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Involvement of Radical Species in the Oxidation of Aminopyrine and 4-Aminoantipyrine by Cumene Hydroperoxide in Rat Liver Microsomes

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SUMMARY

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Spectral changes observed during the microsomal oxidation of aminopyrine by cumene hydroperoxide have demonstrated the formation of the aminopyrine radical prior to, and concomitant with, the formation of antipyrine red. The latter chromophore is the oxidation product of 4-aminoantipyrine in several systems, including microsomes with cumene hydroperoxide, which generate one-electron oxidants. The spin-trap nitrosobenzene strongly inhibited not only the cumene hydroperoxide-supported oxidation of aminopyrine and its metabolite, but also the oxygen utilization of microsomes stimulated by this hydroperoxide. Thus, efficient trapping by nitrosobenzene of reactive organic radicals derived from cumene hydroperoxide inhibits their subsequent reactions, i.e., substrate oxidation and initiation of lipid peroxidation. These cumene hydroperoxide-dependent activities of microsomes were significantly inhibited by micromolar concentrations of metyrapone and also by known substrates of cytochrome P-450, thus demonstrating the essential role of this hemeprotein in these reactions.

INTRODUCTION

There are numerous reports in the literature describing hydroperoxide-supported oxidation activities of liver microsomes; these have established that several classes of substrates of liver microsomal cytochrome P-450 are oxidized to the same products produced in the respective mono-oxygenation reactions of this hemeprotein (1–3). The specificity for the hydroperoxide in these reactions is low and appears to be determined principally by its lipophilicity (1, 2); for example, cumene hydroperoxide is considerably more active than H_2O_2. Moreover, the properties of these "peroxidase" activities of microsomal fractions have been shown to be very similar to those of a purified preparation of liver microsomal cytochrome P-450 (4), providing strong evidence for involvement of this hemeprotein in these reactions. Although it has been proposed (2, 4) that the mechanism of this activity of cytochrome P-450 is very similar to the well-established mechanism of HRP[1] catalysis, there are few published data to support this idea. The most serious criticisms of this hypothesis are the lack of evidence for an intermediate of cytochrome P-450, analogous to Compound I of HRP, which is presumed to transfer an oxene species to the electron donor substrate (2, 4) and the failure to consider any role(s) for radical species of either the hydroperoxide or electron donor substrates.

In an attempt to resolve these questions, we have investigated the proposed analogy with peroxidases by studying the hydroperoxide-supported oxidation of typical cytochrome P-450 substrates catalyzed by other hemeproteins. Kadlubar *et al.* (1) first demonstrated that catalase could catalyze the oxidation of certain tertiary amine substrates of cytochrome P-450 by cumene hydroperoxide (1). Our recent work has established that the oxidative *N*-demethylation of aminopyrine and several other related compounds by such "model" systems involves radical intermediates of these substrates which can be directly detected by room-temperature EPR spectroscopy (5, 6). Moreover, we have used the EPR technique of spin trapping to demonstrate that metmyoglobin initiates the decomposition of cumene hydroperoxide to reactive free radicals (7). More recently, analogous spin-trapping experiments have been performed with a liver microsomal fraction (8), with results altogether similar to those obtained with metmyoglobin and cumene hydroperoxide. Recent evidence has also been reported from other laboratories for the radical oxidation of benzo-

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[1] The abbreviations used are: HRP, horseradish peroxidase; EPR, electron paramagnetic resonance; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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[*a*]pyrene in several hydroperoxide-containing systems (9, 10), including liver microsomes with cumene hydroperoxide (10).

Since the aminopyrine radical can be readily detected by EPR, or spectrophotometrically by its intense violet color (11, 12), it is the ideal probe with which to investigate radical pathways operative in various oxidizing systems. In the present study, definitive evidence has been obtained that the cumene hydroperoxide-supported oxidation of aminopyrine by liver microsomes proceeds by the previously proposed radical mechanism (5):

$$R_2N{-}CH_3 \xrightarrow{-e^-} R_2N{-}CH_3^+\cdot \xrightarrow{-H\cdot} R_2N^+ = CH_2 \xrightarrow{H_2O} R_2NH_2^+ + H_2C{=}O \quad (1)$$

In addition, results obtained with nitrosobenzene, the spin trap used in the recent experiments with microsomes (8), have provided additional evidence that the one-electron oxidants in this reaction are reactive organic radicals derived from cumene hydroperoxide. Although the essential features of the mechanism of aminopyrine *N*-demethylation in this microsomal system are similar to those established for the model hemeprotein-hydroperoxide systems, an important difference has been noted which is attributed to the microsomal membrane.

MATERIALS AND METHODS

Microsomal fractions were prepared from the livers of phenobarbital-pretreated male rats as previously described (13). Cumene hydroperoxide was purified as the sodium salt (14) from a technical-grade product of Matheson, Coleman and Bell, Norwood, Ohio; the purity of the salt was confirmed by iodometric titration (14) and by high-pressure liquid chromatography. Aminopyrine and nitrosobenzene were purchased from Aldrich Chemical Company, St. Louis, Mo. The sources of other drugs used in this study were as follows: metyrapone, Ciba Pharmaceutical Company, Summit, N. J.; 4-aminoantipyrine, Winthrop Laboratories, New York, N. Y.; ethylmorphine (hydrochloride salt), Merck & Company, West Point, Pa.; and benzphetamine (hydrochloride salt), The Upjohn Company, Kalamazoo, Mich. Other common laboratory chemicals used in this study were analytical reagent grade. Metmyoglobin was a homogeneous preparation isolated from beef heart (15). Formaldehyde was assayed by the standard procedure of Nash (16) in aliquots of the reaction mixture which had been quenched by trichloroacetic acid and centrifuged to remove the protein precipitate. Protein concentration was determined by the biuret method (17), with bovine serum albumin as the standard. All spectrophotometric experiments with microsomal solutions were carried out at 22° with an Aminco DW-2 spectrophotometer; other absorbance measurements were made on a Beckman 25 spectrophotometer.

RESULTS

In the presence of aminopyrine and liver microsomes from phenobarbital-treated rats, low concentrations of cumene hydroperoxide stimulated the rapid formation of a broad, prominent absorbance in the visible region (Fig. 1). As shown in Fig. 2, the difference absorbance spectrum recorded 1 min after initiating the reaction closely resembled that of the aminopyrine free radical produced by the HRP-catalyzed oxidation of aminopyrine (12) or generated electrochemically (11). In the absence of aminopyrine, cumene hydroperoxide produced only a small, negative absorbance similar to the inverse absolute spectrum of low-spin cytochrome P-450 (18), which is attributed to destruction of this hemeprotein; it has also been previously reported that purified microsomal cytochrome

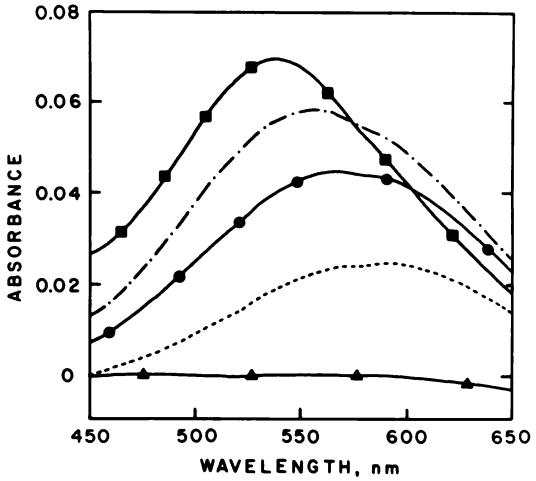

FIG. 1. *Spectral changes occurring during the oxidation of aminopyrine by cumene hydroperoxide in rat liver microsomes*

A solution containing microsomal protein (1 mg/ml) in 50 mM Hepes-Tris buffer, pH 6.5, with 0.15 M KCl, 10 mM $MgCl_2$, and 30 mM aminopyrine was added to both sample and reference cuvettes, and the baseline was recorded (▲——▲). The spectrum was scanned at the indicated times following the addition of 0.25 mM cumene hydroperoxide to the sample cuvette: 15 sec (– – –), 1 min (●——●), 3 min (– · –), and 5 min (■——■).

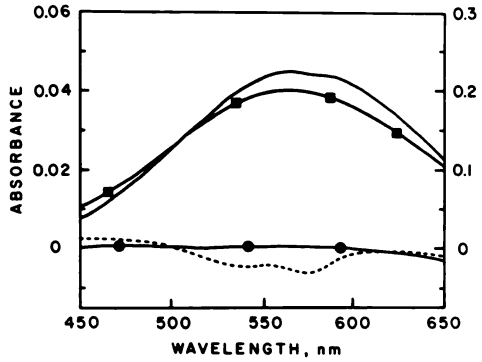

FIG. 2. *Identification of the aminopyrine free radical produced during the microsomal oxidation of aminopyrine*

The baseline (●——●) was recorded with the same solution in both cuvettes; the reaction was initiated by adding the stated concentration of hydroperoxide to the sample cuvette. ——, The microsomal solution described in legend to Fig. 1, scanned 1 min after the addition of 0.25 mM cumene hydroperoxide; ■——■, a solution containing 35 nM HRP and 2 mM aminopyrine in 50 mM Hepes-Tris buffer, pH 6.5, scanned 2 min after the addition of 0.8 mM H_2O_2; – – –, control for the experiment with microsomes, in which aminopyrine was omitted from the reaction mixture. The absorbance scale on the *right* applies to the HRP-containing solution.

P-450 is substantially destroyed by this hydroperoxide in the absence of electron donors (3). The absorbance spectra recorded during the microsomal oxidation of aminopyrine (Fig. 1) appear to contain a small contribution resulting from heme destruction. Moreover, as a function of time, the absorbance maximum shifted from approximately 560 nm in the 1-min scan to 535 nm in the 5-min scan, when the absorbance attained its maximal value; the absorbance subsequently decayed with a further small shift in the wavelength maximum to 527 nm. The absorbance did not develop if microsomes or either substrate were omitted, or if microsomes heated to 90° for 2 min were used. Over the pH range 6.5 to 8.0, the maximal absorbance change decreased markedly. The inhibitory effect of micromolar concentrations of metyrapone on this absorbance change, shown in Fig. 3, established the involvement of cytochrome P-450 in the reaction.

The time-dependent changes in the absorbance spectra observed during the microsomal oxidation of aminopyrine (Fig. 1) suggested that the initial oxidation product was the aminopyrine radical, which served as precursor to the species absorbing near 535 nm. Indeed, under the experimental conditions of Fig. 1, significant *N*-demethylation of aminopyrine occurred. As shown in Fig. 4, good correlations were observed between the kinetics of formaldehyde production and the kinetics of formation and decay of the visible absorbance under various experimental conditions; significantly, both parameters showed parallel inhibition by metyrapone and by hexobarbital, another substrate of microsomal cytochrome P-450. These data indicate that the aminopyrine radical is an intermediate in aminopyrine *N*-demethylation in this microsomal system, in support of the proposed mechanism (5). The possibility that a demethylated product of aminopyrine gave rise to the 535-nm absorbance was suggested by previous studies in which it was shown that both *N*-methyl-4-aminoantipyrine and 4-aminoantipyrine were oxidized by H_2O_2 even more rapidly than was aminopyrine in the presence of HRP (5). As shown in Fig. 5, the HRP-catalyzed oxidation of 4-aminoantipyrine produced an intense absorbance at 525 nm which was unstable. Acidic solutions of ferrous ion containing either cumene hydroperoxide or H_2O_2, both of which effect the one-electron oxidation of aminopyrine (19), also oxidized 4-aminoantipyrine to the violet species. This chromophore has the properties of antipyrine red, a coupling product of two molecules of 4-aminoantipyrine in which one amino group is lost as NH_3 (20). The chromophore is *not* a radical, and attempts to detect a radical species generated during the HRP-catalyzed oxidation of 4-aminoantipyrine were unsuccessful (5). However, since one-electron oxidizing systems convert 4-aminoantipyrine to antipyrine red, it is presumed that a highly unstable radical of this substrate is an intermediate in the oxidation. With the microsomal preparation, oxidation of 4-aminoantipyrine by cumene hydroperoxide also produced antipyrine red (Fig. 5). The dependence of this reaction on pH and its sensitivity to metyrapone inhibition were very similar to those observed for aminopyrine. The substantial shift of the absorbance maximum to the shorter wavelength observed during the microsomal oxidation of aminopyrine by cumene hydroperoxide (Fig. 1) is attributed to oxidation of the metabolite 4-aminoantipyrine, which becomes competitive with aminopyrine oxidation.

Evidence that the microsomal oxidation of aminopyrine, as well as 4-aminoantipyrine, by cumene hydroperoxide involves one-electron oxidants is supported by results of spin-trapping experiments (7, 8). Experiments with nitrosobenzene as the radical trap have shown that both metmyoglobin and rat liver microsomes are more effective than is ferrous ion in initiating the radical decomposition of cumene hydroperoxide (8). With each of these initiators, the same EPR signal was detected,

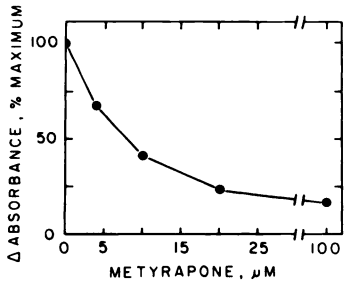

FIG. 3. *Effect of metyrapone on the maximal absorbance measured during the microsomal oxidation of aminopyrine by cumene hydroperoxide*

Experimental conditions were identical with those described in legend to Fig. 1, with metyrapone added at the indicated concentrations. The maximal absorbance increase near 535 nm was measured after the addition of 0.25 mM cumene hydroperoxide.

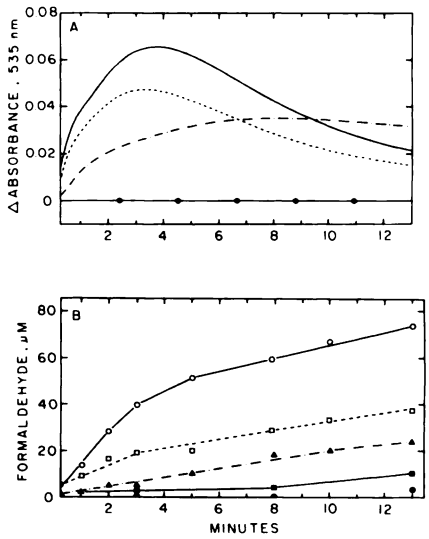

FIG. 4. *Kinetic correlation of the absorbance at 535 nm (A) and formaldehyde measured under identical experimental conditions (B) during the microsomal oxidation of aminopyrine by cumene hydroperoxide*

Experimental conditions were identical with those described in legend to Fig. 1 (——, O——O) or with modifications as indicated: – – –, □– – –□, +5 mM hexobarbital in the reaction mixture; – · –, ▲– · –▲, +20 μM metyrapone in the reaction mixture; ●——●, ●, with microsomes omitted; ●——●, ■——■, with microsomes heated to 90° for 2 min. All reactions were initiated by the addition of 0.25 mM cumene hydroperoxide; temperature 22°.

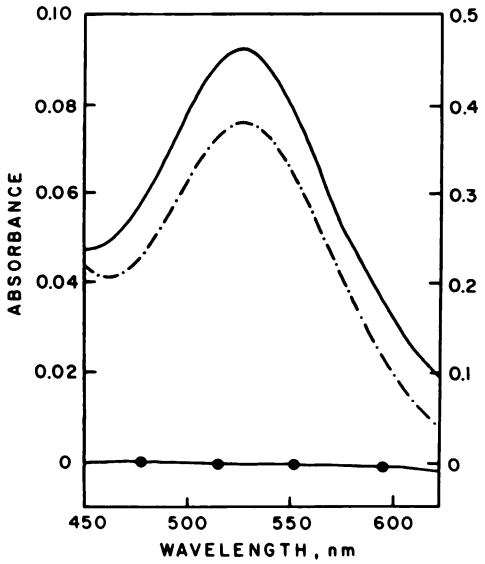

FIG. 5. *Absorbance spectrum resulting from the oxidation of 4-aminoantipyrine by cumene hydroperoxide in rat liver microsomes or by the HRP-H_2O_2 system*

The baseline (●——●) was recorded with the same solution in both cuvettes. The microsomal solution (——) was identical with that described in legend to Fig. 1, with 1.67 mM 4-aminoantipyrine instead of aminopyrine. The solution containing HRP (– · –) was identical with that described in legend to Fig. 2, with 2 mM 4-aminoantipyrine instead of aminopyrine. The maximal absorbance which developed after the addition of the hydroperoxide is shown; the absorbance scale on the *right* applies to the HRP-dependent reaction.

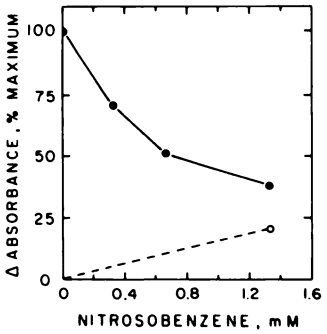

FIG. 6. *Effect of nitrosobenzene on the maximal absorbance measured during the microsomal oxidation of aminopyrine by cumene hydroperoxide*

Experimental conditions were identical with those described in legend to Fig. 1, with nitrosobenzene added at the indicated concentrations (●). The maximal absorbance increase near 535 nm was measured. A measurable absorbance, with no well-defined features, developed in the control experiment (○) for the highest nitrosobenzene concentration employed in which aminopyrine was omitted.

which was identified as either a cumyloxy or a cumyl radical trapped by nitrosobenzene (8). More recently, additional experiments with these systems have indicated that this spin adduct represents a trapped cumyl radical, as Terabe and Konaka (21) also concluded. In their study with nitrosobenzene and cumene hydroperoxide in organic solvents, it was shown that the cumyloxy radical is trapped efficiently by nitrosobenzene to form the short-lived phenylcumyloxynitroxide, which decomposes to nitrobenzene and the cumyl radical (21). Subsequent trapping of this alkyl radical by nitrosobenzene yields the very stable nitroxide reported in that study (21) and also in our related study of aqueous systems containing hemeproteins (8). Numerous other experimental findings support this interpretation, i.e., the origin of the cumyl radical and the high efficiency of trapping of the cumyloxy radical by nitrosobenzene, and will be presented in more detail elsewhere (22). These results have strongly implicated the cumyloxy radical and/or the methyl radical, arising from decomposition of the cumyloxy radical, as the functional oxidants generated in these hemeprotein-cumeme hydroperoxide systems (7, 8). The data in Fig. 6 provide additional support for this proposal: nitrosobenzene significantly inhibited the cumene hydroperoxide-supported oxidation of aminopyrine in liver microsomes. The oxidation of 4-aminoantipyrine to antipyrine red by this system was similarly inhibited by nitrosobenzene. Thus, these results demonstrate that nitrosobenzene is an efficient trap of radical species which participate in the oxidation of aminopyrine and 4-aminoantipyrine.

The effects of other substrates of microsomal cytochrome P-450 on the microsomal oxidation of aminopyrine (Fig. 7) and 4-aminoantipyrine by cumene hydroperoxide were examined; very similar results were observed for both reactions. Hexobarbital was a more potent inhibitor than benzphetamine, but ethylmorphine was only weakly inhibitory. These results are qualitatively consistent with the rates of oxidation of benzphetamine and ethylmorphine by cumene hydroperoxide measured under conditions similar to those of Fig. 7. As shown in Table 1, aminopyrine *N*-demethylation was clearly faster than benzphetamine oxidation, whereas ethylmorphine oxidation was barely measurable. We are now investigating the possibility suggested by Fig. 7 and Table 1 that hexobarbital also undergoes oxidation in this system.

The proposal that the addition of cumene hydroperoxide to liver microsomes produces one-electron oxidants which can be trapped by nitrosobenzene was subjected to another experimental test. The stimulation of oxygen uptake by the addition of low concentrations of various hydroperoxides to liver microsomes has been previously reported and attributed to induced lipid peroxidation (23). However, the electron-abstracting agents were considered to be oxidized, Compound I-like species of cytochrome P-450 rather than organic radicals. Under the experimental conditions of Fig. 1, in the absence of aminopyrine, cumene hydroperoxide caused the rapid and complete depletion of oxygen present in the medium. As observed for aminopyrine oxidation by this system (cf. Fig. 3), micromolar concentrations of metyrapone significantly inhibited this oxygen consumption. Additional experiments provided strong evidence that these two phenomena occurring in the presence of cumene hydroperoxide and involving cytochrome P-450 were closely linked. For example, as shown in Fig. 8, nitrosobenzene, at concentrations similar to those which inhibited aminopyrine oxidation by cumene hydroperoxide (cf. Fig. 6), efficiently inhibited oxygen uptake dependent on the hydroperoxide. Also, rather low concentrations (1.6 mM)

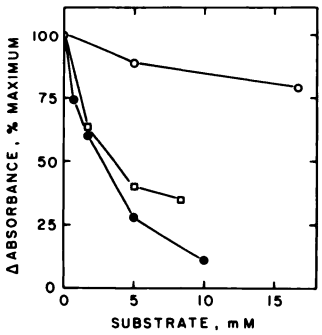

FIG. 7. *Effects of substrates of cytochrome P-450 on the maximal absorbance measured during the microsomal oxidation of aminopyrine by cumene hydroperoxide*

Experimental conditions were identical with those described in legend to Fig. 1, with each substrate added at the indicated concentration: ●, hexobarbital; □, benzphetamine; ○, ethylmorphine. The maximal absorbance increase near 535 nm was measured after the addition of 0.25 mM cumene hydroperoxide.

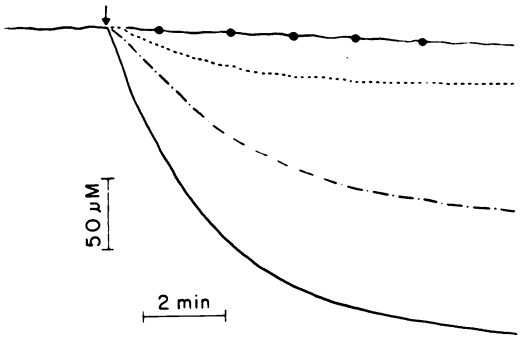

FIG. 8. *Effect of nitrosobenzene on oxygen utilization by rat liver microsomes stimulated by cumene hydroperoxide*

Experimental conditions were identical with those described in legend to Fig. 1 except that aminopyrine was omitted and nitrosobenzene was added at the indicated concentrations: ——, no nitrosobenzene; – · –, 0.33 mM nitrosobenzene; – – –, 0.67 mM nitrosobenzene; ●——●, 1.33 mM nitrosobenzene. The reaction was initiated by adding 0.25 mM cumene hydroperoxide at the indicated point; temperature 25°.

of aminopyrine and 4-aminoantipyrine almost completely inhibited the reaction. Benzphetamine, hexobarbital, and ethylmorphine were less effective inhibitors, although each inhibited the reaction to an extent qualitatively consistent with their observed inhibition of aminopyrine oxidation (cf. Fig. 7). These results support the idea that cytochrome P-450 initiates the decomposition of cumene hydroperoxide to radicals which can be trapped by nitrosobenzene or reduced by aminopyrine or 4-aminoantipyrine. In the absence of these radical trapping agents, the radicals can abstract electrons from unsaturated fatty acid moieties in the membrane, thereby initiating oxygen consumption associated with the radical chain process of lipid peroxidation.

To test the proposal that unsaturated fatty acid moieties were indeed required for this cumene hydroperoxide-dependent oxygen uptake, control experiments were performed with metmyoglobin at the same concentration as cytochrome P-450 employed in these experiments. The oxidative *N*-demethylation of aminopyrine in the presence of metmyoglobin and cumene hydroperoxide has been previously reported (12) to occur at rates comparable to those for the cytochrome P-450-dependent reaction. In the absence of nitrosobenzene, under experimental conditions identical with those of Fig. 8, with 3 μM metmyoglobin substituted for the liver microsomes, no measurable oxygen uptake occurred. When 0.3 mM arachidonic acid was also present, cumene hydroperoxide stimulated oxygen reduction at a rate somewhat smaller than the control rate indicated in Fig. 8. With 1.0 mM arachidonic acid and metmyoglobin, significant oxygen consumption occurred in the absence of added cumene hydroperoxide. This result is attributed to low levels of hydroperoxides in the arachidonic acid, which, above a certain minimal concentration, can sustain efficient initiation of lipid peroxidation with metmyoglobin. Both the cumene hydroperoxide-dependent and -independent reactions were strongly inhibited by nitrosobenzene concentrations as low as 0.5 mM, consistent with the data in Fig. 8. Also, as observed with the microsomal system, aminopyrine and 4-aminoantipyrine were efficient inhibitors of oxygen consumption in these metmyoglobin-containing systems. These reactions were strongly inhibited by ligands of metmyoglobin, such as fluoride and cyanide, but not by metyrapone, a specific ligand of cytochrome P-450.

TABLE 1

Rates of drug N-demethylation by cumene hydroperoxide dependent on rat liver microsomes

The reaction mixtures contained 1 mg of microsomal protein per milliliter, 5 mM substrate, 0.15 M KCl, 10 mM $MgCl_2$, and 1 mM cumene hydroperoxide in 50 mM Hepes-Tris buffer, pH 6.5; temperature 22°.

Drug substrate	Rate of *N*-demethylation
	nmoles/min/mg protein
Aminopyrine	12.7
Benzphetamine	5.4
Ethylmorphine	<1.0

DISCUSSION

The marked inhibition of the several cumene hydroperoxide-dependent reactions of rat liver microsomes characterized in this study by very low concentrations of metyrapone provided strong evidence for cytochrome P-450 involvement in the decomposition of this hydroperoxide. Moreover, the detection of the colored radical species of aminopyrine as a transient intermediate during the cumene hydroperoxide-supported microsomal oxidation of this compound supports the radical mechanism of *N*-demethylation previously proposed for other heme-protein-hydroperoxide systems (5). With microsomes, however, spectrophotometric detection of the radical has distinct advantages over EPR detection because of the instability of the radical and the low concentrations produced. Also, it should be noted that this particular free radical has a very complex hyperfine splitting pattern (5, 11), i.e., the intensity of the EPR signal is spread over a large magnetic field and many resonance lines. As a consequence, the EPR detection sensitivity is effectively decreased by a large factor as compared with simple nitroxide radicals, for example.

Rapid oxidation of the aminopyrine radical, resulting in loss of both methyl substituents on the 4-amino group,

and subsequent rapid oxidation of 4-aminoantipyrine to antipyrine red have been observed during the oxidation of aminopyrine only by a microsomal system. This is attributed to the following factors: (a) aminopyrine, a very water-soluble compound, distributes in both the aqueous and lipid phases of microsomes; (b) the various oxidized species of aminopyrine undergo subsequent oxidation more rapidly than does the parent compound (5); and (c) these metabolites of aminopyrine remain associated with the lipid phase and thus compete effectively with the membrane-associated fraction of aminopyrine for oxidizing species which are generated at or in the membrane (as evidenced by the involvement of cytochrome P-450). These results imply that the membrane itself may play a more important role than has been generally recognized in dictating the specific course of oxidation of a given compound, depending on the relative lipid solubilities and rates of metabolism of the parent compound and its metabolites.

The results of this study have provided additional evidence that cytochrome P-450 initiates a radical decomposition of cumene hydroperoxide which appears to be quite analogous to the reaction of metmyoglobin with this hydroperoxide. Previous experiments had suggested that at least two radicals, the cumyloxy and the methyl, can be formed from cumene hydroperoxide in these systems (7, 8). Although the methyl radical, like other alkyl radicals, can react with O_2 to form a peroxy radical, the control experiments with metmyoglobin demonstrated that this reaction cannot account for the significant O_2 uptake stimulated by the addition of cumene hydroperoxide to microsomes. The properties of both the methyl radical and the cumyloxy radical (7, 24) to function as one-electron oxidants do, however, provide a consistent interpretation of the various phenomena observed in this study: (a) O_2 consumption resulting from initiation of lipid peroxidation; (b) the one-electron oxidation of aminopyrine and 4-aminoantipyrine to colored products; and (c) competition of the unsaturated microsomal lipids, added drug substrates of cytochrome P-450, and the spin trap nitrosobenzene for the reactive oxidants derived from cumene hydroperoxide. Moreover, these radical species are probably responsible for the reported destruction of cytochrome P-450 heme by cumene hydroperoxide (4), which was shown to be inhibited by electron donor substrates of this enzyme. We have also observed in preliminary experiments[2] that both aminopyrine and nitrosobenzene inhibit destruction of metmyoglobin heme initiated by cumene hydroperoxide, presumably by reacting with the cumyloxy and/or methyl radicals.

In many earlier studies of cumene hydroperoxide-supported reactions of liver microsomes (1, 2, 23) and purified cytochrome P-450 (4), probes for reactive radical intermediates were not employed and thus possible roles for these species were not considered. In addition, the proposed analogy of these reactions with H_2O_2-dependent reactions catalyzed by the "true" hemeprotein peroxidases (2, 4) failed to consider the rather high specificity of those peroxidases for their oxidizing substrate and the pronounced differences in chemical reactivity between cumene hydroperoxide and H_2O_2 (24). In a study which appeared after this work was submitted, this analogy was examined more critically (25); it was concluded that (a) Compounds I or II, or mixtures of these, are *not* formed when purified liver microsomal cytochrome P-450 reacts with a large number of organic hydroperoxides and perbenzoic acids and (b) these reactions may, indeed, result in homolytic cleavage of the hydroperoxides. The results of the present study, together with those of recently published spin-trapping experiments (8), have provided considerable evidence for a homolytic, or radical, cleavage of cumene hydroperoxide initiated by microsomal cytochrome P-450.

[2] B. W. Griffin, unpublished results.

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