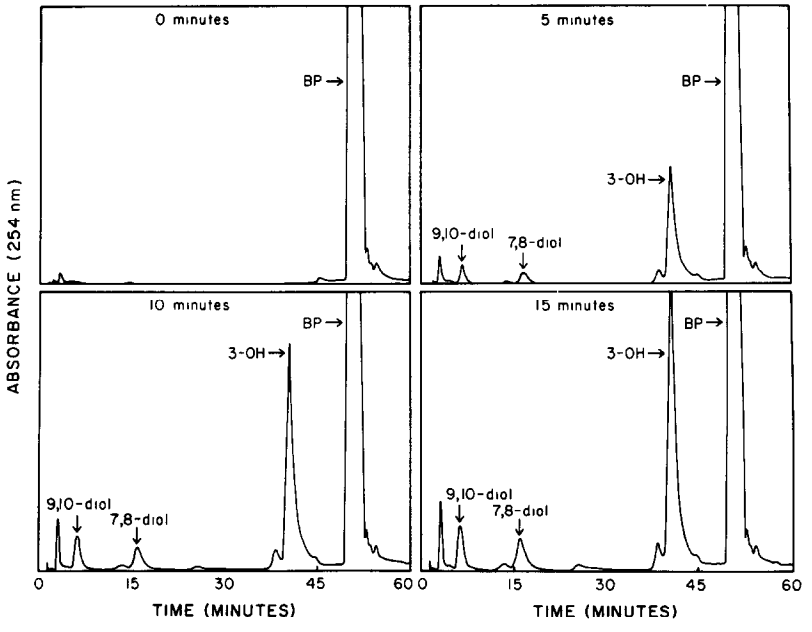

Fig. 1. Time-course of the metabolism of benzo[a]pyrene (BP) by guinea pig adrenal microsomes. Adrenal microsomes (0.25 mg protein/ml) were incubated with [^{3}H]BP and NADPH for 0, 5, 10, or 15 min as described in Materials and Methods. BP and its metabolites were separated by high pressure liquid chromatography and the eluent was monitored by u.v. absorbance at 254 nm. Peaks representing BP and its major metabolites (3-hydroxy-BP, 3-OH; BP-9,10-dihydrodiol, 9,10-diol; and BP-7,8-dihydrodiol, 7,8-diol) are indicated by the arrows.

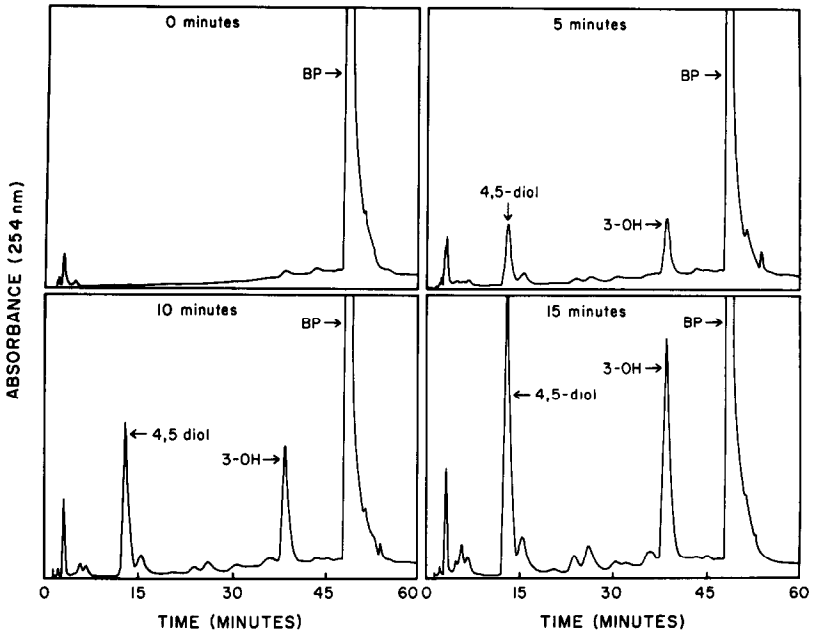

Fig. 2. Time-course of the metabolism of benzo[*a*]pyrene (BP) by guinea pig hepatic microsomes. Conditions were identical to those described for Fig. 1 except that hepatic microsomes (0.5 mg protein/ml) were incubated in place of adrenal microsomes. Peaks representing BP and its major metabolites (3-hydroxy-BP, 3-OH; and BP-4,5-dihydrodiol, 4,5-diol) are indicated by the arrows.

benzo[*a*]pyrene (BP) hydroxylase activity, when measured as the production of fluorescent metabolites (phenols), was far greater in adrenal than in hepatic microsomes (Table 1). However, when total BP metabolism was estimated using the isotopic assay described by Van Cantfort *et al.* [26], the tissue differences in the rates of BP metabolism were smaller than those obtained with the fluorometric assay. The results of the fluorometric assay indicated that the rate of adrenal BP metabolism was approximately four times greater than of hepatic metabolism, but adrenal enzyme activity was only twice as great when measured with the isotopic assay. These observations suggested that phenols (fluorescent metabolites) comprised a larger fraction of the total BP metabolites produced by adrenal microsomes than by liver microsomes. The activity of epoxide hydratase, the enzyme(s) catalyzing the conversion of epoxides to dihydrodiols, was significantly greater in hepatic than in adrenal microsomes.

High pressure liquid chromatography was used to tentatively identify the BP metabolites produced by adrenal and hepatic microsomes. Chromatograms illustrating the time-courses for the production of BP metabolites by adrenal and liver microsomes in the presence of NADPH are shown in Figs. 1 and 2 respectively. Adrenal microsomes converted BP primarily to a metabolite(s) with the same retention time as authentic 3-hydroxy-BP; smaller amounts of the 7,8-diol and 9,10-diol metabolites were also produced (Fig. 1). Liver microsomes, by contrast, converted BP to two major metabolites, the 4,5-diol and 3-hydroxy-BP (Fig. 2). The production of the various BP metabolites by liver and adrenal microsomes

Table 2. Metabolism of benzo]*a*]pyrene (BP) by guinea pig adrenal and hepatic microsomes*

	Adrenal metabolism		Hepatic metabolism	
Metabolite	Rate (pmoles/min × mg protein)	% of total†	Rate (pmoles/min × mg protein)	% of total†
3-Hydroxy-BP	478 ± 58	64 ± 7	125 ± 19‡	34 ± 5‡
9-Hydroxy-BP	42 ± 5	6 ± 1	31 ± 4	9 ± 2
Quinones	29 ± 4	4 ± 1	17 ± 3‡	5 ± 1
BP-7,8-diol	61 ± 7	8 ± 2	13 ± 3‡	4 ± 2
BP-4,5-diol	14 ± 3	2 ± 1	139 ± 18‡	39 ± 5‡
BP-9,10-diol	83 ± 7	10 ± 2	14 ± 3‡	4 ± 2‡

* Values are means ± S.E.; six to eight incubations per value.

† Expressed as a percentage of the total radioactivity or u.v.-absorbing material eluting from the HPLC column before BP (after correction for blanks).

‡ $P < 0.05$ (vs corresponding adrenal value).

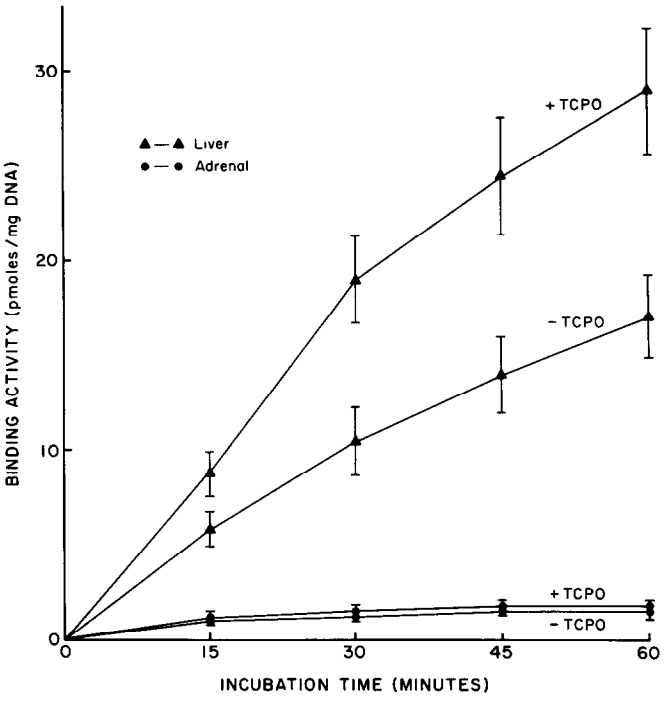

Fig. 3. Effects of trichloropropene oxide (TCPO) on the conversion of [^{3}H]benzo[a]pyrene (BP) to DNA-binding metabolites by guinea pig adrenal and hepatic microsomes. Microsomal preparations were incubated with [^{3}H]BP, calf thymus DNA, and NADPH in the presence or absence of TCPO (1.0 mM). Incubation conditions and covalent binding assays were as described in Materials and Methods. Incubation times were varied from 0 to 60 min and microsomal protein concentrations during incubation were approximately 0.5 mg/ml for both liver and adrenal preparations. All values were corrected for non-specific binding as indicated in Materials and Methods.

during incubation remained approximately linear for at least 15 min (Figs. 1 and 2). Subsequent incubations, therefore, were carried out for 10 min. A summary of the rates of production of adrenal and hepatic BP metabolites is presented in Table 2. Liver microsomes produced approximately equal amounts of 3-hydroxy-BP and BP-4,5-diol, those two metabolites comprising more than 70% of the total metabolites. Other liver metabolites were produced in only small quantities. 3-Hydroxy-BP accounted for nearly 65% of the total adrenal BP metabolites; none of the other adrenal metabolites comprised more than 10% of the total. The proportionately greater production of dihydrodiols by liver than by adrenal microsomes may be attributable, at least in part, to the higher level of epoxide hydratase activity in the liver (Table 1).

To determine if reactive intermediates were produced in the course of adrenal or hepatic BP metabolism, the formation of DNA-binding metabolites was evaluated. When liver microsomes were incubated with [^{3}H]BP and NADPH in the presence of calf thymus DNA, radioactivity became irreversibly bound to the DNA. The amount of binding increased with the duration of incubation for at least 60 min (Fig. 3) and was directly proportional to the concentration of microsomal protein (0.5 to 2.0 mg/ml) in the incubation flask (data not shown). Binding to DNA was not detectable when NADPH was omitted from the incubation flask. Very little binding was detectable when adrenal microsomes (0.5 to 4.0 mg protein/ml) were substituted for hepatic microsomes during the incubation period (Fig. 3).

If epoxides were responsible for the microsome-mediated covalent binding to DNA, inhibition of epoxide hydratase activity might increase the amount of binding by blocking the conversion of epoxides

Table 3. Effects of cyclohexene oxide (CHO) and trichloropropene oxide (TCPO) on adrenal and hepatic microsomal epoxide hydratase activities

	Epoxide hydratase activity*	
Inhibitor	Liver	Adrenal
TCPO		
0 μM	100	100
50 μM	48 ± 3	57 ± 4
200 μM	27 ± 2	33 ± 2
500 μM	14 ± 1	19 ± 2
1000 μM	8 ± 1	10 ± 1
CHO		
0 mM	100	100
0.2 mM	96 ± 8	97 ± 7
1.0 mM	74 ± 6	74 ± 5
10 mM	41 ± 3	36 ± 4
100 mM	10 ± 2	9 ± 2

* Values are mean percent of control ± S.E.; six to eight determinations per value.

to dihydrodiols. Two of the widely used epoxide hydratase inhibitors, trichloropropene oxide (TCPO) and cyclohexene oxide (CHO), had effects on hepatic and adrenal enzyme activities (Table 3). TCPO was the far more potent inhibitor in both tissues. At concentrations producing substantial inhibition of epoxide hydratase, CHO also decreased BP hydroxylase activity (as estimated using the isotopic assay). TCPO, at concentrations as high as 1.0 mM, had virtually no effect on BP hydroxylation. Therefore, because of its greater specificity, TCPO (1.0 mM) was used to evaluate the effects of inhibiting epoxide hydratase on the covalent binding to DNA and on the identity of BP metabolites.

As shown in Fig. 3, when TCPO was incubated with liver microsomes, the amount of covalent binding to DNA was increased at all incubation times. By contrast, TCPO had no significant effect on the low level of covalent binding catalyzed by adrenal microsomes. The effects of TCPO on the patterns of BP metabolism by liver and adrenal microsomes are shown in Fig. 4. TCPO decreased the production of dihydrodiol metabolites by adrenal microsomes, but otherwise had little effect on metabolism. In liver microsomes, TCPO blocked the production of BP-4,5-diol and caused the appearance of a metabolite with a retention time identical to that of authentic BP-4,5-oxide. High pressure liquid chromatographic fractions containing the metabolite were collected, and spectral analyses indicated that its u.v. spectrum was essentially identical to that of the BP-4,5-oxide standard.

DISCUSSION

The importance of metabolism in the cytotoxic, mutagenic, and carcinogenic effects of BP has led to extensive investigation of the pathways involved in BP metabolism. Most studies have focused on hepatic metabolism of BP, in part because of the quantitative importance of the liver in the overall disposition of xenobiotics. However, BP metabolism also occurs in many other tissues, including the adrenal cortex. Many hydrophobic xenobiotics tend to selectively accumulate at lipophilic sites such as the adrenal glands. Adrenal metabolism of BP or other PAH could be detrimental if reactive metabolites are produced, or if either the parent compound or metabolites interfere with the production of steroid hormones. Cytochrome P-450-containing mixed function oxygenases are involved in both the oxidation of BP and steroidogenesis, but the substrate specificity of adrenal microsomal cytochrome(s) P-450 has yet to be resolved.

The results presented in this paper indicate that the BP metabolites produced by guinea pig adrenal microsomes are qualitatively similar to those produced by subcellular fractions from various other tissues. However, the relative proportions of the various adrenal metabolites are somewhat unusual. The major adrenal product is 3-hydroxy-BP, constituting nearly two-thirds of the total metabolites. The production of disproportionately large amounts of this highly fluorescent phenol accounts for the very high rates of adrenal BP hydroxylation when enzyme activity is estimated by the production of fluorescent metabolites. Relatively small amounts of diols were produced by adrenal microsomes, an observation consistent with the low level of epoxide hydratase activity in the guinea pig adrenal. Quinones also constitute a very small fraction of the BP metabolites produced by guinea pig adrenal microsomes.

Adrenals from other species have also been found to metabolize BP. For example, Berry *et al.* [20] found that BP metabolism by human fetal adrenal

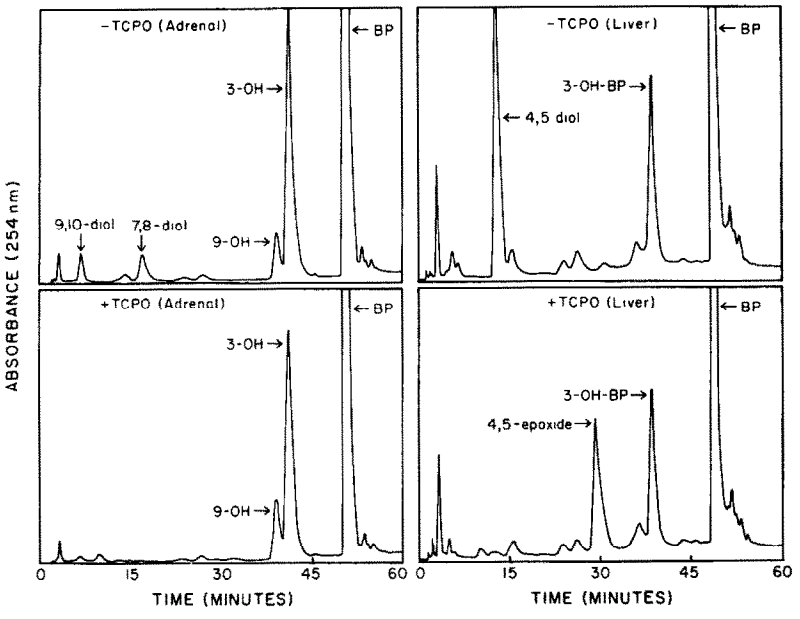

Fig. 4. Effects of trichloropropene oxide (TCPO) on the metabolism of benzo[*a*]pyrene (BP) by guinea pig adrenal and hepatic microsomes. Conditions were identical to those described in the legends of Figs. 1 and 2 except that incubations were done in the presence or absence of TCPO (1.0 mM).

microsomes resulted in the production of 3-hydroxy-BP. However, the rates of BP metabolism, when estimated isotopically following separation of metabolites by high pressure liquid chromatography, were far lower than when products were measured fluorometrically. The reason for the lack of correlation between the two assays was not known. Pelkonen and Karki [18], using thin-layer chromatography, found that human fetal adrenal microsomes converted BP to diols as well as phenols, but metabolite identification was only tentative. Adrenal homogenates from untreated pregnant rats produced 3-hydroxy-BP and 9-hydroxy-BP as the major metabolites of BP, but treatment with 2,3,7,8-tetrachlorodibenzo-*p*-diopin (TCDD) produced a 5-fold increase in the production of 9-hydroxy-BP without affecting 3-hydroxy-BP levels [19]. Menard *et al.* [33] reported that 3-hydroxy-BP, 9-hydroxy-BP, and BP-9,10-diol were the major metabolites of BP produced by rat and mouse adrenals. Rydstrom *et al.* [34] also found that 3-hydroxy-BP and 9-hydroxy-BP were produced by rat adrenal microsomes, each constituting more than 40% of the total metabolites. The results obtained in these relatively few studies indicate that adrenal metabolism of BP, like that in other tissues, varies somewhat from species to species, probably reflecting species differences in the catalytic properties of adrenal microsomal cytochromes P-450. Within species, strain differences may also influence the pattern of adrenal BP metabolism, and may account for some of the apparently divergent reports in the literature.

Hepatic and adrenal metabolism of BP in the guinea pig differed considerably. Liver microsomes produced approximately equal amounts of 3-hydroxy-BP and BP-4,5-diol, each accounting for approximately one-third of the total metabolites. This pattern of metabolism bears little resemblance to those previously described for rat and mouse liver microsomes, but is very similar to that reported for hamster liver [35, 36]. The higher percentage of diols produced by guinea pig liver microsomes than by adrenal microsomes may result in part from the higher hepatic epoxide hydratase activity (Table 1). Of the diols detected, the liver produced primarily the K-region diol (4,5-diol) and the adrenal non-K-region diols (7,8- and 9,10-diols), suggesting that different cytochromes P-450 catalyzed BP metabolism in each tissue. It has been shown that the position-specific oxidation of BP is mediated by different forms of cytochrome P-450 [37].

The pattern of BP metabolism probably explains the high rate of covalent binding to DNA mediated by guinea pig liver microsomes (Fig. 4). The amount of binding was approximately two to three times greater than that mediated by comparable amounts of rat liver microsomal protein (unpublished observations). In addition, Baker *et al.* [38] have reported that S9 fractions from guinea pig livers are more active than those from rat livers in converting BP to mutagenic metabolites. The species differences are probably attributable to the nature of the metabolites rather than the rates of BP oxidation, since BP hydroxylase activity is greater in rat than in guinea pig liver microsomes [38]. It seems likely that BP-4,5-epoxide contributes significantly to both the covalent binding and mutagenic activity resulting from BP metabolism by guinea pig liver microsomes. BP-4,5-epoxide is the immediate precursor to BP-4,5-diol (see Refs. 2–4), a major product of BP metabolism by guinea pig liver microsomes. In addition, it has been found previously that BP-4,5-epoxide has mutagenic and carcinogenic activity and covalently binds to tissue macromolecules [38–40].

BP-4,5-epoxide has been isolated as a product of BP metabolism by rat liver microsomes [41–43] but was not demonstrable in our control incubations (Fig. 4). However, when epoxide hydratase activity was decreased by TCPO, blocking the production of diols, large amounts of BP-4,5-epoxide were isolated. TCPO also increased the amount of covalent binding to DNA, apparently resulting from an increase in the steady-state levels of BP-4,5-epoxide. Other investigators [44–46] have found that epoxide hydratase inhibitors enhance the covalent binding of BP to DNA catalyzed by rat liver microsomes, presumably also the result of increased steady-state levels of epoxides. Interestingly, several epoxide hydratase inhibitors were shown to decrease the total oxidation of BP by rat liver microsomes [29, 44], but we found that TCPO, at concentrations as high as 1.0 mM, did not affect the rate of BP metabolism by guinea pig liver or adrenal microsomes.

In contrast to the high levels of covalent binding mediated by guinea pig liver microsomes, little, if any, binding was catalyzed by adrenal microsomes. Even the addition of TCPO to the adrenal incubations had no effect on the amount of covalent binding to DNA. In other experiments, we were also unable to demonstrate covalent binding to adrenal microsomal protein. Jones *et al.* [47] similarly found that human fetal adrenal microsomes were relatively inactive in converting BP to mutagenic metabolites despite high rates of BP metabolism in such preparations. Cytochrome P-450-containing mixed function oxygenases are found in adrenal mitochondria as well as in microsomes, but mitochondria catalyze little, if any, BP metabolism (unpublished observations). These observations suggest that adrenal metabolism of BP does not generate significant quantities of reactive metabolites although the possibility of binding to some adrenal macromolecule(s) cannot be excluded at this time. 3-Hydroxy-BP, the major BP metabolite produced by both guinea pig and human fetal adrenal microsomes, may be derived in part by rearrangement of an unstable 2,3-epoxide intermediate [48], but direct hydroxylation without epoxide intermediates may also contribute to 3-hydroxy-BP production (see Refs. 2–4). If the latter mechanism dominated in adrenal microsomes, only small amounts of reactive metabolites would be produced. In any case, our observations and those of Jones *et al.* [47] suggest that adrenal metabolism may serve largely to detoxify rather than activate BP. This conclusion is supported by the absence of any known cytotoxic, mutagenic, or carcinogenic effects of BP on the adrenal cortex.

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