Bioconversion and Macromolecular Binding of 2-Amino-4-(5-nitro-2-furyl)thiazole by Cultured Rat Urothelial Cells

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ABSTRACT

Bioconversion and binding of 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT) were examined using cultured rat bladder epithelial cells from weanling male F344 rats. Bladder cells were obtained in large quantities from outgrowths of dissected explants which were grown on collagen gels. Metabolic potential of rat urothelial cells to activate ANFT was evaluated by incubating primary culture cells with [2-14C]ANFT for 48 hr. Metabolites were subsequently analyzed by chromatographic and spectroscopic methods. Thin-layer chromatography of the ethyl acetate:diethyl ether (1:1, v/v) extract of the culture medium revealed two regions of radioactivity with Rf values of 0.12 and 0.60, the former corresponding to ANFT and the latter to one of its metabolites. High-pressure liquid chromatography of the solvent extract revealed two major peaks, with retention times of about 4 and 9 min, corresponding with the metabolite and ANFT, respectively. Low-resolution mass spectrum of the isolated metabolite showed a molecular ion at m/e 181. The metabolite was identified as 1-4-(2-aminothiazolyl)-3-cyano-1-propanone based on its metabolites. High-pressure liquid chromatography of the solvent extract revealed two major peaks, with retention times of about 4 and 9 min, corresponding with the metabolite and ANFT, respectively. Low-resolution mass spectrum of the isolated metabolite showed a molecular ion at m/e 181. The metabolite was identified as 1-4-(2-aminothiazolyl)-3-cyano-1-propanone based on its chromatographic and spectral characteristics in comparison with the synthetic compound. About 24% of the recovered radioactivity from the culture medium was extractable into the organic phase, a majority of which was identified as 1-4-(2-aminothiazolyl)-3-cyano-1-propanone. Analysis of binding to proteins and nucleic acids prepared following exposure of [2-14C]ANFT revealed a 15- and 9-fold greater amount of binding, respectively, in cultures incubated with bladder cells than their homogenates. These data demonstrate that rat bladder cells possess the metabolic capability to reduce ANFT and to generate reactive intermediate(s) that bind to cellular macromolecules.

INTRODUCTION

Carcinogenic activities of numerous 5-nitrofuranylthiazoles analogues have been documented in a number of experimental animals (reviewed in Ref. 8). Among the 5-nitrofuranylthiazoles, FANFT3 is widely used as a model bladder carcinogen. It induced bladder tumors in a variety of experimental animals including dogs (11), rats (13), mice (9, 12), and hamsters (10). Earlier studies on excretion and analyses of metabolites using intact animals (15, 30, 33, 37, 40) or tissues (30, 36) from such susceptible animals revealed that FANFT was deformedylated to ANFT. Administration of ANFT p.o. resulted in carcinomas of the bladder in rats (39). Thus, it was proposed that ANFT might be a proximate carcinogen in FANFT-induced bladder carcinogenesis (32, 39). It was postulated earlier that “activation” of ANFT and related 5-nitrofuranylthiazoles is mediated by hepatic (30, 32-34) or kidney (19, 42, 43) enzymes. Alternatively, these chemicals might be activated directly by urothelial cells by virtue of their intrinsic metabolic capabilities. Recent studies show that urinary bladder cells by themselves are capable of metabolic activation of different classes of carcinogens (1-3, 6, 16, 22, 25, 28, 31, 35, 38, 44). This is evidenced by the presence of certain activating enzymes, such as mixed-function oxidase (25) and nitroreductases (44) in urothelium. Furthermore, metabolism (1-3, 6, 22, 31, 38), mutagenesis (16), and unscheduled DNA synthesis (41) of various carcinogens have also been observed. These results suggest that bladder carcinogens could be activated directly at the target sites. To examine this further, we studied the bioconversion and binding of the rat bladder carcinogen ANFT using urothelial cells cultured by a method developed recently by us (26). The epithelial characteristics of these cells were well studied and reported (26). This method permits us to obtain large quantities of bladder epithelial cells needed for conducting metabolic studies. Since the carcinogenicity and mutagenicity of the 5-nitrofurans have been related to reductive metabolism (reviewed in Ref. 32), we focused on the reduction products formed from ANFT. The results presented here demonstrate the nitroreduction of ANFT to ATCP by cultured rat bladder cells and the binding of the metabolite(s) to proteins and nucleic acids.

MATERIALS AND METHODS

Source of Materials. ANFT, 2-bromoacetyl-5-nitrofuran (Saber Laboratories, Inc., Morton Grove, IL), ethyl acetate and methanol (Burdick and Jackson Laboratories, Inc., Muskegon, MI), thiourea (Aldrich Chemical Co., Inc., Milwaukee, WI), and [14C]thiourea (approximately 50 mCi/mmol; Amersham Corp., Arlington Heights, IL) were purchased from the sources cited. The sources of biochemicals for tissue culture methods were: Ham’s F12 (Grand Island Biological Co., Grand Island, NY); fetal bovine serum (HyClone; Sterile Systems, Inc., Logan, UT); penicillin and streptomycin (Grand Island Biological Co., Inc., Milwaukee, WI), bovine serum (HyClone; Sterile Systems, Inc., Logan, UT); penicillin and streptomycin (Grand Island Biological Co., Inc., Milwaukee, WI), dimethyl sulfoxide; TCA, trichloroacetic acid; HPLC, high-pressure liquid chromatography; TLC, thin-layer chromatography.

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3The abbreviations used are: FANFT, N-(4-<5-nitro-2-furyl)-2-thiazolyl)formamide; ANFT, 2-amino-4-(5-nitro-2-furyl)thiazole; ATCP, 1-(4-(2-aminothiazolyl))-3-cyano-1-propanone; [2-14C]ANFT, 2-amino-4-(5-nitro-2-furyl)[2-14C]thiazole; DMSO, dimethyl sulfoxide; TCA, trichloroacetic acid; HPLC, high-pressure liquid chromatography; TLC, thin-layer chromatography.
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streptomycin (Pfizer, Inc., New York, NY); transferrin (Sigma Chemical Co., St. Louis, MO); epidermal growth factor (Collaborative Research, Inc., Lexington, MA); insulin (Elf Lilly Co. and Indianapoli, IN); and hydrocortisone (Merck Sharpe and Dohme, West Point, PA). Male F344 rats (4 to 6 weeks of age) were obtained from Harlan Sprague-Dawley, Madison, WI. The tissue culture plastic used was 100-mm plates (Flow Laboratories, McLean, VA) and 75-cm² flasks (Coming Science Products Division, Corning, NY).

Synthesis of [2-14C]ANFT. [2-14C]ANFT was synthesized from recrystallized 2-bromocrotyl-5-nitrofen and [14C]thiourea using a procedure described by Sherman and Dickson (27). The final product (6.2 mCi/mmol) was recrystallized in ethanol and determined to be >99% pure, as evaluated by radiochromatographic scans and by HPLC.

Synthesis of ATCP. ATCP was synthesized and purified by a procedure described earlier (30).

Growth of Rat Bladder Epithelial Cells. Rat bladder epithelial cells were cultured using modifications of the procedures developed in this laboratory for the in vitro growth of human urothelium (26). The growth and ultrastructural properties of these primary cultures have been well characterized and appear to be free of stromal contamination. Primary cultures were initiated from explants of dissected rat bladder epithelium and plated on type I collagen gels. The cells were grown using a medium reported earlier (28) supplemented with 5% fetal bovine serum and epidermal growth factor (10 ng/ml). Confluent primary cultures (5 to 6 x 10⁶ viable cells/100-mm plate) were passaged by removal of the explant, dispersal of the epithelial outgrowth with 0.1% EDTA, and replating onto new gels. With this method, 6 to 7 x 10⁶ viable cells could be obtained from each bladder after first passage. Based on the measurement of the effect of differing doses of ANFT on cell viability, 50 μM concentration was chosen for conducting metabolic studies.

Bioconversion of [2-14C]ANFT by Cultured Bladder Cells. Primary bladder cultures were plated onto collagen gels at a density of 0.8 x 10⁶ viable cells/60-mm plate. The cells were allowed to attach for 24 hr, following which 2 ml of fresh medium containing either 1% DMSO or 50 μM [2-14C]ANFT (1% DMSO) were added to each plate. Control plates without the cells were similarly treated. After 48 hr, the medium was removed, and the cells were rinsed twice with ice-cold, sterile 0.25 M sucrose. The medium and the sucrose washings were pooled and centrifuged at 1500 rpm for 10 min. The layer of cells from the collagen gel was dispersed with 0.1% EDTA and counted for viability by the trypan blue exclusion method. Under these conditions, there was no significant effect of ANFT on cell viability at 48 hr (77.8 ± 4.9% (S.E.) for DMSO controls and 81.9 ± 3.8% for ANFT-treated group; n = 3).

Isolation and Characterization of Metabolites from Bladder Cell Cultures. For isolation and characterization of metabolite(s) derived from ANFT, a large number of bladder cells from primary cultures (9 x 10⁶ viable cells; 83% viability) were pooled and plated into a 75-cm² culture flask in a serum-free medium (21) at a cell density of 1.8 x 10⁶ cells/ml. A stock solution of [2-14C]ANFT in DMSO was added to the medium at a final concentration of 50 μM and incubated for 48 hr at 37°. The suspension was then centrifuged, and the cell pellet and flask were washed 3 times with cold 0.25 M sucrose. The cells that remained attached to the flask were dispersed in 0.1% EDTA and added to the washed pellet. The supernatant was analyzed for ANFT metabolites, and the cell pellet was processed for macromolecular binding. The supernatant was extracted 5 times with an equal volume of ethyl acetate:diethyl ether (1:1, v/v), and the organic phase was concentrated to dryness and analyzed by chromatographic and spectroscopic methods.

Analyses of the Metabolite. HPLC was performed on a Micromeritics 7000B chromatograph interfaced with a Varian Varichrom variable wavelength detector. The instrument was fitted with a 25-cm Whatman Partisil PSX 10/25 ODS-3 column. The HPLC was operated at a constant flow rate of 1 ml/min using aqueous methanol as the eluting solvent. TLC was performed on 0.1-mm cellulose plates with 2% methanol in water as the developing solvent. Mass spectral analyses were performed as described earlier (30). UV absorption spectra were obtained using a Beckman 25 recording spectrophotometer.

Nitroreduction of [2-14C]ANFT by Bladder Cell Homogenates. Rat liver homogenate preparations were obtained using methods described earlier (33). Homogenizations of rat bladder cells were carried out at 4° by repeated sonication (12 times, 20 sec each) in 0.05 M Tris buffer (pH 7.7). The homogenate was centrifuged at 10,000 x g for 20 min, and the supernatant was used for the nitroreduction assay. Protein estimations were made by the method of Lowry et al. (18). The nitroreduction assay was conducted anaerobically by a procedure published earlier (33). At the end of 3-hr incubation, an aliquot was extracted and analyzed as described above. Quantitative determinations of the distribution of radioactivity in the organic and aqueous phases were measured in a Tracor Analytic Mark III liquid scintillation counter.

Macromolecular Binding of [2-14C]ANFT. The binding of [2-14C]ANFT to proteins and nucleic acids in cultured cells was measured using a procedure described by Beland et al. (4). The samples were supplemented with 10 mg each of yeast rRNA and bovine serum albumin prior to the phenol extraction step, to facilitate the recovery of macromolecules. Proteins (18), and nucleic acids, by measurement of absorbance at 260 nm (23) were quantitated. Estimates of the recovery of proteins and nucleic acids by these procedures from control samples prepared in the absence of cells were 92 ± 95%, respectively. Binding data were expressed as the percentage of initial radioactivity added to the medium.

The nature of binding of ANFT metabolite(s) to nucleic acids was evaluated by competition with a 1000-fold excess of unlabeled ANFT followed by chromatography, as described earlier (34). Further evaluation of the nature of binding was made by hydrolysis of the nucleic acids with nucleases. The hydrolysis was carried out as described earlier (34) by digestion with snake venom phosphodiesterase and alkaline phosphatase for 24 hr, followed by digestion with RNase A for 18 hr. After neutralization, 0.1 volume of 2 M potassium acetate was added followed by 2 volumes of ethanol. The precipitate was removed by centrifugation. An aliquot of the supernatant was taken for determination of radioactivity.

For measurement of total macromolecular binding by bladder cell or liver homogenates, the reaction mixtures consisted of 1.7 mM NADPH, 87 μM [2-14C]ANFT, and homogenate protein (3 mg/ml). An aliquot of the incubation mixtures was precipitated with cold 40% TCA to a final concentration of 10%. The precipitate was separated by centrifugation at 10,000 x g for 15 min at 4°. The pellet was washed twice consecutively with 10% TCA, ethanol, and then diethyl ether. The dried samples were dissolved in 0.5 mM NaOH, and aliquots were taken for measurement of radioactivity as described above. Using the above method, the recovery of proteins was determined to be 100%.

RESULTS

Bioconversion of [2-14C]ANFT by Cultured Bladder Cells. The ethyl acetate:diethyl ether extract of the cultures treated with [2-14C]ANFT and bladder cells contained 34% of the radioactivity in the organic phase. The remainder of the activity was distributed in the aqueous phase and represents water-soluble metabolite(s) derived from ANFT. In contrast, in the control group incubated in the absence of bladder cells, most of the radioactivity (~94%) was present in the organic phase. The organic extract from the control group showed an absorption maxima at 390 nm corresponding with that of the parent compound (ANFT). The extracts obtained from incubation of [2-14C]ANFT with bladder cells showed a marked decrease (~85%) in absorbance, in comparison with that of the control group. These data suggest that the rat bladder cells can metabolize ANFT to other product(s). This was further confirmed by chromatographic analyses.

Chart 1 shows the HPLC profile of the organic phase of the culture medium. Control samples incubated in the absence of rat...
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bladder cells revealed only one radioactive peak (Chart 1A) corresponding with the parent compound ANFT. In contrast, the extract of cultures obtained following incubation with intact bladder cells grown on collagen gels showed an additional radioactive peak with an elution time of 4 min (Chart 1B).

In order to isolate and characterize the above metabolite, a large number of primary cultured bladder cells (~1 x 10^6), from fifteen 100-mm dishes, were pooled. They were treated with [2-14C]ANFT after plating into a 75-sq cm tissue culture flask to maximize the recovery of the products formed. The metabolites were isolated and analyzed as described in “Materials and Methods.”

Analyses of the Metabolites. Radiochromatographic scans of the TLC of ethyl acetate:diethyl ether extracts of rat urothelial culture medium, obtained 48 hr after exposure to [2-14C]ANFT, showed mainly one peak corresponding with ANFT in the heat-inactivated control samples. However, in the presence of urothelial cells, relatively very little (~10%) of ANFT was detected. A new metabolite with an Rf value of about 0.6 was observed (data not shown), which accounted for a major portion of the radioactivity in the organic phase. Only 24% of the recovered radioactivity was extracted into the organic phase, and the remainder was distributed in the aqueous phase. Similar distribution of radioactivity was observed with bladder cells grown on collagen gels. TLC of the aqueous phase showed the presence of a metabolite that was different from the metabolite observed in the organic extract. The Rf of the aqueous metabolite was about 0.75 (data not shown). Thus, the metabolite detected in the aqueous phase is a major metabolite of ANFT, and its identity remains to be established. The identity of the metabolite corresponding with an Rf value of 0.6 has been determined, and its chromatographic and spectral characteristics are described below.

Results of HPLC analysis of the organic extract prepared from the large batch of rat urothelial culture medium, following exposure to [2-14C]ANFT, under the conditions described above, showed a major peak at an elution time of 4.0 min, similar to that observed with the cells treated on collagen gels (Chart 1A). In contrast, the control samples incubated with heat-inactivated bladder cells showed a major peak at 9.0 min, corresponding with the parent synthetic compound ANFT (Chart 1C).

A low-resolution mass spectrum of the metabolite was obtained following purification on a column of silica gel with ethyl acetate as the eluting solvent (30). The relative abundance of the ions observed on fragmentation is represented in Chart 2. The spectrum showed the molecular ion at m/e 181 (C7H7N3OS) and a base peak at m/e 127 [M—(CH2-CH2-CN)]. Additional fragment ions at m/e 100 and 99 characteristic of the 2-aminothiazole nucleus (C3H3N2S) were also observed. The fragmentation pattern observed is similar to that obtained with synthetic ATCP reported earlier (30).

The UV spectrum of the isolated material had an absorption maxima at 305 nm, similar to that observed with synthetic ATCP (Chart 3). Furthermore, the chromatographic properties, such as Rf values in TLC (30), retention times in HPLC (Chart 1B), and spectral characteristics (Charts 2 and 3) were identical to those of synthetic ATCP. Thus, the metabolite has been identified as ATCP.

Formation of ATCP from ANFT Using Cellular Homogenates. To examine the formation of ATCP in vitro, [2-14C]ANFT
was incubated under anaerobic conditions in the presence of NADPH, using rat liver or urothelial homogenates. Following incubation, the reaction mixtures were extracted 5 times with the ethyl acetate:diethyl ether mixture. Measurement of distribution of label in the incubation mixture of bladder or liver samples showed 33 and 40% of the recovered radioactivity in the organic phases, respectively. TLC analyses showed radioactivity mainly at a region of ANFT in controls. In the group incubated with cellular homogenates, an additional peak (Rf = 0.6), corresponding with ATCP, was observed (data not shown).

HPLC analyses of the extracts of bladder or liver homogenate samples (Chart 4, B and C) also showed a new peak in both absorbance and radioactivity at an elution time of 5.5 min. This peak was different from ANFT and was not observed in control samples (Chart 4A). Detection of radioactivity in this peak suggests that it is a metabolite derived from ANFT. The elution time of the metabolite corresponded with that of synthetic ATCP when run under the same conditions. The majority of the radioactivity in the organic phase of the bladder homogenate sample was present as ATCP (Chart 4B). These results demonstrate nitroreductase-mediated reduction of ANFT to ATCP by rat liver and bladder cells in vitro.

**Binding of [2-14C]ANFT Metabolite(s) to Macromolecules.** The proteins and nucleic acids were isolated from the bladder cells treated with [2-14C]ANFT by extraction with phenol reagent, as described in “Materials and Methods.” The total amount of label bound to proteins and nucleic acids, expressed as the percentage of initial radioactivity added to the culture medium, was 2.1 and 0.1%, respectively. The corresponding values for heat-inactivated controls were 0.14 and 0.01%. The binding of [2-14C]ANFT derivative(s) to nucleic acids was confirmed by testing the sensitivity of this fraction to nuclease treatment.

About 90% of the radioactivity was released as ethanol-soluble material following enzymatic digestion of the nucleic acid preparations with snake venom phosphodiesterase and RNase A. Furthermore, the nature of binding to the nucleic acid fraction was determined by the addition of a 1000-fold excess of unlabeled ANFT and denaturation at 70° for 30 min. The sample was chromatographed on a Sephadex G-25 column and eluted with phosphate buffer (1 mM, pH 7.4). Analysis of the column eluant showed that the label was present only in fractions eluting within 0.4 to 0.9 column volumes corresponding with the nucleic acids. Radioactivity was not detected in the fractions where ANFT was eluted under the same conditions. These results demonstrate that the binding of ANFT to the isolated nucleic acids was not displaced by competition with unlabeled ANFT, thus ruling out reversible, noncovalent interac-

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**Chart 2.** Low-resolution mass spectrum of the metabolite isolated from culture medium following treatment of urothelial cells with ANFT.

**Chart 3.** UV absorption spectrum of synthetic ATCP (---) in methanol, 45 μg/ml, and the metabolite isolated from the culture medium (-----) after treatment of urothelial cells with ANFT.

**Chart 4.** HPLC traces of the ethyl acetate:diethyl ether extract of the reaction mixture [ANFT (100 μM) homogenate protein (4.2 mg/ml):NADPH (1.5 mM)] obtained following incubation of ANFT with heat-inactivated bladder cell homogenate (A), with bladder cell homogenate (B), and with liver homogenate (C). HPLC was operated in a linear gradient mode, from 25% methanol:water to 75% methanol:water, with continuous measurement of absorbance at 305 nm. The gradient time was 15 min, and the flow rate was 1.2 ml/min. Fractions were collected every 30 sec in a stepwise fashion, and radioactivity was determined using a scintillation counter.
Measurements of the total macromolecular binding with rat bladder cell homogenates under anaerobic conditions with NADPH showed about 7-fold greater binding of [2-14C]-ANFT in comparison to the corresponding heat-inactivated controls. Even after solubilization with 0.5 M NaOH or 1 M urea, the extent of binding in bladder cell homogenates under anaerobic conditions of ANFT and its related analogues and their binding to macromolecules suggests that reactive intermediate(s) are formed following incubation of ANFT with intact bladder cells or cellular homogenate preparations.

**DISCUSSION**

The genotoxic and cytotoxic effects of ANFT and related 5-nitrofuranyltiazoles have been reported with certain bacterial and other mammalian systems (20, 24). However, the mechanism(s) of activation of ANFT or its related analogues to toxic, mutagenic, or carcinogenic intermediate(s) remains unclear. Possible mechanisms involving nitroreduction (reviewed in Ref. 32), cooxidation by prostaglandin endoperoxide synthetase (42), or formation of superoxide radical anions (5, 7) have all been proposed. A number of observations indicate that the nitroreduction process is involved in the activation of 5-nitrofurans (reviewed in Ref. 32). Earlier, we reported the formation of ATCP as a nitroreduction product from ANFT and FANFT in vitro and in vivo using liver tissue preparations obtained from mice (30). Recently, ATCP has been reported to be formed from ANFT with rabbit liver and kidney microsomal preparations (19). Although the nitroreduction of ANFT and its related analogues and their binding to macromolecules have been demonstrated in liver and kidney tissues of experimental animals (17, 19, 23, 29, 31–33), the occurrence of such reaction(s) in rat urothelial cells, the target cells for FANFT- and ANFT-induced carcinogenesis, has not been documented until now. The results presented here demonstrate for the first time the bioconversion of ANFT to ATCP in both urothelial cell cultures (Charts 1 to 3) and homogenate preparations (Chart 4). The data also show that the reactive intermediates generated from ANFT are capable of binding to macromolecules. The urothelial cell-mediated binding of [2-14C]ANFT to proteins was greater by 15-fold in comparison to heat-inactivated control samples. Similarly, a 9-fold greater binding to nucleic acids was observed. The strong affinity of binding of the 14C label to macromolecules was evidenced by the retention of radioactivity under various denaturing conditions, including phenol treatment; extraction of the adduct with organic solvents; and by lack of displacement of radioactivity from nucleic acid adducts by unlabelled ANFT.

Measurement of radioactivity bound to the TCA-precipitable fraction following incubation with [2-14C]ANFT indicated that the extent of binding in bladder cell homogenates was about 4.2 nmol/mg protein. Under the same experimental conditions, the binding of [2-14C]ANFT with liver homogenates was of a similar magnitude (2.1 nmol/mg protein). Previous studies using rabbit bladder tissues showed that the bladder microsome contains nitroreductase (44). However, the nitroreductase activity found in urothelial cells could be associated with a number of enzymes such as xanthine oxidase, aldehyde oxidase, NADPH:cytochrome c reductase, or cytosol DT-diaphorase (32). The question as to which of the above enzyme(s) is responsible for activation of ANFT in urothelial cells and their relative activities in bladder and liver tissues remains to be answered.

A number of recent reports suggest that the urinary bladder is capable of metabolizing a variety of chemical carcinogens, such as 4-aminobiphenyl, 2-naphthylamine, 2-acetylaminofluorene, aflatoxin B1, ANFT, and certain nitrosamines (1–3, 6, 16, 22, 25, 28, 31, 35, 38). Activation of some of these chemicals was reported using bladder cell-mediated mutagenesis assays (16) or by binding of metabolites to cellular macromolecules (1–3, 22, 25, 31, 38) or by induction of unscheduled DNA synthesis (41). Furthermore, neoplastic transformation of rat urothelial cells using N-butyl-N-(4-hydroxybutyl)nitrosamine and the major urinary metabolite, N-(butyl-N-3-carboxypropyl)nitrosamine, was also reported (14). Some of the earlier studies used chemicals the carcinogenic activity of which for the bladder has not been established (1–3, 16). To evaluate the activation of a bladder-specific carcinogen, we investigated the metabolism of ANFT by rat bladder epithelial cells, the target tissue for ANFT-induced bladder carcinogenesis. The studies reported here were conducted using a culture system developed for growing large amounts of transitional epithelial cells of the bladder devoid of contamination by stroma and fibroblasts, thus eliminating the role of the latter in metabolism. Using this culture method, 7 x 107 viable cells were routinely obtained from each rat bladder in about 3 weeks. Obtaining such large numbers of cells has allowed us to isolate and identify the metabolite generated from ANFT in vitro.

Activation of the rat bladder carcinogen ANFT is thought to be mediated mainly, if not exclusively, by enzymes in the liver or kidneys (19, 30–34, 37, 38, 42, 43). Proximate metabolites generated by renal or hepatic metabolism are excreted in urine, converted to ultimate carcinogenic forms, and enter bladder epithelial cells to react with critical cellular macromolecules. In contrast, the results presented here demonstrate that the rat bladder has a complement of enzyme(s) capable of metabolizing and activating ANFT to reactive intermediate(s), and that the extent of macromolecular binding was comparable to that observed with liver. Since ANFT has been detected in the urine of rats fed FANFT or ANFT (38), the exposure of the urothelium to ANFT by urinary excretion or also through the vascular system may result in activation directly by the target tissue. Thus, these results support the hypothesis that the urothelium of the bladder itself is capable of metabolic activation of bladder carcinogens (1–3, 16, 22, 28, 41, 44) and may play an independent or cooperative role with the liver or kidneys in the initiation of bladder carcinogenesis.

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