Metabolism of Polycyclic Aromatic Hydrocarbons and Covalent Binding of Metabolites to Protein in Rat Adrenal Gland

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ABSTRACT

The metabolism of the carcinogenic and adrenocorticotrophic polycyclic aromatic hydrocarbon 7,12-dimethylbenz(a)anthracene in rat adrenals was investigated. Both 7,12-dimethylbenz(a)anthracene and benzo(a)pyrene, which also is a well-known carcinogen but has no short-term effects on rat adrenals, appear to be metabolized by one common type of cytochrome P-450-dependent monooxygenase localized in the endoplasmic reticulum. Studies of the kinetic properties of this cytochrome P-450 reveal that the K_m values for 7,12-dimethylbenz(a)anthracene and benzo(a)pyrene are lower than 3 μM. Identification of metabolites indicates that, with both 7,12-dimethylbenz(a)anthracene and benzo(a)pyrene, phenols and diols were formed the relative rates of formation of which were markedly influenced by the epoxide hydrolase inhibitor cyclohexane oxide, suggesting that epoxides are intermediate metabolites. Added or endogenous microsomal glutathione S-transferase B had little or no effect on the distribution of metabolites. A rather selective binding of metabolites of 7,12-dimethylbenz(a)anthracene to soluble and microsomal proteins was demonstrated. The adrenal cytochrome P-450 involved in the conversion of these polycyclic aromatic hydrocarbons appears to be unrelated to those responsible for the synthesis of mineralocorticoids and glucocorticoids from cholesterol. Among androgens and estrogens, estradiol proved to be the metabolite of DMBA3 which, for unknown reasons, may have a relatively selective localization to soluble and microsomal proteins. The assay of these polycyclic aromatic hydrocarbons appears to be unrelated to those responsible for the synthesis of monooxygenases of DMBA3 and 2,2-bis(4-chlorophenyl,2-chlorophenyl)-1,1-dichloroethane but not BP or other known polycyclic hydrocarbons cause necrosis of the 2 inner zones of the cortex, the zona fasciculata and the zona reticularis, possibly mediated by steroid-metabolizing hydroxylases (2, 5, 27, 28, 36). In the rat, the adrenal xenobiotic-metabolizing system is not inducible by polycyclic aromatic hydrocarbons (12, 13, 18, 22). Specific effects of DMBA have also been observed in testes and ovaries (1, 27, 35), and it appears that the polycyclic hydrocarbon-metabolizing cytochrome P-450 systems in all these organs are regulated by pituitary peptide hormones (22, 32). The active adrenocorticotrophic metabolite of DMBA has been proposed to be 7-OHM-12-MBA and/or 3,4-dihydro-3,4-dihydroxy-7-hydroxymethyl-12-methylbenzo(a)anthracene or reactive products of these substances formed after further metabolism (8, 27, 56). The present study forms part of an investigation that aims at elucidating the mechanisms of metabolism and action of carcinogenic polycyclic hydrocarbons in steroidogenic organs, in particular, adrenals and gonads. Evidence is presented to indicate that in rat adrenals DMBA and BP are metabolized by a monooxygenase localized in the endoplasmic reticulum which resembles the liver microsomal cytochrome P-448 system. This metabolism generates reactive metabolites that bind covalently and, in the case of DMBA, rather selectively to soluble and microsomal proteins. A preliminary account of these results has been published elsewhere (36, 44).

INTRODUCTION

Certain aromatic hydrocarbons are well-known and potent carcinogens which, for unknown reasons, may have a relatively high specificity with respect to the target organ, e.g., liver, lung, skin, and mammary glands (for reviews, see Refs. 10, 16, 27, and 34). The general mechanism of action of this class of compounds is believed to involve the formation of an epoxide catalyzed by one or several cytochrome P-450-dependent monooxygenases localized in the endoplasmic reticulum. Reactive epoxides as well as radical derivatives and peroxides are believed to be important intermediates in the generation of cancer cells. Other products are diols, phenols, and conjugates, the relative rates of formation of which are determined by the activities of the monooxygenases, epoxide hydrolase, conjugation systems (e.g., glutathione S-transferases), and nonenzymatic reactions, respectively (7, 14, 15, 23). In some organs, metabolism of chemical carcinogens results in destruction of mitochondria and necrosis rather than cancer, possibly because these organs have a relatively large amount of its monooxygenase systems localized in the mitochondria. However, it is generally agreed that the basic mechanisms responsible for the generation of cancer and necrosis following activation of xenobiotics are similar (4, 30, 47, 53).

An important and thus far unsolved problem in chemical carcinogenesis, particularly in steroid-metabolizing organs, concerns the identity and biological function of the cytochrome P-450 systems responsible for the activation of polycyclic hydrocarbons to reactive intermediates. In adrenals, DMBA and 2,2-bis(4-chlorophenyl,2-chlorophenyl)-1,1-dichloroethane but not BP or other known polycyclic hydrocarbons cause necrosis of the 2 inner zones of the cortex, the zona fasciculata and the zona reticularis, possibly mediated by steroid-metabolizing hydroxylases (2, 5, 27, 28, 36). In the rat, the adrenal xenobiotic-metabolizing system is not inducible by polycyclic aromatic hydrocarbons (12, 13, 18, 22). Specific effects of DMBA have also been observed in testes and ovaries (1, 27, 35), and it appears that the polycyclic hydrocarbon-metabolizing cytochrome P-450 systems in all these organs are regulated by pituitary peptide hormones (22, 32). The active adrenocorticotropic metabolite of DMBA has been proposed to be 7-OHM-12-MBA and/or 3,4-dihydro-3,4-dihydroxy-7-hydroxymethyl-12-methylbenzo(a)anthracene or reactive products of these substances formed after further metabolism (8, 27, 56). The present study forms part of an investigation that aims at elucidating the mechanisms of metabolism and action of carcinogenic polycyclic hydrocarbons in steroidogenic organs, in particular, adrenals and gonads. Evidence is presented to indicate that in rat adrenals DMBA and BP are metabolized by a monooxygenase localized in the endoplasmic reticulum which resembles the liver microsomal cytochrome P-448 system. This metabolism generates reactive metabolites that bind covalently and, in the case of DMBA, rather selectively to soluble and microsomal proteins. A preliminary account of these results has been published elsewhere (36, 44).

MATERIALS AND METHODS

Adrenal glands were removed from male Sprague-Dawley rats (>180 g) after decapitation and kept frozen until used. Fractionation of the glands was carried out essentially as described previously (41). Sub...
cellular fractions were used immediately after preparation or frozen, even though slightly decreased monoxygenase activities were normally observed after freezing. Incubations for estimating DMBA and BP metabolism were carried out in a medium composed of 2 mM isocitrate, 6 mM MgCl₂, 30 μg isocitrate dehydrogenase, 0.4 μg rotenone, 1 to 50 μM \((\text{dimethyl}^{-14}C)[\text{DMBA}]\) or \([\text{14C}]\text{BP}\), 2 mM EDTA, 15 mM potassium chloride, 5 mM potassium phosphate, and 50 mM Tris-HCl (pH 7.0), in a final volume of 0.2, 0.5, or 1 ml. After preincubation for 5 min at 30°, the reaction was started by the addition of 1.5 mM NADPH and stopped after 60 min with 4 ml ethyl acetate. Analysis of the extent of conversion was achieved by 2 extractions of metabolites (and unchanged DMBA or BP) with ethyl acetate containing 0.04% (w/v) butylated hydroxytoluene, followed by thin-layer chromatography on silica gel with toluene as eluant. Alternatively, the conversion was assayed by the distribution method described by van Cantfort et al. (54), which was found to be applicable also for DMBA. The thin-layer and the distribution assays differed by less than ±15%. High-pressure liquid chromatographic analysis of ethyl acetate-extractable DMBA metabolites was carried out by a Waters Associates instrument using a Nucleosil C18 column equilibrated with 35% methanol and eluted with a 35 to 95% methanol gradient during a period of 70 min, including a hold at 50% methanol for 10 min and a second hold at 95% methanol for 10 min. Covalent binding of DMBA metabolites was measured by precipitation of proteins on filter paper discs according to the method of Wallin et al. (55) or after precipitation with 10% (final concentration) trichloroacetic acid, 4 extractions with 6 ml ethyl acetate and 2 extractions with acetone. In the latter case, the residue was dissolved in 10% sodium dodecyl sulfate (pH 9.0) containing 10 mM mercaptoethanol and applied to a 5 to 15% sodium dodecyl sulfate "slab" polyacrylamide gel. After autoradiography, the gel was sliced, and the radioactivity was estimated by scintillation counting. All handling of DMBA, BP, and their metabolites during preparations, incubations, and assays was carried out in the dark or in diffuse light. Radioimmunoassay of estradiol was carried out after exchange of DMBA or BP for 10 μM androstenedione, quenching of the reaction with 4 ml diethyl ether, and separation and evaporation of the organic phase. 21-Hydroxylation of progesterone was assayed with 100 μM \([\text{3H}]\)progesterone (57.2 mCi/mmol). Other conditions were as in the assay of DMBA and BP metabolism, except that the reaction was stopped by addition of 1.5 mM NADPH and stopped after 60 min with 4 ml ethyl acetate. The water phase was extracted once more with ethyl acetate. Analysis of the extent of conversion was achieved by 2 extractions of metabolites (and unreacted DMBA or BP) with ethyl acetate containing 0.04% (w/v) butylated hydroxytoluene, followed by thin-layer chromatography on silica gel with toluene as eluant. Alternatively, the conversion was assayed by the distribution method described by van Cantfort et al. (54), which was found to be applicable also for DMBA. The thin-layer and the distribution assays differed by less than ±15%. High-pressure liquid chromatographic analysis of ethyl acetate-extractable DMBA metabolites was carried out by a Waters Associates instrument using a Nucleosil C₁₈ column equilibrated with 35% methanol and eluted with a 35 to 95% methanol gradient during a period of 70 min, including a hold at 50% methanol for 10 min and a second hold at 95% methanol for 10 min. Covalent binding of DMBA metabolites was measured by precipitation of proteins on filter paper discs according to the method of Wallin et al. 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Other conditions were as in the assay of DMBA and BP metabolism, except that the reaction was stopped by addition of 4 ml chloroform:methanol (4:1); the water phase was extracted once more with chloroform:methanol, and the thin-layer chromatography was developed twice in chloroform:ethyl acetate (4:1). The effects of long-term treatments of male Sprague-Dawley rats were followed by injecting 25 mg DMBA per kg i.p., using a solution of 9 mg DMBA per ml of corn oil, for 6 consecutive days. A control group received the corresponding amount of corn oil only at these occasions. Two rats from each group were sacrificed after 0, 3, 6, and 10 days, respectively. The 4 adrenals were homogenized and sonicated gently (4 times for 10 sec each) in 2 ml of 50 mM Tris-HCl (pH 7.0) containing 0.25 M sucrose. Glutathione S-transferase was measured as glutathione one conjugation with CDNB or DCNB as substrates according to the method of Habig et al. (24). Cytochrome oxidase was measured with reduced cytochrome c (52), and lactate dehydrogenase according to the method of Habig et al. (24). Other biochemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

**RESULTS**

**Subcellular Distribution of Activity.** The mitochondrial and microsomal fractions of rat adrenal cortex were found to convert DMBA at a rate of 44.0 and 138.8 pmol/min/mg protein, respectively. Extensive washing of the mitochondrial fraction decreased the metabolism in this fraction to 27.2 pmol/min/mg protein, which corresponds to about 19% of the microsomal activity. A 100,000 x g supernatant fraction was inactive (Table 1). Since mitochondria under these conditions are contaminated with 10 to 15% microsomes (51), pure mitochondria were assumed to be inactive. For a comparison, the adrenal microsomes also catalyzed conversion of BP at a rate of 120.71 pmol/min/mg protein (cf. Ref. 22) with a similar relative conversion by the mitochondrial fraction as in the case with DMBA. The metabolism of DMBA was linear for at least 120 min (Chart 1A) and with protein in the range of 0.5 to 1.5 mg microsomal protein per ml (Chart 1B). An accurate \(K_m\) proved

**Table 1**

*Subcellular distribution of DMBA metabolism activity*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>No. of experiments</th>
<th>Specific activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria (washed once)</td>
<td>4</td>
<td>44.0 ± 4.6*</td>
</tr>
<tr>
<td>Mitochondria (washed 4 times)</td>
<td>4</td>
<td>27.2 ± 2.0</td>
</tr>
<tr>
<td>Microsomes</td>
<td>4</td>
<td>138.8 ± 6.0</td>
</tr>
<tr>
<td>Soluble</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean ± S.D.

![Chart 1. Time (A) and protein (B) dependence of DMBA metabolism in rat adrenal microsomes. The conditions were as described in "Materials and Methods." Each point represents the mean of 2 experiments. prot, protein.](chart.png)

J. Montelius et al.

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1480

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difficult to determine but was estimated to be lower than 3 μM (not shown).

Analysis of Metabolites. High-pressure liquid chromatographic analysis of reaction products indicated that, with [14C]DMBA as substrate, 3 major labeled metabolites were formed (Table 2), the Rf of which did not coincide with any of the available reference compounds (cf. Ref. 9); in addition, some minor unlabeled and unknown metabolites were formed. The more polar of the labeled metabolites (Rf 0.53) was tentatively identified as a diol or a derivative of a diol. The major unknown hydrophobic metabolite (Rf 0.89) was slightly more hydrophobic than was 12-hydroxy-7-methylbenz(a)anthracene and may be a phenol; little or no hydroxymethyl derivatives were formed. The minor hydrophobic metabolite (Rf 0.91) is presumably not a phenol but may be an aldehyde or a dione (quinone). This identification is supported by the fact that, in the presence of cyclohexane oxide or cyclohexane oxide plus reduced glutathione, the formation of the hydrophilic metabolite was decreased whereas the formation of the major and minor hydrophobic metabolite was increased and unchanged, respectively. This is consistent with the possibility that the hydrophilic metabolite is primarily formed by the action of an epoxide hydrase on an intermediate epoxide metabolite; in the presence of the epoxide hydrase inhibitor cyclohexane oxide (40), the epoxide will undergo a nonenzymatic conversion to a phenol. However, the inhibitor also caused an overall inhibition of the conversion of DMBA which may reflect the interaction of this compound with cytochrome P-450 (cf. Ref. 40). No or few changes were seen in the absence of cyclohexane oxide but in the presence of conjugation systems, e.g., transferase B (Table 2), except for a 50% decrease in the formation of the minor hydrophobic metabolite. Exchange of microsomes for mitochondria in the incubation gave a metabolite pattern which was qualitatively identical to that obtained with microsomes (not shown). This supports the assumption that the mitochondrial activity represents a microsomal contamination and suggests, in addition, that mitochondria do not catalyze a secondary conversion of DMBA metabolites. With [14C]BP as substrate, all metabolites were labeled; these were mainly 3-hydroxybenzo(a)pyrene, 9-hydroxybenzo(a)pyrene, and an unknown metabolite with a Rf close to that of 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, tentatively identified as a diol (Table 3). Quinones and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were also formed. Also in this case cyclohexane caused an overall inhibition and a diol-phenol shift of both the 9,10- and 7,8-diols, indicating 2 possible intermediate epoxide metabolites, but the extent of the increased phenol formation was less pronounced than with DMBA as substrate.

Effect of Inhibitors. Microsomal DMBA metabolism was NADPH dependent and sensitive to carbon monoxide, indicating that the enzyme system(s) involved is cytochrome P-450 dependent (Table 4). Other inhibitors included BP, estradiol, and SU 9055, an inhibitor of 17α-hydroxylase (6). Aminopyrine and metyrapone, a substrate (17) and inhibitor (33), respectively, of cytochrome P-450 from liver microsomes and adrenal mitochondria, were strongly inhibitory, the most potent being 17,20-dihydroxy-9,10-dihydroxybenzo(a)pyrene, tentatively identified that the mitochondrial activity represents a microsomal contamination and suggests, in addition, that mitochondria do not catalyze a secondary conversion of DMBA metabolites. With [14C]BP as substrate, all metabolites were labeled; these were mainly 3-hydroxybenzo(a)pyrene, 9-hydroxybenzo(a)pyrene, and an unknown metabolite with a Rf close to that of 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, tentatively identified as a diol (Table 3). Quinones and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were also formed. Also in this case cyclohexane caused an overall inhibition and a diol-phenol shift of both the 9,10- and 7,8-diols, indicating 2 possible intermediate epoxide metabolites, but the extent of the increased phenol formation was less pronounced than with DMBA as substrate.
BP and vice versa was investigated more carefully. As may be seen in Chart 2, increasing concentration of the inhibiting, i.e., unlabeled, hydrocarbon led to a maximal inhibition of about 75%. With 50 μM DMBA as substrate and BP as inhibitor (Chart 2), half-maximal inhibition was obtained at a BP concentration of about 20 μM. In the reverse situation, with 50 μM BP as substrate, 60 μM DMBA was required for half-maximal inhibition (Chart 2). Inhibition of DMBA and BP metabolism by SU 9055 showed a similar discrepancy; i.e., as compared to DMBA metabolism, BP metabolism required more than 2 times the concentration of SU 9055 to reach 50% inhibition (Chart 3).

A variety of steroids and steroid analogs were also tested as possible inhibitors of DMBA metabolism (Table 5). Although none was particularly potent, progesterone and estradiol were the most efficient among the steroids whereas spironolactone was the most efficient steroid analog. Attempts to characterize the cytochrome P-450 responsible for the adrenal metabolism of polycyclic hydrocarbons by measuring the effects of DMBA on various steroid hydroxylases (not shown) have confirmed the results shown in Table 5, i.e., that the substrates of the side-chain cleavage systems for cholesterol and 17α-hydroxyprogrenolone and the 11β-, 17α-, 18-, and 19-hydroxylase systems had no effect on DMBA metabolism, indicating that none of these hydroxylases are involved in DMBA metabolism. The 21-hydroxylase activity assayed with 100 μM progesterone as substrate was inhibited to 36% by 500 μM DMBA (not shown). However, as judged from the inhibition of DMBA metabolism by progesterone (cf. Table 5), the DMBA-metabolizing cytochrome P-450 has an approximately 20-fold higher affinity for DMBA than for progesterone. If 21-hydroxylase was responsible for DMBA metabolism, one would therefore expect an almost total inhibition of progesterone conversion at a DMBA:progesterone ratio of 5. The fact that this was not seen suggests that 21-hydroxylase is unrelated to DMBA metabolism and that the effects of steroids on DMBA metabolism and vice versa are unspecific. The activity of the aromatase system with androstenedione as substrate was not measurable by radioimmunoassay (less than 0.5 pmol per mg microsomal protein per hr), and the possible effect of DMBA on this system could therefore not be tested (not shown).

Covalent Binding of DMBA Metabolites to Protein. Chart 4 shows 4 different peptides in a microsomal plus soluble adrenal fraction (25,000 × g supernatant) that were demonstrated to be labeled after incubation with [14C]DMBA, electrophoretic separation on a slab polyacrylamide gel, autoradiography, and scanning. The molecular weights of these peptides were about 47,000, 28,000, 25,000, and 13,000. The corresponding experiment with only microsomes gave 5 major labeled peptides with molecular weights of 59,000, 50,000, 47,000, 25,000, and 13,000. These peptides may tentatively be identified as
cytochrome P-450 (M, 59,000 and 50,000; cf. Refs. 20, 22, and 25) and glutathione S-transferases (M, 25,000 and 28,000; cf. Ref. 24). The peptide with a molecular weight of 13,000 comigrates with cytochrome c from beef. For unknown reasons, peptides labeled by covalent binding of BP metabolites could not be separated properly. Omission of NADPH or addition of SU 9055 or carbon monoxide markedly inhibited labeling of protein (Table 6), indicating that generation of protein-bound radioactivity was related to the cytochrome P-450-dependent conversion of [14C]DMBA or [14C]BP to reactive metabolites. Quantitatively, there was no significant difference between DMBA and BP in the generation of protein-bound metabolites. Quantitatively, there was no significant difference between DMBA and BP in the generation of protein-bound metabolites. Quantitatively, there was no significant difference between DMBA and BP in the generation of protein-bound metabolites.

Table 6
Effects of various steroids and steroid analogs on DMBA metabolism in rat adrenal microsomes

<table>
<thead>
<tr>
<th>Conditions</th>
<th>No. of experiments</th>
<th>Specific activity (pmol/min/mg protein)</th>
<th>Inhibition (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes</td>
<td>14</td>
<td>77.7 ± 9.7b</td>
<td>2.1 NSc</td>
<td></td>
</tr>
<tr>
<td>+ Cholesterol</td>
<td>4</td>
<td>76.0 ± 8.7</td>
<td>3.7 NS</td>
<td></td>
</tr>
<tr>
<td>+ Pregnenolone</td>
<td>4</td>
<td>74.7 ± 11.5</td>
<td>16.0 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>+ Progestosterone</td>
<td>4</td>
<td>65.3 ± 11.6</td>
<td>9.3 NS</td>
<td></td>
</tr>
<tr>
<td>+ Decoycorticosterone</td>
<td>4</td>
<td>70.4 ± 6.9</td>
<td>9.0 NS</td>
<td></td>
</tr>
<tr>
<td>+ Corticosterone</td>
<td>4</td>
<td>70.7 ± 5.0</td>
<td>9.0 NS</td>
<td></td>
</tr>
<tr>
<td>+ 17-Hydroxyprogrenolone</td>
<td>4</td>
<td>77.3 ± 4.4</td>
<td>0.5 NS</td>
<td></td>
</tr>
<tr>
<td>+ 17-Hydroxyprogesterone</td>
<td>4</td>
<td>73.2 ± 8.6</td>
<td>5.8 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>+ Testosterone</td>
<td>4</td>
<td>69.1 ± 5.3</td>
<td>11.0 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>+ Androstendione</td>
<td>4</td>
<td>67.1 ± 6.8</td>
<td>13.6 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>+ 19-Hydroxytestosterone</td>
<td>4</td>
<td>69.5 ± 11.6</td>
<td>10.4 &lt;0.05</td>
<td></td>
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<tr>
<td>+ 19-Hydroxyandrostenedione</td>
<td>4</td>
<td>66.9 ± 7.6</td>
<td>13.8 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>+ 17-Estradiol</td>
<td>4</td>
<td>62.8 ± 7.9</td>
<td>19.2 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>+ Estrone</td>
<td>4</td>
<td>69.6 ± 6.5</td>
<td>10.3 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>+ Estradiol</td>
<td>4</td>
<td>66.8 ± 9.8</td>
<td>14.0 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>+ 3-Desoxysteone</td>
<td>4</td>
<td>86.5 ± 3.8</td>
<td>3.0 NS</td>
<td></td>
</tr>
<tr>
<td>+ d-Equilenin</td>
<td>4</td>
<td>65.0 ± 1.2</td>
<td>16.3 &lt;0.01</td>
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</tr>
<tr>
<td>+ Stilbestrol</td>
<td>4</td>
<td>73.2 ± 3.6</td>
<td>6.0 NS</td>
<td></td>
</tr>
<tr>
<td>+ Spironolactone</td>
<td>4</td>
<td>54.4 ± 5.3</td>
<td>29.9 &lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Effects of various steroids and steroid analogs on DMBA metabolism in rat adrenal microsomes

All steroids and steroid analogs were added at a final concentration of 500 μM. The concentration of DMBA was 50 μM.

Effect of DMBA on Adrenal Cortex Enzymes In Vivo. At the start of DMBA administration.

DISCUSSION
The present data show that the adrenocorticolytic agent
DMBA is metabolized by one or more P-450-dependent monooxygenase systems localized in the endoplasmic reticulum. The mitochondrial fraction containing the bulk of cytochrome P-450 (23) was inactive. In the presence of BP, which is not an adrenocorticolytic agent (27) but which has been shown to be metabolized by adrenals (13, 18, 22, 56), the DMBA metabolism was inhibited to a maximum of 75%. Since this interaction also occurred in the reverse case, i.e., BP metabolism was inhibited to 75% by DMBA, it may be concluded that the 2 hydrocarbons are metabolized predominantly by one common cytochrome P-450. The residual activity, which was inhibited by SU 9055 close to 100% independently of the substrate used, may involve the same cytochrome P-450, in which case the incomplete inhibition by a competing substrate may be due to a lack of sufficient substrate accessibility in the presence of too high concentrations of hydrocarbons. Thus, contributions of more than one cytochrome P-450 to the overall DMBA metabolism appear unlikely but cannot be excluded at the present stage.

Both DMBA and BP appear to be metabolized to diols and phenols, which together with the striking diol-phenol transitions occurring in the presence of cyclohexane oxide may be taken as strong evidence for the involvement of epoxide intermediates. This is also consistent with a previous demonstration of the presence of epoxide hydrase in this tissue (39). However, the detailed metabolite patterns for DMBA and BP are different. DMBA apparently generates only one type of diol. Inhibition of epoxide hydrase leads to the conversion of this metabolite to the second major but more hydrophobic metabolite, consequently proposed to be a phenol derivative. The third main metabolite is also hydrophobic but is influenced only by glutathione S-transferase from rat liver and is therefore probably not derived from an epoxide. Interestingly enough, it has been shown (37) that glutathione S-transferase catalyzes the formation of glutathione conjugates with quinones. The minor hydrophobic metabolite may therefore be tentatively identified as a quinone, which may be involved in the subsequent reactions to epoxide, diol, and phenol; a definite identification of unknown metabolites will have to await a mass spectrometric analysis. Nevertheless, the data allow the conclusion that rat adrenals, in contrast to rat liver (cf. Refs. 29, 48, and 49), do not form significant amounts of the potent adrenocorticolytic and carcinogenic agent 7-OHM-12-MBA (8, 27, 57).

With regard to BP metabolism, it is interesting to note that adrenal microsomes produce a metabolite pattern that resembles that of 3-methylcholanthrene-induced liver microsomes (46, 48, 49), suggesting the involvement of a C-type of cytochrome P-450 (Ref. 3; see also Refs. 20, 22, and 25).

The presence of both, 9,10- and 7,8-dihydroxy-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an intermediate in the overall adrenal BP metabolism. It may also be concluded that the selective adrenocorticolytic effect of DMBA as compared to BP presumably is not due to any major qualitative differences, i.e., with respect to intermediate epoxides, phenols, and diols, or quantitative differences in metabolite patterns between the 2 hydrocarbons.

As to the identity and biological function of the DMBA-metabolizing system, the slightly inhibitory effects of high concentrations of steroids, especially progesterone and estrogens, presumably represent unspecific interactions with the DMBA-metabolizing system. This conclusion is further substantiated by the fact that the affinities of steroid hydroxylases for their steroid substrates appear to be in the μM range (31, 45). However, it cannot be excluded at the present time that 21-hydroxylase contributes to the metabolism of DMBA. A role of a steroid hydroxylase in DMBA metabolism is supported by the finding that SU 9055, a potent inhibitor of 17α-hydroxylase (6) and liver BP metabolism (44), also inhibits DMBA metabolism. Such a mechanism is in agreement with a previous report (cf. Ref. 19 but see Ref. 11) on the relationship between metabolism of steroids and xenobiotics in the guinea pig. It is obvious, however, that the specificity of the hydrocarbon-metabolizing system(s) in the guinea pig is very different from that in the rat. Direct effects of estradiol on DMBA-induced adrenal necrosis have been demonstrated earlier (26, 50).

That covalent binding of DMBA metabolites to adrenal protein apparently occurs essentially without the formation of 7-OHM-12-MBA as a major metabolite suggests that 7-OHM-12-MBA is not an obligatory intermediate in the generation of reactive metabolites. However, since 7-OHM-12-MBA is generated by the liver (49) and is metabolized by the adrenal (49), it is still possible that, in vivo, adrenal necrosis is caused by metabolites of 7-OHM-12-MBA generated in the adrenal. Moreover, prevention of necrosis by induction of liver cytochrome P-448 by, e.g., BP or 3-methylcholanthrene, which causes an increased ring hydroxylation of DMBA as well as hydroxylation of methyl groups (13, 29, 48, 49), may involve different types of protective mechanisms. Ring-hydroxylated derivatives may compete with methyl-hydroxylated derivatives for further adrenal metabolism, and/or adrenal metabolism of liver products may be inhibited through a direct and competing binding to the cytochrome P-450 by the inducer.

The extensive binding to microsomal and soluble proteins may indicate that the seemingly specific and primary destruction of mitochondria that precedes necrosis (2) is a secondary event possibly caused by a direct effect of microsomal metabolites. Alternatively, a mitochondrial metabolism of DMBA or DMBA metabolites has been considered (cf. Ref. 2). However, the present results seem to exclude at least a quantitatively important mitochondrial pathway for DMBA and/or its metabolites. This conclusion is substantiated by recent and preliminary results that indicate that 7-OHM-12-MBA is not metabolized when incubated with mitochondria. Both the in vitro and the in vivo data may suggest that inactivation of a glutathione S-transferase through covalent modification may contribute to the toxic effects of DMBA on rat adrenals. Whether there are any differences between DMBA and BP in this respect which may provide a partial explanation for the specific adrenocorticolytic effect of DMBA is presently being investigated.

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Metabolism of Polycyclic Aromatic Hydrocarbons in Rat Adrenals


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