Metabolism and Binding of Benzo(a)pyrene and 2-Acetylaminofluorene by Short-Term Organ Cultures of Human and Rat Bladder

Brian P. Moore, R. Marian Hicks, Margaret A. Knowles, and Susan Redgrave

INTRODUCTION

Despite the development of many new experimental systems to study carcinogenesis in vitro in human tissues, the bulk of carcinogen testing is still done with experimental animals. In order to make some assessment of the validity of extrapolating carcinogenicity data from animals to humans, it is useful to compare the rates and products of metabolism of carcinogens by human and animal tissues in vitro. For many years, it was thought that most carcinogens were metabolically converted to active intermediates by mixed-function oxidases and other enzymes in the liver and that the metabolites produced subsequently interacted covalently with critical macromolecules in target organs (48). Comparatively recently, however, as more sensitive techniques have become available, metabolism of carcinogens by their peripheral target tissues has been detected. Tissue preparations from many different human peripheral organs including lung (4, 13), bronchial mucosa (6, 22, 25, 26), esophagus (27), mammary gland (23), pancreatic duct (28), endometrium (46), gastrointestinal mucosa (2, 3, 5), and skin (44) are known to be capable of metabolizing and binding representative carcinogens from different chemical classes.

The data associating human bladder cancer with exposure to certain industrial chemicals, particularly arylamines such as 2-naphthylamine and benzidine, are extremely strong and have led to legislative restriction on the use of such chemicals in many countries (11, 31). It has been assumed, but is not proven, that carcinogenesis in the bladder depends on metabolic activation of these carcinogens elsewhere in the body (12, 35, 38). The bladder is, of course, a storage organ for urine which undoubtedly contains the excreted metabolites of many compounds formed elsewhere, notably in the liver and kidneys. For example, glucuronide conjugates of BP, 2-naphthylamine, and AAF are all excreted via the urine (20, 34, 40). Some may well be sufficiently unstable to spontaneously generate active intermediates capable of initiating carcinogenesis without further metabolism in situ (34, 40), whereas others may require further enzymatic activation. This is undoubtedly possible since both urine and the lysosomes of the superficial urothelial cells contain β-glucuronidase (9, 42) which could recover the glucuronides to potential proximate carcinogens. Furthermore, microsomes prepared from bovine bladder mucosa possess cytochrome P-450 and are capable of metabolizing an arylamine bladder carcinogen, 4-amino-biphenyl (53), and whole cells from a similar source have been used to activate precarcinogens in an in vitro mutagenicity test (45). Dog bladder epithelial cells have also been shown to metabolize a presumed bladder-specific carcinogen, 2-amino-4(5-nitro-2-furyl)thiozole, to active species that bind to protein (61). The experiments reported here show that the unmethylated carcinogens BP and AAF can both be metabolized by human bladder tissue in vitro to the same derivatives as are.

The ability of organ cultures of normal human and rat bladder to metabolize the polycyclic hydrocarbon, benzo(a)pyrene (BP), and the arylamine, 2-acetylaminofluorene, has been studied. Cultures were maintained for 0 to 6 days in a chemically defined medium before incubation with [3H]BP (0.3 to 0.5 μM) or 2[14]C]acetaminofluorene (18 to 25 μM) for 24 hr. Ethyl acetate-soluble and water-soluble metabolites were produced from both compounds by both species. The ethyl acetate extracts from [3H]BP-treated human cultures contained 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, and 3-hydroxybenzo(a)pyrene. Rat bladder cultures produced similar metabolites but in slightly different proportions. Ethyl acetate-soluble products of 2[14]C]acetaminofluorene from human cultures contained 7-hydroxy-2-acetylaminofluorene, 9-hydroxy-2-acetyaminofluorene, 2-aminofluorene, and N-hydroxy-2-acetyaminofluorene. Rat bladder cultures produced similar metabolites, but 2-amino- fluorene was found in relatively higher proportion. Hydrolysis of the water-soluble products produced from both carcinogens gave ethyl acetate-extractable derivatives. These hydrolyzable glucuronide conjugates were relatively more abundant following metabolism of the carcinogens by the rat than by the human cultures.

Covalent binding to DNA occurred with [3H]BP in both human (19.7 ± 1 pmol/mg DNA) and rat cultures (22.8 ± 8.6 pmol/mg DNA). As with other human tissues, considerable variation (50-fold) was observed between individuals.

The results demonstrate that both human and rat bladder epithelium can metabolize known potent carcinogens and, in the case of BP, can effect covalent binding between the products of metabolism and the urothelial cell DNA. In theory, carcinogenesis in the urinary bladder could thus be initiated by the case of BP, can effect covalent binding between the product of metabolism and the urothelial cell DNA. In theory, carcinogenesis without further metabolism in situ (34, 40), whereas others may require further enzymatic activation. This is undoubtedly possible since both urine and the lysosomes of the superficial urothelial cells contain β-glucuronidase (9, 42) which could recover the glucuronides to potential proximate carcinogens. Furthermore, microsomes prepared from bovine bladder mucosa possess cytochrome P-450 and are capable of metabolizing an arylamine bladder carcinogen, 4-amino-biphenyl (53), and whole cells from a similar source have been used to activate precarcinogens in an in vitro mutagenicity test (45). Dog bladder epithelial cells have also been shown to metabolize a presumed bladder-specific carcinogen, 2-amino-4(5-nitro-2-furyl)thiozole, to active species that bind to protein (61). The experiments reported here show that the unmethylated carcinogens BP and AAF can both be metabolized by human bladder tissue in vitro to the same derivatives as are.

\(^{3}\) The abbreviations used are: BP, benzo(a)pyrene; AAF, 2-acetylaminofluorene; N-OH-AAF, N-hydroxy-2-acetylaminofluorene; HPLC, high-performance liquid chromatography; 9-OH-AAF, 9-hydroxy-2-acetylaminofluorene; 7-OH-AAF, 7-hydroxy-2-acetylaminofluorene; 5-OH-AAF, 5-hydroxy-2-acetylaminofluorene; 3-OH-AAF, 3-hydroxy-2-acetylaminofluorene; 1-OH-AAF, 1-hydroxy-2-acetylaminofluorene; AF, 2-amino-7,8-diol, trans-10-dihydro-9,10-dihydroxybenzo(a)pyrene; 9,8-diol, trans-9,10-dihydro-9,10-dihydroxybenzo(a)pyrene; 4,5-diol, trans-4,5-dihydroxy-4,5-dihydroxybenzo(a)pyrene; 9-OH-BP, 9-hydroxybenzo(a)pyrene; 3-OH-BA, 3-hydroxybenzo(a)pyrene; 7,10/8,9-tetrol, tetrahydrodiol, tetrahydrodiol, tetrahydrobenzo(a)pyrene in which the 10-hydroxy group is cis and the 8-hydroxy and 9-hydroxy groups are trans to the reference 7-hydroxy group (other tetroles and triols are similarly designated).

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\(^{2}\) To whom requests for reprints should be addressed.

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produced by other tissues such as the liver (36, 65). BP is normally regarded as a carcinogen for the skin and lung. However, since cigarette smoke also contains BP and since cigarette smoking is also epidemiologically linked to an increased risk for bladder cancer, the metabolism of BP and its conjugates by the urinary bladder may be of etiological significance. AAF, though a proven bladder carcinogen in rodents and dogs (1, 8, 12, 17), is not known to be carcinogenic for humans. However, its metabolism is a model for many arylamines which are proven human bladder carcinogens. The possibility that carcinogens such as these may be excreted in the urine (54, 55) and metabolized and activated by normal human urothelium has considerable implications for the dye and chemical industries producing potentially carcinogenic compounds, many of which are derivatives of known carcinogens such as tolidine, benzidine, and dianisidine.

**MATERIALS AND METHODS**

**Chemicals.** [3H]BP (37 to 40 Ci/mmol) and 9-[^14C]AAF (55 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, England. [3H]-CN-OOH-AAF (6 mCi/mmol) was generously donated by Dr. F. Beland, National Center for Toxicological Research, Jefferson, Ark. [3H]BP was purified before use by eluting through a Sep-Pak silica gel (Waters Associates, Hartford, Cheshire, England) with benzene. [3H]-AAF was used as supplied (98% pure as measured by thin-layer chromatography). Authentic metabolites of BP and AAF were obtained from the Chemical Repository through Dr. David Longfellow of the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. All compounds were protected from light and stored at −20°C.

**Bladder Tissues.** Cup biopsies were obtained from consenting male and female patients undergoing routine cystoscopic examination. Biopsies were placed in ice-cold buffered medium immediately after removal from the patient and subsequently cultured within 3 hr. Portions of tissue adjacent to those cultured were assessed histologically after fixation and staining with hematoxylin and eosin. Samples with little or no normal urothelium were discarded. Half-bladders from F344 rats were used for comparison.

**Culture.** Cup biopsies were, as far as possible, trimmed free of muscle and connective tissue under a binocular microscope and cultured as 0.5-cm fragments (2.2 to 27.6 mg) in 2 ml of Ham’s Medium F-12 supplemented with 10% calf serum, hydrocortisone (1 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and ferric sulfate (0.45 μg/ml). Rat bladder tissue was cultured similarly excepting that Waymouth’s Medium MN 752/1 containing identical supplements was used in place of Ham’s F-12. These 2 media were used because they have proved to be the most successful in preserving normal morphology when human or rat bladders are maintained in long-term cultures (up to 70 days) (43). However, for carcinogen metabolism experiments, the published procedure was modified by culturing directly onto scratched plastic Petri dishes (5 cm) and incubating on a rocking platform (10 cycles/min) in an airtight gas chamber (95% air:5% CO2) which facilitated the exchange of carcinogen from the medium. [3H]BP (0.3 to 0.5 μCi) or [3H]Arctonol (18 to 25 μCi) was added to the media from stock solutions in dimethyl sulfoxide (final concentration, <0.2%) and was incubated with the cultures for 24 hr during the first or second days of culture (0 to 24 and 24 to 48 hr, respectively) or for 24 hr after 6 days (6 to 7 days). After incubation with carcinogen, the tissues and media were separated and stored at −20°C until analyzed. Control incubations, from which tissue was omitted, were included with each experiment and showed that BP and AAF were similarly stable in both media and that only low levels of unidentified oxidative metabolites were produced nonenzymatically.

**Biochemical Studies.** Media from [3H]BP-treated cultures were extracted once with 2 volumes of ethyl acetate:acetone (2:1) and twice with 2 volumes of ethyl acetate. After the extract was dried over anhydrous sodium sulfate and then evaporated to dryness in a vacuum, the residue, redissolved in 1 ml benzene, was applied to a Waters Sep-Pak silica gel. Unmetabolized BP was eluted with 5 ml benzene, and metabolites were eluted with 8 ml methanol. Samples of the methanolic fraction were filtered, dried in a vacuum, and subjected to HPLC on a Zorbax octadecysilane column (250 x 6.2 mm; DuPont Instruments) using a 60% methanol in water to 100% methanol linear gradient (40 min) at a flow of 1 ml/min, essentially as described by Yang et al. (65). The chromatograph was used with a Laboratory Data Control Analyst 7800 equipped with 2 constantpump, a Spectromonitor III detector, and a gradient master controller.

The medium from [3H]AFP-treated cultures was processed similarly except that the organic phase obtained after 2 extractions with 2 volumes of ethyl acetate was, after drying, applied directly to HPLC. Chromatographic separation was achieved by a modification of the procedure described by Thorgeirsson and Nelson (59) using a Zorbax C-8 column (250 x 4.2 mm; DuPont Instruments). A 30-min linear gradient from 60% buffer:40% solvent (buffer, 0.1 M Tris (pH 8.5); solvent, methanol:propanol (9:1) to 35% buffer:65% solvent at a flow of 1 ml/min separated 9-OH-AAF, 7-OH-AAF, 5-OH-AAF, 3-OH-AAF, and 1-OH-AAF from AF and the parent compound. N-OH-AAF was eluted with 100% solvent at the end of the linear gradient.

Fractions of 0.5 ml were collected from both HPLC procedures for 55 min, and the radioactivity was determined in a Packard 4600 liquid scintillation counter with 3 ml Fissolution (Fisons, Loughborough, United Kingdom) scintillation cocktail. Radioactive products were identified by comparison with the UV absorption peaks of authentic metabolites added during the work-up procedure.

DNA was extracted from bladder cultures that had been digested with protease K (BDH Chemicals, Poole, Dorset, United Kingdom) using hydroxyapatite chromatography (7) as described by Aitrevs et al. (3). DNA was quantified from its UV absorption spectrum recorded between 230 and 300 nm using a Pye Unicam SP1800 spectrophotometer and material bound by liquid scintillation counting.

Water-soluble conjugated metabolites of [3H]BP or [3H]AAF, which remained in the culture media after extraction with organic solvent, were hydrolyzed by incubation with equal volumes of β-glucuronidase (5000 units/ml) (Ketodase, Warner-Chilcott Laboratories) or with aryl-sulfatase (50 units/ml) (Sigma Chemical Co. Ltd.) containing 40 mEq-saccharic acid-1,4-lactone in 0.1 M sodium acetate buffer, pH 5.0 (51). The products released by the enzyme treatment were reextracted into ethyl acetate and analyzed in a similar way to that described for the free organic solvent-soluble products.

**RESULTS**

**Metabolism of [3H]BP by Human and Rat Bladder Cultures.** [3H]BP was metabolized to organic solvent-soluble and water-soluble products by cultures of rat and human bladder tissue. Less organic solvent-soluble metabolites were formed by rat bladder cultures than human bladder cultures, but they were qualitatively similar showing only minor differences in the proportions of individual metabolites produced. A product that cochromatographed with 9,10-diol was the major metabolite from both species (Table 1) but was produced to a slightly higher degree in rat cultures than in human cultures. 9,10-Diol is often a major metabolite in culture systems (4, 6, 13, 23, 51) possibly because it is either a poor substrate for UDP-glucuronyl transferase (52) or less well bound by some tissue macromolecules (60) and is thus easily excreted from cells. However, caution must be exercised in the interpretation, because in the chromatography system used, 9,10-diol cochromatographs with 2 other potential metabolites, 7,8,9-triol and...
7,9,10/8-tetrol. The production of polar metabolites, e.g., sulfates, tetrols, and triols that chromatographed early on the HPLC gradient, and of 7,8-diol and mono-hydroxy metabolites was relatively higher in human than in rat cultures (Table 1). Figures for the production of 4,5-diol, 9-OH-BP, and quinones, although given in Table 1, were not significantly above background levels produced by 24-hr incubation of [3H]BP with culture medium only.

A larger proportion of [3H]BP was metabolized to water-soluble products by rat bladder cultures (32 ± 2.5% of initial radioactivity) than by human bladder cultures (16.9 ± 8.2% of initial radioactivity for all human bladder cultures). This can be attributed to the larger quantities of tissue in the experiments with rat bladder (44.2 ± 10.2 mg) than in those with human bladder (13.8 ± 7 mg), although when calculated in absolute terms, human bladder produced more water-soluble metabolites per gram of tissue (Table 1). This result, however, should be treated with due caution because it is uncertain if there is a direct relationship between the weight of tissue used and amount of metabolites produced. Approximately 15 to 20% of the water-soluble radioactive products from human cultures and a slightly higher proportion from rat cultures, 20 to 30%, were susceptible to enzymatic hydrolysis with β-glucuronidase to yield ethyl acetate-extractable materials. Insufficient radioactive product was released for HPLC analysis of the deconjugated metabolites from human cultures, but the material released from rat cultures could be analyzed. Major peaks of radioactive materials were detected that cochromatographed with 3-OH-BP and 9-OH-BP (28 to 30%), 4,5-diol (6 to 7%), 7,8-diol (5.3 to 6.8%), and unidentified products at the start of the HPLC gradient, probably 7,10/8,9-tetrol and 7/8,9,10-tetrol (18 to 20%). Only a relatively small proportion of the radioactivity released (<3.5%) was associated with quinones which chromatograph between the monohydroxybenzo(a)pyrenes and the parent compound. Treatment of these media with arylsulfatase released a similar proportion of the total radioactive material (23%) to ethyl acetate-soluble products. HPLC of the released material showed a very different pattern of metabolites. In this case, almost all the radioactivity (>75%) cochromatographed early on the HPLC gradient in the tetrol region with little or no activity above background levels associated with 4,5-diol (0.3%), 7,8-diol (0.2%), or monohydroxybenzo(a)pyrenes (2.5%). Control experiments run in parallel, in which buffer only was incubated with the water-soluble metabolites, failed to release significant amounts of material to ethyl acetate-extractable products (<5% of total) which when subjected to HPLC gave a minor peak of radioactivity that chromatographed in the monohydroxybenzo(a)pyrene region. These results, with monohydroxybenzo(a)pyrenes as the main glucuronide conjugates, were essentially similar to those obtained previously with other rat tissues when enzymatic deconjugation was also used to analyze the water-soluble metabolites of BP (36, 51).

Although the patterns of metabolism remained fairly constant in human cultures with a remarkably consistent proportion of water-soluble metabolites (79.0 ± 15.0%), a considerable individual variation was observed in total metabolism (4.2 to 36.6% of total radioactivity). This variation could not be related to the age of the culture (0 to 24 hr, 1 to 2 days, or 6 to 7 days) but may have been affected by the considerable variation in amounts and viability of human tissue cultured. Less variation was observed in rat bladder cultures, either in the overall metabolism (25 to 35% of total radioactivity) or in the proportion of water-soluble metabolites (93.6 ± 2.8%).

### Table 1

<table>
<thead>
<tr>
<th>No. of animals or patients</th>
<th>Water-soluble metabolites (nmol/hr/g wet wt)</th>
<th>Organic-soluble metabolites (nmol/hr/g wet wt)</th>
<th>Early chromatographing products ( % of total organic-soluble metabolites)</th>
<th>4,5-diol (% of total organic-soluble metabolites)</th>
<th>7,8-diol (% of total organic-soluble metabolites)</th>
<th>9-OH-BP (% of total organic-soluble metabolites)</th>
<th>3-OH-BP (% of total organic-soluble metabolites)</th>
<th>Quinones (% of total organic-soluble metabolites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 24-48 hr</td>
<td>6.0 ± 0.2 (5)</td>
<td>0.44 ± 0.17</td>
<td>0.03 ± 0.02</td>
<td>12.5 ± 0.7</td>
<td>33.6 ± 10.0</td>
<td>&lt;1.0</td>
<td>9.7 ± 7.6</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>0-24 hr</td>
<td>4.1 ± 1.0</td>
<td>0.76 ± 0.8</td>
<td>0.1 ± 0.04</td>
<td>20.2 ± 7.2</td>
<td>20.5 ± 5.5</td>
<td>&lt;1.5</td>
<td>13.5 ± 3.6</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>Human 24-48 hr</td>
<td>9.0 ± 0.5</td>
<td>0.97 ± 0.5</td>
<td>0.13 ± 0.08</td>
<td>20.8 ± 3.7</td>
<td>17.5 ± 5.7</td>
<td>&lt;0.9</td>
<td>9.6 ± 2.9</td>
<td>3.3 ± 1.4</td>
</tr>
<tr>
<td>6-7 days</td>
<td>3.1 ± 0.7</td>
<td>0.95 ± 0.6</td>
<td>0.16 ± 0.12</td>
<td>18.4 ± 7.4</td>
<td>21.9 ± 7.7</td>
<td>&lt;1.4</td>
<td>9.8 ± 3.7</td>
<td>3.1 ± 2.2</td>
</tr>
<tr>
<td>All human</td>
<td>16.1 ± 0.7</td>
<td>0.85 ± 0.6</td>
<td>0.15 ± 0.08</td>
<td>19.7 ± 5.7</td>
<td>21.1 ± 6.8</td>
<td>&lt;1.4</td>
<td>10.9 ± 3.4</td>
<td>3.0 ± 1.6</td>
</tr>
</tbody>
</table>

a Includes 4 possible tetrols and sulfate conjugates.
b Mean ± S.D.
Carcinogen Metabolism in Bladder Cultures

Human and rat bladder cultures metabolized [\(^{14}\)C]AAF to ethyl acetate-soluble and water-soluble metabolites. Ethyl acetate-extractable metabolites that cochromatographed with 7-OH-AAF, 9-OH-AAF, AF, and N-OH-AAF were produced by cultures of both species but to differing extents (Table 3). The hydroxylation products, 7-OH-AAF and 9-OH-AAF, made up a larger proportion of the ethyl acetate-soluble metabolites from human cultures (approximately 26%) than from rat cultures (8%). This difference may be partially attributed to the greater degree of secondary metabolism to form conjugates which occurred in the rat cultures. AF, a product of deacetylation, which was not apparently metabolized to glucuronide conjugates, was relatively more abundant in the extracts from rat bladder than those from human bladder. The production of N-OH-AAF, which elutes from the HPLC as a rather broad peak well behind all other radioactive materials including the parent compound, was determined after correction by subtraction of equivalent fractions from the radioactive profiles of control cultures. This, however, may result in underestimation of its production.

Rat cultures produced considerably more water-soluble products (6.7 ± 2.5% of starting material) than did human cultures (1.6 ± 0.8%), although, as was also apparent in the [\(^{3}\)H]BP experiments, the actual rate of production was greater in human bladder cultures (Table 3). Seventy to 80% and 48 to 67% of the water-soluble metabolites from rat and human bladder cultures, respectively, were hydrolyzed to ethyl acetate-soluble products by β-glucuronidase. Control incubations with buffer alone released much smaller quantities of radioactivity-soluble products by β-glucuronidase. Control incubations failed to release significant amounts of radioactive materials above those of control levels from rat cultures but yielded levels approximately 10% higher than did controls from human cultures.

### Table 2

<table>
<thead>
<tr>
<th>No. of animals or patients</th>
<th>Carcinogen binding (pmol/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>6</td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>0–24 hr</td>
<td>5</td>
</tr>
<tr>
<td>24–48 hr</td>
<td>6</td>
</tr>
<tr>
<td>6–7 days</td>
<td>3</td>
</tr>
<tr>
<td>All human</td>
<td>14</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

### Table 3

<table>
<thead>
<tr>
<th>No. of animals or patients</th>
<th>Total metabolites (nmol/hr/g wet wt)</th>
<th>Water-soluble metabolites (nmol/hr/g wet wt)</th>
<th>Organic-soluble metabolites (nmol/hr/g wet wt)</th>
<th>7-OH-AAF (% of organic-soluble metabolites)</th>
<th>9-OH-AAF (% of organic-soluble metabolites)</th>
<th>N-OH-AAF (% of organic-soluble metabolites)</th>
<th>AF (% of organic-soluble metabolites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>7</td>
<td>6.7 ± 2.7</td>
<td>2.0 ± 0.6</td>
<td>4.6 ± 2.4</td>
<td>9.7 ± 0.4</td>
<td>5.3 ± 2.0</td>
<td>6.9 ± 4.0</td>
</tr>
<tr>
<td>Human</td>
<td>9</td>
<td>22.5 ± 19.6</td>
<td>5.7 ± 8.1</td>
<td>16.8 ± 13.0</td>
<td>15.0 ± 7.0</td>
<td>11.2 ± 3.6</td>
<td>5.9 ± 3.6</td>
</tr>
</tbody>
</table>

* Tissue quantity, 66.8 ± 22.7 mg.
* Mean ± S.D.
* Tissue quantity, 13.4 ± 6 mg.

DISCUSSION

The experiments reported here demonstrate that the normal enzyme complement of human and rat bladder is capable of metabolizing 2 carcinogens, a polycyclic hydrocarbon, BP, and an arylamine, AAF, to the same metabolites that are produced by metabolism in other organs including the liver. BP, a ubiquitous carcinogenic constituent of tobacco smoke, is normally regarded as a skin and respiratory tissue carcinogen (30) but was included in the present study because of the epidemiolog-
ical link between cigarette smoking and an increased relative risk of developing bladder cancer (15). The pathways of BP metabolism reported here, including that which leads eventually to the production of the ultimate carcinogen benzo(a)-pyrene-7,8-dihydrodiol-9,10-epoxide (58), are similar to those found previously in a number of tissues from humans and experimental animals (4–6, 13, 23, 28, 51) and demonstrate the ability of the urothelium to activate this carcinogen. The considerable interindividual variations in rates of metabolism and DNA binding by human bladder parallel the variability in BP metabolism (4, 5, 14) and DNA binding (2, 24, 27) demonstrated for other human tissues.

AAF was demonstrated to be carcinogenic for experimental animals in the 1940s, and fortunately, its development as a potential insecticide was discontinued. It has, however, been used widely as a model compound for studying the metabolism of aromatic amines. It is both a hepatocarcinogen and a bladder carcinogen for mice, rats, rabbits, and dogs (1, 8, 12, 17). Metabolic activation of AAF by the liver proceeds by at least 2 steps, the first of which is a cytochrome P-450-dependent N-hydroxylation (16). The product formed, N-OH-AAF, is a substrate for sulfotransferase, N,O-acetyltransferase, membrane-bound deacetylase, and UDP-glucuronyltransferase, all of which have been implicated in the formation of carcinogenic or mutagenic products (47, 48). In addition, AAF is metabolized by aromatic hydroxylation forming 1-OH-AAF, 3-OH-AAF, 5-OH-AAF, and 7-OH-AAF; by aliphatic hydroxylation forming 9-OH-AAF; and by deacetylation to AF (49). Production of all these metabolites by tissue fractions of human, rat, and cotton rat liver has been confirmed recently (18, 19, 56). With the exception of 1-OH-AAF, the same metabolites were identified as free or conjugated compounds following metabolism of AAF by rat and human bladder cultures. The major glucuronide conjugates formed by the rat bladder cultures, those of 7-OH-AAF, 5-OH-AAF, and 3-OH-AAF, are also produced from AAF by hepatocytes (18) and are excreted in the urine of the same species (49, 54, 63). However, in contrast to findings with rat hepatocytes (18), AF was not converted to a glucuronide conjugate by either rat or human bladder cultures but remained entirely as the free metabolite. Rat urine contains only small amounts of the free amine after AAF feeding (64), but it is also known that AF can be reacetylated and converted to ring and N-hydroxylated products (49). Its formation in substantial quantities by the bladder culture systems of both species suggests that deacetylation could be important in bladder carcinogenesis, for, if N-OH-AAF were metabolized by this pathway, the highly mutagenic compound N-hydroxy-2-aminofluorene would be formed (33, 57, 62).

Although only trace levels of material cochromatographing with N-OH-AAF were released by β-glucuronidase from the water-soluble metabolites of AF, N-OH-AAF was clearly metabolized to a glucuronide conjugate by rat and human cultures when they were presented with the free metabolite. The O-glucuronide of N-OH-AAF, a urinary metabolite of AAF (32, 49), is a weakly electrophilic agent which may be directly important in carcinogenesis (34). However, the ability of bladder tissues to readily perform deacetylation reactions could result in the formation of the highly reactive O-glucuronide of N-hydroxyminofluorene from this material (10, 35). Kadlubar et al. (37) have suggested recently that N-hydroxycarboxyamines, such as those of 1- and 2-naphthyamine, are generally con-

jugated with glucuronide via the nitrogen atom rather than via the oxygen to produce stable isolatable products. In mild acidic conditions, these materials are hydrolyzed to the parent hydroxycarboxyamines which can react covalently with DNA to form adducts (38–40). If bladder epithelium could, as our results suggest, reconvert these hydroxycarboxyamines back to their stable glucuronides, it could reduce their effective toxicity. Individual sensitivity to these carcinogens may therefore be related to individual variation in the levels of urothelial UDP-glucuronyltransferase.

Further experiments are in progress to determine whether other more toxic metabolites are formed by the bladder. Formation of sulfate esters [which have been found from AAF in rat urine (21)], even at very low levels, could also be important in bladder carcinogenesis. Until now, it has been assumed that activation of bladder carcinogens is mediated by enzyme systems in the liver or kidneys (12, 34, 37, 66). The subsequent covalent binding to urothelial DNA and resultant initiation of cancer have been presumed to occur spontaneously (38, 39) or by a single final activation step (50). The results reported here demonstrate that the bladder has a complement of enzymes capable of completely metabolizing 2 carcinogens, BP and AAF, to their proximate intermediates. The bladder must therefore be presumed capable of metabolizing carcinogens which may be excreted per se in urine (41, 54, 55), may be released in the urine by the action of hydroxylases (9, 42), or may be actually formed in the urine as a result of bacterial activity (29). It may well depend on the individual carcinogen and its route of entry into the body whether metabolism by the urothelium or by the liver is the more etiologically significant pathway. The overall similarity in metabolism of these 2 carcinogens by the human and rat bladder lends additional support to the use of rodent experimental models for the study of human bladder carcinogens.

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REFERENCES


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Brian P. Moore, R. Marian Hicks, Margaret A. Knowles, et al.


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