Interspecies Comparisons of Benzo(a)pyrene Metabolism and DNA-Adduct Formation in Cultured Human and Animal Bladder and Tracheobronchial Tissues


ABSTRACT

Cultured bladder and tracheobronchial explants from human, monkey, dog, hamster, and rat were used to study the metabolism, covalent binding to DNA, and DNA-adduct formation of [3H]benzo(a)pyrene (BP). Both organs from all species formed large amounts (40 to 70% of total 3H in the medium after 24 hr of incubation) of polar material and small amounts (<1% for animal tissues and 1 to 5% for human tissues) of organic solvent-extractable (unconjugated) metabolites, with the balance (14 to 38%) being unmetabolized BP. Patterns of unconjugated BP metabolites revealed qualitatively similar metabolism in the various species, with (±)-7β,8α,9α,10β-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene, trans-9,10-dihydro-9,10-dihydrobenzo(a)pyrene, trans-4,5-dihydro-4,5-dihydroxybenzo(a)pyrene, trans-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, phenols, and quinones constituting identifiable metabolites. Relative proportions of these metabolites differed between organs and between species. Total metabolism was highest in human tissues and lowest in rat tissues. Purification of [3H]BP-DNA from the explants revealed that covalent binding (μmol of BP per mol of DNA) was significantly higher in human bladder [6.4 ± 3.3 (S.D.)] and bronchus (±1.0 to 2.0) than in the corresponding animal bladder (0.8 to 2.4) and tracheobronchial (0.8 to 3.4) tissues. Hydrolysis of [3H]BP-DNA to the nucleoside level showed that, in most cases, 7R- and 7S-enantiomer of N2-(7β,8α,9α,10β-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrenyl)deoxyguanosine was the major DNA adduct formed, with smaller amounts of the corresponding 7S enantiomer and of N2-(7α,8α,9β,10α-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrenyl)deoxyguanosine. Two unidentified adducts (possibly deoxyctydylate adducts) were formed by both tissues of all species and, in dog and rat bladder explants, one of these constituted the largest proportion of [3H]BP-DNA adducts. A product, tentatively designated to be an N2-[7α,8α,9β,10α-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrenyl]deoxyguanosine, was formed by rat tissues only and not by tissues from the other species. It is concluded that bladder and tracheobronchial tissues of the human, monkey, dog, hamster, and rat metabolize BP in vitro in a qualitatively similar manner and that only a small percentage of the metabolites can be detected in both tissues of all species, the most abundant one, in most cases, being the adduct derived from the most carcinogenic of the known BP metabolites. Further, the distribution of BP-DNA adducts is specific and reproducible for each species.

INTRODUCTION

At present, data from animal tumorigenicity bioassays are still considered the most reliable basis for estimating the carcinogenic risk of humans to chemicals. Many carcinogens, including BP, require metabolic activation before their carcinogenicity is expressed. Therefore, a crucial element in determining the relative carcinogenic potential of a particular chemical in a given species rests with the propensity of that species to metabolically activate the chemical to a reactive metabolite that can covalently interact with cellular macromolecules, including DNA.

Cigarette smoking is associated with cancer of the human lung (39) and bladder (11). BP, a component of cigarette smoke (40), produces tumors in various organs, including the trachea and bronchus, of a number of animal species (16). The present study was designed to compare the in vitro metabolism and DNA-adduct formation of BP by cultured explants of human and animal tracheobronchial and bladder tissues. The explant culture system is particularly suitable for this purpose, since it is a whole-cell system, and interspecies comparisons based on identical experimental conditions can be made. Moreover, both bladder and tracheobronchial tissues exhibit excellent prolonged morphological viability in this system (36, 38), so that interspecies differences in rates of metabolism cannot be ascribed to the degree of viability of the tissue.

In these studies, we chose to compare the human to the monkey, since the capacity of these 2 primate species to metabolize BP has been amply demonstrated (3, 15, 24) but not under identical experimental conditions. In addition, the comparison was extended to the dog, since the dog has been used as an...
animal model for both lung and bladder cancer (8, 20, 21, 25, 30). Finally the rat and hamster were chosen for comparison, since they have been used extensively as models for respiratory carcinogenesis (23, 31), and both species have been used as models for bladder cancer (14, 35).

MATERIALS AND METHODS

Chemicals. Generally tritiated BP (20 to 30 Ci/mmol) was purchased from Amersham/Searle Corporation (Arlington Heights, Ill.) and diluted with nonradioabeled BP (Eastman Organic Chemicals, Rochester, N. Y.) to a final specific activity of 3 to 5 Ci/mmol. The mixture was purified by elution from a Sep-Pak (Waters Associates, Milford, Mass.) with 80% methanol in water. Each preparation was checked for chemical and radiochemical purity by HPLC and used only if greater than 96% pure. Purified \[^{3}H\]BP was dissolved in dimethyl sulfoxide: methanol (4:1) and stored at 4° at a concentration of 0.1 mCi/mg. Following enzymic digestion to the deoxyribose:DNA preparation eluted from the Sep-Pak. The unmetabolized BP was removed from the column by evaporation under a stream of N\(_2\) at 41°, and the residue was reconstituted in 3 mCi of 0.1 m sodium acetate buffer (pH 4.6) containing aryl sulfatase (10 units/ml; type VII; Sigma Chemical Co., St. Louis, Mo.) and \(\beta\)-glucuronidase (72 units/ml; type VII; Sigma) and incubated at 37° for 24 hr. Following incubation, samples were extracted twice with 2 volumes of ethyl acetate:acetone (2:1). The organic extracts were pooled, dried under N\(_2\) at 41°, and analyzed by HPLC as described above. For the analysis of unconjugated BP metabolites, an ethyl acetate:acetone extract was prepared from pooled (4 to 7 ml) media, after which the residue was reconstituted with 0.4 ml of benzene and transferred to a normal-phase Sep-Pak. The unconjugated BP was removed from the column by elution with 2.5 ml of benzene. BP metabolites were then eluted with 5 ml of methanol into the original (benzene-rinsed) test tube. After the evaporation of the methanol under N\(_2\), the residue was reconstituted in 250 ml of water: methanol (1:1) and analyzed by HPLC using the system described above. Nontabeled authentic BP metabolites were added before chromatography.

Tissue Specimens. Grossly normal bladder and bronchial tissues were taken from 16 patients at autopsy within 2 to 6 hr of the time of death. Tissues from patients with sepsis, metastatic cancer, serum hepatitis, or a history of tuberculosis were not used in these studies. Tracheal and bladder specimens were obtained from 4 to 6-week-old male Syrian golden hamsters and 5 to 8-week-old male CDF rats, purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Bronchus and bladder specimens were taken from mongrel dogs obtained from the Division of Laboratory Animal Medicine (Medical College of Ohio). Bladder specimens were obtained from rhesus monkeys (Macaca mulatta) supplied through the courtesy of the Animal Facility at the University of Michigan (Ann Arbor, Mich.). After death (humans) or sacrifice (animals), the tissues were removed and transported to the tissue culture laboratory in ice-cold L-15 medium. Under aseptic conditions, bronchial specimens were trimmed of adherent lung tissue. Tracheal specimens were cut longitudinally along their entire length to expose the surface of the epithelium. Bladder specimens were trimmed of muscle and connective tissue, while the epithelial layer was left intact. Samples of each specimen were fixed in phosphate-buffered 4% formaldehyde:1% glutaraldehyde (36) and embedded in Epon for assessment by light microscopy after staining with toluidine blue.

Explant Culture and Incubation with \[^{3}H\]BP. Bronchial and bladder specimens and trachea were cut into approximately 0.3 to 0.5-cm pieces and placed on the etched surface of 60-mm plastic tissue culture dishes (1 to 4 explants/dish) with the epithelium oriented toward the gas-liquid interface (37). Attempts were made to culture approximately the same amount of epithelial tissue per dish. Culture conditions were as described before (36). On the third day of explant culture, \[^{3}H\]BP (3 to 5 Ci/mmol) was added to the culture medium at a concentration of 1 \(\mu\)mol (approximately 10 \(\mu\)Ci/dish). After incubation with carcinogen for 24 hr, the medium and tissues were separated, and the media were stored immediately at -20°. The explants were rinsed twice with cold phosphate-buffered saline containing 0.1% glucose and were then pooled and stored at -20°.

Isolation of \(^{3}H\)BP-labeled DNA and BP-DNA Adduct Analysis. DNA was isolated from the explants using hydroxylapatite chromatography, as described previously in detail (36). Overall recoveries of DNA (from homogenate to pure DNA) varied from 36 to 58%. The purified \(^{3}H\)BP-DNA preparation eluted from the hydroxylapatite was dialyzed against water and redissolved in 0.1 \(\mu\)l Tris buffer, pH 7.0, containing 10 mM MgCl\(_2\) and 1 mM ZnCl\(_2\). Following enzymic digestion to the deoxyribonucleoside level (DNase, snake venom phosphodiesterase, and \(\alpha\)-thymidine phosphatase; Worthington Biochemical Corp., Freehold, N. J.) (36), the BP-DNA adducts were separated from the unmodified deoxyribonucleosides by extraction into 1 volume of n-butyl alcohol (water-saturated). In all cases, >80% of the total amount of radioactive material in the hydrolysate partitioned into the n-butyl alcohol phase. The butanol was evaporated under a stream of N\(_2\) at 37°, and the adduct residue was dissolved in 1 ml of 50% methanol. The radioactive adducts were then separated by HPLC as described before (36).

Analysis of BP Metabolites. Metabolite analysis was carried out using a Waters Model 272 HPLC (Waters Associates) system equipped with the M730 data module, radial compression module (RCM-100), UV detector, and Model 440 UV detector. The metabolites were separated on a Waters 8- \(\times\) 100-mm C-18 reverse-phase cartridge column (5-\(\mu\)m particle size). Fractions were collected, and the radioactivity in each fraction was quantitated by standard liquid scintillation techniques. For the analysis of whole medium, 1 volume of medium was mixed with an equal volume of methanol, and 500 \(\mu\)l of the mixture were injected onto the column at 22° and eluted with a 40% methanol in water:100% methanol linear gradient at 2 ml/min for 40 min. This approach always revealed a large amount of early-eluting (Fractions 2 to 6, 10 ml) radioactive material. These fractions were combined and evaporated to dryness under N\(_2\) at 41°, and the residue was reconstituted in 3 ml of 0.1 m sodium acetate buffer (pH 4.6) containing aryl sulfatase (10 units/ml; type VIII; Sigma Chemical Co., St. Louis, Mo.) and \(\beta\)-glucuronidase (72 units/ml; type VII; Sigma) and incubated at 37° for 24 hr. Following incubation, samples were extracted twice with two volumes of ethyl acetate:acetone (2:1). The organic extracts were pooled, dried under N\(_2\) at 41°, and analyzed by HPLC as described above. For the analysis of unconjugated BP metabolites, an ethyl acetate:acetone extract was prepared from pooled (4 to 7 ml) media, after which the residue was reconstituted in 0.4 ml of benzene and transferred to a normal-phase Sep-Pak. The unconjugated BP was removed from the column by elution with 2.5 ml of benzene. BP metabolites were then eluted with 5 ml of methanol into the original (benzene-rinsed) test tube. After the evaporation of the methanol under N\(_2\), the residue was reconstituted in 250 ml of water: methanol (1:1) and analyzed by HPLC using the system described above. Nontabeled authentic BP metabolites were added before chromatography.

RESULTS

In preliminary experiments, it was found that the morphology of tracheobronchial and bladder explants from all species was well maintained through at least 5 days in culture. By high-resolution light microscopy, there was excellent preservation of the normal mucociliary epithelium of the lung explants, and the integrity of the bladder epithelium was well maintained. In addition, basal and mucus-producing epithelial cells in lung explants and epithelial cells in bladder explants from all species incorporated \(^{3}H\)thymidine into their nuclei during 5 days in culture, and at a relatively constant rate from Days 2 to 5.4

HPLC analysis of whole media from cultured tracheobronchial and bladder tissues revealed, in all cases, 2 major peaks of radioactive material (Table 1). The first of these eluted with the column void volume (Fractions 2 to 6, 40 to 50% methanol in water) and therefore constituted polar material. The second peak, eluting in Fractions 49 to 51, cochromatographed with authentic BP in all cases and thus represented unmetabolized substrate. Radioactive material eluting between these 2 peaks was termed the unconjugated fraction, since many of the known unconjugated metabolites of BP eluted in this area. When indi-

4 G. D. Stoner, Unpublished data.
individual samples were analyzed, these fractions were pooled, and the total amount of radioactive material in these fractions was termed the unconjugated fraction (Table 1). In all cases, the largest proportion (38.7 to 70.4%) of radioactive material was associated with the polar fraction, with smaller proportions (14.1 to 37.6%) in the BP fractions. Unconjugated material accounted for less than 1% of the total for both tissues of all animal species, while this fraction was 2- to 10-fold higher in the human tissues (1 to 5%). In all cases, recoveries of radioactive material from the column were >75% of the total chromatographed. The fate of the balance (<25%) is unknown, because fractions were collected until the elution of BP was complete. We observed similar recoveries in earlier studies (36). Control incubations (medium with [3H]BP only) revealed negligible radioactivity in either the polar or the unconjugated fraction, indicating the stability of [3H]BP under these conditions. Human bladder was more active with respect to BP metabolism (70.4% polar material) than were the animal bladder tissues (38.7 to 59.3% polar material). Dog and human bronchial tissue exhibited comparable rates of BP metabolism (67.2 to 67.5% polar material), which differed in that an unknown product was the major metabolite, with lower but comparable amounts of trans-9,10-dihydrodiol, trans-4,5-dihydrodiol, phenols, and quinones (Table 2). This unknown eluted between trans-9,10-dihydrodiol and trans-4,5-dihydrodiol and was well separated from these 2 dihydrodiols. It is possible that this unknown metabolite is identical to (7,9/8)-triol, which has been reported to elute at this position on the chromatogram (42). Similar to the situation with bladder explants, human bronchial explants formed more BP metabolites (1515 pmol/mg of DNA; Table 2) than did the animal tissues (140.9 to 384 pmol/mg of DNA). For each animal species, the total amount of BP metabolites formed by tracheobronchial tissue was comparable to that formed by bladder tissue (Table 2). Similarly, the distribution of individual BP metabolites produced by bladder explants of the various animal species was comparable to that produced by tracheobronchial explants of the corresponding species (Table 2).

We demonstrated previously that the rate of covalent binding of [3H]BP to bladder and bronchial DNA was linear for time periods of up to 24 hr (36). The human bladder explants produced higher levels of BP:DNA adducts (6.4 µmol BP/mol of deoxyribonucleotide) than did the dog and rodent bladder explants (0.8 to 1.1 µmol BP/mol of deoxyribonucleotide; p < 0.05, Duncan multiple range test; Table 3). Binding to human bronchial explants was significantly higher (3.1 µmol/mol of deoxyribonucleotide) than was that to tracheobronchial explants of the dog and rat (0.8 to 1.8 µmol/mol of deoxyribonucleotide) but only at the p < 0.01 level of significance (Table 3). The results of these analyses are shown in Table 3. Because of the large variation in the amounts of BP metabolites formed, quantitative interspecies comparisons could not be made. It appears, however, that human bladder explants produced more BP metabolites (3342 pmol/mg of DNA) than did any of the animal species (92.5 to 368 pmol/mg of DNA; Table 2) and that trans-9,10-dihydrodiol was the major metabolite in this tissue. Whether (7,9,10/8)-tetrol and/or (7/8,9)-triol coelute with trans-9,10-dihydrodiol, as has been reported before (3, 5, 28), is not known, since these standards were not used in the present study. The rat bladder differed in that an unknown product was the major metabolite, with lower but comparable amounts of trans-9,10-dihydrodiol, trans-4,5-dihydrodiol, phenols, and quinones (Table 2). This unknown eluted between trans-9,10-dihydrodiol and trans-4,5-dihydrodiol and was well separated from these 2 dihydrodiols. It is possible that this unknown metabolite is identical to (7,9/8)-triole, which has been reported to elute at this position on the chromatogram (42). Similar to the situation with bladder explants, human bronchial explants formed more BP metabolites (1515 pmol/mg of DNA; Table 2) than did the animal tissues (140.9 to 384 pmol/mg of DNA). For each animal species, the total amount of BP metabolites formed by tracheobronchial tissue was comparable to that formed by bladder tissue (Table 2). Similarly, the distribution of individual BP metabolites produced by bladder explants of the various animal species was comparable to that produced by tracheobronchial explants of the corresponding species (Table 2).
Duncan multiple range test). Significantly different from values for dog bronchus and rat trachea (p < 0.1; Duncan multiple range test).

JIM final concentration). Covalent binding of [3H]BP to cellular DNA was determined as described in "Materials and Methods." Tentatively has been designated BPDE:dA (Charts 1 and 2). Hamster tissues were exceptional in that both bladder and tracheobronchial explants appeared to form relatively large amounts of BPDE ll:dG. The rat appeared to form relatively large amounts of BPDE II:dG. The rat was the only species producing a late-eluting adduct, which tentatively has been designated BPDE:dA (Charts 1 and 2). However, since no standard BPDE:dA was used, the identification of this adduct awaits confirmation.

DISCUSSION

The abundant formation of highly polar metabolites formed from [3H]BP by bladder and tracheobronchial explants confirms our previous results obtained with human bladder and bronchus (36) and extends these observations to several animal species.

### Table 2

Pattern of unconjugated (organic solvent-soluble) BP metabolites formed by cultured bladder and tracheobronchial tissues

<table>
<thead>
<tr>
<th>BP Metabolite</th>
<th>Human</th>
<th>Hamster</th>
<th>Dog</th>
<th>Monkey</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7,10/8,9)-Tetrol</td>
<td>577 ± 726</td>
<td>103 ± 77</td>
<td>40.2 ± 7.3</td>
<td>40.9 ± 4.6</td>
<td>8.1 ± 3.0</td>
</tr>
<tr>
<td>trans-9,10-Dihydrodiol</td>
<td>1455 ± 2114</td>
<td>124 ± 145</td>
<td>33.0 ± 27.1</td>
<td>34.2 ± 6.1</td>
<td>13.8 ± 6.4</td>
</tr>
<tr>
<td>Unknowna</td>
<td>346 ± 234</td>
<td>76.0 ± 114</td>
<td>23.1 ± 19.3</td>
<td>8.3 ± 3.7</td>
<td>40.8 ± 11.4</td>
</tr>
<tr>
<td>trans-4,5-Dihydrodiol</td>
<td>22.4 ± 26.7</td>
<td>16.8 ± 14.9</td>
<td>18.8 ± 15.4</td>
<td>5.5 ± 3.9</td>
<td>11.4 ± 13.8</td>
</tr>
<tr>
<td>trans-7,8-Dihydrodiol</td>
<td>338 ± 537</td>
<td>4.2 ± 2.4</td>
<td>8.8 ± 6.2</td>
<td>13.6 ± 1.6</td>
<td>4.1 ± 3.4</td>
</tr>
<tr>
<td>Phenols and quinonesc</td>
<td>604 ± 825</td>
<td>43.9 ± 61.7</td>
<td>59.9 ± 47.9</td>
<td>86.3 ± 24.0</td>
<td>14.1 ± 10.9</td>
</tr>
<tr>
<td>Total</td>
<td>3342 ± 4141</td>
<td>368 ± 411</td>
<td>183 ± 101</td>
<td>168 ± 24</td>
<td>92.5 ± 38.7</td>
</tr>
<tr>
<td>Trachea-bronchus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7,10/8,9)-Tetrol</td>
<td>319 ± 132</td>
<td>156 ± 42</td>
<td>6.1 ± 59.3</td>
<td>NDd</td>
<td>25.3 ± 15.6</td>
</tr>
<tr>
<td>trans-9,10-Dihydrodiol</td>
<td>397 ± 435</td>
<td>82.9 ± 14.4</td>
<td>40.5 ± 42.2</td>
<td>ND</td>
<td>30.4 ± 16.7</td>
</tr>
<tr>
<td>Unknowna</td>
<td>369 ± 197</td>
<td>81.6 ± 89.7</td>
<td>38.2 ± 56.2</td>
<td>ND</td>
<td>55.0 ± 17.1</td>
</tr>
<tr>
<td>trans-4,5-Dihydrodiol</td>
<td>10.9 ± 18.9</td>
<td>12.4 ± 2.0</td>
<td>32.5 ± 37.0</td>
<td>ND</td>
<td>10.5 ± 13.2</td>
</tr>
<tr>
<td>trans-7,8-Dihydrodiol</td>
<td>148 ± 199</td>
<td>10.7 ± 8.9</td>
<td>42.9 ± 30.1</td>
<td>ND</td>
<td>4.9 ± 3.8</td>
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<tr>
<td>Phenols and quinonesc</td>
<td>271 ± 296</td>
<td>40.6 ± 42.7</td>
<td>82.3 ± 67.2</td>
<td>ND</td>
<td>14.8 ± 11.7</td>
</tr>
<tr>
<td>Total</td>
<td>1515 ± 875</td>
<td>384 ± 170</td>
<td>298 ± 291</td>
<td>140.9 ± 11.7</td>
<td></td>
</tr>
</tbody>
</table>

a Mean ± S.D.
b The unknown possibly represents (7,9/8)-triol.
c Because of incomplete separations, these metabolites were grouped together.
d ND, not determined.

### Table 3

DNA binding in bladder and tracheobronchial explants

<table>
<thead>
<tr>
<th>Species</th>
<th>Bladder</th>
<th>Trachea-bronchus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>6.4 ± 5.3 (17)</td>
<td>3.4 ± 2.0 (16)</td>
</tr>
<tr>
<td>Monkey</td>
<td>2.4 ± 1.2 (5)</td>
<td>0.8 ± 0.4 (4)</td>
</tr>
<tr>
<td>Dog</td>
<td>1.0 ± 0.6 (4)</td>
<td>1.8 ± 1.2 (6)</td>
</tr>
<tr>
<td>Rat</td>
<td>0.8 ± 0.6 (6)</td>
<td>1.8 ± 1.2 (6)</td>
</tr>
<tr>
<td>Hamster</td>
<td>1.1 ± 0.4 (5)</td>
<td>3.4 ± 3.0 (5)</td>
</tr>
</tbody>
</table>

a Mean ± S.D.

Numbers in parentheses, number of explants.

Significantly different from values for monkey bladder (p < 0.1) and dog, rat, and hamster bladder (p < 0.05; Duncan multiple range test).

Significantly different from values for dog bronchus and rat trachea (p < 0.1; Duncan multiple range test).

be considered semiquantitative, however, since, in most cases, 7S-BPDE I:dG was not well separated from the 7R-BPDE I:dG adduct (Charts 1 and 2). Moreover, the structure 7S-BPDE I:dG cannot be assigned with certainty to this peak, because other BPDE:dG adducts, such as those substituted at Position 9 of the BP molecule, may also elute in this region (13, 19). Similarly, tentative chromatographic evidence for the presence of the BPDE II:dG adduct was obtained in most bladder tissues (Chart 1), but the amount of this adduct could not be quantitated. Except for the human tissue, the bladder explants always formed more of Unknown I than of Unknown II.

Contrary to the situation observed with the bladder, 7R-BPDE I:dG was the most abundant adduct formed by tracheobronchial explants of all species (Chart 2). Hamster tissues were exceptional in that both bladder and tracheobronchial explants appeared to form relatively large amounts of BPDE II:dG. The rat was the only species producing a late-eluting adduct, which tentatively has been designated BPDE:dA (Charts 1 and 2). However, since no standard BPDE:dA was used, the identification of this adduct awaits confirmation.
The small proportion of unconjugated metabolites found in the medium (Table 1) confirms our previous findings (36) with human bladder and bronchial explants. It is possible that, even though the amount of radioactivity associated with the tissues was always less than 0.5% of the total, some unconjugated metabolites remained bound (covalently or noncovalently) to tissue macromolecules other than DNA (e.g., RNA and proteins), but this was not quantitated. The relative amounts of unconjugated BP metabolites formed by human bladder explants (Table 2) are similar to those obtained by Moore et al. (28), who, using a similar experimental approach, also found that tetroles and the trans-9,10-dihydrodiol were major metabolites with lesser amounts of the other dihydrodiols. In contrast to the explants, human bladder epithelial cell lines produce predominantly trans-9,10-dihydrodiol and trans-7,8-dihydrodiol and relatively small amounts of tetroles (2). In both this study and in an earlier study (28), 2 other potential BP metabolites, (7,9,10/8,9)-tetrole and (7/8,9)-triol, were not used as standards, so that the adequacy of their separation from trans-9,10-dihydrodiol, which is often difficult to achieve (5, 28), could not be verified. Therefore, the quantitation of trans-9,10-dihydrodiol has to be interpreted with this reservation. While Moore et al. (28) found that the pattern of BP metabolites formed by human bladder explants is quite similar to that formed by rat bladder explants, our results show that the rat bladder forms more of an unknown, which elutes after trans-9,10-dihydrodiol (Table 2). Whether this is due to slight differences in culture conditions or to the different rat strains used remains to be established. Hamster, dog, and monkey bladder explants are all capable of metabolizing BP, albeit to different extents (Table 2).

Patterns of unconjugated BP metabolites formed by human bronchial explants (Table 2) are similar to those reported in earlier studies (3, 5) in that tetroles and trans-9,10-dihydrodiol are the major metabolites, with much smaller amounts of other dihydrodiols. Also, in both studies, phenols and quinones represent a substantial portion of the unconjugated metabolites formed. Compared to the rat trachea, the hamster trachea appears to form larger amounts of (7,10/8,9)-tetrole, trans-9,10-dihydrodiol (Table 2). This pattern has been observed before in tracheal tissues from these 2 rodent species (5, 27) and may be related to their differing susceptibilities to BP-induced respiratory cancer (32). Our results are the first experimental evidence that the dog bronchus has the capacity to metabolize BP to proximate carcinogenic forms (Table 2). It is interesting to note that repeated injections of BP into the submucosa of the dog bronchus does not result in squamous cell carcinoma (although premalignant lesions are observed), even when such dogs are followed for up to 34 months (8, 25). Only when the s.c. implanted bronchial autograft is treated with BP (20, 21) can invasive squamous cell carcinoma be demonstrated in this dog organ.

As we found before (36), BP binding to human bladder DNA is higher than that to human bronchial DNA (Table 3). In another study, similar in design to the present study (28), it was found that human and rat bladder DNA bind similar amounts of BP, while the results of both the present study (Table 3) and another study involving cultured cells (2) indicate that rat bladder DNA binds lower amounts of BP than does human bladder DNA. BP binding to human bronchial DNA and hamster tracheal DNA is similar (Table 3). This is in contrast to other findings, which indicate that binding to hamster tracheal DNA is higher (5). It is possible that some of the interspecies differences in the levels...
of BP-DNA adduct formation observed in this and other studies are due to factors other than intrinsic metabolic capacities.

In agreement with our previous results (36) obtained with human bladder and bronchial explants, 7R-BPDE adducts are the major adduct formed between BP and DNA from both tissues of most species (Charts 1 and 2). This adduct also constitutes the major component of [3H]BP-DNA isolated from explants of human and bovine bronchus (18), human colon and esophagus (3), and rodent trachea (5). In all of these studies, much lower amounts of 7S-BPDE adducts are found. An exception to this phenomenon is the rat trachea in explant culture (5), which forms equivalent amounts of the 7R- and 7S-BPDE adducts. Unfortunately, in this study, the chromatographic resolution of 2 enantiomeric adducts was incomplete (Charts 1 and 2); therefore, their individual quantities could not be obtained. Explants of monkey and hamster bladder and hamster trachea form an adduct which has been tentatively assigned the structure BPDE II:dG, since it coeluets with the synthetic standard. While this adduct is not completely separated from the 7R-BPDE adduct (Charts 1 and 2), the amounts are always less than the amounts of the combined BPDE I adducts (Table 3). This situation has been observed before, both with similar experimental systems (3, 5, 34) and with other systems, including mouse skin (22), rat liver microsomes (41), and cultured mouse embryo fibroblasts (33). Thus, the stereoselective synthesis (and persistence) of BPDE I enantiomers appears to be a fairly general phenomenon. Exceptions are human alveolar tumor cells (7) and baby hamster kidney cells (33), both of which form comparable amounts of the BPDE I:dG and BPDE II:dG adducts after incubation with BP.

A late-eluting adduct appears to be specific for rat tissues (Charts 1 and 2). This adduct has a retention time which is similar to that reported for BPDE:Da formed by rat colon explants (4) and rat tracheal explants (5). However, formation of BPDE:Da adducts by hamster tracheal epithelial cells (13) and primary hamster embryo cells (17) has also been reported, and it appears that these adducts may be excised rapidly (12, 13). Thus, it is possible that the rat is relatively less efficient in its ability to remove these adducts.

In all of the species and tissues studied, 2 unknown adducts (I and II, Charts 1 and 2) are formed by the explants. These appear to correspond to Unknown Adducts I and II formed by human bronchial explants after incubation with BP (5). Even though BPDE:dc adducts elute in this region (i.e., before BPDE I:dG adducts) of the chromatogram (6, 19), the considerable overlap between the HPLC profiles of the synthetic adducts formed between BP and the deoxycytidine homopolymer and between BP and the deoxyguanosine homopolymer (13, 19) preclude assignment of tentative structures to Unknown Adducts I and II isolated in the present study. Alternatively, it is possible that Unknown II is an adduct formed between BPDE II and DNA (6) or that Unknown I (and/or Unknown II) represents BPDE I adducts to the O6 or N7 of guanine (29). Considering the patterns of BP:DNA adducts for the various species (Charts 1 and 2), it is clear that these patterns are specific for each species and that the different patterns are probably related to different rates of formation and/or removal of adducts.

ACKNOWLEDGMENTS

We thank Drs. P. J. Golabek, A. F. Goheara, N. J. Budd, and M. Alousi for assistance in obtaining human tissues and J. Ungerleider of the University of Michigan for providing the monkey bladder tissues. We also thank B. Greener for assistance in the preparation of the manuscript.

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