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Prostaglandin Hydroperoxidase-Catalyzed Activation of Certain *N*-Substituted Aryl Renal and Bladder Carcinogens

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Certain carcinogens are thought to induce renal and bladder cancer following metabolic activation. We propose a model system for this activation and provide supporting experimental evidence. This model proposes that renal and bladder carcinogens' entry into the urinary tract is facilitated, that carcinogens are activated by the prostaglandin hydroperoxidase activity of prostaglandin endoperoxide synthetase (PES), and that activation results in covalent binding to nucleic acids which can initiate carcinogenesis. Benzidine and the 5-nitrofuran HMN were shown to inhibit uptake of organic anions and cations, respectively. Carcinogen binding to DNA was dependent upon specific unsaturated fatty acid substrates and prevented by specific inhibitors of PES, i.e., aspirin. Activation with organic peroxides or H_2O_2 was inhibited by antioxidants but not aspirin. Horseradish peroxidase (HRP) metabolized benzidine but not ANFT. Acetaminophen and the 5-nitrofurans ANFT and HMN prevented PES ^{14}C-benzidine metabolism. However, only acetaminophen inhibited HRP metabolism of benzidine. The only aerobic metabolism we have observed of 5-nitrofurans is PES-catalyzed. Aspirin (0.5% in the diet) inhibited rat bladder hyperplastic lesions induced by feeding 0.1% or 0.2% FANFT for 6 or 12 weeks. Aspirin reduced bladder prostaglandin synthesis and PES metabolism of FANFT. After one year of an ongoing long-term study, gross examination reveals bladder tumors in 85% of the rats fed 0.2% FANFT and in only 37% of the rats fed FANFT plus 0.5% aspirin.

Introduction

We have developed an experimental model to explain the initiation by certain chemicals of renal and bladder cancer. This model is based on the hypothesis that these urinary tract carcinogens are activated by the hydroperoxidase activity of prostaglandin endoperoxide synthetase (PES). Activation results in initiation of the carcinogenic process. We have used the 5-nitrofuran carcinogens 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT) and *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) and the aromatic amine benzidine to develop this model. In particular, advantage was taken of a well-defined rat model in which urinary bladder cancer is induced with FANFT (*1*). We will present the experimental evidence which supports our model and examine the possible involvement of other peroxidases in the initiation of urinary tract carcinogenesis.

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Characteristics of Prostaglandin Endoperoxide Synthetase

Purified PES enzyme (Fig. 1) has been shown to consist of two separate activities: fatty acid cyclooxygenase and prostaglandin hydroperoxidase (*2*). Fatty acid cyclooxygenase is responsible for the initial bisdioxygenation of the unsaturated fatty acid. For arachidonic acid, this product is prostaglandin G_2, a 15-hydroperoxy prostaglandin cyclic endoperoxide. The hydroperoxidase activity is responsible for cleavage of the 15-hydroperoxy group. With prostaglandin G_2 as substrate, the product is the 15-hydroxy cyclic endoperoxide prostaglandin H_2. Prostaglandin H_2 is the common substrate for synthesis of prostaglandins and thromboxanes. PES has been shown to exist as a dimer with each individual subunit having a molecular weight of 72,000 (*3*).

Certain characteristics of the cyclooxygenase and hydroperoxidase activities of PES allow separate examination of each activity. Aspirin has been shown to irreversibly inhibit fatty acid cyclooxygenase by acetylation of a serine moiety (*3*). Other nonsteroidal anti-inflammatory drugs, in addition to as-

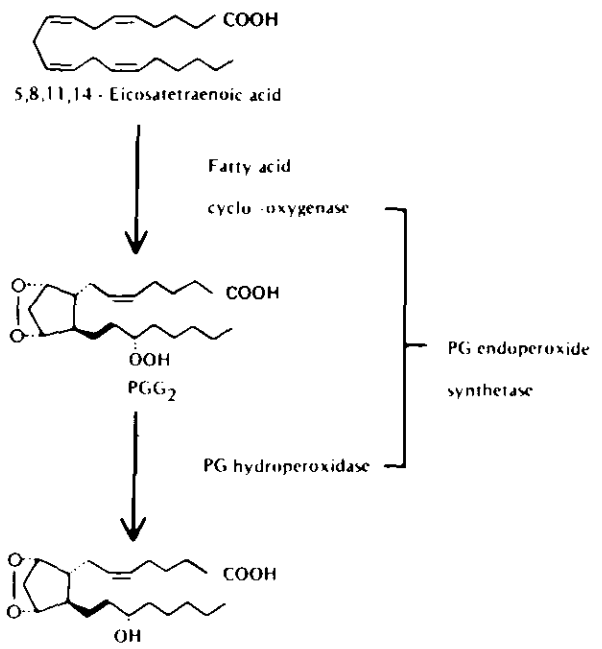

FIGURE 1. Reactions catalyzed by prostaglandin endoperoxide synthetase.

pirin, inhibit fatty acid cyclooxygenase. The substrate requirements of fatty acid cyclooxygenase are very specific. All unsaturated fatty acid substrates have in common the location of a triene system which terminates at the sixth carbon from the alkyl terminus (*4*). Arachidonic acid is thought to be the major *in vivo* substrate. The fatty acid cyclooxygenase reaction requires molecular oxygen (*5*) and heme (*6*). By contrast, prostaglandin hydroperoxidase is not inhibited by nonsteroidal anti-inflammatory drugs and will utilize a broad range of substrates. The hydroperoxidase enzyme can only use ferric heme, while cyclooxygenase can use either Fe^{3+}-heme or Mn^{2+}-Fe^{3+}-heme (*6*). PES has been shown to be uniquely distributed within the kidney (*7*) with the most activity in the renal medulla (*8*). Immunohistofluorescence studies which localize PES subcellularly have demonstrated that the enzyme system is associated with the endoplasmic reticulum and nuclear membrane (*9*).

The hydroperoxidase activity of PES is responsible for the cooxidative metabolism of carcinogens. Cooxidative metabolism has been demonstrated with microsomes, intact tissue slices and *in vivo*. Major classes of compounds metabolized by PES include aromatic amines (*10*), aromatic amides (*11*), 5-nitrofurans (*10*), and polycyclic aromatic hydrocarbons (*12*). Cooxidative metabolism of 7,8-dihydroxy-7,8- dihydrobenzo(a)pyrene (*12*) and the aromatic amines benzidine and 2-naphthylamine (*13*) has been shown to result in the formation of mutagens.

Metabolism and Activation of Benzidine and 5-Nitrofurans by Microsomal Prostaglandin Endoperoxide Synthetase

Benzidine and the 5-nitrofuran urinary tract carcinogens are not thought to be proximal carcinogens because they initiate carcinogenesis at a site distant from their entry into the body. Therefore, these carcinogens require activation to elicit their carcinogenic effects. Using ^{14}C-ANFT and bladder epithelial microsomes (*14*), PES-catalyzed activation was assessed by covalent binding to protein and DNA (Table 1). Binding determined in the absence of arachidonic acid was not different from binding in the absence of microsomes. Addition of arachidonic acid caused a large increase in ANFT metabolism. Indomethacin completely inhibited arachidonic acid-dependent binding. There was no measurable binding when DNA was added at the end of the standard incubation for an additional 5 min (not shown). This suggests that a short-lived reactive intermediate of ANFT is formed during the reaction. 15-Hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE)-mediated metabolism is consistent with the prostaglandin hydroperoxidase component of PES catalyzing activation of ANFT. 15-HPETE-mediated metabolism was not prevented by indomethacin but was prevented by the antioxidant vitamin E. Previous studies have examined mixed-function oxidase-catalyzed metabolism of ANFT in renal cortex and of FANFT in renal cortex and bladder transitional epithelial tissue. There was no measurable mixed-function oxidase-dependent binding in any of

Table 1. Covalent binding of [^{14}C]ANFT to protein and DNA catalyzed by prostaglandin endoperoxide synthetase prepared from rabbit bladder transitional epithelial microsomes.

Conditions	Binding, nmole/mg protein/5 min	
	Aqueous, TCA precipitable	DNA bound
Complete system[a]	0.16 ± 0.02	0.014 ± 0.002
− Arachidonic acid	N.D.[b]	N.D.
− Microsomes	0.002 ± 0.002	0.003 ± 0.002
+ Indomethacin (50 μM)	N.D.	N.D.
15-HPETE (30 μM)	0.23 ± 0.02	0.026 ± 0.003
+ Indomethacin (100 μM)	0.23 ± 0.004	0.024 ± 0.005
+ Vitamin E (50 μM)	N.D.	N.D.

[a]The complete system consists of 0.5 mg rabbit bladder transitional epithelial microsomes, 0.024 *mM* [^{14}C]ANFT, 0.0012 *mM* methemoglobin, 0.06 *mM* arachidonic acid, and the indicated concentration of test agents in a total volume of 0.25 mL. Where indicated, 15-HPETE was used instead of arachidonic acid. All inhibitors were preincubated for 2 min at 25°C. Data are expressed by mean ± S.E. (n = 3 to 8) (*14*).

[b]Not detected.

those tissues. The only aerobic metabolism of ANFT or FANFT observed in these tissues is PES catalyzed. Prostaglandin hydroperoxidase-catalyzed binding of ^{14}C-benzidine to protein and DNA has also been reported (*15*). These results are consistent with PES playing a role in 5-nitrofuran and benzidine-induced renal and bladder carcinogenesis.

The dose-response effect of indomethacin on renal inner medullary microsomal synthesis of PGE_2 and cooxidation of ^{14}C-benzidine was used to assess the relationship between renal prostaglandin synthesis and cooxidation (Fig. 2). Two separate incubation conditions were used to assess these effects (*16*). One incubation contained 30 μM ^{14}C-benzidine and 25 μM unlabeled arachidonic acid. The other contained 25 μM ^{14}C-arachidonic acid and 30 μM unlabeled benzidine. Cooxidation was assessed as ^{14}C-benzidine bound to TCA precipitable material and prostaglandin synthesis as ^{14}C-prostaglandin E_2 isolated by thin-layer chromatography. The similarity in the indomethacin ID_{50} values is consistent with PES-catalyzed prostaglandin E_2 synthesis and covalent binding of benzidine to protein.

The benzidine analogs 3,3′-dimethylbenzidine (*o*-tolidine) and 3′,3′-dimethoxybenzidine (*o*-dianisidine) are also thought to be carcinogenic (*17*). We have investigated the metabolism of these compounds by PES. Each compound exhibited spectral changes consistent with metabolism by PES. The spectral changes catalyzed by horseradish peroxidase and PES were similar. These results are consistent with the prostaglandin hydroperoxidase component of PES catalyzing the metabolism of a variety of aromatic amines.

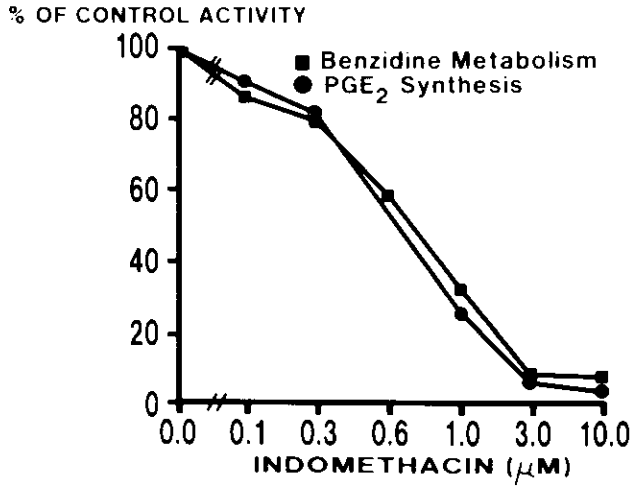

FIGURE 2. Effect of indomethacin on PGE_2 synthesis and benzidine metabolism. Reaction mixtures contained 0.14 mg of microsomal protein, 0.1 *M* phosphate buffer (pH 7.8), 0.025 *mM* arachidonic acid and 0.03 *mM* benzidine in a final volume of 0.1 mL. Microsomes and reaction mixture were preincubated for 2 min at room temperature before a 10-min incubation at 37°C. For determination of PGE_2 synthesis, [^{14}C]-arachidonic acid was used. For determination of benzidine metabolism, [^{14}C]benzidine was used. The rates of PGE_2 synthesis and total benzidine metabolism (both TCA-precipitable and non-TCA-precipitable aqueous fractions) were 0.64 and 0.15 nmole/mg/min, respectively. These rates each correspond to 100% of their respective control activity. Values represent the mean of four determinations (*16*).

Demonstration of Intact Tissue Metabolism of ^{14}C-Benzidine by Prostaglandin Endoperoxide Synthetase

If PES-catalyzed metabolism of urinary tract carcinogens is an important step in initiation of the carcinogenic process, this metabolism should be demonstrable with intact tissue. ^{14}C-Benzidine metabolism by renal medullary tissue slices was used as a test system (Fig. 3). Slices were subjected to two successive incubations with Krebs-Ringer bicarbonate buffer containing 1 mg/mL each of glucose and BSA (*18*). Both 0.15 *mM* arachidonic acid and 25 μM ^{14}C-benzidine were present in only the final incubation. Other test agents were present in both incubations. Metabolism was assessed by binding to TCA-precipitable material. Benzidine metabolism was indicated by an increase in radioactivity over the blank which contained heated tissue slices or slices incubated anaerobically. ^{14}C-Benzidine binding was increased by arachidonic acid. While inhibitors of PES prevented this increase, SKF-525A and metyrapone, inhibitors of mixed-function oxidases, were not effective. These results are consistent with an experiment demonstrating *in vivo* PES-catalyzed metabolism of ^{14}C-benzidine by dog kidney (*19*). ^{14}C-Benzidine was administered by retrograde perfusions into the renal pelvis through a ureteral catheter. Benzidine metabolism in subsequent urinary collections was prevented by meclofenamic acid. These results are consistent with microsomal, intact tissue and *in vivo* metabolism of urinary tract carcinogens by PES.

Role of Peroxidases in Chemical Carcinogenesis

Hemeprotein peroxidases are present in mammalian tissues and catalyze metabolic activation of carcinogens. Both Forrester (*20*) and Bartsch (*21*) demonstrated the conversion of *N*-hydroxy-2-acetylaminofluorene to 2-nitrosofluorene and *N*-acetylaminofluorene. Later Bartsch demonstrated that

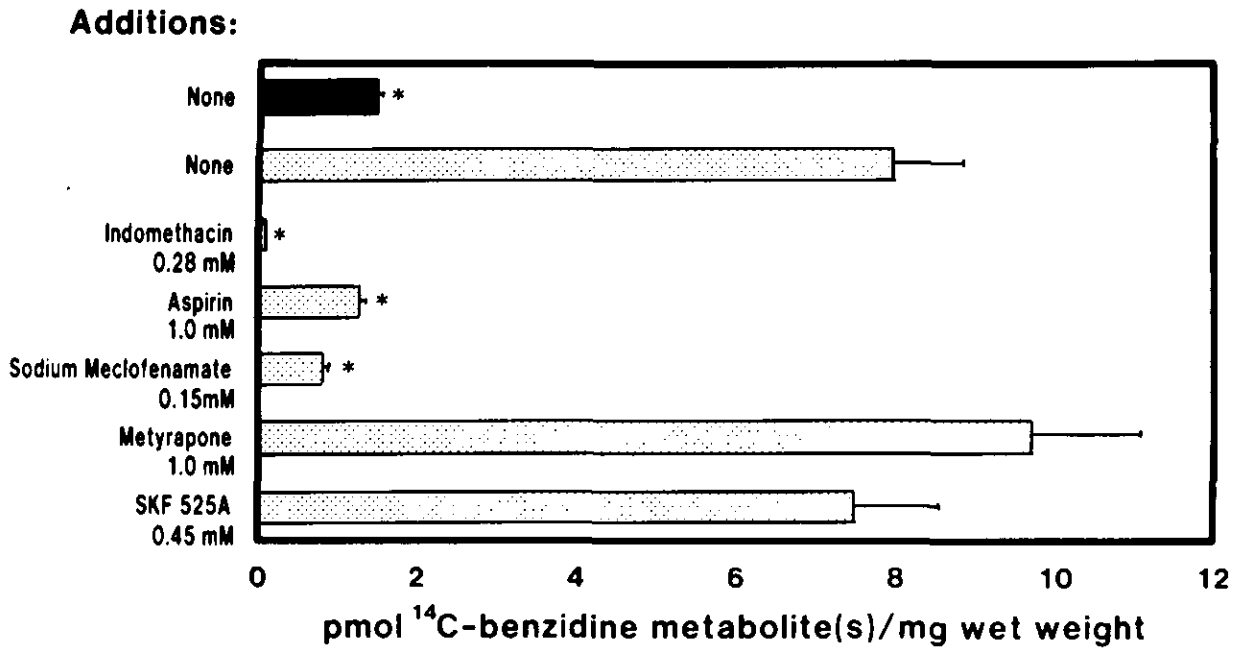

FIGURE 3. Effect of different agents on [^{14}C]benzidine binding to renal medullary slices. Both 0.15 *mM* arachidonic acid and 0.025 *mM* [^{14}C]benzidine were present only during the second incubation. ■ No arachidonic acid, □0.15 *mM* arachidonic acid. Other test agents were present during both incubations. [^{14}C]Benzidine binding to TCA precipitable material was determined. Values are mean ± S.E. of three or more determinations. The asterisk denotes $p<0.05$ compared to 0.15 *mM* arachidonic acid alone (*18*).

not only horseradish peroxidase but also the mammalian enzymes myeloperoxidase and lactoperoxidase catalyzed this reaction (*22*). In the uterus, diethylstilbestrol is thought to be metabolized to its ultimate carcinogen by peroxidase (*23*). We have demonstrated PES-catalyzed activation of diethylstilbestrol (*10*). However, two distinct peroxidases have been reported in uterine tissue (*24*) and the relationship of these peroxidases to PES is not known. Differences in substrate specificity (*25*) and in the products formed (*26*) by peroxidases have been reported. A better understanding of the specificity of peroxidase-catalyzed activation of urinary tract carcinogens would improve interpretations of intact tissue and whole animal experiments and provide a stronger basis for current experimental models.

Comparison of Effects of Prostaglandin Endoperoxide Synthetase and Horseradish Peroxidase on 5-Nitrofuran and Aromatic Amine Metabolism

The catalysis of ^{14}C-benzidine and ^{14}C-ANFT binding to TCA-precipitable material was examined with ram seminal vesicle PES and horseradish peroxidase. Binding was indicated by an increase in radioactivity over blank values obtained with samples containing 2 mg/mL bovine serum albumin but not enzyme. Both PES and horseradish peroxidase metabolized benzidine (Table 2). Arachidonic acid—but not HPETE-mediated metabolism—was prevented by aspirin. The lack of effect of salicylate, the deacetylated metabolite of aspirin, suggests that aspirin inhibition is due to acetylation of the fatty acid cyclooxygenase component of PES. At 0.2 *mM* KCN, horseradish peroxidase but not PES-catalyzed metabolism was inhibited. The antioxidant vitamin E prevented binding of ^{14}C-benzidine catalyzed by both peroxidases. Both peroxidases catalyze binding of benzidine to DNA as well as protein (not shown).

To further assess the specificity of the peroxidase enzymes, metabolism of ANFT was determined (Table 3). PES catalyzed binding of ANFT but horseradish peroxidase did not. As previously shown, ANFT binding was consistent with metabolism by prostaglandin hydroperoxidase component of PES. PES-catalyzed binding of ^{14}C-ANFT to DNA has been demonstrated (Table 1).

The mechanism of benzidine metabolism by horseradish peroxidase and prostaglandin endoperoxide synthetase was further investigated using electron paramagnetic resonance. As shown in

Table 2. Peroxidase-catalyzed binding of ^{14}C-benzidine to TCA precipitable material.[a]

	Binding, nmole/mg protein/min		
	Prostaglandin endoperoxide synthetase		Horseradish peroxidase ($\times 10^{-3}$);[b] substrate
Inhibitors	Arachidonate 0.13 *mM*	HPETE 0.05 *mM*	H_2O_2, 0.3 *mM*
None	26.9 ± 3.4	29.3 ± 3.5	38.5 ± 3.5
Cyanide (0.2 *mM*)	27.2 ± 3.1	30.1 ± 2.4	10.4 ± 0.4
Aspirin (1.2 *mM*)	1.1 ± 0.2	30.1 ± 1.2	44.7 ± 4.5
Salicylate (2 *mM*)	21.8 ± 2.1	26.8 ± 1.3	—
Vitamin E (0.05 *mM*)	14.8 ± 1.8	18.1 ± 0.32	17.9 ± 0.9

[a]Complete reaction mixture contained solubilized seminal vesicle microsomes or horseradish peroxidase, 2 mg/mL bovine albumin, 0.06 *mM* ^{14}C-benzidine, substrate and inhibitors as indicated in a final volume of 0.25 mL and were incubated at 37°C for 3 min. Reactions were stopped and extracted with ethyl acetate before addition of 0.6 *M* TCA. Data expressed as mean ± S.D. (n = 3).

[b]Actual value is obtained by multiplying by 10^3.

Table 3. Peroxidase-catalyzed binding of ^{14}C-ANFT to TCA precipitable material.[a]

	Binding, nmole/mg protein/min		
	Prostaglandin endoperoxide synthetase		Horseradish peroxidase substrate
Inhibitors	Arachidonate 0.13 *mM*	HPETE 0.05 *mM*	H_2O_2, 0.3 *mM*
None	5.7 ± 0.4	5.6 ± 0.6	N.D.
Cyanide (0.2 *mM*)	5.3 ± 0.4	6.3 ± 1.3	—
Aspirin (12 *mM*)	N.D.	5.9 ± 1.1	—
Salicylate (2 *mM*)	4.7 ± 0.8	5.1 ± 0.9	—
Vitamin E (0.05 *mM*)	2.3 ± 0.6	2.0 ± 0.2	—

[a]Complete reaction mixture and recovery of TCA precipitable material was the same as described in Table 2 but with 0.02 *mM* ^{14}C-ANFT as label. Data expressed as mean ± S.D. (n = 3).

Figure 4, a benzidine radical was observed with horseradish peroxidase and H_2O_2. This radical was dependent on the presence of both H_2O_2 and horseradish peroxidase. Acetaminophen at 200 μM completely inhibited radical formation by 200 μM benzidine. However, 200 μM ANFT had no effect. This free radical mechanism of benzidine metabolism was also observed with prostaglandin endoperoxide synthetase and arachidonic acid (Fig. 5). Radical formation was prevented by addition of indomethacin. Both horseradish peroxidase and prostaglandin endoperoxide synthetase appear to metabolize benzidine by a similar mechanism. Our results are consistent with recent reports (*27, 28*) demonstrating 3,5,3′,5′-tetramethylbenzidine radical formation with horseradish peroxidase and prostaglandin hydroperoxidase.

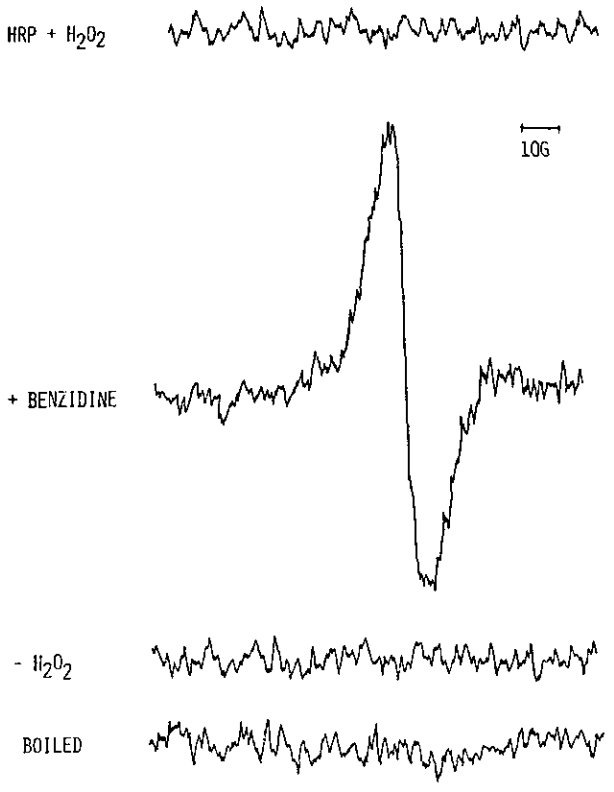

FIGURE 4. Radical formed at room temperature with 100 ng/mL HRP, 100 μM H_2O_2 and 200 μM benzidine in 0.1 *M* acetate buffer pH 5.0. Measurements were performed over a 400 G scan range in region around g = 2 with a modulation amplitude of 10, a modulation frequency of 100 kHz and a microwave power of 20 mW. The instrument was a Varian E-109E EPR spectrometer.

A model describing the relationship of PES and other peroxidases in the activation of carcinogens is illustrated in Figure 6. This model is consistent with the results above. Three types of peroxidatic reactions are envisioned. Type 1 reactions occur with carcinogens that are metabolized only by the hydroperoxidase component of PES. PES-catalyzed activation of 5-nitrofurans is a type 1 reaction. Type 2 reactions occur with carcinogens that are metabolized by both prostaglandin hydroperoxidase and other peroxidases. Aromatic amines and diethylstilbestrol activation appears to occur by a type 2 reaction. In contrast to the other peroxidases, prostaglandin hydroperoxidase is part of a complex which generates its own hydroperoxide substrate, prostaglandin G_2. Type 3 reactions would occur with certain carcinogens which are metabolized by other peroxidases and not PES. There is not a known type 3 reaction at this time. This model suggests a more expanded role for peroxidative activation of carcinogens than previously conceptualized.

Mechanisms by Which Renal and Bladder Carcinogens are Concentrated Within the Urinary Tract

Target tissue metabolism is an important part of our proposed model for explaining initiation of chemical carcinogenesis in bladder and kidney. Therefore, entry of these compounds into the kidney and their concentration within the urinary tract is very important in the carcinogenic process. Organic acid and base transport by renal cortical slices were investigated by using ^{131}I-hippuran and ^{14}C-tetraethylammonium, respectively (Table 4). Since both transport systems are energy dependent, their dramatic inhibition by anaerobic incubation conditions suggests that they are valid model systems (*29*). The 5-nitrofuran 3-hydroxymethyl-1-[(3-(5-nitro-2-furyl)-allydidene)amino]-hydantoin (HMN) inhibited organic acid but not base transport. HMN inhibition of organic acid transport was shown to be dose dependent and reversible (not shown). This suggests that the 5-nitro group of HMN functions as a carboxylate anion. Benzidine inhibited organic base but not acid transport. These results are consistent with transport of 5-nitrofurans and aromatic amines by organic acid and base transport systems, respectively, in the renal cortex. In addition to transport by the organic ion systems, drugs and xenobiotics are concentrated in urine by water reabsorption. Under physiological conditions, greater than 98% of the glomerular filtrate is removed from the lumen of the renal tubule (water reabsorption). Therefore, substrates which are not reabsorbed by the tubule will reach high concentrations in the urinary space (*30*). These specific transport and concentrating properties of the kidney are further emphasized in view of the fact that the kidney receives 20-25% of the cardiac output at rest.

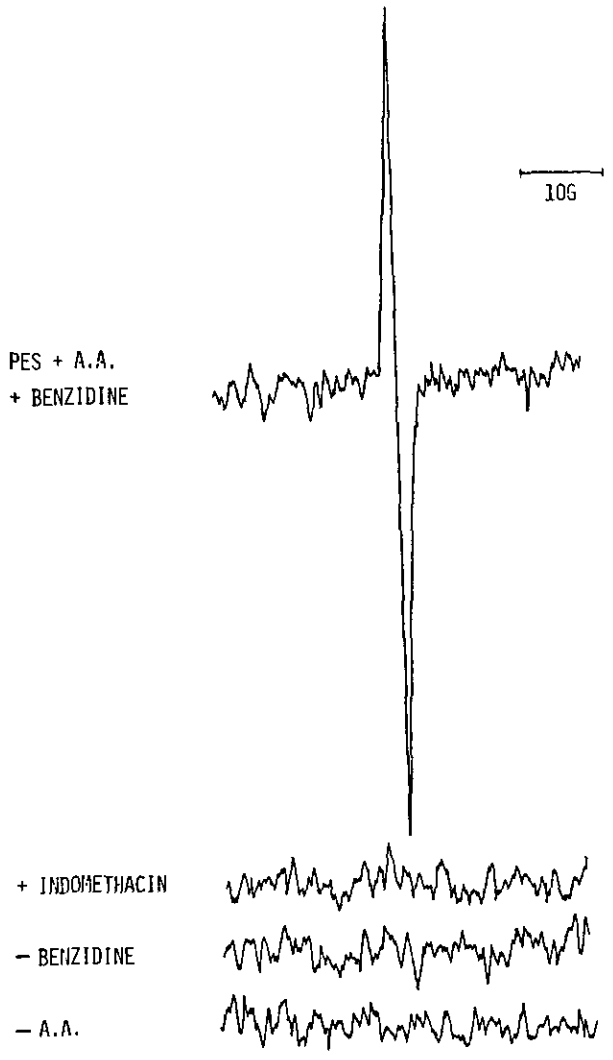

FIGURE 5. Radical formed at room temperature with 70 μg of solubilized prostaglandin hydroperoxidase, 500 μM arachidonic acid and 200 μM benzidine in 0.1 M phosphate buffer pH 6.0. Measurements were performed over a 200 G scan range in region around $g = 2$ with a modulation amplitude of 1.0, a modulation frequency of 100 kHz and a microwave power of 20 mW. The instrument was a Varian E-109E EPR spectrometer.

Table 4. Effect of HMN and benzidine on organic acid and base transport.

Conditions[a]	Organic acid (S:M)[b]	Organic base (S:M)
95% N_2:5% CO_2	2.9 ± 0.4[a]*	1.1 ± 0.1*
Control	10.4 ± 1.2	12.3 ± 0.3
Diluent (0.13% DMSO)	11.7 ± 1.2	12.1 ± 0.2
HMN (0.5 mM)	4.9 ± 0.2*	13.1 ± 0.5
Benzidine (0.5 mM)	9.8 ± 0.9	8.9 ± 0.4*

[a]Control represents transport of either [^{131}I]hippuran (organic acid) or [^{14}C]tetraethylammonium (organic base) in the absence of test agents. Unless otherwise indicated, slices were incubated with a gas phase of 95% O_2:5% CO_2 (n = 3 or more) (*29*).

[b]Mean ± S.E.

*$p < 0.01$ compared to corresponding control value.

A FANFT Rat Feeding Study: Evidence for Prostaglandin Endoperoxide Synthetase Involvement in the Initiation of the Carcinogenic Process

The preceding results suggest that PES may be involved in the initiation of certain chemical-induced

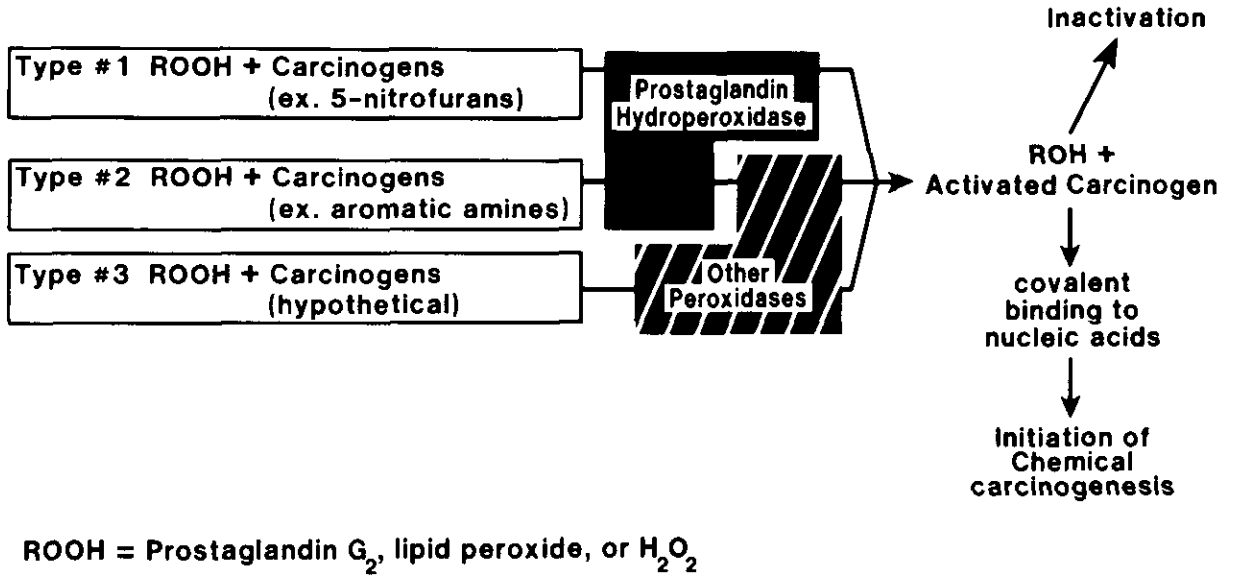

FIGURE 6. Proposed model for peroxidatic activation of carcinogens.

Table 5. Effect of aspirin on bladder lesions induced by 6 or 12 weeks of feeding FANFT.[a]

				Rats with bladder lesions[b]							
		No. of rats		Hyperplasia		Ropy microridges		Uniform microvilli		Pleomorphic microvilli	
Group	Chemicals	6 wk	12 wk	6 wk	12 wk	6 wk	12 wk	6 wk	12 wk	6 wk	12 wk
1	0.2% FANFT	8	5	6	5	6	5	4	3	2	3
2	0.2% FANFT + aspirin	8	6	2*	2**	2*	3	1***	2	0	2
3	0.1% FANFT	5	5	1	2	3	5	2	5	0	1
4	0.1% FANFT + aspirin	5	6	0	0	2	0†	1	0†	0	0
5	Aspirin	3	4[c]	0	0	0	0	0	0	0	0
6	Control	3	8[c]	0	0	0	0	0	0	0	0

[a]From Cohen et al. (*31*).
[b]Hyperplasia was assessed by light microscopy, and the other lesions were assessed by scanning electron microscopy.
[c]two rats from each of groups 5 and 6 were killed 24 weeks after the beginning of the experiment; the other rats from these groups were killed after 12 weeks.
*Group 1 versus group 2, $p < 0.07$.
**Group 1 versus group 2, $p < 0.05$.
***Group 1 versus group 2, $p < 0.15$.
†Group 3 versus group 4, $p < 0.025$.

carcinogenic processes in kidney and bladder. Therefore, if PES-catalyzed activation of carcinogens is prevented, initiation of the carcinogenic process may be prevented. In a short-term study, FANFT was fed to male 5-week-old Fischer rats as 0.2 or 0.1% of the diet with or without 0.5% aspirin (*31*). Other rats were fed either aspirin in the diet or a control diet without added chemicals. At the end of 6 and 12 weeks of feeding, rats were killed from each group and the bladder examined by light microscopy and scanning electron microscopy. The results, summarized in Table 5, demonstrate that early preneoplastic lesions induced by FANFT are inhibited by co-administration of aspirin.

A long-term experiment is in progress to determine if the bladder carcinomas induced by FANFT are inhibited by aspirin administration. The rats were fed FANFT and/or aspirin for 12 weeks and then fed control diet until the end of the experiment. The rats fed 0.2% FANFT alone and 0.2% FANFT plus 0.5% aspirin have been killed (69 weeks after the start of the experiment). Gross examination reveals bladder tumors in 18 of 21 (85%) rats fed FANFT and in 10 of 27 (37%) rats

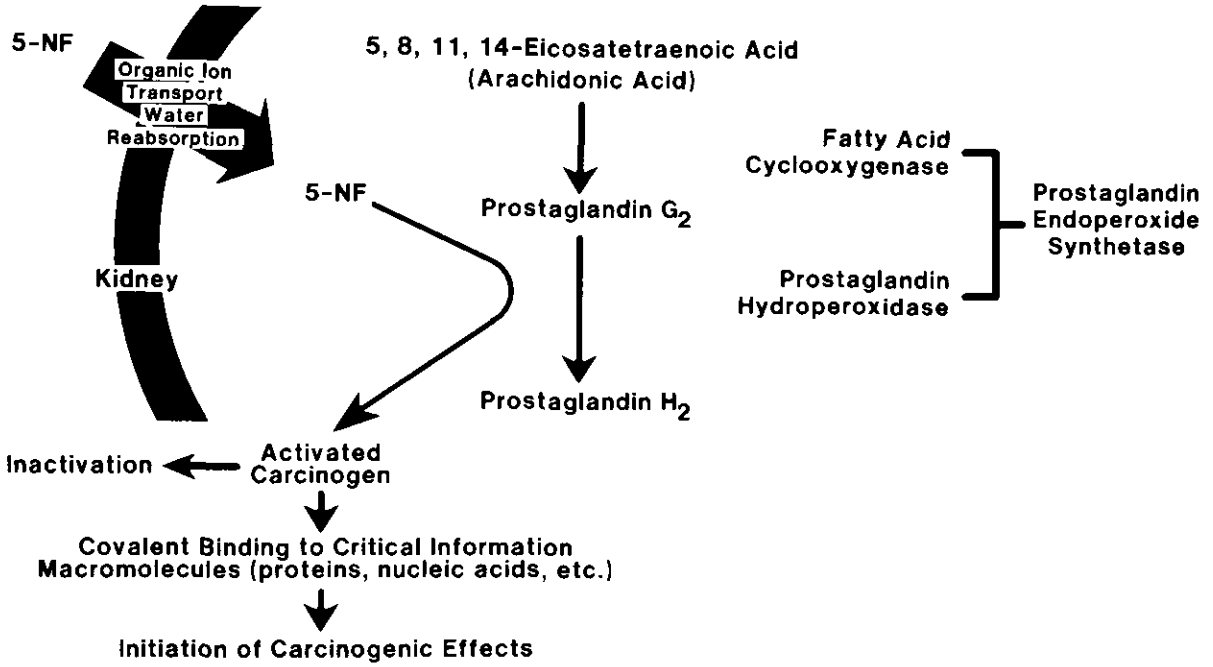

FIGURE 7. Model describing initial steps in 5-nitrofuran (5-NF)-induced bladder cancer.

fed FANFT plus aspirin ($p < 0.004$). In addition, nine rats fed FANFT plus aspirin developed forestomach tumors whereas none developed in rats fed FANFT without aspirin. Of the rats with forestomach tumors, only three also had a bladder tumor. The microscopic evaluation of these tissues is in progress.

Description of a Model System for Initiation of 5-Nitrofuran-Induced Bladder Carcinogenesis

A scheme describing the initiation of 5-nitrofuran-induced bladder carcinogenesis is shown in Figure 7. Target tissue metabolism is a salient feature of this model. Bladder carcinogens are thought to enter the kidney by facilitated transport and/or to be concentrated by water reabsorption. The latter may partially explain why these 5-nitrofurans cause tumors in bladder but not liver. Carcinogens are activated by the hydroperoxidase activity of PES to electrophiles which covalently bind to macromolecules such as DNA. This results in the initiation of the carcinogenic process. We have experimental evidence to support each step in this model: (1) organic acid transport of 5-nitrofurans (*29*); (2) prostaglandin hydroperoxidase-catalyzed covalent binding of carcinogens to tissue macromolecules (*14*); and (3) prevention of the expression of FANFT-induced lesions by aspirin in rat feeding studies (*31*).

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