MOLECULAR PHARMACOLOGY, 21:133–141

Metabolism of *N*-Alkyl Compounds during the Biosynthesis of Prostaglandins

N-Dealkylation during Prostaglandin Biosynthesis

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Received June 24, 1981; Accepted September 16, 1981

SUMMARY

The microsomal fraction of ram seminal vesicles (RSV), when fortified with arachidonic acid, catalyzed the dealkylation of various *N*-methyl compounds. These included an analogous series of monomethyl- and dimethyl-substituted anilines as well as the drugs aminopyrine and benzphetamine. In contrast, *S*-alkyl and *O*-alkyl compounds were poor substrates for dealkylation by RSV microsomes fortified with fatty acid. RSV microsomal *N*-dealkylation was completely dependent on enzyme and arachidonic acid and could be inhibited by the prostaglandin synthetase inhibitors indomethacin, phenylbutazone, and flufenamic acid as well as by anaerobic conditions. Butylated hydroxyanisole also inhibited the reaction, whereas SKF-525A and metyrapone, which are inhibitors of cytochrome P-450-dependent *N*-dealkylation, did not. In addition to arachidonic acid, *N*-dealkylation was elicited by 15-hydroperoxyarachidonic acid, *tert*-butyl-hydroperoxide, and hydrogen peroxide; these latter reactions were not inhibited by either prostaglandin synthetase inhibitors or anaerobic conditions but did require the presence of microsomal protein. The time course of RSV *N*-dealkylation, which paralleled O_2 consumption by this tissue (an indicator of prostaglandin biosynthesis) implied arachidonic acid-dependent irreversible self-inactivation of catalytic activity. Apparently, oxidizing agents are formed during the interaction of hydroperoxide intermediates of prostaglandin biosynthesis with prostaglandin synthetase, with the oxidizing agents then causing both substrate *N*-dealkylation and destruction of the enzyme. The metabolism of *N*-alkyl compounds during the biosynthesis of prostaglandins may provide an additional xenobiotic oxidation pathway to cytochrome P-450-dependent monooxygenases.

INTRODUCTION

Recent investigations have shown that a number of biosynthetic steps are involved in the formation of prostaglandins from the fatty acid precursor, arachidonic acid. The initial reaction is dioxygenation of arachidonic acid to the cyclic hydroperoxy-endoperoxide, PGG_2.[4] PGG_2 is reduced to PGH_2, a hydroxy-endoperoxide, which is subsequently converted to the physiologically important prostaglandins, thromboxanes and prostacyclin (1). Both reactions involved in the formation of PGH_2 from arachidonic acid are catalyzed by prostaglandin synthetase,[5] an enzyme present in almost all mammalian cell types (2).

Prostaglandin synthetase can utilize a wide variety of reducing cofactors to provide the requisite pair of electrons involved in the reduction of PGG_2 to PGH_2 (3–5). Although most of these cofactors undergo simple dehydrogenation, several compounds have oxygen inserted into their carbon framework during the course of the reaction. These latter compounds include the carcinogenic polycyclic aromatic hydrocarbons benzo[*a*]pyrene,

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[4] The abbreviations used are: PGG_2 and PGH_2, prostaglandins G_2 and H_2; 15-HPAA, 15-hydroperoxyarachidonic acid; RSV, ram seminal vesicle; cumene OOH, cumene hydroperoxide; *t*-butyl-OOH, *tert*-butyl-hydroperoxide; AA, arachidonic acid; PCMA, *p*-chloro-*N*-methylaniline; HPLC, high-pressure liquid chromatography.

[5] Prostaglandin synthetase appears to consist of two enzymatic components, each catalyzing a separate reaction; fatty acid cyclooxygenase (prostaglandin endoperoxide synthetase, EC 1.14.99.1), which converts arachidonic acid to PGG_2, and a hydroperoxidase, which reduces PGG_2 to PGH_2. It is the latter activity which appears to be responsible for the xenobiotic co-oxidations observed during prostaglandin biosynthesis.

0026-895X/82/010133-09$02.00/0

benzo[*a*]pyrene 7,8-dihydrodiol, and 7,12-dimethylbenzanthracene (6, 7); the anti-inflammatory agent oxyphenbutazone (6); the oxygen trap diphenylisobenzofuran (8); and the chemiluminescence indicator luminol (6). The oxygenation of these xenobiotics is very rapid in the presence of both enzyme and arachidonic acid, does not occur with omission of either, and is inhibited by indomethacin, a known inhibitor of prostaglandin biosynthesis. Prostaglandin synthetase will also convert several of the above compounds to their oxygenated derivatives when 15-HPAA or other organic hydroperoxides are substituted for arachidonic acid (6, 8, 9). Significantly, in the case of the polycyclic aromatic hydrocarbons, oxidation of these compounds during prostaglandin biosynthesis generates metabolities which covalently bind to both DNA and protein (10) and are mutagenic to *Salmonella typhimurium* (11).

Prostaglandin synthetase may thus play an important role in the oxidative metabolism of xenobiotics, a property almost exclusively attributed to the cytochrome P-450-dependent monooxygenases of liver and other tissues. Besides substrate oxygenation, however, it is not known whether prostaglandin synthetase also catalyzes other drug-metabolizing activities characteristic of the monooxygenases, such as *N*- and *O*-dealkylation, *N*-oxidation, or deamination. It has been recently demonstrated that certain plant hemeproteins with peroxidase activity, including chloroperoxidase, horseradish peroxidase, and pea seed peroxygenase, can catalyze *O*- and *N*-dealkylations and hydroxylations in the presence of organic hydroperoxides or H_2O_2 (12–15). In addition, cytochrome P-450 itself may function as a peroxidase, oxidizing various compounds when hydroperoxides are substituted for NADPH and O_2 (16–19). Therefore, prostaglandin synthetase, a hydroperoxide-generating enzyme with peroxidatic activity, may provide an alternative pathway to the cytochrome P-450-dependent monooxygenases with regard to oxidative drug metabolism.

This communication describes our investigation of the prostaglandin synthetase-dependent metabolism of foreign compounds using an enzyme preparation from ram seminal vesicles. This tissue was chosen because it has extremely high prostaglandin biosynthetic capacity and very low cytochrome P-450-dependent monooxygenase activity. The substrate specificity, cofactor requirements, and kinetics of xenobiotic oxygenation by RSV microsomal prostaglandin synthetase were examined. Our results indicate that RSV microsomes, when fortified with arachidonic acid or organic hydroperoxides, dealkylate various *N*-alkyl compounds at significant rates which are comparable to those reported for rat liver microsomes fortified with NADPH. In addition, the mechanisms by which these tissues catalyze *N*-dealkylation appear to be very different.

EXPERIMENTAL PROCEDURES

Materials. Arachidonic acid (99% pure) was obtained from Nu Chek Preparations, Inc. (Elysian, Minn.). 15-Hydroperoxyarachidonic acid was prepared from arachidonic acid using soybean lipoxygenase (Sigma Chemical Company, St. Louis, Mo.) according to published methods (20). Indomethacin, NADPH, butylated hydroxyanisole, glutathione (reduced), phenylbutazone, and flufenamic acid were also obtained from Sigma Chemical Company. *p*-Chloro-*N*-methylaniline was purchased from Calbiochem (San Diego, Calif.). SKF-525A was obtained from Smith Kline & French Laboratories (Philadelphia, Pa.). Benzphetamine, 7-ethoxycoumarin, 7-ethoxyresorufin, and metyrapone [2-methyl-1,2-bis(3-pyridyl)-1-propanone] were generously provided by Dr. J. Fouts, National Institute of Environmental Health Sciences. Aminocarb (4-dimethylamino-*m*-tolyl-methylcarbamate) and Methiocarb [4-[methylthio]-3,5-xylyl-*N*-methylcarbamate] were obtained from Mobay Chemical Corporation (Kansas City, Mo.). Cumene hydroperoxide was obtained from ICN-K&K Laboratories (Plainview, N. Y.) and was purified by alumina chromatography prior to use. *t*-Butyl-OOH was obtained from Pennwalt Corporation (Buffalo, N. Y.). All other reagents used were obtained from either Aldrich Chemical Company (Milwaukee, Wisc.) or Eastman Kodak (Rochester, N. Y.)

Enzyme preparation. Ram seminal vesicles were obtained from a local slaughterhouse, trimmed of excess fat and connective tissue, and stored at −70° until use. Microsomes were prepared from the vesicles as previously described (7) and used on the same day. Microsomal prostaglandin synthetase activity was determined by measuring the AA-dependent oxygen uptake using a Clark-type electrode; RSV microsomes with low enzymatic activity were discarded. Attempts to detect cytochrome P-450 spectrally in RSV microsomes (see below) following reduction with dithionite and carbon monoxide gassing (21) revealed no detectable absorbance at or near 450 nm at protein concentrations of up to 3 mg/ml.

Male Sprague-Dawley rats (200–250 g, Sprague-Dawley, Madison, Wisc.), male guinea pigs (300–400 g, Charles River Breeding Laboratories Inc., Wilmington, Mass.), male New Zealand White rabbits (2.5–2.7 kg, Dutchland Laboratories Inc., Denver, Pa.), and female A/J mice (20–25 g Jackson Laboratories, Bar Harbor, Me.) were provided with food and water ad libitum until immediately prior to sacrifice. Animals were killed by decapitation, and the livers, lungs, and kidneys were quickly removed and washed in ice-cold 1.15% KCl. All subsequent procedures were performed at 4°. After being weighed, the tissues were coarsely chopped with scissors and a 25% (v/v) homogenate was made in 66 mM Tris-HCl buffer (pH 7.4), using a Polytron-type homogenizer. Microsomes were then prepared by differential centrifugation as previously described (22, 23). Microsomes were resuspended in 0.25 M sucrose containing 5.4 mM EDTA at a final protein concentration of approximately 20 mg/ml and frozen at −70° under N_2 until use. Protein concentration was measured by the method of Lowry *et al.* (24) using bovine albumin as a standard. The cytochrome P-450 concentration was determined from the reduced CO difference spectrum by the method of Omura and Sato (21) using an Aminco DW-2a spectrophotometer (American Instrument Company, Silver Spring, Md.) in the split-beam mode. An extinction coefficient of 91 mM^{-1} cm^{-1} between 450 and 490 nm was used.

Incubation procedures. Microsomal protein was sus-

pended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4) containing 15 μmoles of semicarbazide and 10 μmoles of $MgCl_2$. Substrates were then added to the incubation mixture in 20 μl of acetone; the final concentration of these substrates was 1 mM. The reactions were initiated with 0.8 μmole of AA and were run in a Dubnoff incubator with shaking for 1 min at either 20° or 37°. Separate experiments were performed in which the incubation times or the concentrations of PCMA, RSV microsomes, or AA were varied (see Results). Metabolic blanks were run using heat-denatured microsomes or by omitting AA. The reactions were terminated by the addition of 0.7 ml of 15% $ZnSO_4$, followed by 0.7 ml of saturated $Ba(OH)_2$. Formaldehyde formation was then determined according to the method of Nash (25). 7-Ethoxycoumarin and 7-ethoxyresorufin-*O*-deethylase activities were measured using previously published methods (26, 27).

During this investigation, we observed that the *N*-demethylase activity of RSV microsomes was quite unstable. Excessive Polytron homogenization of ram seminal vesicles, lengthy microsomal preparation times, or just exposing RSV microsomes kept on ice for periods greater than 2 hr resulted in a drastic reduction of enzymatic activity. This decrease in enzymatic activity paralleled a similar reduction in AA-dependent RSV microsomal O_2 uptake. Experiments utilizing RSV microsomes were therefore planned in such a way that all incubations were completed within 45 min of the final resuspension of the microsomal pellets.

HPLC analysis was performed using a Waters Associates liquid chromatograph (Milford, Mass.) consisting of two M6000A pumps, M440 UV detector, U6K injector, M660 gradient programmer and a C-18 μ Bondapak reverse-phase column (300 × 3.9 mm). After termination of the reaction with $ZnSO_4$, a 25-μl aliquot of the incubation mixture was directly injected onto the HPLC. PCMA and metabolites were then separated by gradient elution at room temperature. The initial solvent composition was 15% methanol and 85% water, changing to a final solvent concentration of 60% methanol and 40% water at a rate of 3%/min. The flow rate used was 2.0 ml/min. Compounds appearing in the column eluates were detected by monitoring the UV absorbance at 254 nm. Identification was made by comparison of the retention times to that of authentic standards.

All experiments were carried out in duplicate or triplicate and the data presented are either average values or the means ± standard error.

RESULTS

Xenobiotic metabolism by RSV microsomes. In initial experiments, a series of structurally diverse chemicals was employed to determine the substrate specificity of RSV microsomal prostaglandin synthetase. The results shown in Table 1 indicate that various *N*-methyl aniline compounds underwent dealkylation in the presence of RSV microsomes and AA to produce formaldehyde. In general, rates of *N*-demethylation were greater with unsubstituted monomethyl and dimethyl compounds than with the substituted analogues. Of the various derivatives, substitution with the NO_2 group had the greatest

TABLE 1

N-Demethylation of various xenobiotics by RSV microsomes

Reaction mixtures contained 0.2 mg of RSV microsomes, 15 μmoles of semicarbazide, 10 μmoles of $MgCl_2$, and 2 μmoles of substrate suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with 0.4 μmole of AA and were terminated after 1 min at 37°. Formaldehyde formation was determined as described under Experimental Procedures.

Compound	Rate of *N*-demethylation[a]
	nmoles HCHO formed/min/mg protein
Monomethyl-substituted	
N-Methylaniline	40.3 ± 1
N-Methyl-*p*-chloroaniline	33.5 ± 4
N-Methyl-*o*-nitroaniline	ND[b]
N-Methyl-*p*-nitroaniline	ND
N-Methyl-*o*-toluidine	22.4 ± 1
N-Methyl-*m*-toluidine	39.9 ± 1
N-Methyl-*p*-toluidine	10.7 ± 1
N-Methyl-*o*-phenylenediamine	3.5 ± 1
N-Methyl-*p*-phenylenediamine	16.5 ± 1
N-Methyl-*n*-nitrosoaniline	5.9 ± 1
Dimethyl-substituted	
N,N-Dimethylaniline	35.3 ± 3
N,N-Dimethylaminobenzoic acid	13.5 ± 1
N,N-Dimethyl-*p*-nitroaniline	6.0 ± 1
N,N-Dimethyl-*p*-phenylenediamine	10.2 ± 1
Miscellaneous	
Aminopyrine	53.8 ± 2
Aminocarb (4-dimethylamino-*m*-tolyl-methylcarbamate	56.4 ± 1
Benzphetamine	52.9 ± 2
p-Nitroanisole	ND
1,2,4-Trimethoxybenzene	ND
Methiocarb	ND
7-Ethoxycoumarin	ND
7-Ethoxyresorufin	ND

[a] Values represent the means ± standard error of at least three separate experiments.

[b] ND, not detectable.

inhibitory effect on dealkylation catalyzed by RSV microsomes. In addition to these *N*-alkyl aniline analogues, the drugs aminopyrine and benzphetamine as well as the insecticide aminocarb were *N*-demethylated by RSV microsomes and AA. Metabolism of the above *N*-alkyl compounds was dependent on RSV microsomes, which could not be replaced by boiled enzyme. The reaction was also dependent on the presence of AA; no other components were needed for dealkylation by this tissue.

In addition to *N*-dealkylation, other drug-metabolizing activities characteristic of the hepatic cytochrome P-450-dependent monooxygenase system were examined using RSV microsomal prostaglandin synthetase. The *S*-demethylation of the insecticide methiocarb, *O*-demethylation of *p*-nitroanisole or 1,2,4-trimethoxybenzene, and *O*-deethylation of 7-ethoxycoumarin or 7-ethoxyresorufin were not catalyzed by RSV microsomes fortified with AA. Although attempts were made to optimize incubation conditions, dealkylation did not occur with any of these compounds. The results indicate that RSV microsomal dealkylation, in comparison to cytochrome P-450-

catalyzed dealkylation, is quite specific; hepatic monooxygenases are known to catalyze all of the above described reactions.

As shown in Table 2, RSV microsomal *N*-demethylation was profoundly inhibited when indomethacin, a classic inhibitor of prostaglandin biosynthesis, was preincubated with microsomal protein before the addition of AA. In the cases of PCMA, *N,N*-dimethylaniline, benzphetamine, and aminocarb, formaldehyde formation was completely abolished by preincubation of enzyme with 500 μM indomethacin; inhibition was not as complete when aminopyrine was employed as a substrate. The observed inhibitory effect of indomethacin was much less pronounced when the inhibitor and AA were added concurrently to the incubation mixture.

Kinetics of PCMA metabolism by RSV microsomes. Incubation of PCMA with RSV microsomes and AA resulted in the formation of formaldehyde and *p*-chloroaniline. No other major metabolites of PCMA were detected by HPLC analysis. PCMA was therefore chosen as a model substrate to examine the parameters of RSV microsomal *N*-demethylation because this compound was metabolized at high rates to products for which exist simple and rapid assay methods for the determination of either formaldehyde or *p*-chloroanaline.

Formaldehyde formation from PCMA catalyzed by RSV microsomes was characterized by an initial burst lasting 10–20 sec, then proceeded more slowly, reaching a plateau after 4–5 min (Fig. 1). For convenience, the amount of formaldehyde formed during the 1st min of incubation was used to calculate initial velocities. Figure 1 also shows that the rate of RSV microsomal *N*-demethylation of PCMA was dependent on the incubation temperature; rates obtained at 20° were only 50% of those at 37°. When RSV microsomal protein was boiled prior to incubation, product formation was not observed.

TABLE 2

Inhibition of prostaglandin synthetase-dependent N-demethylation by indomethacin

Reaction mixtures contained 0.2 mg of RSV microsomal protein, 15 μmoles of semicarbazide, 10 μmoles of $MgCl_2$, and 2 μmoles of substrate suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with 0.4 μmole of AA and were terminated after 1 min at 37°. When added, indomethacin (final concentration 500 μM) was preincubated with the reaction mixture for 5 min at 4° before the addition of AA. Formaldehyde formation was determined as described under Experimental Procedures.

Compound	Indomethacin	Formaldehyde formation[a]
		nmoles/min/mg RSV protein
PCMA	−	57.3
	+	ND[b]
N,N-Dimethylaniline	−	42.1
	+	ND
Benzphetamine	−	52.9
	+	ND
Aminopyrine	−	53.8
	+	13.6
Aminocarb	−	56.4
	+	ND

[a] Values represent the means of two or more experiments.
[b] ND, not detectable.

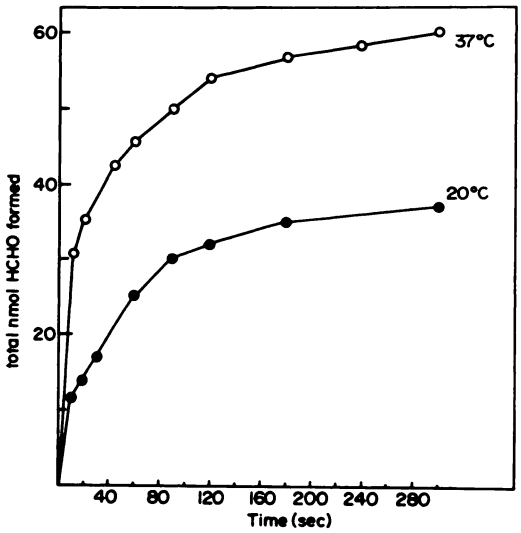

FIG. 1. *Time course of HCHO formation from PCMA by RSV*

Reaction mixtures contained 0.2 mg of RSV microsomes, 2 μmoles of PCMA, 15 μmoles of semicarbizide, and 10 μmoles of $MgCl_2$ suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with the addition of 0.8 μmole of AA and were terminated at the times indicated. Formaldehyde formation was determined as described under Experimental Procedures. ●——●, RSV microsomes at 20°; ○——○, RSV microsomes at 37°.

Regarding the effect of PCMA concentration on the *N*-demethylase activity of RSV microsomes, *N*-demethylation rates were found to increase in a linear fashion with PCMA concentrations up to 100 μM; further increases in the substrate concentration had relatively little effect on the rate of *N*-demethylation (data not shown). However, K_m and V_{max} values for RSV microsomal PCMA dealkylation could not be obtained in this study because of the nonlinearity of substrate demethylation with respect to time (see Fig. 1).

Effect of protein concentration on PCMA metabolism. Figure 2 illustrates the effect of RSV microsomal protein concentration on the rate of PCMA *N*-demethylation. Only small amounts of RSV microsomal protein were

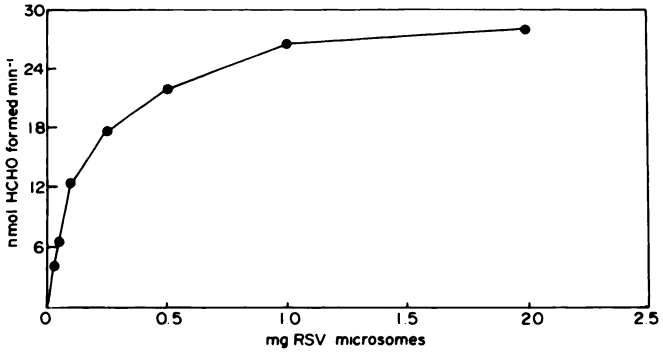

FIG. 2. *Effect of protein concentration on HCHO formation from PCMA by RSV*

Reaction mixtures contained the indicated amounts of RSV microsomes, 2 μmoles of PCMA, 15 μmoles of semicarbazide, and 10 μmoles of $MgCl_2$ suspended in 2 ml of 66 mM Tris-HCl buffer (ph 7.4). Reactions were initiated with 0.8 μmole of AA or 5 μmoles of NADPH and were terminated after 1 min at 37°. Formaldehyde formation was determined as described under Experimental Procedures.

required to catalyze the formation of substantial amounts of formaldehyde from PCMA; rates of product formation at a protein concentration of 0.15 mg/ml were about 50% of those at 2 mg/ml. Increasing the RSV microsomal protein concentration above this latter value had practically no effect on *N*-demethylation rates.

RSV microsomal N-demethylation using various hydroperoxides. The ability of several structurally diverse compounds to catalyze RSV microsomal PCMA metabolism was examined (Table 3). Of the compounds tested, the highest rates of PCMA *N*-demethylation were obtained using AA. However, appreciable RSV microsomal *N*-demethylase activity was also observed when the organic hydroperoxides 15-HPAA, and *t*-butyl-OOH as well as hydrogen peroxide were used in place of AA. Microsomal protein had to be present in order for PCMA *N*-demethylation to occur; hydroperoxide alone was not effective. Cumene hydroperoxide, which can replace NADPH in cytochrome P-450-catalyzed *N*-demethylation reactions, was not effective in catalyzing RSV microsomal PCMA *N*-demethylation. In addition, no product was formed when either NADPH or PGH_2 was employed. Unfortunately, we could not include PGG_2, the naturally occurring hydroperoxide derived from AA, in this group of compounds because it proved very difficult to synthesize and was not available from other sources.

Inhibition of PCMA N-Demethylation. Figure 3 illustrates the inhibition of the PCMA *N*-demethylase activity of RSV microsomes as a function of indomethacin concentration. The concentration of indomethacin necessary to inhibit 50% of the original enzyme activity (I_{50}) was 15 μM; this value corresponds closely with the indomethacin I_{50} determined for prostaglandin biosynthesis in RSV microsomes (28). The indomethacin I_{50} was dependent on both the arachidonic acid and microsomal protein concentration but was independent of the concentration of PCMA when this compound was present in amounts exceeding 75 μM.

N_2 and CO were also effective inhibitors of RSV microsomal PCMA *N*-demethylase activity (Table 4). However, anaerobic conditions employing either N_2 or CO had no effect on PCMA metabolism when 15-HPAA was substituted for AA. These results indicate that oxygen is not directly involved in the RSV microsome-catalyzed reaction but is required for the synthesis of the hydroperoxy intermediate, PGG_2. The failure of CO to inhibit 15-HPAA-catalyzed RSV microsomal PCMA metabolism also suggests that the ferrous (Fe^{2+}) form of heme in

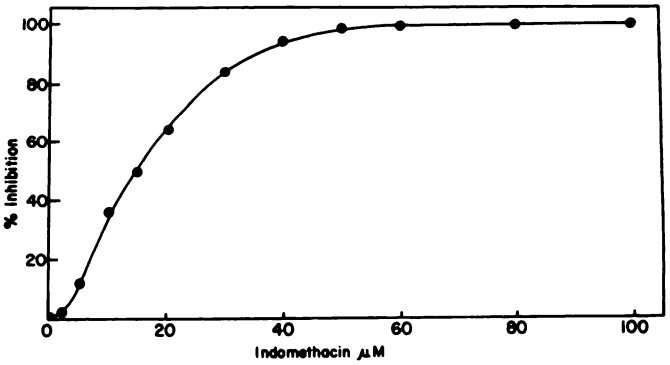

FIG. 3. *Indomethacin inhibition of RSV microsomal PCMA N-demethylation*

Reaction mixtures contained 0.2 mg of RSV microsomes, 15 μmoles of semicarbazide, 10 μmoles of $MgCl_2$, 2 μmoles of PCMA, and the indicated concentrations of indomethacin suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with the addition of 0.8 μmole of AA and were terminated after 1 min at 37°. Formaldehyde formation was determined as described under Experimental Procedures.

TABLE 3

N-Demethylation of PCMA by RSV microsomes using various cofactors

Reaction mixtures contained 0.2 mg of RSV microsomes, 2 μmoles of PCMA, 15 μmoles of semicarbazide, and 10 μmoles of $MgCl_2$ suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with the addition of cofactor and were terminated after 1 min at 37°. Formaldehyde formation was determined as described under Experimental Procedures.

Cofactor added[a]	Formaldehyde formation[b]
	nmoles/min/mg protein
None	0
AA	40.4
15-HPAA	25.2
PGH_2	ND
Cumene OOH	ND
t-Butyl-OOH	39.5
H_2O_2	48.4
NADPH	0

[a] Amounts of cofactors added were: AA, 0.8 μmole; 15-HPAA, 0.15 μmole; Cumene OOH, 2 μmoles; PGH_2, 0.8 μmole; *t*-butyl-OOH, 2 μmoles; H_2O_2, 0.5 μmole; NADPH, 5 μmoles.

[b] Values represent the means of at least two separate experiments.

[c] ND, not detectable.

TABLE 4

Inhibition of RSV microsomal PCMA N-demethylation

Reaction mixtures contained 0.2 mg of RSV microsomes, 15 μmoles of semicarbazide, 10 μmoles of $MgCl_2$, and 2 μmoles of PCMA suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). The compounds listed were preincubated with microsomal protein for 5 min at 4° before the addition of cofactor. The reactions were terminated after 1 min at 37° and formaldehyde was determined as described under Experimental Procedures.

Agent	Formaldehyde formation[a]	
	RSV microsomes/AA[b]	RSV microsomes/15-HPAA[b]
None	100	100
N_2[c]	55	96
CO[c]	60	99
Cyanide (1 mM)[d]	81	95
SKF-525A (1 mM)	98	95
Metyrapone (1 mM)	121	100
Indomethacin (0.1 mM)	8	99
Phenylbutazone (0.1 mM)	72	101
Flufenamic acid	13	100
Butylated hydroxyanisole (0.5 mM)	3	96
Glutathione (1 mM)	112	101

[a] Product formation is expressed relative to "none" (=100).

[b] Concentrations of cofactors used were: AA, 400 μM; 15-HPAA, 120 μM.

[c] Gases were gently bubbled through the reaction mixture for 1 min before the addition of cofactor.

[d] Values in parentheses indicate the final concentration of compound used.

prostaglandin synthetase is not involved in the reaction. [It should be pointed out here that neither anaerobic conditions nor CO is capable of inhibiting cytochrome P-450-catalyzed *N*-dealkylation in the presence of certain hydroperoxides (16).] Cyanide, which is capable of serving as a ligand to the heme iron atom in the ferric (Fe^{3+}) form, was also ineffective as an inhibitor of PCMA *N*-demethylation of RSV microsomes, in the presence of either AA or 15-HPAA.

SKF-525A and metyrapone, known inhibitors of many hepatic monooxygenase reactions, did not inhibit RSV microsomal PCMA *N*-demethylation (Table 4). In fact, metyrapone slightly stimulated this reaction when AA was used as a cofactor. Conversely, indomethacin, phenylbutazone, and flufenamic acid, all of which are inhibitors of prostaglandin biosynthesis, inhibited RSV microsomal PCMA metabolism in the presence of AA but had little effect when 15-HPAA was utilized as cofactor, indicating that they inhibit the reaction by interfering with the synthesis of PGG_2 by the cyclooxygenase component of prostaglandin synthetase (2). Glutathione, which has been reported to stimulate prostaglandin biosynthesis (29) had no effect on RSV microsomes or PCMA *N*-demethylation. Butylated hydroxyanisole also dramatically inhibited AA-dependent RSV microsomal *N*-demethylase activity. This compound has been previously reported to inhibit RSV microsomal drug oxidations (30, 31).

Release of oxidants during AA metabolism by RSV microsomes. As described previously, the formation of formaldehyde during RSV microsomal PCMA *N*-demethylation was characterized by an initial burst lasting 10–20 sec, then proceeded more slowly and finally ceased after 4–5 min. The *N*-demethylase activity of RSV microsomes could not be restored after this time by adding more substrate or cofactor; only the addition of fresh microsomal protein to the incubation mixture restored enzymatic activity. Apparently, this phenomenon was limited not only to PCMA, for we obtained similar results using other substrates (aminopyrine, *N,N*-dimethylaniline, and aminocarb) which were *N*-demethylated by RSV microsomes. Inactivation of RSV microsomal enzyme activity paralleled a similar time-related decrease in prostaglandin biosynthesis by this tissue.[6] Egan *et al.* (32) have shown that both enzymatic components of prostaglandin synthetase are subject to AA-dependent self-inflicted deactivation, which is due to indirect attack on the enzyme by oxidants released during the reduction of PGG_2 to PGH_2. To determine whether oxidants were formed during AA metabolism and were responsible for the deactivation of the *N*-demethylase activity of RSV microsomes, we first examined the effect of active prostaglandin biosynthesis on rat liver microsomal *p*-nitroanisole *O*-demethylation. Table 5 shows that addition of RSV microsomes together with AA inhibited the *O*-demethylation of *p*-nitroanisole by rat liver microsomes fortified with NADPH (*p*-nitroanisole is not demethylated by RSV microsomes). This inhibition was proportional to the amount of RSV microsomal protein added, and neither RSV microsomes nor AA were capable of producing this effect alone. The inhibition of rat liver microsomal *O*-demethylase activity was apparently due to the destruction of cytochrome P-450 in the microsomes, as determined by measurements of the Soret band of the reduced hemeprotein-CO difference spectrum at 450 nm. In other experiments, incubation of PCMA with both RSV and rat liver microsomes and the necessary cofactors resulted in the formation of significantly less formaldehyde than the sum of the amounts formed by each tissue separately (Table 6). This lack of product additivity with the two microsomal systems was not due to consumption of PCMA, because saturating amounts of substrate were used, and was not due to end-product inhibition, since $PGF_{2\alpha}$, PGE_2, and PGD_2 do not inhibit the *N*-demethylase activity of either tissue. Finally, we observed the same type of time-related decrease in RSV microsomal PCMA *N*-demethylase activity when 15-HPAA was substituted for AA as a cofactor in the reaction. These data indicate that oxidants are released dur-

[6] J. M. Lasker and K. Sivarajah, unpublished observations.

TABLE 5

Effect of prostaglandin biosynthesis on the O-demethylation of p-nitroanisole by rat liver microsomes

Reaction mixtures contained 1.25 mg of rat liver microsomes (0.8 nmole of cytochrome P-450 per milligram) plus varied amounts of RSV microsomes, 1 μmole of *p*-nitroanisole, 7.5 μmoles of semicarbazide, and 5 μmoles of $MgCl_2$ suspended in 1 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with 0.8 μmole of AA plus 5 μmoles of NADPH and were run for 5 min at 37°. Formaldehyde formation was measured as described under Experimental Procedures.

Amount of RSV microsomes added	Formaldehyde formation[a]	% of control
mg	*nmoles/min/mg microsomal protein*	
0	5.12 ± 0.7	100
0.25	2.80 ± 0.4	55
0.50	1.91 ± 0.3	37
1.0	1.39 ± 0.1	27
2.0	0.85 ± 0.1	17

[a] Values represent the means ± standard error of three separate experiments.

TABLE 6

Product additivity of RSV and rat liver microsomal PCMA N-demethylation

Reaction mixtures contained the indicated amounts of microsomal protein, 15 μmoles of semicarbazide, 10 μmoles of $MgCl_2$, and 2 μmoles of PCMA suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with the addition of 0.8 μmole of AA and/or 5 μmoles of NADPH and were terminated after 5 min at 37°. Formaldehyde formation was determined as described under Experimental Procedures.

Microsomal protein added	Amount	Formaldehyde formation	
		Observed	Expected[a]
	mg	*nmoles/mg RSV or rat liver microsomes/5 min*	
RSV	1	25.8	—
RSV	3	45.5	—
Rat liver	0.6	10.5	—
Rat liver	1.8	29.4	—
RSV + rat liver	1 + 1.8	37.3	55.2
RSV + rat liver	3 + 0.6	49.1	56.0

[a] Expected values represent the sum of the observed rates of formaldehyde formation by the two different microsomal preparations.

TABLE 7

N-Demethylation of PCMA by lung and kidney medullary microsomes

Reaction mixtures contained 2 mg of microsomal protein, 15 μmoles of semicarbazide, 10 μmoles of $MgCl_2$, and 2 μmoles of substrate suspended in 2 ml of 66 mM Tris-HCl buffer (pH 7.4). Reactions were initiated with 0.8 μmole of AA and were terminated after 1 min at 37°. When added, indomethacin (final concentration 100 μM) was preincubated with the reaction mixture for 5 min at 4° before the addition of AA. Formaldehyde formation was determined as described under Experimental Procedures.

Tissue	Indomethacin	Formaldehyde formation[a]
		nmoles/min/mg protein
Guinea pig lung	−	6.5
	+	ND[b]
Mouse lung	−	4.0
	+	ND
Rabbit kidney medulla	−	6.2
	+	ND

[a] Values represent the mean of two or more experiments.
ND, not detectable.

ing the reduction of hydroperoxides by RSV microsomes which can inactivate functional proteins, including those proteins responsible for their formation. Although we did not attempt to identify the oxidizing species, this area deserves further investigation.

Prostaglandin synthetase-dependent PCMA N-demethylation by renal and pulmonary tissues. In addition to seminal vesicles, other mammalian tissues such as lung and kidney possess significant prostaglandin synthetase activity. We therefore examined whether prostaglandin synthetase-dependent *N*-demethylation could also occur in these tissues. As shown in Table 7, microsomes derived from mouse lung, guinea pig lung, and rabbit kidney medulla catalyzed PCMA *N*-demethylation when fortified with AA. Preincubation of microsomal protein with 200 μM indomethacin completely inhibited prostaglandin synthetase-dependent co-oxidation in all of these tissues. Although the rates of PCMA *N*-demethylation obtained with lung and kidney medullary microsomes were somewhat less than that observed with RSV microsomes, the former tissues possess less than 1% of the prostaglandin synthetase activity of vesicular tissue (33).

DISCUSSION

Our results show that various *N*-alkyl compounds are enzymatically cleaved to their corresponding dealkylated derivatives in a system which promotes the biosynthesis of prostaglandins. The microsomal fraction of ram seminal vesicles, when fortified with AA, catalyzed the demethylation of various monomethyl- and dimethyl-substituted aromatic anilines as well as several drugs and pesticides with *N*-methyl moieties. Such xenobiotic dealkylations are common to the cytochrome P-450-dependent monooxygenases present in liver and other tissues. However, the prostaglandin synthetase-dependent system did not exhibit the broad substrate specificity reported for the hepatic monooxygenase system (34) with regard to dealkylation. (This difference in substrate specificity may be a result of the different dealkylation mechanisms employed in each case and is further discussed below.) Nevertheless, rates of prostaglandin synthetase-dependent *N*-dealkylation were comparable, on a per milligram of protein basis, to those reported with the rat liver microsomal monooxygenase system.

Prostaglandin synthetase-dependent metabolism of drugs and other foreign chemicals has important implications with regard to the disposition of such compounds, since the biosynthesis of prostaglandins occurs in many cell types (1). Although the majority of experiments described in this study utilized an enzyme system derived from seminal vesicles (whose size, anatomical location, and sexual dependence would preclude a major role for this organ in xenobiotic metabolism), significant prostaglandin synthetase-dependent *N* demethylase activity was also observed in guinea pig lung, mouse lung, and rabbit kidney medulla. These latter tissues are intimately involved in the disposition of foreign chemicals. In a previous study (33), we demonstrated that microsomes derived from human lung, guinea pig lung, mouse skin, and rabbit kidney medulla catalyzed the AA-dependent co-oxidation of benzo[*a*]pyrene 7,8-dihydrodiol to benzo[*a*]pyrene epoxide I, the ultimate carcinogenic metabolite of the parent hydrocarbon. In addition, Zenser *et al.* (31, 35) have reported that the urinary bladder carcinogens *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide and benzidine are metabolized by rabbit kidney medullary microsomes fortified with AA to products which covalently bind to nucleic acids. The renal inner medullary tissue is the last tissue in contact with urine prior to its entry into the urinary space. Clearly, additional studies are warranted to ascertain further the pharmacological and/or toxicological importance of prostaglandin synthetase-dependent xenobiotic co-oxidation *in vivo*.

N-dealkylation catalyzed by prostaglandin synthetase appears to be due to the peroxidative activity of this enzyme. Oxidative conversion of AA to the hydroperoxy endoperoxide PGG_2 by the cyclooxygenase component of prostaglandin synthetase (2) was a prerequisite for *N*-dealkylation. Inhibition of PGG_2 formation by cyclooxygenase inhibitors (indomethacin, phenylbutazone, and flufenamic acid or by anaerobic conditions also inhibited *N*-dealkylation. However, these treatments had no effect on the *N*-dealkylation reaction when the hydroperoxy compound 15-HPAA was substituted for AA. Neither 15-HPAA nor the other organic hydroperoxides examined were the sole catalysts but required the presence of RSV microsomal protein to effect *N*-dealkylation. The fact that these hydroperoxides mimicked the action of AA, whereas PGH_2 did not, further indicates the importance of the hydroperoxy moiety in the *N*-dealkylation reaction. It is of interest that several hemeproteins with peroxidase activity, such as myoglobin, catalase, and chloroperoxidase, have also been reported to catalyze *N*-dealkylations in the presence of hydroperoxides (12, 13, 15, 36, 37).

In addition to NADPH, various hydroperoxy compounds can also support cytochrome P-450-dependent drug oxidations (16–19). The possibility therefore exists that the *N*-dealkylations observed during prostaglandin biosynthesis were due to the interaction of PGG_2 with

cytochrome P-450 in the RSV microsomes. However, evidence argues against this possibility. First, we were unable to detect spectrally any cytochrome P-450 in the RSV microsomes following dithionite reduction and CO gassing. Second, SKF-525A and metyrapone, which are inhibitors of cytochrome P-450-dependent *N*-dealkylation (38), were not inhibitory to *N*-dealkylation by RSV microsomes fortified with either AA or organic hydroperoxides. Third, there was no NADPH-dependent *N*-dealklylation by RSV microsomes; this nucleotide cofactor would have elicited *N*-dealkylation had any cytochrome P-450 (and NADPH cytochrome P-450 reductase) been present in the RSV microsomal preparations.

The nature of the oxidizing agent formed during the interaction of hydroperoxides with prostaglandin synthetase is a matter of speculation. If it is typical of other peroxidatic enzymes, the interaction of hydroperoxides such as PGG_2 with the enzyme would be expected to generate PGH_2 and a Compound I-type derivative of prostaglandin synthetase. This enzyme derivative would be two oxidizing equivalents above the resting state of the enzyme (39). Sequential one-electron reductions of the enzyme with reducing equivalents provided by dehydrogenation (electron abstraction) of a suitable substrate would complete the catalytic cycle. In this case, the oxidizing agent catalyzing *N*-dealkylation would be protein-derived. It should be noted here that Lasker and co-workers (40) have reported that the incubation of aminopyrine with prostaglandin synthetase and AA results in the formation of a free radical cation and iminium cation by sequential one-electron oxidations, the latter of which are hydrolyzed to formaldehyde and the demethylated amine. On the other hand, prostaglandin synthetase could reduce hydroperoxides by only one electron initially, producing an alkoxy free radical and an enzyme derivative one oxidizing equivalent above the resting state. The oxidizing agent formed here would be either hydroperoxide or protein-derived. In either case, the ability of a compound to serve as a reductant of the oxidizing agent would determine the over-all substrate specificity of prostaglandin synthetase. A dehydrogenation mechanism of *N*-dealkylation for prostaglandin synthetase (40) would be very different from the *N*-dealkylation mechanism suggested for cytochrome P-450; in the latter case, oxygen purportedly attacks the *N*-alkyl group, forming an unstable carbinolamine compound which breaks down spontaneously to the aldehyde and the dealkylated amine (41).

In conclusion, the microsomal fraction of sheep vesicular glands contains an enzyme system capable of metabolizing a number of structurally diverse *N*-alkyl compounds when incubated with either arachidonic acid or lipid hydroperoxides. The metabolism of *N*-alkyl compounds during the biosynthesis of prostaglandins may provide an additional oxidation pathway to the cytochrome P-450-dependent monooxygenases. Such alternative metabolic pathways could conceivably play a significant role in xenobiotic disposition, especially in those tissues rich in prostaglandin synthetase activity and lacking monooxygenase activity. In addition, these investigations have raised the intriguing questions of which cellular constituents are oxidized during prostaglandin biosynthesis *in vivo* and how this is involved in cellular physiology and/or pathology.

ACKNOWLEDGMENTS

We are grateful to Dr. Ronald P. Mason for helpful discussions, to Mr. Thomas Norkus and Mr. Philip R. Timmons for excellent research assistance, and to Mrs. Jean Gordner for preparation of the manuscript.

REFERENCES

1. Moncada, S., and J. R. Vane. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol. Rev.* **30**:293–331 (1979).
2. Samuelsson, B., M. Goldyne, E. Granstrom, M. Hamburg, S. Hammarstrom, and C. Malmsten. Prostaglandins and thromboxanes. *Annu. Rev. Biochem.* **47**:997–1029 (1978).
3. Sih, C. J., C. Takeguchi, and P. Foss. Mechanism of prostaglandin biosynthesis. III. Catecholamines and serotonin as coenzymes. *J. Am. Chem. Soc.* **92**:6670 (1970).
4. Takeguchi, C., and C. J. Sih. A rapid spectrophotometric assay for prostaglandin synthetase: application to the study of non-steroidal anti-inflammatory agents. *Prostaglandins* **2**:169–184 (1972).
5. Lands, W. E. M., P. R. Lettellier, L. H. Rome, and J. Y. Vanderhock. Inhibition of prostaglandin biosynthesis. *Adv. Biosci.* **9**:15–28 (1973).
6. Marnett, L. J., P. Wlodawer, and B. Samuelsson. Co-oxygenation of organic substrates by the prostaglandin synthetase of sheep vesicular gland. *J. Biol. Chem.* **250**:8510–8517 (1975).
7. Sivarajah, K., H. Mukthar, and T. E. Eling. Arachidonic acid-dependent metabolism of (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP 7,8-diol) to 7,10/8,9 tetrols. *F. E. B. S. Lett.* **106**:17–20 (1979).
8. Marnett, L. J., M. J. Bienkowski, and W. R. Pagels. Oxygen 18 investigation of the prostaglandin synthetase-dependent co-oxidation of diphenylisobenzofuran. *J. Biol. Chem.* **254**:5077–5082 (1979).
9. Marnett, L. J., and G. A. Reed Peroxidatic oxidation of benzo[*a*]pyrene and prostaglandin biosynthesis. *Biochemistry* **18**:2923–2929 (1979).
10. Sivarajah, K., M. W. Anderson, and T. E. Eling. Metabolism of benzo[*a*] pyrene to reactive intermediates via prostaglandin biosynthesis. *Life Sci.* **23**: 2571–2578 (1978).
11. Marnett, L. J., G. A. Reed, and D. J. Dennison. Prostaglandin synthetase-dependent activation of 7,8-dihydroxy-7,8-dihydro-benzo[*a*]pyrene to mutagenic derivatives. *Biochem. Biophys. Res. Commun.* **82**:210–216 (1978).
12. Corbett, M. D., and B. R. Chipko. Peroxide oxidation of indole to oxindole by chloroperoxidase catalysis. *Biochem. J.* **183**:269–276 (1979).
13. Griffin, B. W., and P. L. Ting. Mechanism of *N*-demethylation of aminopyrine by hydrogen peroxide catalyzed by horseradish peroxidase, metmyoglobin and protohemin. *Biochemistry* **17**:2206–2211 (1978).
14. Ishimaru, A., and I. Yamazaki. Hydroperoxide-dependent hydroxylation involving "H_2O_2-reducible hemeprotein" in microsomes of pea seeds. *J. Biol. Chem.* **252**:6118–6124 (1977).
15. Hollenberg, P., and L. P. Hager. The P-450 nature of the carbon monoxide complex of ferrous chloroperoxidase. *J. Biol. Chem.* **248**:2630–2633 (1973).
16. Rahimtula, A. D., and P. J. O'Brien. Hydroperoxide-dependent *O*-dealkylation reactions catalyzed by liver microsomal cytochrome P-450. *Biochem. Biophys. Res. Commun.* **62**:268–275 (1975).
17. Rahimtula, A. D., and P. J. O'Brien. The peroxidase nature of cytochrome P-450 in Microsomes and Drug Oxidations (V. Ullrich, I. Roots, A. Hildebrandt, R. W. Estabrook, and A. H. Conney, eds.) Pergamon Press, New York, 210–214 (1977).
18. Nordbleom, G. D., R. E. White, and M. J. Coon. Studies on hydroperoxide dependent substrate hydroxylation by purified liver microsomal cytochrome P-450. *Arch. Biochem. Biophys.* **175**:524–533 (1976).
19. Hrycay, E. G., and P. J. O'Brien. Cytochrome P-450 as a microsomal peroxidase utilizing a lipid peroxidase substrate. *Arch. Biochem. Biophys.* **147**:14–27 (1971).
20. Funk, M. O., R. Isaac, and N. A. Porter. Preparation and purification of lipid hydroperoxides from arachidonic and linolenic acids. *Lipids* **11**:113–117 (1976).
21. Omura, T., and R. Sato. The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239**:2370–2384 (1964).
22. Dent, J. G., M. E. Graichen, S. Schnell, and J. M. Lasker. Constitutive and induced hepatic microsomal cytochrome P-450 monooxygenase activities in male Fischer 344 and CD rats: a comparative study. Toxicol. Appl. Pharmacol. **52**:45–53 (1980).
23. Zenser, T. V., M. B. Mattammal, and B. B. Davis. Differential distribution of the mixed function oxidase activities in rabbit kidney. *J. Pharmacol. Exp. Ther.* **207**:719–725 (1978).
24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
25. Nash, T. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* **55**:416–421 (1953).
26. Ullrich, V., and P. Weber. The *O*-dealkylation of 7-ethoxy-coumarin by liver

microsomes: a direct fluorometric test. *Hoppe-Seyler's Z. Physiol. Chem.* **353:** 1171–1177 (1972).
27. Burke, D. M., and R. T. Mayer. Ethoxyresorufin: direct fluorometric assay of a microsomal *O*-dealkylation which is differentially inducible by 3-methylcholanthrene. *Drug Metab. Dispos.* **2:**583–588 (1974).
28. Moncada, S., P. Needleman, S. Bunting, and J. R. Vane. Prostaglandin endoperoxide and thromboxane generating systems and their selective inhibition. *Prostaglandins* **12:**323–334 (1976).
29. Nugteren, D. H., E. Hazelhof. Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim. Biophys. Acta* **326:**448–461 (1973).
30. Marnett, L. J., G. A. Reed, and J. T. Johnson. Prostaglandin synthetase dependent benzo(a)pyrene oxidation: products of the oxidation and inhibition of their formation by antioxidants. *Biochem. Biophys. Res. Commun.* **79:** 569–576 (1977).
31. Zenser, T. V., M. B. Mattammal and B. B. Davis. Metabolism of *N*-[4-(5-nitro-2-furyl)-2-thioazolyl]formamide by prostaglandin endoperoxide synthetase. *Cancer Res.* **40:**114–118 (1980).
32. Egan, R. W., P. H. Gale, and F. A. Kuehl, Jr. Reduction of hydroperoxides in the prostaglandin biosynthetic pathway by a microsomal peroxidase. *J. Biol. Chem.* **254:**3295–3302 (1979).
33. Sivarajah, K., J. M. Lasker, and T. E. Eling. Prostaglandin synthetase-dependent co-oxidation of (±)-benzo(*a*)pyrene-7,8-dihydrodiol by human lung and other mammalian tissues. *Cancer Res.* **41:**1834–1839 (1981).
34. Nebert, D. W., and N. M. Jensen. The Ah locus: genetic regulation of the metabolism of carcinogens, drugs and other environmental chemicals by cytochrome P-450-mediated monooxygenases. *CRC Crit. Rev. Biochem.* **6:** 401–437 (1979).
35. Zenser, T. V., M. B. Mattammal, and B. B. Davis. Co-oxidation of benzidine by renal medullary prostaglandin cyclo-oxygenase. *J. Pharmacol. Exp. Ther.* **211:**460–464 (1979).
36. Griffin, B. W. Evidence for a free radical mechanism of *N*-demethylation of *N,N*-dimethylaniline and an analog by hemeprotein-H_2O_2 systems. *Arch. Biochem. Biophys.* **190:**850–853 (1978).
37. Kadlubar, F. F., K. C. Mortons, and D. M. Ziegler. Microsomal-catalyzed hydroperoxide-dependent C-oxidation of amines. Biochem. *Biophys. Res. Commun.* **54:**1255–1261 (1973).
38. Hildebrandt, A. G. The binding of metyrapone to cytochrome P-450 and its inhibitory action on microsomal hepatic mixed-function oxidation reactions. *Biochem. Soc. Sympt.* **34:**79–102 (1972).
39. Dolphin, D., A. Forman, D. C. Barg, J. Fajer, and R. H. Felton. Compounds I of catalase and horseradish peroxidase: π-cation radicals. *Proc. Natl. Acad. Sci. U. S. A.* **68:**614–618 (1971).
40. Lasker, J. M., K. Sivarajah, R. P. Mason, B. Kalyanaraman, M. B. Abou-Donia, and T. E. Eling. A free radical mechanism of prostaglandin synthetase-dependent aminopyrine demethylation. *J. Biol. Chem.* **256:**7764–7767 (1981).
41. Brodie, B. B., J. R. Gillette, and B. N. LaDu. Enzymatic metabolism of drugs and other foreign compounds. *Annu. Rev. Biochem.* **27:**427–463 (1958).

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