Ann. Rev. Pharmacol. Toxicol. 1983. 23:481–503

SUICIDAL DESTRUCTION OF CYTOCHROME P-450 DURING OXIDATIVE DRUG METABOLISM

Paul R. Ortiz de Montellano and Maria Almira Correia

Departments of Pharmaceutical Chemistry and Pharmacology, and the Liver Center, University of California, San Francisco, California 94143

INTRODUCTION

Cytochrome P-450 enzymes catalyze three general types of oxidative reactions: (*a*) insertion of an oxygen atom into the sigma bond between a hydrogen and a heavier atom (hydroxylation), (*b*) addition of an oxygen atom across the two ends of a π-bond (epoxidation), and (*c*) addition of an oxygen atom to a nonbonding electron pair (oxidation). A limited number of reductive reactions are also catalyzed by cytochrome P-450 monooxygenases under appropriate conditions. All cytochrome P-450 enzymes appear to operate by closely related catalytic mechanisms that hinge on activation of molecular oxygen by the prosthetic heme iron atom, although the hemoproteins differ, among other things, in their size, amino acid sequence, cellular and organ location, and substrate specificity. Some cytochrome P-450 enzymes, such as the sterol hydroxylases located both in the adrenal gland and in the liver, are primarily engaged in the metabolism or biosynthesis of critical physiological substrates, whereas others are primarily devoted to the metabolism of lipophilic drugs and other xenobiotics. The cytochrome P-450 class of enzymes is subject to a variety of regulatory mechanisms that are in turn meshed to the regulation of tributary pathways, such as that of hepatic heme biosynthesis. Therefore, the inhibition or destruction of cytochrome P-450 enzymes by exogenous substrates is of concern not only because it can alter the metabolism of other substances but also because it can interfere with essential physiological processes.

Cytochrome P-450 enzymes can be inhibited by competition between substrates for residence in the active site; by generalized destruction, usually associated with lipid peroxidation; by catalytic activation of substrates to

species that tightly but reversibly coordinate with the heme iron atom; by the generation of metabolites that react with the protein structure; or, finally, by catalytic oxidation of substrates to intermediates that bind covalently to the prosthetic heme group. Recent reviews of several of these inhibitory processes are available (1–5). This review is devoted to the mechanism, and the consequences, of the inactivation of cytochrome P-450 enzymes by N-alkylation of the prosthetic heme moiety, an area in which major advances have been made since the last comprehensive review (6).

THE ACTIVE SUBSTRATES

Olefins

The destruction of cytochrome P-450 by what is now known to be self-catalyzed prosthetic heme alkylation was first observed with homoallylic amides. The most intensively investigated of these substrates is 2-isopropyl-4-pentenamide (AIA), a compound that has served as a pilot for most of the developments in the field. Barbiturates with 5-allyl substituents, of which secobarbital is an example, have also received a great deal of attention. Early work with these agents has been reviewed in detail by De Matteis (6). A relatively recent addition to this class of substrates is 2,2-diethyl-4-pentenamide (novonal), a sedative-hypnotic currently in use in Europe (7). The presence of a double bond separated by two carbon atoms from an amide group in essentially all the agents first observed to cause cytochrome P-450 destruction and "green pigment" formation suggested that a critical relationship might exist between this structural feature and the destructive activity. This premise, reinforced by the finding that destructive activity is lost on reduction of the double bond (8, 9), was explored in 1980 with a series of compounds in which the homoallylic amide group was transformed in turn to a methyl ester, a methyl ketone, a methyl ether, and, eventually, to a hydrocarbon terminus (10). The observation that all of these structures retain destructive activity led to the realization that the destructive potential resides in the unsaturated carbon-carbon bond itself rather than in a more complex substructure. This is confirmed by the finding that several terminal olefins, including ethylene, destroy cytochrome P-450 by prosthetic heme alkylation (10). As expected, the saturated hydrocarbons are inactive. Olefins with alkoxy or halide substituents, notably 2,2,2-trifluoroethyl vinyl ether (fluroxene) (11, 12) and more recently vinyl fluoride, 1,1-difluoroethylene, and vinyl bromide (13), have also been shown to be active. It is likely that vinyl chloride, a documented human carcinogen that destroys cytochrome P-450 and heme (14, 15), also acts through prosthetic heme alkylation, although direct evidence for this is not yet available. The olefins that have been shown to destroy cytochrome P-450 through prosthetic heme alkylation are listed in Table 1.

Table 1 Substrates that inactivate cytochrome P-450 by heme alkylation[a]

Olefins	Acetylenes (continued)
Sedormid (57)	(1-Methoxycyclohexyl) acetylene (21)[c]
2-Isopropyl-4-pentenamide (AIA) (9, 26, 27, 36, 45)	3-(2,4-Dichlorophenoxy)-1-propyne (21)
2,2-Diethyl-4-pentenamide (Novonal) (7)[b]	3-(4-Nitrophenoxy)-1-propyne (21)
2-Phenyl-4-pentenamide (8)[c]	3-Phenoxy-1-propyne (21)
Secobarbital (9, 58)	4-Phenyl-1-butyne (21)
Aprobarbital (9)[c]	3-Phenyl-1-propyne (21)
Allobarbital (8, 9, 59)[c]	Phenylacetylene (21)
Methyl 2-isopropyl-4-pentenoate (10,45)	Biphenylacetylene (23)[c]
3-Isopropyl-5-hexen-2-one (10)	Cyclohexylacetylene (21)[c]
Methyl 2-isopropyl-4-pentenyl ether (10)	2-(2-Propynyl)-4-pentynamide (19)
4-Ethyl-1-hexene (10, 40)	Acetylene (13, 19, 21)
Ethylene (10, 39, 41)	Propyne (42)
Propene (41)	1-Heptyne (24)[c]
1-Heptene (10)	1-Octyne (41, 60)
1-Octene (41)	1-Decyne (24)
3-Methyl-1-octene (10)	1-Tridecyne (24)[c]
Fluroxene (11, 12, 13)	Ethchlorvynol (22)
Vinyl fluoride (13)	Hexapropymat (16)[c]
Vinyl bromide (13)	Heteroatomic substrates
Vinyl chloride (14, 15)[c]	3,5-*Bis*(carbethoxy)-2,4,6-trimethyl-1,4-dihydropyridine (DDC) (26, 27, 29)
Vinylidene fluoride (13)	3,5-*Bis*(carbethoxy)-2,6-dimethyl-4-ethyl-1,4-dihydropyridine (DDEP) (29, 30)
Triallyl cyanurate (59)[c]	3,5-*Bis*(carbethoxy)-2,6-dimethyl-4-propyl-1,4-dihydropyridine (30)
Acetylenes	3,5-*Bis*(carbethoxy)-2,6-dimethyl-4-isobutyl-1,4-dihydropyridine (30)
Norethisterone (17, 18)	1-Aminobenzotriazole (25)
Ethinyl estradiol (17)	Benzyl cyclopropyl amine (31, 32, 33)[c]
Norgestrel (18)[c]	2-Phenylethylhydrazine (34)[b]
Danazol (19)	Phenylhydrazine (35)
1-Ethinylcyclohexanol (18, 21)	
1-Ethinylcyclopentanol (21)	
3-Methyl-1-pentyn-3-ol (21)[c]	

[a] Except where indicated, the porphyrin derived from the heme adduct with each of the following compounds has been characterized at least by its electronic absorption spectrum.

[b] The alkylated porphyrin obtained with this compound has been characterized in the laboratory of one of the authors (unpublished work).

[c] Heme alkylation by this compound has been inferred but has not yet been confirmed by the isolation of an alkylated porphyrin derivative.

The available data is too sparse for a credible structure-activity relationship to be defined. It is nevertheless possible to make a number of tentative generalizations. The most evident is that a double bond suffices to bring about autocatalytic destruction of cytochrome P-450, but the presence of such a bond in a substrate does not automatically convey destructive activity. Among the olefinic substrates that cause no detectable loss of cyto-

chrome P-450 on incubation with phenobarbital-induced rat liver microsomes are styrene (10), 2-methyl-1-heptene (10), 3,3-dimethyl-1-hexene (10), oxprenolol (16), alprenolol (16), and carbamazepine (16). 1,2-Disubstituted olefins, specifically cyclohexene and both geometric isomers of 2-nonene and 3-hexene, appear not to alkylate prosthetic heme (10). The role of isozyme specificity in these negative results cannot be factored out at this time (see discussion of isozyme specificity). It nevertheless appears that the presence of two carbon substituents attenuates or suppresses destructive activity, as does steric congestion at the allylic position (i.e. 3,3-dimethyl-1-hexene) or electron delocalization (i.e. styrene). More definitive and extensive experiments are required, however, to substantiate these tentative conclusions.

Acetylenes

The discovery that acetylenes are catalytically oxidized to intermediates that destroy cytochrome P-450 stems from work in two distinct areas. One, the study of the 17-ethinyl sterol components of birth control pills, led to the demonstration by White & Muller-Eberhard in 1977 that norethisterone and ethinyl estradiol cause a time-dependent, irreversible loss of cytochrome P-450 that is paralleled by formation of hepatic "green pigments" (heme adducts) (17). This finding has been extended to two other clinically used ethinyl sterols, norgestrel (18) and danazol (19). The second indicator of the destructive activity of acetylenic groups was provided by the development, without knowledge of the specific mechanism involved, of a variety of insecticide synergists with an acetylenic function as the common denominator. Two of these compounds were shown in 1971 to mediate rapid in vivo loss of hepatic cytochrome P-450 (20). This loss has more recently been shown by in vitro experiments to reflect destruction of preexisting enzyme (21). A broad range of terminal acetylenes has been shown to exhibit this activity (Table 1). The intrinsic activity of the carbon-carbon triple bond is most clearly established by the destructive action of acetylene itself (13, 19, 21). In addition to the ethinyl sterols, two other clinically used acetylenic agents, ethchlorvynol and hexapropymat, are now known to destroy rat hepatic cytochrome P-450 (16, 22).

A survey of the active acetylenic compounds establishes that the intrinsic destructive activity of the triple bond is less readily masked than that of a double bond. Phenylacetylene (21) and biphenylacetylene (23), for example, are active, whereas styrene (10) is not. A trisubstituted propargylic position does not suppress destructive activity (note the ethinyl sterols), whereas a trisubstituted allylic position appears to do so (10). It is notable that disubstituted acetylenes like 2-heptyne exhibit high destructive activity but, unlike terminal acetylenes, do not give rise to isolable prosthetic heme adducts

(21). Several terminal acetylenes have nevertheless been found that do not cause detectable destruction of the phenobarbital-induced rat enzyme, including the monoamine oxidase inhibitor pargyline and the sedative-hypnotic ethinamate (16, 24). The reasons for the inactivity of the latter compounds, particularly in view of the structural similarity between hexapropymat (active) and ethinamate (inactive), remain to be elucidated.

Heteroatomic Substrates

Two heterocyclic systems have been identified that are oxidized by cytochrome P-450 to species that alkylate the prosthetic heme group. Rational analysis of the requirements for prosthetic heme alkylation led to the examination of 1-aminobenzotriazole, the prototype of a new class of destructive agents (25). The other heterocycle with destructive activity has a long history as an inhibitor of ferrochelatase and consequently of porphyrin biosynthesis. The ability of 3,5-*bis*-carbethoxy-2,4,6-trimethyl-1,4-dihydropyridine (DDC) to lower cytochrome P-450 levels in vivo was noted some time ago (26, 27) but was not connected until recently with the destruction of preexisting enzyme. The observation that DDC causes the formation of a trace of hepatic green pigment (28), however, led to the demonstration that DDC, but more particularly its homologues with a longer substituent at the 4-position, are suicide substrates for cytochrome P-450 (29, 30). The 4-ethyl (DDEP), 4-propyl, and 4-isobutyl derivatives exhibit high destructive activity, the 4-methyl derivative (DDC) low activity, and the compounds with a 4-phenyl or without a 4-substituent no activity. Paradoxically, the compounds with a 4-isopropyl or 4-benzyl substituent are characterized by high destructive activity but, unlike the other derivatives, give no detectable prosthetic heme adducts. These results suggest that a primary carbon substituent without conjugating groups is required for prosthetic heme alkylation to be observed.

Cyclopropylamines, notably benzyl cyclopropyl amine, have been reported to suicidally inactivate cytochrome P-450 (31, 32). Preliminary results suggest that the destruction mediated by these substrates may, in part, be due to heme alkylation (33). If so, cyclopropylamines represent a third class of substrates that derive their destructive activity from metabolic oxidation of a nitrogen atom.

Recent evidence suggests that hydrazines may represent a fourth class of nitrogen-based suicide substrates for cytochrome P-450 monooxygenases. The antidepressant phenelzine (2-phenylethylhydrazine) and phenylhydrazine have been reported to irreversibly lower the cytochrome P-450 and heme concentrations of hepatic microsomes and to cause the formation of unidentified "green" pigments (34, 35).

MECHANISMS OF PROSTHETIC HEME ALKYLATION

Autocatalytic Role of the Enzyme

The destruction of cytochrome P-450 by prosthetic heme alkylation is coupled, in every instance, with catalytic turnover of the destructive agent by the target enzyme. The catalytic role of the enzyme has been most thoroughly documented in the case of AIA, but support exists for similar involvement of the enzyme in other examples. It has been demonstrated with AIA that (*a*) NADPH and oxygen are required for destruction to occur (36, 9, 37), (*b*) destruction is attenuated by inhibitors of either cytochrome P-450 itself (36) or of cytochrome P-450 reductase (37), (*c*) destruction follows pseudo-first order kinetics, as required for a suicidal event (37), and (*d*) the destruction of purified, reconstituted cytochrome P-450 occurs only in the presence of functional enzyme and is isozyme-specific (38). Similar but less complete evidence is available for fluroxene (11, 12, 38), norethisterone (17, 38), vinyl chloride (if prosthetic heme alkylation is involved) (14, 15), 1-aminobenzotriazole (25, 38), and 3,5-*bis*-carbethoxy-2,6-dimethyl-4-ethyl-1-,4-dihydropyridine (DDEP) (29, 30). In most other cases it has been established, as a minimum, that NADPH is required for in vitro destruction.

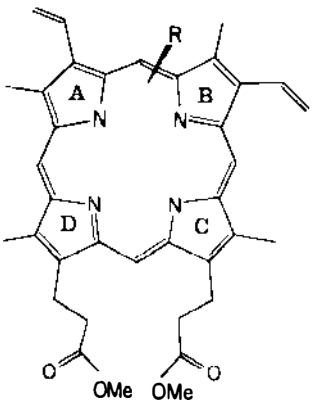
Structures of the Prosthetic Heme Adducts

The heme adducts formed with the following agents have been isolated and their structures have been unambiguously determined by complete (UV, MS, NMR) spectroscopic characterization: ethylene (39, 40), propene (41), octene (41), acetylene (13), propyne (42), octyne (41), fluroxene (13), vinyl fluoride (13), ethchlorvynol (22), DDC (43, 44), and DDEP (29). Strong evidence supports the structure assigned to the adduct formed with 1-aminobenzotriazole (25), but a reliable NMR spectrum is not yet available to confirm the assignment. The absorption and mass spectra of the adducts obtained with many of the other agents in Table 1 have furthermore been recorded and in all cases but those of AIA (45) and norethisterone (18), where the secondary elimination of ammonia or water appears to have occurred, are those expected if the adducts are directly analogous to the appropriate well-characterized prototypes.

The adducts are obtained, due to the isolation procedure, as the dimethyl esterified iron-free porphyrins. All of the isolated adducts are N-alkylated derivatives of protoporphyrin IX, the porphyrin framework of prosthetic heme, in which the N-alkyl group derives from the destructive substrate. The adducts with olefins are formally obtained by addition of a porphyrin nitrogen to the terminal carbon and of a hydroxyl group to the internal

carbon of the double bond in the substrate. If a good leaving group is present on the carbon to which the hydroxyl is added, as it is in fluroxene and vinyl fluoride (13), the leaving group is eliminated and the resulting carbonyl derivative is obtained. The adducts with acetylenes are generated by a similar process except that ketone (or aldehyde) products are invariably obtained because the new hydroxyl function is at the enol oxidation state, owing to the second π-bond of the acetylenic group. The adducts obtained with dihydropyridine substrates reflect transfer of the 4-substituent from the substrate to a nitrogen of protoporphyrin IX. The structures of adducts with olefins, acetylenes, and dihydropyridine derivatives that have been unambiguously established are summarized in Table 2. The structure of the adduct isolated from 1-aminobenzotriazole-treated rats has yet to be confirmed by NMR, but almost certainly is formally obtained by bridging two

Table 2 Structures of the isolated prosthetic heme adducts



Substrate	N-Alkyl group R	Pyrrole ring nitrogen alkylated	References
Ethylene	$-CH_2CH_2OH$	D	39
Propene	$-CH_2CHOHCH_3$	D	41
Octene	$-CH_2CHOH\ (CH_2)_5\ CH_3$	D	41
Acetylene	$-CH_2CHO$	several	13
Fluroxene	$-CH_2CHO$	several	13
Vinyl fluoride	$-CH_2CHO$	several	13
Propyne	$-CH_2COCH_3$	A	42
Octyne	$-CH_2CO\ (CH_2)_5\ CH_3$	A	41
Ethchlorvynol	$-CH_2COC\ (CH_2CH_3)\ (OH)\ (CH = CHCl)$	all	22
DDC	$-CH_3$	all	43, 44
DDEP	$-CH_2CH_3$	all	29

vicinal nitrogens in protoporphyrin IX with a vicinally disubstituted phenyl ring (25).

Four regioisomers are possible for each of the adducts because the four nitrogens of protoporphyrin IX are not equivalent. Recent results have established that the alkylation process, at least with some of the substrates, is regiospecific. The alkylation regiospecificity, where it is known, is indicated in Table 2. It is important to note that the data in Table 2 was obtained with phenobarbital-induced rats because it is possible that the regiospecificity of alkylation by a given substrate will depend on the isozyme of cytochrome P-450 involved in the reaction.

Identity of the Reactive Species

OLEFINS The prosthetic heme adducts with olefins formally are obtained by chemical addition of the porphyrin nitrogen to the terminal carbon of the corresponding epoxide metabolite. This mechanism for alkylation of the prosthetic heme group is definitively ruled out, however, by the following independent lines of evidence: (*a*) the epoxides of several active olefins do not destroy cytochrome P-450 when incubated with hepatic microsomes even though spectroscopic and kinetic evidence confirms that they reach the active site of the enzyme (10, 16, 45); (*b*) the porphyrin nitrogen adds to the unsubstituted terminus of fluroxene (13), whereas addition to the trifluoroethoxy-substituted end of the corresponding epoxide would be expected (46); and (*c*) recent evidence establishes that the oxygen and the nitrogen add in a *cis*-fashion rather than in the *trans* arrangement expected for an epoxide (47). Two epoxides, those obtained from trifluoroethyl allyl ether (48) and alclofenac (4-allyloxy-3-chlorophenyl acetic acid) (49), have nevertheless been reported to destroy cytochrome P-450 by NADPH-independent mechanisms. No evidence exists in either case that the enzyme is destroyed through prosthetic heme alkylation. It is likely that the destruction associated with these epoxide substrates is due to a different mode of action. Two possibilities are protein alkylation or a membrane perturbation such as that presumably responsible for the NADPH-independent destructive action of 1-heptene, an olefin that, in the presence of NADPH, in fact mediates normal prosthetic heme alkylation (10).

The catalytically-driven alkylation of prosthetic heme by olefins, in view of the fact that epoxide metabolites are not involved, requires that an intermediate in the oxidation sequence prior to the epoxide react with the heme. The nature of this intermediate is not yet known, but is subject to the constraints imposed by the following observations: (*a*) both the epoxidation and heme alkylation associated with *trans*-1-(^{2}H)-1-octene, a typical olefin, proceed with retention of the olefin stereochemistry (50); (*b*) the

alkylation of prosthetic heme by linear olefins occurs with high regioselectivity at the nitrogen of pyrrole ring A (Table 2); and (*c*) studies of the absolute chirality of oxygen addition to the internal carbon of the double bond in both the epoxide and the heme adduct indicate that one orientation of the olefin relative to the heme face gives rise to both products. Oxygen addition to the opposite face of the π-bond does occur, but gives rise only to the epoxide and not to the heme adduct (50). Retention of stereochemistry during both epoxidation and heme adduct formation is only consistent with parallel, probably concerted, epoxidation and heme alkylation or, alternatively, partitioning into both products of a common intermediate unable to undergo rotation about the carbon-carbon sigma bond of the original olefin because of stereoelectronic interactions with the heme. If parallel mechanisms are in operation, the commitment of a given catalytic event to epoxidation or heme alkylation must be made at the substrate-binding stage (before oxygen transfer is initiated). If the alternative mechanism involving partitioning of a common intermediate prevails, the fact that the heme adducts with fluroxene and vinyl fluoride require catalytic addition of oxygen to the heteroatom-substituted carbon suggests that a radical rather than a cation mechanism is involved (13). The data now available is most comfortably rationalized by a nonconcerted mechanism involving a radical species, but is not yet sufficiently definitive to rule out the alternatives. The mechanism of prosthetic heme alkylation by olefins and acetylenes has recently been reviewed in some detail (50).

ACETYLENES Oxidation of carbon-carbon triple bonds by cytochrome P-450 does not give the corresponding unsaturated epoxides (oxirenes) but rather products derived from rearrangement of these highly unstable structures to ketenes or alpha-ketocarbenes (50). The heme adducts obtained, however, are not those expected from reaction of heme with the ketene rearrangement products (Table 2), because the oxygen in the adducts is located on the internal rather than on the terminal carbon predicted by addition to a ketene. The alkylation therefore requires (*a*) reaction with the oxirene itself before it rearranges, (*b*) reaction with an intermediate in the oxidation sequence that precedes the oxirene (see discussion of olefins), or (*c*) concerted oxygen transfer and heme alkylation.

HETEROATOMIC SUBSTRATES The alkylation of prosthetic heme by 4-alkyl-1,4-dihydropyridine analogues differs from that mediated by olefins and acetylenes in that only a fragment of the substrate, the 4-alkyl group, is incorporated into the isolated porphyrin adduct. The requirement for oxidative turnover of the substrate and the fact that the nitrogen can be fully substituted (and thus cannot be activated by N-hydroxylation) suggests that

the nitrogen undergoes a one-electron oxidation (29, 30). The resulting radical cation then aromatizes by extrusion of the 4-alkyl group as a radical. Spin-trapping experiments have recently confirmed that the 4-ethyl group is released as a radical during the oxidation of DDEP by cytochrome P-450 (30). Strong evidence therefore exists for the one-electron oxidation of DDEP, and presumably other related substrates, to radical cation intermediates. If benzyl cyclopropyl amine destroys cytochrome P-450 by prosthetic heme alkylation, the destructive process probably also reflects oxidation of the nitrogen to a radical cation that subsequently ring opens to a carbon radical alkylating species (32, 33).

The chemical oxidation of 1-aminobenzotriazole by a variety of chemical agents yields benzyne (51). The biological oxidation of this substrate by chloroperoxidase, a one-electron oxidant, has also been shown recently to give benzyne (52). The observation that the heme adduct obtained with this agent can be rationalized by addition of benzyne across two nitrogens of protoporphyrin IX (followed by autoxidative rearomatization) thus probably reflects a mechanistic as well as formal derivation (25).

The destruction of hemoglobin by phenylhydrazine, first noted a century ago, has recently been shown to involve catalytic formation of an iron-phenyl intermediate that subsequently rearranges to the corresponding N-phenyl heme derivative (53, 54). It is likely that the destruction of cytochrome P-450 by phenylhydrazine involves a similar mechanism because phenylhydrazine reacts in the same way with hemin itself (55). The iron-phenyl intermediate in the destruction of hemoglobin by phenylhydrazine is presumably formed by combination of the prosthetic heme with phenyl radicals generated during oxidation of the substrate (53). The formation of 2-phenylethyl radicals from phenelzine could similarly rationalize the alkylation of prosthetic heme by this agent.

ISOZYME SPECIFICITY

The isozyme specificity of substrates that destroy cytochrome P-450 by prosthetic heme alkylation has been investigated in a limited number of cases with purified, reconstituted forms of the enzyme. The principal fraction purified from phenobarbital-induced rat liver has been shown to be inactivated by AIA (38, 56), 1-ethinylcyclopentanol (38), 1-aminobenzotriazole (38), fluroxene (38, 48), and benzyl cyclopropyl amine (32). Vinyl chloride has also been found to inactivate this form of the enzyme, although, as noted, direct evidence is not yet available for prosthetic heme alkylation (14). In contrast, the principal isozyme isolated from rats induced with 3-methylcholanthrene is not inactivated by AIA (38) even though it is still susceptible to the action of fluroxene and 1-aminobenzo-

triazole (38). The isozyme induced in rats by beta-naphthoflavone is also destroyed by fluroxene (48). The inactivity of AIA with the 3-MC-inducible enzyme reflects the fact that it is not accepted as a substrate rather than that the 3-MC isozyme is more resistant to destruction (38). Earlier studies with microsomes from rats induced with phenobarbital and 3-methylcholanthrene [see (38, 48) for appropriate citations] are in accord with the purified enzyme studies.

CONSEQUENCES OF SUICIDAL INACTIVATION OF CYTOCHROME P-450

Impairment of Cytochrome P-450 Function

Suicidal inactivation of hepatic cytochrome P-450 is expected a priori to impair not only further metabolism of the inactivating substrate but also that of any coadministered drug(s). In turn, this impairment could alter the disposition, retard the elimination, and possibly prolong the duration of pharmacological action of the drug(s) in question. If the drug has a relatively small therapeutic index and is predominantly metabolized by hepatic cytochrome P-450, its impaired elimination could result in toxicity. Although the potential for such toxicity has long been recognized, very few of the suicidal inactivators of cytochrome P-450 (Table 1) have actually been examined for their effects on the metabolism of drugs in vivo or in vitro (Table 3). Not surprisingly, the few inactivators examined conform to a rather predictable pattern of impairment of drug metabolism due to their characteristic selective destruction of particular cytochrome P-450 isozymes. AIA, the most extensively studied inactivator, preferentially inactivates the principal phenobarbital-inducible cytochrome P-450 species of rat liver and impairs not only its own metabolism (61, 62) but also that of ethylmorphine, aminopyrine, hexobarbital, and benzphetamine (63–67), all preferred substrates of that particular isozyme. The metabolism of p-chloro-N-methylaniline, a prototype substrate of the 3-methylcholanthrene (3MC)-inducible isozyme, is only minimally affected by AIA (64). On the other hand, fluroxene, a substrate that inactivates both the phenobarbital and 3-MC-inducible isozymes, is correspondingly nondiscriminatory in its impairment of oxidative metabolism (63).

Although impairment of drug-metabolism is an acute manifestation of the suicide-inhibitor, it must be recognized that with repeated administration, the inhibitor, in common with most lipophilic substrates of cytochrome P-450, may also induce the cytochrome and consequently enhance its own metabolism and that of coadministered drugs. Both the destructive and the inductive potentials of an inactivating drug therefore need to be taken into consideration in its clinical evaluation.

Table 3 Some substrates of cytochrome P–450 subject to metabolic impairment by suicide inactivators

Inactivator	Preferred cyt P–450 species	Substrates subject to metabolic impairment[a]
AIA	PB-inducible[b]	AIA (61, 62) Aminopyrine (65) Benzphetamine (67) Benzpyrene (63) Ethoxyresorufin (63) Ethylmorphine (64, 65) Hexobarbital (66) 3-Methyl-4-monomethyl-azobenzene (26) p-Nitroanisole (63) Propranolol (68)
Cyclopropylamines	PB-inducible[b]	Aminopyrine (31)
DDC	PB-inducible	3-Methyl-4-monomethyl-aminoazobenzene (26)
Ethinyl estradiol	PB-inducible	Ethylmorphine (110)
Fluroxene	PB and 3-MC inducible	Benzpyrene (63) Ethoxyresorufin (63) Ethylmorphine (63) p-Nitroanisole (63)
Griseofulvin		3-Methyl-4-monomethyl-aminoazobenzene (26)
Norethisterone	?	Androstenedione (69)
Phenelzine	?	Aminopyrine (34) Meperidine (111)
Vinyl chloride	PB-inducible	Benzphetamine (14)

[a] References indicated in parentheses.
[b] PB, phenobarbital; 3-MC, 3-methylcholanthrene; has been ascertained from data with purified isozymes (14, 32, 38, 48).

In addition to the impact of an inactivator on the rate of metabolism, its effect on the normal pattern of metabolites of a coadministered drug may be important. AIA not only dramatically enhances the bioavailability of propranolol in dogs by inhibiting its cytochrome P-450-dependent oxidation to naphthoxylactic acid, for instance, but also, as a result, increases the fraction of the drug that is glucuronidated (68). In the case of propranolol, impairment of one metabolic pathway enhances the activity of an innocuous alternative one. However, a suicide inactivator could just as well shift the metabolism of a co-administered drug into a toxic pathway.

Finally, drug-mediated inactivation of cytochrome P-450 may also disrupt other physiological functions. Cytochrome P-450 inactivation in steroidogenic tissues, for instance, could impair steroid synthesis and create

a hormonal imbalance. In fact, the acetylenic estrogen norethisterone has been shown to inhibit cytochrome P-450-dependent estrogen synthetase (aromatase) activity in vitro (69), but the sequelae of such inhibition in vivo are yet to be determined.

Heme-stripping of Cytochrome P-450

Selective alkylation of the prosthetic heme moiety of cytochrome P-450 in the course of oxidative metabolism of a destructive substrate generates a relative excess of apocytochrome P-450 that can be functionally reconstituted with fresh heme. Reconstitution after AIA-induced heme alkylation has been demonstrated in vivo by administration of exogenous heme (62, 64, 70) or hemoglobin (71) to rats, and, in vitro, by heme addition to liver homogenates or isolated perfused livers from AIA-treated rats (72, 73). Heme-mediated reconstitution of the cytochrome restores its mixed function oxidase activity (62, 64, 66) and partially reverses the associated impairment in drug metabolism and elimination (62, 66). These findings indicate that AIA-induced loss of cytochrome P-450 heme releases its apocytochrome ostensibly intact and functionally reconstitutable. Reconstitution is incomplete, however, in that even though exogenous heme can double the residual cytochrome P-450 content, this amount represents at best only 70% of the original cytochrome P-450 destroyed (L. Bornheim and M. A. Correia, unpublished observations). Moreover, reconstitution of the isoenzyme responsible for ethylmorphine N-demethylation is likewise incomplete (64). Incomplete reconstitution may reflect impaired substrate access to the reconstituted cytochrome P-450, irreversible damage to a fraction of the specific apoprotein, or disrupted cytochrome P-450-flavoprotein reductase coupling. Failure of suicide inactivators to affect the reductase activity, determined with cytochrome c as the external electron donor, suggests that the flavoprotein itself is unscathed (74, 75). Whether the inability of the reconstituted cytochrome P-450 to be fully reactivated is due to interference of the residual heme adduct in the microsomal membrane, or to AIA-induced damage to the apoprotein and/or the critical membrane phospholipid milieu, remains to be determined. Indeed, it has been reported that AIA promotes microsomal lipid peroxidation of rat liver microsomes in vitro (76, 77). Lipid peroxidative damage to the microsomal membrane may thus occur during in vitro incubations of liver preparations from AIA-treated rats, even though no evidence of AIA-induced lipid peroxidation in vivo was obtained when serum malonaldehyde and pulmonary ethane evolution, two specific indices of lipid peroxidation, were monitored hourly in phenobarbital-pretreated rats given AIA (400 mg/Kg, s.c.) for 8 h (M. A. Correia and R. F. Burk, unpublished observations).

Heme-mediated reversal of cytochrome P-450 inactivation, although in-

complete, nevertheless rapidly and significantly accelerates the plasma disappearance rate and considerably shortens the half-life of the inactivating drug (62, 64, 66). Heme-mediated repair may thus represent a promising therapeutic modality for the enhancement of drug elimination in patients following deliberate or accidental poisoning with drugs such as the allylbarbiturates, ethchlorvynol, or novonal.

Effects on Hepatic Heme Synthesis

Cytochrome P-450, among hepatic hemoproteins, is unquestionably the most avid consumer of heme synthesized in the liver (78). It is hardly surprising therefore that drug-mediated alkylation of its prosthetic heme markedly depletes hepatic heme and consequently represses δ-aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme in heme synthesis (79). Quite independently of this action, alkylating agents such as AIA and norethisterone are inherently good inducers of ALAS (17, 26, 79) and possibly of apocytochrome P-450 (79). The synergism between these effects is found to provoke an acute demand for hepatic heme synthesis that translates into a marked induction of ALAS. The resulting strain on the heme biosynthetic pathway triggers porphyria in individuals genetically predisposed to this metabolic disorder. In these individuals, the porphyrinogenic potential of such drugs may be appreciable even at nominally low doses. Of equal, if not greater, clinical concern is the synergistic interaction that might result in genetically susceptible individuals during combination therapy with a heme-alkylating drug and one or more drugs that are potent inducers of ALAS and/or apocytochrome P-450. Mechanistic aspects of the porphyrinogenic action of several suicide inactivators have been discussed in detail by De Matteis (79, 80).

More recently, a second mechanism by which heme alkylation can markedly perturb heme synthesis has been elucidated. The heme adducts can, in some instances, directly inhibit the biosynthetic pathway (81–83). Thus, the alkylated porphyrins obtained with DDEP and its 4-methyl analog (DDC) are potent inhibitors of ferrochelatase (81, 83–86), the terminal enzyme in the heme-synthetic pathway. By itself such a block in heme synthesis may be insufficient to induce porphyria (87), but with porphyrinogenic promotion it could critically contribute to the porphyrinogenic scenario. Ferrochelatase inhibition by the potent N-alkylated porphyrin generated by DDC, a relatively poor cytochrome P-450 inactivator when compared to AIA, may explain its relatively superior porphyrinogenic potential (26, 27, 88). It is intriguing, however, that N-alkylated adducts of cytochrome P-450 heme derived from DDC, but not AIA, ethylene, or secobarbital, inhibit heme synthesis at the ferrochelatase level (83). Struc-

tural differences in the N-alkylated isomers that are formed explain this discrepancy at least in part (86, 89). Indeed, the A and B pyrrole-ring N-ethylated porphyrins from DDEP are much more potent inhibitors of ferrochelatase than the corresponding C- and D-regioisomers (86).

In contrast to the DDC-mediated inhibition of ferrochelatase, hepatic and renal ALA-dehydratase activity in hamsters and hens is stimulated by DDC administration (90). However, as in the case of ALAS-stimulation, this activity appears to result from the derepression caused by ferrochelatase inhibition rather than a direct effect of the drug or its porphyrin adduct. It is unknown to what extent other enzymes in the heme synthetic pathway may be affected by the suicide substrates or by their alkylated heme adducts or subsequent metabolites.

The question of the tissue specificity of cytochrome P-450 inactivation and its sequelae on heme synthesis remains largely unexplored. Most studies to date have been understandably confined to the liver, as the liver is strategically located for uptake, clearance, and metabolism of most drugs and has not only the highest cytochrome P-450 content but also a relatively high heme turnover. The few studies that have examined extrahepatic effects have revealed that these tissues are somewhat variable in their response to cytochrome P-450 destructive drugs. For instance, neither DDC nor AIA stimulates rat adrenal ALAS at dosages causing 4–10-fold increases in the hepatic enzyme activity (91), whereas AIA stimulates renal ALAS, albeit to a small extent, in rats (92) and chicks (93). DDC, on the other hand, inhibits ferrochelatase and stimulates ALA-dehydratase activity not only in the liver but also in the renal cortex and medulla of hamsters and hens (90). Organ as well as species differences thus appear to exist. The extrahepatic effects of a given inactivator, therefore, may be determined not only by its relative tissue distribution and the extent to which it is metabolized by specific cytochrome P-450 isozymes in the given tissue, but also by the efficiency with which alkylated heme adducts generated in the liver and other tissue sites are delivered to the tissue in question.

Effects on Hepatic Heme and Hemoproteins other than Cytochrome P-450

Since by definition cytochrome P-450 is instrumental in the metabolic activation of its suicide substrates, it is not surprising that it is the principal target of the reactive metabolites. Cytochrome b_5, in contrast, although localized in the microsomal membrane in close proximity to cytochrome P-450, appears to escape unscathed (17, 26, 64, 75, 94, 95). Selective loss of microsomal cytochrome P-450 heme thus largely accounts for the 50–60% decrease of newly labelled isotopic heme observed in rat liver microsomes within just one hour of AIA administration (94–96). The

accompanying striking loss (80%) of cytosolic heme, however, is highly surprising (95). This heme loss could, in principle, be due to AIA-induced heme depletion of the cytosolic hemoprotein, tryptophan pyrrolase (97). DDC, griseofulvin, and DDEP also reduced the activity of this hemoprotein [(97), D. Littman and M. A. Correia, unpublished observations]. However, since tryptophan pyrrolase represents only a minor fraction of the entire hepatic hemoprotein complement, and utilizes only 1.5% of the heme synthesized in the liver (78, 98), it cannot account for a heme loss of such magnitude. The most plausible explanation is that drug-mediated alkylation and loss of the cytochrome P-450 prosthetic heme permits the apocytochrome to utilize heme from the "free" or uncommitted regulatory heme pool with which the cytochrome is apparently in intimate contact (70, 79, and references therein). Functional reassembly in turn would permit the reconstituted cytochrome to enter a fresh suicidal cycle. Recycling of the apocytochrome thus would effectively deplete the cytosolic heme pool and could account for the dramatic loss of cytosolic heme. The finding that radiolabelled green pigments can be isolated from incubations of rat liver microsomes and norethisterone following addition of isotopically labelled hemin, but not of its nonreconstitutable congener diisopropylhemin, supports such a possibility (73). Additional evidence for a recycling process is provided by the report that the biliary yield of norethisterone green pigments in rats far exceeds the original hepatic content of cytochrome P-450 (99).

The observed impairment of hepatic tryptophan pyrrolase in rats following treatment with various suicide inhibitors may consequently reflect depletion of the "free" heme pool with which the hemoprotein is closely attuned (100). Because the hemoprotein regulates tryptophan metabolism, its impairment could considerably increase the flux of tryptophan into alternative pathways, such as into the formation of 5-hydroxytryptamine (5-HT), a potent neuroeffector in the CNS and gastrointestinal tract. Whether the observed neurological and gastrointestinal manifestations, the symptomatic hallmarks of porphyric attacks in humans, can be ascribed to such an alteration in tryptophan metabolism remains to be determined. In accordance with this hypothesis, DDEP administration to rats does indeed markedly lower their hepatic tryptophan pyrrolase activity and enhance their brain tryptophan, 5-HT, and 5-HIAA levels. The exogenous administration of heme, which normalizes the "free" heme pool, not only restores hepatic tryptophan pyrrolase activity in these animals but also reverses the alterations of tryptophan metabolism (D. Littman and M. A. Correia, unpublished observations). An instructive parallel exists between these findings and the fact that, in humans, heme administration dramatically reverses the neurological and gastrointestinal symptoms of hepatic porphyria.

In contrast to the acute decrease in microsomal and cytosolic heme produced by heme alkylators, mitochondrial heme is only minimally affected (95). In fact, the hepatic mitochondrial content of cytochromes a + a_3, b, and c in rats is not altered by AIA-treatment (92). Moreover, hepatic mitochondrial cytochrome oxidase and succinic dehydrogenase activities are unaffected even after prolonged (45 day) daily treatment of rats with Sedormid (101). The slight loss of mitochondrial heme actually observed may only reflect increased efflux of newly synthesized heme elicited by depletion of the "free" heme pool rather than destruction of mitochondrial cytochromes. Suicide inactivators of cytochrome P-450 impair hepatic catalase activity (8, 101–104). Indeed, rat hepatic catalase was the first hemoprotein reported to be deranged in animals treated with Sedormid (101), a drug now recognized as a suicide inactivator of cytochrome P-450. However, the loss of rat hepatic catalase occurs with time rather than acutely and results, as originally suggested, from reduced formation of the hemoprotein rather than from its inactivation (105, 106). Drug-induced depletion of the free heme pool may be largely responsible for the decreased synthesis of catalase.

Fate of Green Pigments

Very little is known about the metabolic disposition of the alkylated heme adducts once they are formed. The AIA-induced loss of labelled heme from liver microsomes is matched by a corresponding gain of total non-heme radioactivity in the cytosol (95), which indicates that the alkylated heme adduct is eventually released from its apocytochrome. This is confirmed by the demonstrated reconstitution of the apocytochrome in presence of fresh heme. Recent studies with norethisterone-treated rats indicate that alkylated heme adducts are excreted largely unchanged, i.e. still retaining their prosthetic iron, in the bile (but not urine) over a 48 h period (99). Conjugation of the adducts with endogenous substrates apparently does not occur. This is in marked contrast to the requirement for glucuronidation prior to excretion of bilirubin, the normal heme catabolite (107 and references therein).

Whether the heme adducts undergo metabolic degradation in the liver is unknown. The normal 1:1 stoichiometric ratio of CO expired to bilirubin formed is reportedly increased to nearly 2:1 in AIA-treated rats (108). In view of decreased degradation of heme to bilirubin in these rats, the source of the additional CO may be the heme adducts. Oxidation of the adducts at the methene bridges could yield CO and the corresponding tetra- or dipyrroles, although, to date, no such metabolites have been reported following AIA-treatment of rats. Furthermore, no radiolabelled CO from heme specifically labelled at the methene-bridges is evolved in vitro under conditions where AIA-mediated destruction of microsomal cytochrome

P-450 is demonstrable (109). Since these studies were carried with microsomal (and postmitochondrial) preparations, the possibility of in vivo oxidative cleavage of the adducts in liver organelles other than the endoplasmic reticulum, or in organs other than the liver, cannot presently be ruled out. However, in view of the reported structural intactness of the biliary and microsomal green pigments (98), their oxidative cleavage in the liver may be a negligible pathway.

SIGNIFICANCE

Drug-mediated cytochrome P-450 inactivation raises two major concerns of clinical significance. The first of these is that drug-drug interactions may result during therapy with multiple drugs if one or more of them is a suicide inactivator of cytochrome P-450. Impaired metabolic disposition, if it results in enhanced or prolonged pharmacological action, may place the patient in jeopardy.

The second clinical concern associated with drug-mediated cytochrome P-450 inactivation is the creation of an acute heme-deficient state. Such acute heme deficiency, particularly in the presence of agents that increase the demand for heme, may trigger porphyric attacks in individuals with a genetically determined latent or overt block in heme synthesis. The risk for induction of porphyria is augmented in the presence of co-drugs that, although innocuous per se, are nevertheless promoters of porphyrinogenesis. Finally, the block in heme synthesis created by the cytochrome P-450 heme adduct of some inactivators may be sufficient to precipitate porphyria even in normal individuals in an acute drug-induced heme-deficient state. The above clinical considerations notwithstanding, cytochrome P-450 inactivation represents a formidable approach to rational drug design. Drug-mediated enzyme inactivations may be ingeniously exploited to alter specific cytochrome P-450-dependent physiological pathways or in the prolongation of drug action (as in insecticide synergists). In addition, the use of suicide inhibitors of cytochrome P-450 has permitted a more intimate understanding of the mechanisms of drug oxidation and of the structural assembly and dynamics of the enzyme itself.

ACKNOWLEDGEMENTS

Ms. Virginia Hayes is gratefully acknowledged for typing parts of the manuscript. Supported by NIH grants GM 25515 and AM 30297 (P.R.O.M.) and AM 26506 (M.A.C.). P.R.O.M. is a fellow of the Alfred P. Sloan foundation.

Literature Cited

1. Testa, B., Jenner, P. 1981. Inhibitors of cytochrome P-450s and their mechanism of action. *Drug Metab. Rev.* 12:1–117
2. Netter, K. J. 1980. Inhibition of oxidative drug metabolism. *Pharmacol. Ther.* 10:515–35
3. Franklin, M. R. 1977. Inhibition of mixed-function oxidations by substrates forming reduced cytochrome P-450 metabolic-intermediate complexes. *Pharmacol. Ther. A* 2:227–45
4. Hodgson, E., Philpot, R. M. 1974. Interaction of methylenedioxyphenyl (1,3-benzodioxole) compounds with enzymes and their effects on mammals. *Drug Metab. Rev.* 3:231–301
5. Halpert, J., Neal, R. A. 1981. Inactivation of rat liver cytochrome P-450 by the suicide substrates parathion and chloramphenicol. *Drug Metab. Rev.* 12:239–59
6. De Matteis, F. 1978. Loss of liver cytochrome P-450 caused by chemicals. In *Heme and Hemoproteins, Handbook of Experimental Pharmacology,* ed. F. De Matteis, W. N. Aldridge, 44:95–127. New York: Springer-Verlag. 449 pp.
7. Brinkschulte-Freitas, M., Uehleke, H. 1979. The effects of 2,2-diethylallylacetamide on hepatic cytochromes in rats and in vitro. *Arch. Toxicol.* 42: 137–46
8. Abbritti, G., De Matteis, F. 1972. Decreased levels of cytochrome P-450 and catalase in hepatic porphyria caused by substituted acetamides and barbiturates: importance of the allyl group in the molecule of the active drugs. *Chem.-Biol. Interact.* 4:281–86
9. Levin, W., Sernatinger, E., Jacobson, M., Kuntzman, R. 1972. Destruction of cytochrome P-450 by secobarbital and other barbiturates containing allyl groups. *Science* 176:1341–43
10. Ortiz de Montellano, P. R., Mico, B. A. 1980. Destruction of cytochrome P-450 by ethylene and other olefins. *Mol. Pharmacol.* 18:128–35
11. Ivanetich, K. M., Marsh, J. A., Bradshaw, J. J., Kaminsky, L. S. 1975. Fluroxene (2,2,2-trifluoroethyl vinyl ether) mediated destruction of cytochrome P-450 in vitro. *Biochem. Pharmacol.* 24:1933–36
12. Marsh, J. A., Bradshaw, J. J., Sapeika, G. S., Lucas, S. A., Kaminsky, L. S., Ivanetich, K. M. 1977. Further investigations of the metabolism of fluroxene and the degradation of cytochromes P-450 in vitro. *Biochem. Pharmacol.* 26:1601–6
13. Ortiz de Montellano, P. R., Kunze, K. L., Beilan, H. S., Wheeler, C. 1982. Destruction of cytochrome P-450 by vinyl fluoride, fluroxene, and acetylene. Evidence for a radical intermediate in olefin oxidation. *Biochemistry* 21:1331–39
14. Guengerich, F. P., Strickland, T. W. 1977. Metabolism of vinyl chloride: destruction of the heme of highly purified liver microsomal cytochrome P-450 by a metabolite. *Mol. Pharmacol.* 13:933-1004
15. Ivanetich, K. M., Aronson, I., Katz, I. D. 1977. The interaction of vinyl chloride with rat hepatic microsomal cytochrome P-450 in vitro. *Biochem. Biophys. Res. Commun.* 74:1411–18
16. Ortiz de Montellano, P. R., Mico, B. A., Beilan, H. S., Kunze, K. L. 1981. Olefins as suicide inhibitors of cytochrome P-450. In *Molecular Basis of Drug Action,* ed. T. Singer, R. Ondarza, pp. 151–66. New York: Elsevier
17. White, I. N. H., Muller-Eberhard, U. 1977. Decreased liver cytochrome P-450 in rats caused by norethindrone or ethynyloestradiol. *Biochem. J.* 166: 57–64
18. Ortiz de Montellano, P. R., Kunze, K. L., Yost, G. S., Mico, B. A. 1979. Self-catalyzed destruction of cytochrome P-450: covalent binding of ethynyl sterols to prosthetic heme. *Proc. Natl. Acad. Sci. USA* 76:746–49
19. White, I. N. H. 1978. Metabolic activation of acetylenic substituents to derivatives in the rat causing the loss of hepatic cytochrome P-450 and haem. *Biochem. J.* 174:853–61
20. Skrinjaric-Spoljar, M., Matthews, H. B., Engel, J. L., Casida, J. E. 1971. Reponse of hepatic microsomal mixed-function oxidases to various types of insecticide chemical synergists administered to mice. *Biochem. Pharmacol.* 20:1607–18
21. Ortiz de Montellano, P. R., Kunze, K. L. 1980. Self-catalyzed inactivation of hepatic cytochrome P-450 by ethynyl substrates. *J. Biol. Chem.* 255:5578–85
22. Ortiz de Montellano, P. R., Beilan, H. S., Mathews, J. M. 1982. Alkylation of the prosthetic heme in cytochrome P-450 during oxidative metabolism of the sedative-hypnotic ethchlorvynol. *J. Med. Chem.* 25:1174–79
23. Ortiz de Montellano, P. R., Kunze, K. L. 1980. Occurrence of a 1,2-shift during enzymatic and chemical oxidation

of a terminal acetylene. *J. Am. Chem. Soc.* 102:7373–75
24. White, I. N. H. 1980. Structure-activity relationships in the destruction of cytochrome P-450 mediated by certain ethynyl-substituted compounds in rats. *Biochem. Pharmacol.* 29:3253–55
25. Ortiz de Montellano, P. R., Mathews, J. M. 1981. Autocatalytic alkylation of the cytochrome P-450 prosthetic haem group by 1-aminobenzotriazole. Isolation of an NN-bridged benzyne-protoporphyrin IX adduct. *Biochem. J.* 195:761–64
26. Wada, O., Yano, Y., Urata, G., Nakao, K. 1968. Behaviour of hepatic microsomal cytochromes after treatment of mice with drugs known to disturb porphyrin metabolism in liver. *Biochem. Pharmacol.* 17:595–603
27. Waterfield, M. D., Del Favero, A., Gray, C. H. 1969. Effect of 1,4-dihydro-3,5-dicarbethoxycollidine on hepatic microsomal heme, cytochrome b_5, and cytochrome P-450 in rabbits and mice. *Biochim. Biophys. Acta* 184:470–73
28. Tephly, T. R., Gibbs, A. H., De Matteis, F. 1979. Studies on the mechanism of experimental porphyria produced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. *Biochem. J.* 180:241–44
29. Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L. 1981. N-Alkylprotoporphyrin IX formation in 3,5-dicarbethoxy-1,4-dihydrocollidine-treated rats. *J. Biol. Chem.* 256:6708–13
30. Augusto, O., Beilan, H. S., Ortiz de Montellano, P. R. 1982. The catalytic mechanism of cytochrome P-450. Spin-trapping evidence for one-electron substrate oxidation. *J. Biol. Chem.* 257:11288–95
31. Hanzlik, R. P., Kishore, V., Tullman, R. 1979. Cyclopropylamines as suicide substrates for cytochrome P-450. *J. Med. Chem.* 22:759–61
32. Macdonald, T. L., Karimullah, Z., Burka, L. T., Peyman, P., Guengerich, F. P. 1982. Mechanism of cytochrome P-450 inhibition by cyclopropylamines. *J. Am. Chem. Soc.* 104:2050–52
33. Hanzlik, R. P., Tullman, R. H. 1982. Suicidal inactivation of cytochrome P-450 by cyclopropylamines. Evidence for cation-radical intermediates. *J. Am. Chem. Soc.* 104:2048–50
34. Muakkassah, S. F., Yang, W. C. T. 1981. Mechanism of the inhibitory action of phenelzine on microsomal drug metabolism. *J. Pharm. Exp. Ther.* 219:147–55
35. Jonen, H. G., Werringloer, J., Prough, R. A., Estabrook, R. W. 1982. The reaction of phenylhydrazine with microsomal cytochrome P-450: catalysis of heme modification. *J. Biol. Chem.* 257:4404–11
36. De Matteis, F. 1971. Lose of heme in rat liver caused by the porphyrogenic agent 2-allyl-2-isopropylacetamide. *Biochem. J.* 124:767–77
37. Ortiz de Montellano, P. R., Mico, B. A. 1981. Destruction of cytochrome P-450 by allylisopropylacetamide is a suicidal process. *Arch. Biochem. Biophys.* 206:43–50
38. Ortiz de Montellano, P. R., Mico, B. A., Mathews, J. M., Kunze, K. L., Miwa, G. T., Lu, A. Y. H. 1981. Selective inactivation of cytochrome P-450 isozymes by suicide substrates. *Arch. Biochem. Biophys.* 210:717–28
39. Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L., Mico, B. A. 1981. Destruction of cytochrome P-450 by ethylene. Structure of the resulting prosthetic heme adduct. *J. Biol. Chem.* 256:4395–99
40. Ortiz de Montellano, P. R., Kunze, K. L., Mico, B. A. 1980. Destruction of cytochrome P-450 by olefins: N-alkylation of prosthetic heme. *Mol. Pharmacol.* 18:602–5
41. Kunze, K. L., Mangold, B. L. K., Wheeler, C. W., Beilan, H. S., Ortiz de Montellano, P. R. 1983. The cytochrome P-450 active site. Regiospecificity of prosthetic heme alkylation by olefins and acetylenes. *J. Biol. Chem.* In press
42. Ortiz de Montellano, P. R., Kunze, K. L. 1981. Cytochrome P-450 inactivation: structure of the prosthetic heme adduct with propyne. *Biochemistry* 20:7266–71
43. Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L. 1981. N-Methylprotoporphyrin IX: chemical synthesis and identification as the green pigment produced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine treatment. *Proc. Natl. Acad. Sci. USA* 78:1490–94
44. Kunze, K. L., Ortiz de Montellano, P. R. 1981. N-Methylprotoporphyrin IX. Identification by NMR of the nitrogen alkylated in each of the four isomers. *J. Am. Chem. Soc.* 103:4225–30
45. Ortiz de Montellano, P. R., Yost, G. S., Mico, B. A., Dinizo, S. E., Correia, M. A., Kambara, H. 1979. Destruction of cytochrome P-450 by 2-isopropyl-4-pentenamide and methyl 2-isopropyl-4-pentenoate: mass spectrometric charac-

terization of prosthetic heme adducts and nonparticipation of epoxide metabolites. *Arch. Biochem. Biophys.* 197:524–33

46. Adams, S. M., Murphy, J. M., Kaminsky, L. S. 1981. Molecular orbital studies of the metabolism of fluroxene and analogous fluorinated ether anesthetics. *Mol. Pharmacol.* 20:423–28
47. Ortiz de Montellano, P. R., Mangold, B. L. K., Kunze, K. L., Wheeler, C. W., Reich, N. O. 1983. Stereochemistry of cytochrome P-450 cata yzed epoxidation and prosthetic heme alkylation. *J. Biol. Chem.* In press
48. Murphy, M. J., Dunbar, D. A., Guengerich, F. P., Kaminsky, L. S. 1981. Destruction of highly purified cytochromes P-450 associated with metabolism of fluorinated ether anesthetics. *Arch. Biochem. Biophys.* 212:360–69
49. Brown, L. M., Ford-Hutchinson, A. W. 1982. The destruction of cytochrome P-450 by alclofenac: possible involvement of an epoxide metabolite. *Biochem. Pharmacol.* 31:195–99
50. Ortiz de Montellano, P. R. 1983. Alkenes and alkynes. In *Bioactivation of Foreign Compounds,* ed. M. W. Anders. New York: Academic. In press
51. Campbell, C. D., Rees, C. W. 1969. Reactive intermediates. Part III. Oxidation of 1-aminobenzotriazole with oxidants other than lead tetraacetate. *J. Chem. Soc. C* pp. 752–56
52. Mathews, J. M., Ortiz de Montellano, P. R. 1982. Oxidative metabolism to 1-aminobenzotriazole to a benzyne intermediate. *Fed. Proc.* 41:1541
53. Augusto, O., Kunze, K. L., Ortiz de Montellano, P. R. 1982. N-Phenylprotoporphyrin IX formation in the hemoglobin-phenylhydrazine reaction: evidence for a protein-stabilized iron-phenyl intermediate. *J. Biol. Chem.* 257:6231–41
54. Ortiz de Montellano, P. R., Kunze, K. L., Augusto, O. 1982. Hemoprotein destruction. Iron-nitrogen shift of a phenyl group in a porphyrin complex. *J. Am. Chem. Soc.* 104:3545–46
55. Ortiz de Montellano, P. R., Kunze, K. L. 1981. Formation of N-phenylheme in the hemolytic reaction of pheny hydrazine with hemoglobin. *J. Am. Chem. Soc.* 103:6534–36
56. Loosemore, M. J., Wogan, G. N., Walsh, C. 1981. Determination of partition ratios for al y isopropylacetamide during suicidal processing by a phenobarbital-induced cytochrome P-450 isozyme from rat liver. *J. Biol. Chem.* 256:8705–12
57. Schwartz, S., Ikeda, K. 1955. Studies of porphyrin synthesis and interconversion with special reference to certain green porphyrins in animals with experimenta hepatic porphyria. In *Ciba Foundation Symposium on Porphyrin Biosynthesis and Metabolism,* ed. G. E. W. Wolstenholme, E. C. P. Millar, pp. 209–28. London: Churchill. 308 pp.
58. De Matteis, F., Gibbs, A. H., Cantoni, L., Francis, J. 1980. Substrate-dependent irreversible inactivation of cytochrome P-450: conversion of its haem into modified porphyrins. In *Environmental Chemicals, Enzyme Function, and Human Disease, Ciba Found. Symp. 76,* pp. 119–39. Excerpta Medica
59. Ioannides, C., Parke, D. V. 1976. The effect of allyl compounds on hepatic microsomal mixed function oxidation and porphyrinogenesis. *Chem. Biol. Interact.* 14:241–49
60. White, I. N. H. 1982. Metabolic activation of saturated a dehydes to cause destruction of cytochrome P-450 in vitro. *Chem. Biol. Interact.* 39:231–43
61. Smith, A. 1976. The metabolism of 2-allyl-2-isopropylacetamide in vivo and in the isolated perfused rat iver. *Biochem. Pharm.* 25:2429–42
62. Farrell, G. C., Schmid, R., Kunze, K. L., Ortiz de Montellano, P. R. 1979. Exogenous heme restores in vivo functiona capacity of hepatic cytochrome P-450 destroyed by allylisopropylacetamide. *Biochem. Biophys. Res. Commun.* 89:456–63
63. Bradshaw, J. J., Ziman, M. R., Ivanetich, K. M. 1978. The degradation of different forms of cytochrome P-450 in vivo by fluroxene and allylisopropylacetamide. *Biochem. Biophys. Res. Commun.* 85:859–66
64. Farrel , G. C., Correia, M. A. 1980. Structural and functional reconstitution of hepatic cytochrome P-450 in vivo: reversa of ally isopropylacetamide mediated destruction of the hemoprotein by exogenous heme. *J. Biol. Chem.* 255:10128–33
65. Deloria, L., Low, J., Mannering, G. J. 1980. Loss and recovery of hepatic mono-oxygenase activities after the administration of allylisopropylacetamide (AIA) to mice. In *Microsomes, Drug Oxidations and Chemical Carcinogenesis,* ed. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, 2:861–64. New York: Academic. 618 pp.

66. Farrell, G. C., Gollan, J. L., Correia, M. A., Schmid, R. 1981. Heme enhances hexobarbital metabolism in perfused rat liver after drug-mediated destruction of cytochrome P-450. *J. Pharm. Exp. Ther.* 218:363–67
67. Bonkowsky, H., Sinclair, J., Healey, J., Sinclair, P. 1982. Restoration of functional hepatic cytochrome P-450 by incubation with heme. *Am. Gastro. Assoc.* Abstr. 85 (2):1223
68. Silber, B., Mico, B. A., Ortiz de Montellano, P. R., Dols, D. M., Riegelman, S. 1981. In vivo effects of the cytochrome P-450 suicide subtrate 2-isopropyl-4-pentenamide (allylisopropylacetamide) on the disposition and metabolic pattern of propranolol. *J. Pharm. Exp. Ther.* 219:125–33
69. Osawa, Y., Yarborough, C., Osawa, Y. 1982. Norethisterone, a major ingredient of contraceptive pills, is a suicide inhibitor of estrogen biosynthesis. *Science* 215:1249–51
70. Correia, M. A., Farrell, G. C., Schmid, R., Ortiz de Montellano, P. R., Yost, G. S., Mico, B. A. 1979. Incorporation of exogenous heme in hepatic cytochrome P-450. *J. Biol. Chem.* 254:15–17
71. Wyman, J. F., Gollan, J. L., Farrell, G. C., Correia, M. A. 1981. Hemoglobin-heme incorporation into hepatic hemoproteins. *Fed. Proc.* 40:718
72. Bhat, K. S., Sardana, M. K., Padmanaban, G. 1977. Role of heme in the synthesis and assembly of cytochrome P-450. *Biochem. J.* 164:295–303
73. Correia, M. A., Farrell, G. C., Olson, S., Wong, J. S., Schmid, R., Ortiz de Montellano, P. R., et al. 1981. Cytochrome P-450 heme moiety: the specific target in drug-induced alkylation. *J. Biol. Chem.* 256:5466–70
74. Levin, W., Jacobson, M., Sernatinger, E., Kuntzman, R. 1973. Breakdown of cytochrome P-450 heme by secobarbital and other allyl-containing barbiturates. *Drug Metab. Disp.* 1:275–85
75. Ivanetich, K. M., Lucas, S., Marsh, J. A., Ziman, M. R., Katz, I. D., Bradshaw, J. J. 1978. Organic compounds: their interaction with and degradation of hepatic microsomal drug-metabolizing enzymes in vitro. *Drug. Metab. Disp.* 6:218–25
76. Schacter, B. A., Marver, H. S., Meyer, U. A. 1973. Heme and hemoprotein catabolism during stimulation of microsomal lipid peroxidation. *Drug Metab. Disp.* 1:286–92
77. Maines, M. D. 1980. Evidence for the catalytic activity of endogenous iron in the lipid peroxidative destruction of heme by allylisopropylacetamide in the rat liver. *Int. J. Biochem.* 12:781–85
78. Meyer, U. A., Schmid, R. 1978. The Porphyrias. In *The Metabolic Basis of Inherited Disease,* ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, pp. 1166–1220. New York: McGraw Hill. 4th ed. 1862 pp.
79. De Matteis, F. 1978. Hepatic porphyrias caused by 2-allyl-2-isopropylacetamide, 3,5-diethoxy-carbonyl-1,4-dihydrocollidine, griseofulvin and related compounds. In *Heme and Hemoproteins, Handbook of Experimental Pharmacology,* ed. F. De Matteis, W. N. Aldridge, 44:129–55. New York: Springer-Verlag. 449 pp.
80. Smith, A. G., De Matteis, F. 1980. Drugs and the hepatic porphyrias. *Clin. Haematol.* 9:339–425
81. Tephly, T. R., Gibbs, A. H., De Matteis, F. 1979. Studies on the mechanism of experimental porphyria produced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. Role of a porphyrin-like inhibitor of protohaem ferrolyase. *Biochem. J.* 180:241–44
82. De Matteis, F., Gibbs, A. H., Tephly, T. R. 1980. Inhibition of protohaem ferrolyase in experimental porphyria: isolation and partial characterization of a modified porphyrin inhibitor. *Biochem. J.* 188:145–52
83. De Matteis, F., Gibbs, A. H. 1980. Drug-induced conversion of liver heme into modified porphyrins. Evidence for two classes of products. *Biochem. J.* 187:285–88
84. Ortiz de Montellano, P. R., Kunze, K. L., Cole, S. P. C., Marks, G. S. 1980. Inhibition of hepatic ferrochelatase by the four isomers of N-methyl-protoporphyrin IX. *Biochem. Biophys. Res. Commun.* 97:1436–42
85. Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L. 1981. N-alkylprotoporphyrin IX formation in 3,5-dicarbethoxy-1,4-dihydrocollidine-treated rats. Transfer of the alkyl group from the substrate to the porphyrin. *J. Biol. Chem.* 256:6708–13
86. Ortiz de Montellano, P. R., Kunze, K. L., Cole, S. P. C., Marks, G. S. 1981. Differential inhibition of hepatic ferrochelatase by the isomers of N-ethylprotoporphyrin IX. *Biochem. Biophys. Res. Commun.* 103:581–86
87. Cole, S. P. C., Marks, G. S., Ortiz de Montellano, P. R., Kunze, K. L. 1982. Inhibition of ferrochelatase by N-methylprotoporphyrin IX is not accompanied by δ-aminolevulinic acid synthetase induction in chick embryo liver cell

culture. *Can. J. Physiol. Pharmacol.* 60: 212–15

88. De Matteis, F., Abbriti, G., Gibbs, A. H. 1973. Decreased liver activity of protoporphyrin-metal chelatase in hepatic porphyria caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. *Biochem. J.* 134:717–27
89. De Matteis, F., Jackson, A. H., Gibbs, A. H., Rao, K. R. N., Alton, J., et al. 1982. Structural isomerism and chirality of N-monosubstituted protoporphyrins. Possible relevance to binding of N-methyl protoporphyrin by ferrochelatase and to orientation of heme in cytochrome P-450. *FEBS Lett.* 142: 44–48
90. Schwartz, S., Stephenson, B., Sarkar, D., Freyholtz, H., Runge, W. 1976. Development and implications of experimental renal porphyria. In *Porphyrins in Human Diseases*, ed. M. Doss, pp. 370–79. Basel: Karger. 512 pp.
91. Condie, L. W., Baron, J., Tephly, T. R. 1976. Studies on adrenal δ-aminolevulinic acid synthetase. *Arch. Biochem. Biophys.* 172:123–29
92. Barnes, R., Jones, M. S., Jones, O. T., Porra, R. J. 1971. Ferrochelatase and δ-aminolevulate synthetase in brain, heart, kidney, and liver of normal and prophyric rats. *Biochem. J.* 124:633–37
93. Yoda, B., Schacter, B. A., Israels, L. G. 1974. Induction of δ-aminolevulinic acid synthetase in the kidney of chicks treated with porphyrinogenic drugs. *Biochem. Biophys. Acta.* 372:478–81
94. Levin, W., Jacobson, M., Kuntzman, R. 1972. Incorporation of radioactive δ-aminolevulinic acid into microsomal cytochrome P-450: selective breakdown of the hemoprotein by allylisopropylacetamide and carbon tetrachloride. *Arch. Biochem. Biophys.* 148:262–69
95. De Matteis, F. 1973. Drug-induced destruction of cytochrome P-450. *Drug Metab. Disp.* 1:267–74
96. Meyer, U. A., Marver, H. S. 1971. Chemically induced porphyria: increased microsomal heme turnover after treatment with allylisopropylacetamide. *Science* 171:64–66
97. Badawy, A. A.-B., Evans, M. 1973. The effects of chemical prophyrinogens and drugs on the activity of rat liver tryptophan pyrrolase. *Biochem. J.* 136:885–92
98. Meyer, U. A., Marver, H. S. 1971. Enhancement of the fractional catabolic rate of microsomal heme in chemically induced porphyria. *S. Afr. J. Lab. Clin. Med.* pp. 175–77 (Special issue)
99. White, I. N. H. 1982. Biliary excretion of green pigments produced by norethindrone in the rat. *Biochem. Pharm.* 31:1337–42
100. Bissell, D. M., Hammaker, L. E. 1977. Effect of endotoxin on tryptophan pyrrolase and δ-aminolevulinate synthetase: evidence for an endogenous regulatory heme fraction in rat liver. *Biochem. J.* 166:301–4
101. Schmid, R., Figen, J. F., Schwartz, S. 1955. Experimental porphyria: studies of liver catalase and other heme enzymes in Sedormid porphyria. *J. Biol. Chem.* 217:263–74
102. Granick, S., Urata, G. 1963. Increase in activity of δ-aminolevulinic acid synthetase in liver mitochondria induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine. *J. Biol. Chem.* 238: 821–27
103. De Matteis, F. 1967. Disturbances of liver porphyrin metabolism caused by drugs. *Pharmacol. Rev.* 19:523–57
104. Sweeney, G., Freeman, K. B., Rothwell, D., Lai, H. 1972. Decrease in hepatic cytochrome P-450 and catalase following allylisopropylacetamide: the effects of concomitant hemin administration. *Biochem. Biophys. Res. Commun.* 47:1366–71
105. Price, J. E., Sterling, W. R., Tarantola, V. A., Hartley, R. W., Rechcigl, M. 1962. The kinetics of catalase synthesis and destruction in vivo. *J. Biol. Chem.* 237:3468–75
106. Kawamata, F., Sakurai, T., Higashi, T. 1975. Biosynthesis of liver catalase in rats treated with allylisopropylacetylcarbamide. *J. Biochem.* 78:969–74
107. Schmid, R., McDonagh, A. F. 1978. Hyperbilirubinemia. See Ref. 78, pp. 1221–57
108. Landaw, S., Callahan, E. W. Jr., Schmid, R. 1970. Catabolism of heme in vivo: comparison of simultaneous production of bilirubin and carbon monoxide. *J. Clin. Invest.* 49:914–25
109. De Matteis, F., Gibbs, A. H., Unseld, A. 1977. Loss of heme from cytochrome P-450 caused by lipid peroxidation and 2-allyl-2-isopropylacetamide. *Biochem. J.* 168:417–22
110. Mackinnon, M., Sutherland, E., Simon, F. 1978. Qualitative alteration in hepatic microsomal cytochrome P-450 apoproteins associated with bile duct ligation, and the administration of ethinyl estradiol, phenobarbital and 3-methylcholanthrene. *Biochem. Pharmacol.* 27:29–35
111. Jounela, A. J. 1968. Effect of phenelzine on the rate of metabolism of pethidine. *Am. Med. Exp. Biol. Fenn.* 46:531–35