Benzo(a)pyrene and 7,12-Dimethylbenz(a)anthracene Metabolism and DNA Adduct Formation in Primary Cultures of Hamster Epidermal Cells

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ABSTRACT

Primary cultures of hamster epidermal cells exposed to hydrocarbon, 1 µg/ml, rapidly metabolized [3H]benzo(a)pyrene and [14C]7,12-dimethylbenz(a)anthracene to ethyl acetate-water-soluble metabolites. By 24 hr, only 13.6% of the organic solvent-soluble radioactivity recovered in the medium was unchanged [3H]benzo(a)pyrene, and only 5.9% was unchanged [14C]7,12-dimethylbenz(a)anthracene. With both hydrocarbons, the major water-soluble metabolites found extracellularly were conjugated with glucuronic acid; these were primarily phenolic derivatives.

Metabolites cochromatographing with 7,8-dihydro-7,8-dihydrobenzo(a)pyrene or trans-3,4-dihydro-3,4-dihydroxy-7,12-dimethylbenz(a)anthracene were not detectable in high-pressure liquid chromatographic profiles of organic solvent-soluble intracellular and extracellular metabolites. However, analysis of [3H]benzo(a)pyrene and [3H]7,12-dimethylbenz(a)anthracene:DNA adducts indicated that these putative proximate carcinogenic metabolites were formed in these cells and subsequently metabolized to DNA-binding products. The results suggest that metabolic incompetence may not be an explanation for the relative resistance of the hamster to epidermal carcinogenesis by polycyclic hydrocarbons.

INTRODUCTION

PAH, such as B(a)P and DMBA, are metabolized to a wide variety of organic solvent-soluble and water-soluble metabolites (22, 29, and references therein) and require metabolic activation for their observed biological effects (30, 54). Of particular interest is the formation of diol-epoxides, for current evidence suggests that bay-region diol-epoxides may be ultimate carcinogenic forms for some PAH (38). It is now apparent that, to better understand the mechanisms by which PAH induce their biological effects, intact cell systems containing a full complement of both oxidative and conjugative enzymes should be utilized, rather than cell homogenates or microsomal preparations (14, 15, 28, 50, 51). It is also important to study the pathways of hydrocarbon metabolism and activation in target as well as nontarget tissues, because of the differences between cells from various tissues and species in both the types and ratios of metabolites formed (15, 25, 40, 41, 44, 51).

Although the metabolic activation and tumor-initiating activity of various PAH have been widely investigated in mouse epidermis, few such studies have been done in the epidermis of other rodents such as hamsters. The hamster represents an interesting animal for such studies, since numerous investigations have not conclusively demonstrated 2-stage epidermal carcinogenesis in this species (12, 20, 31, 49, 53). We have analyzed the metabolism and DNA-binding capacity of B(a)P and DMBA in Syrian hamster epidermal cell cultures. Recently, Sun et al. (57) reported that these cells can be malignantly transformed by N-methyl-N'-nitro-N-nitrosoguanidine. Further studies in our laboratory (55) demonstrated that they can also be transformed by B(a)P, although there is not a dose-response relationship. We now report that hamster epidermal cells in vitro rapidly metabolize B(a)P and DMBA to a variety of organic solvent- and water-soluble derivatives as well as to DNA-bound products. The results suggest that metabolic incompetence may not be an explanation for the relative resistance of the hamster to epidermal carcinogenesis by PAH.

MATERIALS AND METHODS

Chemicals. L-Ascorbic acid, 2,6-di-tert-butyl-p-cresol, D-saccharic acid-1,4-lactone, β-glucoronidase (Escherichia coli type IX, EC 3.2.1.31), aryl sulfatase (limpet type V, EC 3.1.6.1), DNase I (bovine pancreas, EC 3.1.4.5), snake venom phosphodiesterase (Crotalus atrox; EC 3.1.5.1), alkaline phosphatase (E. coli type III; EC 3.1.3.1), and 4-(p-nitrobenzyl)pyridine were purchased from the Sigma Chemical Co., St. Louis, Mo. B(a)P (Gold Label) was obtained from the Aldrich Chemical Co., Milwaukee, Wis.; benzene:ethanol (9:1) as solvent. [G-3H]B(a)P (17.4 to 65 Ci/mmol) and [G-3H]DMBA (42 to 46 Ci/mmol) were supplied by Amersham-Searle Corp., Arlington Heights, III., and diluted with benzene:toluene (±)-anf/-[3H] BPDE, (±)-syn-[G-3H] BPDE and other derivatives of B(a)P were acquired through the Carcinogenesis Program of the National Cancer Institute. Metabolites and derivatives of DMBA were purchased from Worthington Biochemical Corp., Freehold, N. J. All

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other chemicals utilized were of the highest purity available.

Cell Cultures. Pure cultures of primary epidermal cells were derived from newborn Syrian hamsters (LVG, Charles River Breeding Laboratories, Wilmington, Mass.) as described previously (55, 57). For metabolism experiments, 2 x 10⁶ cells were seeded in T-25 plastic flasks (Corning), and for DNA-binding experiments, 15 to 30 x 10⁶ cells were seeded into 150-mm flasks. Cultures were grown at 37° in an enriched Waymouth’s medium containing 10% fetal bovine serum (57). The cultures were refed on Day 3 and exposed to hydrocarbon when confluent (usually Day 6). At this time, the cultures had begun to show stratification (~2 to 4 cell layers) but contained only a few cornified envelopes (55). The medium was removed and replaced with fresh medium containing [³H]B(a)P, [¹⁴C]DMBA, or [³H]DBA at concentrations of 0.1 or 1 μg/ml. Cells and media were then harvested at various time points thereafter.

Extraction of Intra- and Extracellular Metabolites of B(a)P or DMBA. The procedures for extraction of intra- and extracellular metabolites of B(a)P (23) and DMBA (25) have been described in detail. Briefly, the cells and media from duplicate flasks were collected separately and pooled. In one set of experiments, cell monolayers were washed twice with PBS prior to removal from the flasks, and these washings were added to the media samples. Next, 1 ml of PBS was added, and the cells were scraped from the dishes. The dishes were washed twice with 0.5 ml of PBS, and these washings were added to the scraped cell suspension. The cells were homogenized using a Polytron PT10 homogenizer (Brinkmann Instruments, Westbury, N. Y.) at Setting 6 for 10 sec. Since the washing procedure may cause some leaching of B(a)P metabolites from the cells, in other experiments, the medium was removed, and the cells were collected without prior washing. These cells were resuspended with appropriate volumes of PBS and homogenized. In both cases, the cell homogenates and medium samples were extracted with ethyl acetate:acetone (2:1) as described (25).

To analyze for the presence of glucuronide and sulfate conjugates, medium samples were treated with appropriate enzymes prior to organic solvent extraction (7, 17). For the determination of glucuronide conjugates, 1 ml of medium was combined with 1 ml of 0.1 M potassium phosphate buffer (pH 6.8) and incubated with β-glucuronidase (2000 Fishman units/ml) for 2 hr at 37°. The addition of 20 mM o-saccharic acid completely inhibited the release of glucuronide conjugates under these conditions. Ascorbic acid (1 mg/ml) or 2,6-di-tert-butyl-p-cresol (1 mg/ml) was routinely included in the incubations to prevent spontaneous oxidation of phenols to quinones. For the determination of sulfate conjugates, 1 ml of medium was combined with 1 ml of 0.1 M potassium phosphate buffer (pH 5.0) and incubated with β-glucuronidase (2000 Fishman units/ml) for 4 hr at 37°. Both reactions were terminated by adding 2 volumes of ice-cold ethyl acetate:acetone (2:1), and then the extracts were done as described above. The amount of material released by β-glucuronidase or sulfate treatment of the medium was determined by subtracting chromatographic profiles of the organic solvent-soluble metabolites from untreated medium samples from the chromatographic profiles of β-glucuronidase- or sulfate-treated medium samples. In some instances, glucuronides were determined by another method described elsewhere (25).

DNA Adduct Formation. For DNA-binding experiments, approximately 2 x 10⁶ cells were treated with [³H]B(a)P or [³H]DMBA at final concentrations of 0.1 μg/ml of medium for 24 hr. The medium was removed, the cells were scraped from the flasks, and the DNA was isolated from the cell pellets by the procedure of Baird and Diamond (8). The extracted DNA was dissolved to an approximate concentration of 1 mg/ml and quantitated either spectrophotometrically at 260 nm assuming E₁₉₀ = 260 or by the method of Burton (18) using calf thymus DNA as standard. The DNA was hydrolyzed using DNase I, snake venom phosphodiesterase, and alkaline phosphatase sequentially as described previously (4). The DNA hydrolysates were subsequently analyzed by using: (a) a long column of Sephadex LH-20 (90 cm; Pharmacia Fine Chemicals, Piscataway, N. J.) eluted with a linear gradient from 30 to 100% methanol-in-water gradient as described by Baird and Brookes (4); or (b) HPLC.

HPLC. All HPLC analyses were performed using an Altex Model 312 MP liquid chromatograph equipped with an UltraspHERE octadecylsilane column (4.6 mm x 25 cm). Metabolites of B(a)P were separately using a methanol-water gradient system as described previously (23). For the analysis of DMBA metabolites, the column was maintained at ambient temperature with a constant flow rate of 1 ml/min. The gradient system consisted of 3 steps: (a) 50 to 70% methanol-in-water gradient (linear, 20 min); (b) 10-min hold at the 70% methanol-in-water concentration; and (c) 70 to 90% methanol-in-water gradient (linear, 20 min). For the analysis of B(a)P:DNA adducts, a modification of the method described by Weinstein et al. (60) was utilized as outlined by Ashurst et al. ⁶

For all analyses, individual fractions were collected directly into scintillation vials immediately after injection of 20- to 50-μl samples into the column. Scintillation fluid (ACS II; Amershams Earle) was added to the vials, and the samples were counted in a Packard Tri-Carb liquid scintillation counter as described previously (25). Metabolites of B(a)P and DMBA were tentatively identified, where possible, by cochromatography with known marker metabolites. The B(a)P:DNA adducts were tentatively identified, where possible, by cochromatography with known marker adducts, prepared as described previously (1, 2).

RESULTS

Metabolism of [³H]B(a)P. As shown in Table 1, [³H]B(a)P was rapidly metabolized by primary hamster epidermal cells; after 24 hr of exposure, only 13.6% of the organic solvent-soluble material in the medium was unmetabolized hydrocarbon. At all time points monitored, approximately 55% of the total water-soluble metabolites in the medium were glucuronide conjugates. The amount of radioactive material associated with the cells at each time point represented approximately 20.1, 6.8, and 5.3% of the total radioactivity recovered at the 6-, 24-, and 48-hr exposure periods, respectively.

The chromatographic profiles of the ethyl acetate:acetone-soluble metabolites of [³H]B(a)P recovered extracellularly are shown in Chart 1. Relatively small quantities of the following tentatively identified metabolites were detected after 6 hr (Chart 1a): trans-B(a)P-9,10-diol; 9-OH-B(a)P; and 3-OH-B(a)P. The identity of these peaks is based on cochromatography with purified reference markers. By 24 and 48 hr (Chart 1b, 48-hr profile not shown), little or no radioactive material chromatographed in regions corresponding to these 3 metabolites. At no time was a distinct peak corresponding to trans-B(a)P-7,8-diol detected, although a broad, diffuse peak of radioactive material eluted between Fractions 30 and 60 at the later times. With the samples from the 24- and 48-hr exposure periods, large amounts of radioactivity eluted at or with the solvent front (Chart 1b, 48-hr sample not shown). Further analysis indicated that some of this represented glucuronide conjugates extracted from the medium by ethyl acetate:acetone (see below). Chart 1c illustrates the pattern of ethyl acetate:acetone-extractable metabolites obtained following treatment of a 24-hr medium sample with β-glucuronidase. Phenolic metabolites [particularly metabolites cochromatographing with 3-OH-B(a)P] represented the majority of the released material.

The major intracellular ethyl acetate:acetone-soluble metab-

olites after a 6-hr exposure to [3H]B(a)P cochromatographed with 9-OH-B(a)P and 3-OH-B(a)P, in addition to material eluting with the solvent front (Chart 2). For the purposes of the present investigation, we have assumed the content of the 2 phenolic peaks to be primarily 9- and 3-OH-B(a)P, respectively. However, with the HPLC system used, other phenolic metabolites (e.g., 1- and 7-hydroxybenzo(a)pyrene) would be expected to elute in these 2 major peaks (33). We cannot rule out the possibility that the metabolite peaks corresponding to 9- and 3-OH-B(a)P contain other phenolic metabolites as well. Small quantities of metabolites cochromatographing with the 9,10-diol also were observed. By 24 hr of exposure (profile not shown), only small quantities of material cochromatographing with the 9,10-diol were detected, and little or no phenolic metabolites were present. It should be noted that intracellularly, the ratio of the unconjugated phenols to the B(a)P-9,10-diol was much greater than one, whereas extracellularly, it was less than one.

With the experimental and HPLC procedures used, sulfate esters would be extracted into ethyl acetate:acetone, and these metabolites would elute at or near the solvent front (17, 19). Large amounts of radioactive material did elute in this region, especially at the later exposure times (Chart 1). However, when medium samples were treated with aryl sulfatase prior to extraction, the radioactivity in this region was not reduced, and sulfate conjugates of B(a)P metabolites were not detected. Interestingly, treatment of the medium with β-glucuronidase did greatly reduce the large amount of radioactivity eluting with the solvent front (compare Chart 1, b and c). This suggested that glucuronide conjugates of B(a)P metabolites were being partially or completely extracted by ethyl acetate:acetone. Baird et al. (6) reported a similar finding with medium samples from hamster embryo cell cultures exposed to [3H]B(a)P. They demonstrated that under acidic conditions, glucuronide conjugates could be quantitatively extracted (uncleaved) from the medium with ethyl acetate.

To further substantiate that we were extracting glucuronides from our medium samples, we used a protocol similar to that described previously (25). Briefly, medium samples were diluted with 0.1M potassium phosphate buffer (pH 7.5) and extracted twice with ethyl acetate:acetone. Nitrogen was bubbled through the remaining aqueous phase to remove residual organic solvent. The aqueous phase was then incubated with β-glucuronidase and extracted as described above. When medium samples were treated in this way, the percentage of material eluting with the solvent front in HPLC runs at 6, 24, and 48 hr was reduced by 1.1, 21.8, and 14.6%, respectively.

Metabolism of [14C]DMBA. DMBA also was rapidly metabolized by primary hamster epidermal cells. In cultures exposed to [14C]DMBA (1 μg/ml), only 5.9% of the organic solvent-soluble radioactivity recovered in the medium remained as unmetabolized hydrocarbon at 24 hr (Table 1). The amount of radioactive material released into the organic solvent by treatment of the medium with β-glucuronidase was 38, 53, and 56% of the total water-soluble metabolites at 6, 24, and 48 hr,
respectively. The percentage of the total radioactive material which was cell associated was approximately 14.6, 6.4, and 3.9% at 6, 24, and 48 hr, respectively.

The ethyl acetate:acetone-soluble metabolites of [14C]DMBA found extracellularly after 6 hr were tentatively identified as: trans-DMBA-8,9-diol; 7-OHM-12-MBA; 12-OHM-7-MBA; and DMBA phenols (Chart 3a). Several additional metabolite peaks (e.g., Peak Fractions 27, 57, and 68) did not cochromatograph with any of the reference markers. At 24 hr (Chart 3b), a large quantity of radioactive material eluted with the solvent front, as in the B(a)P profiles. Also as with B(a)P, no distinct peak corresponding to trans-DMBA-3,4-diol could be detected at any time point, although a rather diffuse peak of radioactive material eluted between Fractions 70 and 80 [24- and 48-hr (not shown) hr exposures]. As with B(a)P, the majority of the material released after treatment of a 24-hr medium sample with β-glucuronidase (Chart 3c) was phenolic metabolites of DMBA, primarily those cochromatographing with 2-, 3-, 9-, or 10-OH-DMBA. Furthermore, the amount of radioactive material eluting with the solvent front was considerably reduced (compare Chart 3, b and c), indicating that phenol glucuronides of DMBA also were being extracted into ethyl acetate:acetone. The modified extraction protocol described above for B(a)P was then utilized to accurately determine the amount of β-glucuronidase-releasable material for the DMBA samples. As with the B(a)P-treated cultures, no sulfate esters of DMBA metabolites could be detected at any of the time points.

The pattern of ethyl acetate:acetone-soluble intracellular metabolites after a 6-hr exposure to [14C]DMBA (data not shown) was similar to the profile of extracellular metabolites, except that the ratios of individual metabolites differed. As shown for B(a)P (Charts 1 and 2), phenolic metabolites represented a greater percentage of the total intracellular than of the extracellular metabolites. A major difference was the absence of the unknown metabolite at Peak Fraction 68.

Covalent Binding to DNA. To determine the ability of the hydrocarbons to bind covalently to the DNA of hamster epidermal cells, confluent cultures were treated for 24 hr with [3H]B(a)P or [3H]DMBA (0.1 μg/ml), and the DNA was isolated from the cell pellets as described in "Materials and Methods." The specific activity of binding for DMBA was approximately 5.7 times greater than for B(a)P (6.81 versus 1.19 pmol hydrocarbon per mg DNA). For further analysis, DNA hydrolysates were chromatographed on Sephadex LH-20 columns (1.5 x 90 cm) using methanol:water gradients. Three distinct B(a)P:DNA adduct peaks eluted after the p-(nitrobenzyl)pyridine marker (Fraction 85) (Chart 4a). With DMBA, 4 distinct DNA adduct peaks eluted after the UV marker (Chart 4b). In both of the profiles shown in Chart 4, considerable amounts of radioactive material eluted from the columns in the first 40 fractions. This early eluting material has not been fully characterized but probably represents DNA fragments resistant to hydrolysis and a certain amount of normal deoxyribonucleosides in which tritium exchange has occurred (47). For the LH-20 profiles shown in Chart 4, the early eluting material represented 14.4 and 34.4% (Chart 4, a and b, respectively) of the total radioactivity eluted from the columns.

Fractions corresponding to the 3 peaks from the B(a)P:DNA

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Table 1

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Total water-soluble metabolites</th>
<th>β-Glucuronidase-releasable metabolites</th>
<th>Unchanged hydrocarbon</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>10.3</td>
<td>12.7</td>
<td>5.6</td>
</tr>
<tr>
<td>24</td>
<td>49.5</td>
<td>33.4</td>
<td>28.0</td>
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<tr>
<td>48</td>
<td>52.8</td>
<td>43.4</td>
<td>29.0</td>
</tr>
</tbody>
</table>

* Determined by extraction of the medium with ethyl acetate:acetone (expressed as a percentage of the total radioactivity recovered in the medium).
* Expressed as a percentage of the total radioactivity recovered in the medium.
* Expressed as a percentage of the total ethyl acetate:acetone-extractable radioactivity recovered in the medium (determined from HPLC profiles).
Hydrocarbon Metabolism in Hamster Epidermal Cells

Chart 4. Sephadex LH-20 chromatographic elution profiles of DNA adducts from primary hamster epidermal cells treated with [3H]B(a)P (a) or [3H]DMBA (b). Cultures were treated with hydrocarbon (0.1 μg/ml) for 24 hr, at which time DNA was isolated and then degraded to deoxyribonucleosides as described in "Materials and Methods." DNA hydrolysates were applied to a 1.5 x 90-cm column packed with Sephadex LH-20 and eluted with a 30 to 100% linear methanol-in-water gradient. Two hundred fractions of 4.3 ml each were collected, and the radioactivity in a 1 ml sample from each fraction was determined. Arrow, position of the UV-absorbing marker 4-(p-nitrobenzyl)pyridine. The total dpm added to the column were 80,730 dpm (a, 0.206 mg DNA) and 510,600 dpm (b, 0.811 mg DNA).

DISCUSSION

We have demonstrated that primary cultures of hamster epidermal cells rapidly metabolize B(a)P and DMBA, 2 PAH that are consistently used as examples of nonsubstituted and methyl-substituted PAH. Both hydrocarbons were essentially completely metabolized during a 24-hr exposure period, but few or no radioactive peaks corresponding to trans-B(a)P-7,8-diol or trans-DMBA-3,4-diol were detected in profiles of organic solvent-extractable metabolites at any of the time points monitored. The profiles of ethyl acetate:acetone-soluble metabolites found intracellularly and extracellularly were qualitatively similar for both hydrocarbons. However, as we (23, 25) and others (17, 39, 44) have observed, the distribution of individual metabolites of B(a)P and DMBA differed between cells and media. For example, a much greater percentage of free phenols was found intracellularly, whereas higher percentages of certain diols were found in the medium.

Glucuronide conjugates are major water-soluble metabolites of B(a)P and DMBA produced by several tissues. For example, the major glucuronic acid conjugates produced by isolated rat hepatocytes are Fraction I (i.e., unidentified polar material) and 3-OH-B(a)P (17, 39). In cultures of hamster trachea, the major glucuronic acid conjugate is formed with 9-OH-B(a)P, and

LH-20 run (Chart 4a) were collected and analyzed by HPLC. Chart 5a is a composite chromatogram of the HPLC runs for each LH-20 peak. LH-20 Peak 1 consisted of one component (Peak A) which cochromatographed with a minor deoxyribonucleoside adduct obtained from reacting (±)-anti-BPDE with calf thymus DNA in vitro (see Chart 5b). LH-20 Peak 2 consisted of 3 components (Chart 5a, Peaks B to D). Peak B cochromatographed with the major reaction product obtained by reacting (±)-anti-BPDE with calf thymus DNA (Chart 5b). This adduct has been characterized by others as N²-(10S,7R,8S,9R-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene)pyrimdinyl):dG (7R anti-BPDE:trans-dG) (reviewed in Ref. 48). Peak B also cochromatographed with one of the (±)-syn-BPDE:DNA adducts (Chart 5c), and we cannot rule out at present the possibility that this peak is or contains a syn-BPDE:DNA adduct. Peaks C and D (Chart 5a) cochromatographed with 2 of the 4 reaction products obtained by reacting (±)-syn-BPDE with calf thymus DNA (Chart 5c). LH-20 Peak 3 consisted of 2 components (Chart 5a, Peaks E and F). These cochromatographed with 2 of the 4 possible deoxyadenosine adducts obtained by reacting (±)-anti-BPDE with calf thymus DNA (Ref. 36; Chart 5b). The DMBA:DNA adducts could not be further analyzed by HPLC because diol-epoxide derivatives needed for preparing marker adducts are unavailable.
smaller quantities of glucuronides are formed with B(a)P-4,5-diol and 9,10-dihydroxy-B(a)P (45). Hamster embryo cells produce glucuronides primarily with 9-OH-B(a)P and smaller quantities of the 3-OH-B(a)P glucuronide (7, 44). They also produce glucuronides of 3- and 4-OH-DMBA (5). Our study shows that primary hamster epidermal cells effectively produce glucuronide conjugates of PAH metabolites. With B(a)P, the primary glucuronide conjugate was with 3-OH-B(a)P, but significant quantities of the 9-OH-B(a)P glucuronide also were found. It should be noted that these are tentative identifications based on cochromatography with purified reference materials. With DMBA, β-glucuronidase treatment of medium samples released metabolites cochromatographing with 2-, 3-, 9-, or 10-OH-DMBA and 4-OH-DMBA. Interestingly, the spectra of β-glucuronidase-releasable metabolites of DMBA and B(a)P were quite similar to those observed with primary cultures of mouse epidermal cells (23, 25). In contrast, Kuroki et al. (43) observed that primary cultures of human epidermal cells effectively metabolized B(a)P but detected few, if any, glucuronide or sulfate conjugates. We have never found sulfate conjugates of B(a)P or DMBA metabolites in the primary cultures of hamster epidermal cells or in primary cultures of mouse epidermal cells exposed to these PAH (23, 25).

Despite the inability to detect by HPLC trans-B(a)P-7,8-diol or trans-DMBA-3,4-diol in organic extracts of cell and medium samples, these metabolites were apparently formed, as demonstrated by our DNA-binding experiments. For B(a)P, current evidence indicates that further metabolism of (-)-trans-B(a)P-7,8-diol accounts for the greatest percentage of DNA-binding products in a variety of tissues (reviewed in Ref. 48). For example, DNA adduct studies using human and bovine bronchial explants (3, 32, 37); mouse embryo fibroblasts (16); human mammary epithelial cells (56); tracheobronchial tissues from mice, rats, and hamsters (3); human hepatoma cells (21); and mouse skin (1, 32, 42) indicate that the major B(a)P:DNA adduct arises from reaction of (+)-anti-BPDE with the exocyclic amino group of guanine. Previous reports by Baird and Diamond (9) and Ivanovic et al. (35) demonstrated that hamster embryo fibroblasts produce a wide spectrum of B(a)P:DNA adducts, including those derived from (-)-anti-BPDE as well as syn-BPDE. Their results also demonstrated that the relative distribution of individual adducts depended on length of exposure to B(a)P and on culture conditions (9, 11, 35). Shinohara and Cerutti (52) also reported that baby hamster kidney cells produced a different ratio of B(a)P:DNA adducts than did secondary mouse embryo fibroblasts.

In the present study, a number of different B(a)P:DNA adducts were observed using HPLC. Peak B (Chart 5a), which cochromatographed with the (+)-anti-BPDE:dG adduct and a minor syn-BPDE:dG adduct, represented approximately 33% of the total B(a)P:DNA adducts formed. Two other major adducts were observed (Peaks A and C), which represented approximately 25 and 28%, respectively, of the total DNA adducts. We have tentatively identified Peak A as (-)-anti-BPDE:dG. This is based on reports from other laboratories (16, 35) and recent results from our laboratory obtained by reacting pure (+)- or (-)-anti-[3H]BPDE with calf thymus DNA. Peak C cochromatographed with one of the major syn-BPDE:dG products. These results indicate that hamster epidermal cell cultures utilize a variety of B(a)P metabolic pathways that result in DNA-binding products. In addition to the (+)-anti-BPDE enantiomer, significant amounts of the syn-BPDE diastereomer are formed, suggesting that the microsomal system of monoxygenases in these cells is less stereoselective and stereospecific than in other cell types in the metabolism of B(a)P. Other possible mechanisms for the different B(a)P:DNA adduct profiles compared to other cell types include different rates of repair of specific DNA adducts or differences in culture conditions.

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* J. DiGiovanni, unpublished studies.
Hydrocarbon Metabolism in Hamster Epidermal Cells

Although the exact identities of the DMBA:DNA adducts are unknown, current evidence suggests that ring hydroxylation in positions 1, 2, 3, and 4 is essential for the binding of DMBA to DNA of mouse skin (13, 58, 59) or rodent embryo cells (10, 26, 27, 34, 46). Our metabolism and DNA-binding studies with DMBA are consistent with this: the LH-20 elution profile of DMBA:DNA adducts contained 4 distinct peaks (Peaks I to IV) which eluted after the UV marker (Chart 4b). This profile is nearly identical to those which we (24) and others (13, 58, 59) have obtained after treating mouse epidermis with DMBA.

It is apparent that hamster epidermal cells in culture possess the ability to process and metabolize PAH. Some hamster cells (including epidermal and embryonic) appear to be unique in the ability to catalyze the formation of a much wider variety of B(a)P:DNA adducts than other tissues and species. Only further experimentation can provide answers to the question of whether qualitative or quantitative differences in the metabolism of polycyclic aromatic hydrocarbons: Chemistry and Biological Effects, pp. 471-488. Columbus, Ohio: Battelle Press, 1980.

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