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Perspective

Metabolic Activation and Drug Toxicity

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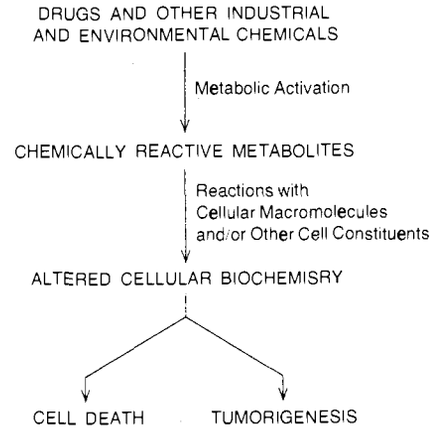
Introduction

Metabolism studies are an integral part of the testing procedures that are applied to evaluate the safety and efficacy of drugs, food additives, pesticides, and other industrial chemicals. Such studies generally provide a better understanding of a drug's mode of action, toxicity, and interactions with other drugs and sometimes provide insight into biochemical reaction mechanisms that are involved in biotransformation and toxicity. Hopefully, the insight that is provided by such studies can be used to increase the efficacy of the drug. In general terms, drug efficacy can be increased by increasing its potency, selectivity, and duration of action or by decreasing the probability of undesirable toxic reactions. The purpose of this paper is to examine the relationship between metabolism and toxicity for selected drugs and to describe some approaches for circumventing these drug-induced toxicities.

The biotransformation of relatively inert chemicals to highly reactive metabolites is commonly referred to as "metabolic activation", and is now recognized to be an obligatory initial event in several kinds of chemical-induced toxicities.[2] Reactive metabolites can be formed by most, if not all, of the enzymes that are involved in drug metabolism.[3] In some cases a single enzymatic reaction is involved, and in other cases several enzymatic and/or chemical reactions are involved in the production of an "ultimate" toxic metabolite. The toxic metabolites that are formed may interact with cells in numerous ways, such as by covalently binding to cellular constituents and/or by stimulating peroxidation and decomposition of cellular lipids. Scheme I is a very simplistic scheme for the role of metabolic activation in cellular toxicity.

Scheme I. Role of Metabolic Activation in Cellular Toxicity



There is a substantial body of information that is available on the metabolic reactions that produce reactive metabolites and on the chemical nature of the metabolites. However, considerably less is known about how these metabolites interact with cellular constituents, and virtually nothing is known about how the metabolite interaction with cellular constituents causes cell death or proliferation. Therefore, this paper will primarily focus on mechanisms of reactive metabolite formation, the chemical nature of toxic metabolites that are formed from selected drugs, and the approaches that a medicinal chemist might use to circumvent the formation of such metabolites.

Mechanisms of Reactive Metabolite Formation

Enzymes That Catalyze the Reactions. Drugs and other chemicals that do not occur normally in the body are metabolized by a wide variety of enzymes. In 1959, Williams[4] classified the reactions that are catalyzed by

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(2) For additional background the reader is referred to several books and reviews: (a) "Biological Reactive Intermediates", D. J. Jollow, J. J. Kocsis, R. Snyder, and H. Vainio, Eds., Plenum Press, New York, 1977; "Biological Reactive Intermediates II: Chemical Mechanisms and Biological Effects", Plenum Press, New York, in press. (b) "Reviews in Biochemical Toxicology", Vol. 1 and 2, E. Hodgson, J. R. Bend, and R. M. Philpot, Eds., Elsevier/North-Holland, New York, 1979 and 1980. (c) E. C. Miller and J. A. Miller, *Pharmacol. Rev.*, 18, 805 (1966). (d) P. N. Magee, *Essays Biochem.*, 10, 105 (1974). (e) J. R. Gillette, J. R. Mitchell, and B. B. Brodie, *Annu. Rev. Pharmacol.*, 14, 271 (1974). (f) S. D. Nelson, M. R. Boyd, and J. R. Mitchell, in "Drug Metabolism Concepts", D. M. Jerina, Ed., American Chemical Society, Washington, DC, 1977, p 155.

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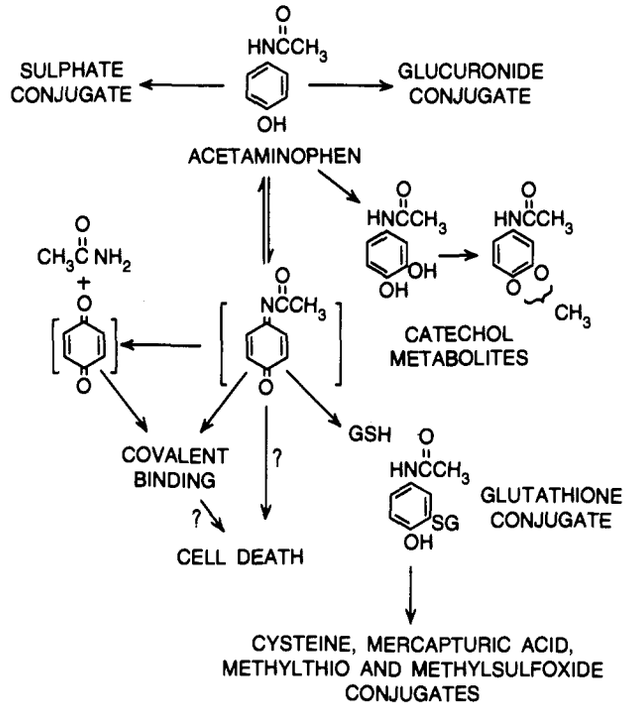
Scheme II. Metabolism of Acetaminophen

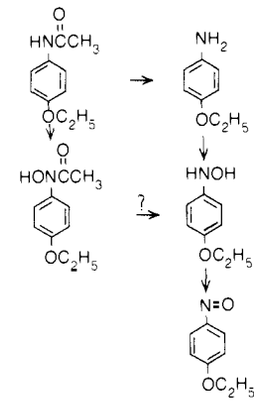


these enzymes into two general phases. Phase I reactions include oxidations, reductions, and hydrolyses, whereas phase II reactions are broadly defined as conjugation reactions and include glucuronidation, sulfation, acylation, methylation, and conjugation with glutathione. Most drugs are metabolized to more polar compounds that are rapidly removed from the body, and some are transformed to therapeutically active metabolites. However, the enzymes that catalyze these processes also catalyze the formation of reactive, toxic metabolites from some drugs. Clearly, the differing structural features of the drug and its metabolites determine whether or not the metabolic reaction produces a detoxification product or a potentially toxic metabolite, and not differences in the nature of the catalytic reaction.

Examples of Drugs That Are Metabolized to Reactive Products. In order to better define the structural features of drug molecules that promote reactive metabolite formation, we must first examine the structures of chemicals that are known to be metabolically activated to toxic substances, and we must examine the metabolic reactions that are involved in the activation process. Chart I depicts the structures of several drugs whose toxicities have been linked to metabolic activation.

Acetanilides. Various toxicological consequences have been observed in man and animals following the use of the para-substituted acetanilide drugs acetaminophen, phenacetin, and practolol (Chart I). The classical studies of Mitchell and co-workers[5] demonstrated that the widely used analgesic acetaminophen is oxidized by hepatic microsomal cytochrome P-450 to a reactive metabolite that, under the conditions employed in their assay, irreversibly binds to hepatic proteins. In situations where the amount of acetaminophen that proceeds by this oxidative pathway is large enough to overwhelm protective mechanisms,

Scheme III. A Partial Scheme for the Metabolism of Phenacetin



binding levels increase and hepatic cell death ensues (Scheme II). Evidence for the electrophilic nature of the reactive metabolite comes from studies that demonstrate a protective role for the nucleophilic sulfhydryl-containing tripeptide, glutathione,[6] and from studies that elucidate the structure of the acetaminophen–glutathione adduct.[7] Additional studies have provided strong support for *N*-acetyl-*p*-benzoquinone imine (NAPQI) as the initial oxidation product and major ultimate toxic metabolite that is formed from acetaminophen.[8] Although an intermediate N-hydroxylation product was initially proposed,[5b] recent investigations strongly indicate that NAPQI is formed in a direct oxidation reaction of acetaminophen by P-450 and peroxidases.[9] NAPQI can then undergo nucleophilic addition reactions, hydrolysis to acetamide and *p*-benzoquinone, and reduction back to acetaminophen (Scheme II). Thus, several lines of evidence support a specific role for the generation of a reactive electrophilic metabolite of acetaminophen by hepatic microsomal P-450 as an obligatory initial event in liver necrosis caused by acetaminophen.

Acetaminophen can also cause kidney damage and ocular damage.[10] Although results of various investigations indicate that metabolic activation is required to illicit the toxic response, a clear definition has not been made either as to which enzymes are involved in forming a toxic

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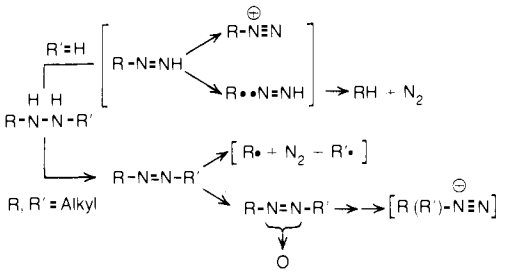
product or as to the nature of the toxic product.[11] Whereas cytochrome P-450 is localized more in the renal cortex, prostaglandin synthetase is localized more in the renal medulla, and both enzymes are capable of oxidizing acetaminophen to reactive metabolites.[11d,f] Furthermore, as the dose of acetaminophen increases, a greater proportion of the dose of the drug is hydrolyzed by amidases to *p*-aminophenol, a known nephrotoxin.[11e] Therefore, it has not been established which pathways or metabolites are most responsible for kidney damage caused by acetaminophen, although it has been fairly well established that the reactive metabolite(s) is generated in the organ that is damaged and is not transported to the target organ from another tissue.[11a,12]

Phenacetin (4′-ethoxyacetanilide) is a congener of acetaminophen with similar therapeutic activity. This mild analgesic–antipyretic agent has been removed from most drug formulations because of peculiar toxicities that have been associated with abusive use of analgesic mixtures that contain phenacetin.[10a] In contrast to acetaminophen, phenacetin does not cause liver injury in man, but it does cause a special type of interstitial nephritis called analgesic nephropathy and may also be responsible for the development of renal pelvic tumors. Unfortunately, phenacetin has not been found to cause these special kinds of kidney damage in animals. This illustrates a major dilemma in chemical toxicology. The lack of a good animal model for a toxic reaction that is observed in man makes it very difficult to establish a role for metabolic activation.

The metabolism of phenacetin in man and animals is well characterized and several reactive metabolites are formed (Scheme III). *N*-Hydroxyphenacetin was prepared by Calder et al.[13] almost a decade ago and was postulated to be a proximate nephrotoxin. Subsequent studies have shown that *N*-hydroxyphenacetin is a hepatocarcinogen in rats,[14] and it forms two mutagenic metabolites, *p*-ethoxyphenylhydroxylamine and *p*-ethoxynitrosobenzene, by deacetylation and further oxidation.[15] Furthermore, glucuronidation and sulfation of *N*-hydroxyphenacetin form hydroxamic acid conjugates that spontaneously decompose to a reactive intermediate that has all of the chemical characteristics of NAPQI.[16] In addition to these pathways, oxidation of phenacetin to arylating and alkylating intermediates via 3′,4′-epoxidation has been proposed to account for oxygen incorporation from molecular oxygen into the 4′-position of phenacetin.[17]

Scheme IV. A Partial Scheme Describing the Metabolism of Monosubstituted Hydrazines and 1,2-Dialkyl-Substituted Hydrazines to Reactive Metabolites



Although reactive metabolites of phenacetin are formed by numerous routes, the only toxic reaction that is caused by phenacetin in man that has been successfully modeled in animals is methemoglobin formation.[18] Inasmuch as deacetylation of phenacetin must precede methemoglobin formation[19] and because selective deuteration of the ethoxy group methylene carbon decreases the rate of oxidative dealkylation of phenacetin to acetaminophen and enhances the production of methemoglobin,[20] it is speculated that oxidative metabolites of *p*-phenetidine cause the methemoglobinemia. This hypothesis is consistent with reports that many aromatic nitroso and hydroxylamino compounds oxidize hemoglobin.[21] Thus, O-ethylation of acetaminophen has rendered an analogue that also forms reactive metabolites; however, the metabolite spectrum is different, and the toxicities associated with phenacetin abuse are quite different than those associated with acetaminophen abuse.

Another congener of acetaminophen, practolol, is a cardioselective β-blocking drug that was never introduced in the U.S. because it gave rise to skin and eye lesions in some patients.[22] The radiolabeled drug has been found to be oxidatively metabolized to a reactive product that binds irreversibly to tissue proteins.[23] Although it has been postulated that the altered protein may be antigenic and that the toxicity associated with the use of practolol arises from immunological reactions,[24] direct experimental evidence for this hypothesis is lacking and may be difficult to obtain in laboratory animals whose immune system is quite different than that of man.

Hydrazines. The studies with practolol suggest that some drugs can cause serious toxic effects when administered in therapeutic doses. Investigations of the toxic reactions caused by several hydrazide and hydrazine drugs further implicate metabolic activation as a cause of tissue injury after therapeutic doses of a drug. Possible modes of metabolism of hydrazines to reactive intermediates are

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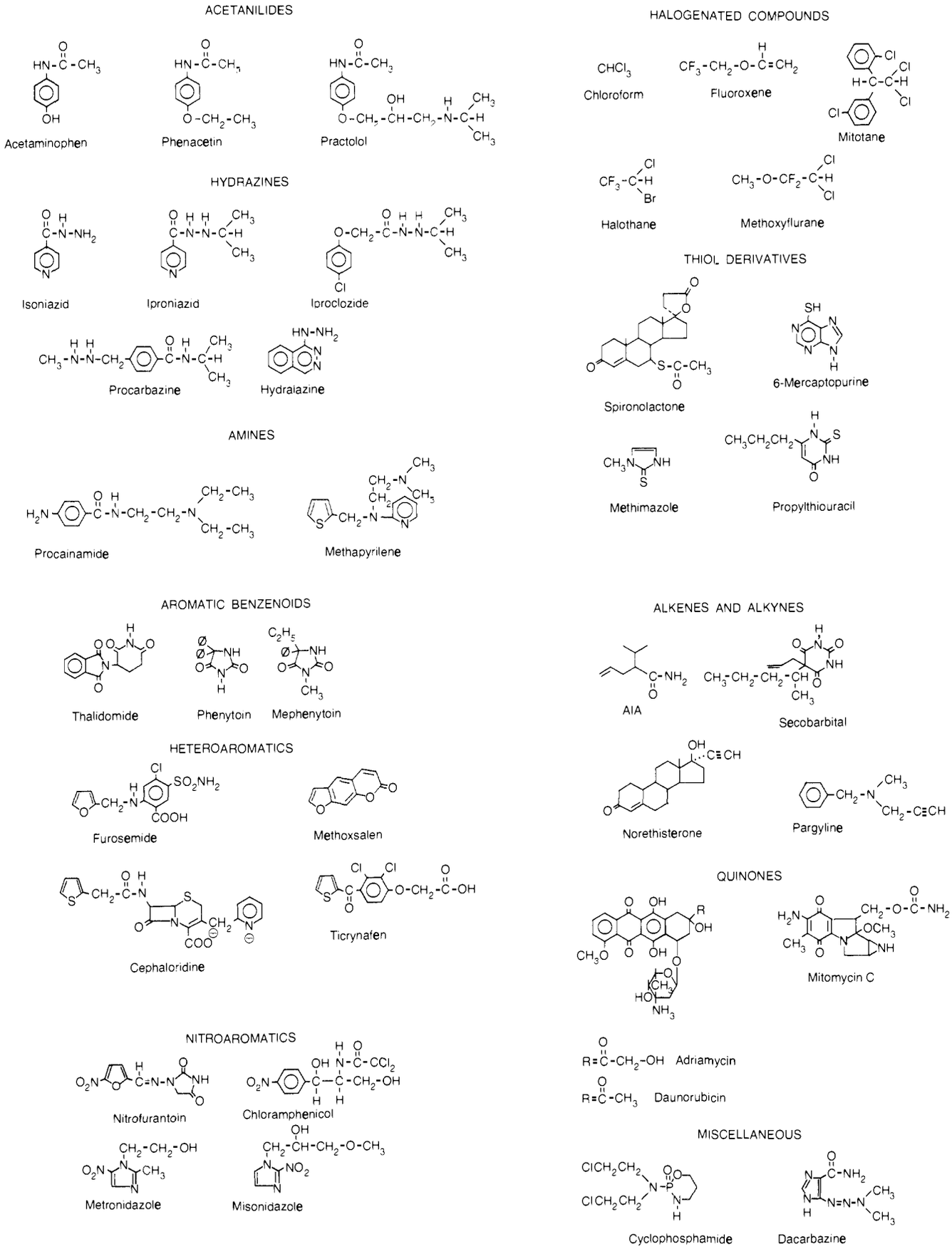
Chart I. Chemical Classes and Structures of Drugs Whose Toxicities Have Been Linked to Metabolic Activation



depicted in Scheme IV and are based on research with the following drugs.

Isoniazid (Chart I), a widely used antitubercular drug, can cause liver damage in some patients, and prospective studies of several thousand patients receiving isoniazid prophylactically revealed that serious hepatotoxicity was associated with those patients who had a rapid acetylator phenotype.[25] Further studies provided evidence that a

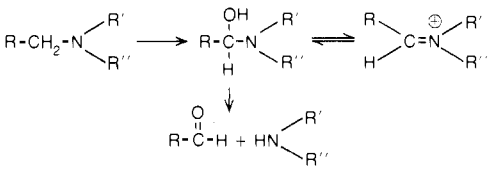
metabolite of isoniazid, acetylhydrazine, is oxidized by a liver microsomal oxygenase to a reactive acetylating agent, the formation of which parallels hepatic injury in rats.[26] Although acetylhydrazine is also a metabolite that is formed from isoniazid in man, a direct relationship between its further metabolism and susceptibility to hepatic injury has not been established.[27]

Iproniazid (Chart I) is a MAO inhibitor antidepressant that was removed from clinical therapy because of a high incidence of hepatocellular injury associated with its use.[28] Iproniazid is hydrolyzed to isopropylhydrazine, which is a potent hepatotoxin in rats.[29] The hepatotoxicity of iproniazid parallels microsomal enzyme-mediated oxidation of isopropylhydrazine to a reactive metabolite that becomes covalently bound to liver proteins and that forms propane.[26b,29] Propane formation requires that isopropylhydrazine be oxidized to an intermediate that generates a propyl radical. Thus, oxidation of monosubstituted hydrazines to diazenes by P-450 and a microsomal FAD-containing monooxygenase has been proposed[26,30] inasmuch as diazenes readily decompose homolytically to yield alkanes and nitrogen.[31] In fact, radicals have been detected in the microsomal oxidation of hydrazines,[32] and nitrogen evolution has been recorded.[33]

Interestingly, other hydrazine antidepressants, including iproclozide (Chart I), have been found to cause fulminant hepatitis in several patients.[34] The structural feature of iproclozide which is common to iproniazid is the isopropylhydrazine moiety, and isopropylhydrazine probably is a major hydrolytic metabolite of iproclozide.

Procarbazine (Chart I) is a 1,2-disubstituted hydrazine antitumor agent that is used in several combination chemotherapy regimens.[35] Like many other 1,2-disubstituted hydrazines, procarbazine itself has been found to be mutagenic, carcinogenic, and teratogenic in several assay systems in vitro and in vivo in rodents and nonhuman primates, and there is evidence that it is toxic in man as well.[35,36] Although direct evidence is lacking, it is likely that metabolic activation of procarbazine is required for both its therapeutic activity and toxicity. Procarbazine is oxidatively metabolized both in vitro and in vivo to its azo and azoxy derivatives, as is the potent model carcinogen 1,2-dimethylhydrazine.[37] The azo metabolite is capable of generating radical intermediates, and the azoxy isomers can potentially be further oxidized to diazonium species. The role that these metabolites play in the activity and toxicity of procarbazine remains to be assessed.

Scheme V. Proposed Scheme Showing Intermediates and Products of Oxidative N-Dealkylation



The major adverse effect of the widely used antihypertensive drug hydralazine (Chart I) is an immunological syndrome that resembles systemic lupus erythematosus (SLE) or rheumatoid arthritis.[38] Like other monosubstituted hydrazines, hydralazine is polymorphically acetylated in man, and the development of SLE occurs almost exclusively in slow acetylators who produce lesser amounts of acetylated metabolites and larger amounts of oxidized metabolites.[39] Recently, it has been demonstrated that oxidation of radiolabeled hydralazine by hepatic microsomal P-450 also produces a reactive metabolite that binds to tissue macromolecules.[40] Thus, the possibility has been raised that such an adduct may be antigenic.

Amines. Procainamide (Chart I) is a widely used antiarrhythmic drug which gives rise to an SLE syndrome indistinguishable from that caused by hydralazine and similarly preponderant in the slow acetylator phenotype.[41] Evidence has been presented for the formation of a reactive metabolite of procainamide that is catalyzed by a cytochrome P-450 monooxygenase enzyme, and the metabolite is thought to be the N-hydroxylated compound.[42] *N*-Acetylprocainamide, the major metabolite of procainamide, formed considerably less reactive metabolite under the conditions of the covalent binding assay that was employed. This result is highly significant, since (1) SLE has not been observed in patients receiving *N*-acetylprocainamide, (2) antibodies to nuclear antigens, a serologic feature of SLE development, are formed much more slowly in patients receiving *N*-acetylprocainamide, and (3) *N*-acetylprocainamide is an active antiarrhythmic agent.[43] Thus, the metabolism studies that have been discussed have led to the introduction of a safer antiarrhythmic drug and have implicated reactive metabolite formation as a cause of an immunological toxicity.

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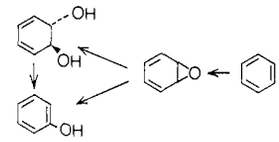
(43) (a) R. Lahita, J. Kluger, D. E. Drayer, D. Koffler, and M. M. Reidenberg, *N. Engl. J. Med.*, **301**, 1382 (1979). (b) D. Drayer, J. Kluger, M. Reidenberg, and R. Lahita, *Drug Metab. Rev.*, **10**, 239 (1979).

Methapyrilene (Chart I), a histamine H_1-receptor antagonist, was a constituent of many sleeping aids and antihistaminics until it was identified as a potent hepatocarcinogen in rats.[44] Morphometric analysis of electron micrographs from rat liver showed extensive ultrastructural changes in periportal hepatocytes, with significant increases in the number of mitochondria, and the administration of radiolabeled methapyrilene resulted in selective concentration of bound activity in the mitochondria of periportal hepatocytes. Electrophilic imminium ions have been trapped with cyanide ion during the metabolism of methapyrilene by rabbit liver microsomal preparations in studies which paralleled the trapping of reactive imminium ion metabolites formed from nicotine.[45] Based on these studies, any drug that undergoes oxidative dealkylation would be expected to generate reactive imminium ions, presumably via dehydration of initially formed carbinolamines (Scheme V). Such a mechanism has been proposed, in fact, to account for suicide inactivation of P-450 by cyclopropylamines, although it is now believed that radical cation intermediates of the cyclopropylamino group are the immediate metabolites that react irreversibly to destroy the P-450.[46] Although such metabolites may be formed, and may react with nucleophilic groups on biological macromolecules, the question of their significance in perpetrating toxicological events is unresolved at the present time, since no study has correlated this metabolic event to a toxic end point.

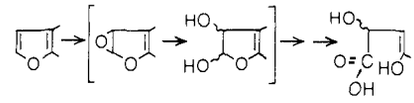
Benzenoid Aromatics. Several benzenoid aromatic compounds are metabolized by cytochrome P-450 to arene oxides, highly reactive products with toxic potential.[47] Indirect evidence has recently been published which supports a role for arene oxide metabolites in initiating various toxic reactions of some drugs.

Thalidomide (Chart I) was identified as a teratogen over 20 years ago.[48] Species-specific embryotoxicity and metabolism is marked inasmuch as the drug is metabolized to phenol metabolites in rabbits, an animal species that is susceptible to the toxicity, and is not metabolized (at least measurably) to phenol metabolites by rats, an animal species that is not susceptible to thalidomide-induced embryotoxicity.[49] Evidence for the formation of a toxic arene oxide metabolite of thalidomide has come from studies in vitro that employed microsomal preparations from susceptible (rabbit, monkey, and man) and nonsusceptible species (rat) and human lymphocytes as a target tissue for the generated toxin.[50] Results correlated well with the embryotoxicity observed in vivo, and the probable role of an arene oxide metabolite in cytoxicity was determined by using an inhibitor of epoxide hydrolase and by adding purified epoxide hydrolase to the incubation media. Consistent with the hypothesis was the finding that hexahydrothalidomide (the saturated hexyl analogue) was neither cytotoxic nor teratogenic. The only anomolous result was that phthalimide itself is nontoxic and yet maintains the aromatic structure.

Scheme VI. Simplified Scheme for Dihydrodiol and Phenol Formation from Benzenoid Compounds



Scheme VII. Simplified Scheme for the Formation of Oxidized Products of Furans



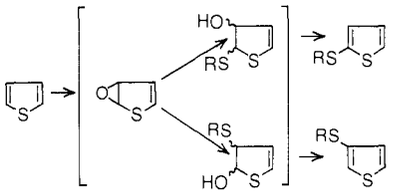
The cytotoxicity of the anticonvulsant drugs, phenytoin and mephenytoin (Chart I) have also been examined in the microsome–lymphocyte system, and indirect evidence was obtained for the intermediacy of arene oxide metabolites.[51] Phenytoin is a widely used anticonvulsant drug which is teratogenic in animals[52] and is a suspected teratogen in man.[53] On rare occasions it has caused overt hepatotoxic effects in man.[54] The teratogenicity of phenytoin in mice has been correlated to reactive metabolite formation by comparing the extent of covalent binding of radiolabeled drug in gestational tissues and the extent of teratogenic injury.[55] The parameters paralleled one another; for example, pretreatment of the mother with an inhibitor of epoxide hydrolase, 1,2-epoxy-3,3,3-trichloropropane, increased both the incidence of phenytoin-induced cleft palate and the extent of covalent binding of radiolabel in the newborn. This result is consistent with the hypothesis that arene oxide formation is an initial step in phenytoin-induced teratogenicity. That this may also be the case in the newborn human is supported by the finding that a dihydrodiol metabolite of phenytoin has been detected in the newborn human when exposed in utero to the drug.[56] Phenols and dihydrodiols are both products of arene oxides (Scheme VI).

Heteroaromatics. Several furans, thiophenes, and pyrroles are metabolically activated to products that can cause liver, lung, and/or kidney necrosis.[57] A few drugs that contain these heteroaromatic structures cause similar toxic reactions in man and laboratory animals. Based on their aromatic character, we might expect that arene oxides would be likely oxidative metabolites of furan, thiophene, and pyrrole. Although there is evidence for such reactive metabolites, at least for furans and thiophenes, none have been prepared or observed probably because of their rapid rearrangement to more stable products.

Furosemide (Chart I), an important diuretic agent, can cause hepatic necrosis in the mouse when administered in

(44) (a) W. Lijinsky, M. D. Reuber, and B. N. Blackwell, *Science*, **209**, 817 (1980). (b) H. M. Reznik-Schüller and W. Lijinsky, *Arch. Toxicol.*, **49**, 79 (1981).
(45) (a) R. Ziegler, B. Ho, and N. Castagnoli, Jr., *J. Med. Chem.*, **24**, 1133 (1981). (b) T.-L. Nguyen, L. D. Gruenke, and N. Castagnoli, Jr., *ibid.*, **22**, 259 (1979).
(46) R. P. Hanzlik, V. Kishore, and R. Tullman, *J. Med. Chem.*, **22**, 759 (1979), and personal communication.
(47) D. A. Jerina and J. W. Daly, *Science*, **185**, 573 (1974).
(48) W. G. McBride, *Lancet*, **2**, 1350 (1961).
(49) H. Schumacher, R. L. Smith, and R. T. Williams, *Br. J. Pharmacol.*, **25**, 338 (1965).
(50) G. B. Gordon, S. P. Spielberg, D. A. Blake, and V. Balasubramanian, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2545 (1981).
(51) S. P. Spielberg, G. B. Gordon, D. A. Blake, E. D. Mellits, and D. S. Bross, *J. Pharmacol. Exp. Ther.*, **217**, 386 (1981).
(52) R. D. Harbison and B. A. Becker, *Teratology*, **2**, 305 (1969).
(53) S. R. Meadow, *Lancet*, **2**, 1296 (1968).
(54) T. J. Lee, C. N. Carney, J. L. Lapis, T. Higgins, and H. J. Fallon, *Gastroenterology*, **70**, 422 (1976).
(55) F. Martz, C. Failinger III, and D. A. Blake, *J. Pharmacol. Exp. Ther.*, **203**, 231 (1977).
(56) M. G. Horning, C. Stratton, A. Wilson, E. C. Horning, and R. M. Hill, *Anal. Lett.*, **4**, 537 (1971).
(57) (a) R. J. McMurtry and J. R. Mitchell, *Toxicol. Appl. Pharmacol.*, **42**, 285 (1977). (b) M. R. Boyd, *CRC Crit. Rev. Toxicol.*, **9**, 103 (1980). (c) P. W. Jennings, J. C. Hurley, S. K. Reeder, A. Holian, P. Lee, C. N. Caughlan, and R. D. Larsen, *J. Org. Chem.*, **41**, 4078 (1976). (d) C. C. J. Culvenor, J. A. Edgar, M. V. Jago, A. Outteridge, J. E. Peterson, and L. W. Smith, *Chem.-Biol. Interact.*, **12**, 299 (1976).

Scheme VIII. Simplified Scheme for the Formation of Mercapturic Acid Metabolites of Thiophenes

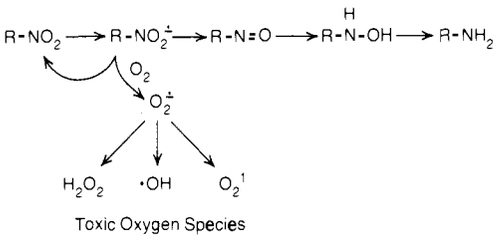


large doses,[58] and reports of hepatotoxicity caused by furosemide in man have appeared.[59] Furosemide is oxidized by hepatic cytochrome P-450 dependent monooxygenases to a reactive metabolite that becomes covalently bound to liver protein, and the extent of binding correlates with the extent of liver necrosis.[59b] Studies that utilized specific labeling of furosemide with both stable isotopes and radiosotopes showed that the binding to proteins occurred through the furan ring.[59c] Furthermore, binding was enhanced by the epoxide hydrolase inhibitor 1,2-epoxy-3,3,3-trichloropropane, thus implicating a furan epoxide as the reactive metabolite. Consistent with this hypothesis was the lack of hepatotoxicity and binding of the tetrahydrofuranyl analogue of furosemide.

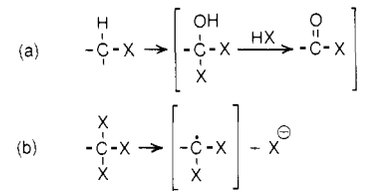
Methoxsalen (Chart I) and other furocoumarins (psoralens) are photochemotherapeutic agents used in the treatment of psoriasis, vitiligo, and mycosis fungoides.[60] The psoralens intercalate with DNA, and the intercalated drug forms cross-links with DNA when irradiated with UV light (320–400 nm).[61] This process can also lead to cytotoxic and mutagenic events. In addition, the furocoumarins are extensively metabolized in man and laboratory animals by oxidation of the furan ring to metabolites suggestive of the intermediacy of furan epoxides (Scheme VII).[62] Thus, an assessment of benefit vs. risk must be carefully made before initiating therapy with the psoralens.

The thiophene nucleus is found in several available and investigational drugs.[63] Although various toxic effects are associated with the use of thiophene-containing drugs, no convincing evidence has been published that illustrates a requirement for metabolic activation of the thiophene moiety in toxicity. Methapyrilene (see Chart I, amines) is a hepatocarcinogen that contains the thiophene structure, and the published work on this drug has already been discussed. Cephaloridine (Chart I) is a known renal toxin in man, and it has been proposed that cytochrome P-450 mediated oxidation in rat kidney is required for the renal tubular necrosis caused by this cephalosporin in the rat.[57a] However, this hypothesis is largely based on the effects of two known inhibitors of cytochrome P-450 which may have other effects on kidney transport mechanisms. Ticrynafen (Chart I) is a widely acclaimed diuretic–uricosuric

Scheme IX. Proposed Scheme for the Reductive Metabolism of Nitro Aromatics to Toxic Products



Scheme X. Oxidation (a) and Reduction (b) of Halogenated Alkyl



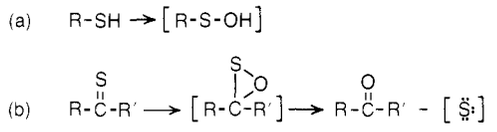
agent that was marketed in May 1979 and recalled in January 1980 after several reports of hepatic and renal injury appeared.[64] Toxicological studies in rats revealed that ticrynafen was mildly hepatotoxic at high dose levels in some animals; however, no attempt was made to determine the pathogenesis of the toxicity.[65]

Although thiophene-containing drugs cause various toxicities, the only studies that implicate a role for metabolic activation of the thiophene ring in toxic reactions have been carried out with model compounds.[57a] Thiophene itself is hepatotoxic, and mercapturic acid metabolites of thiophene (Scheme VIII) have been identified in the urine of both rats and rabbits treated with thiophene.[66] This provides a priori evidence for the formation of an epoxide metabolite of thiophene. However, further work is necessary to confirm and extend these findings.

Nitroaromatics. Metabolic activation of nitroaromatic compounds is distinct from that of other aromatic compounds. The primary pathways of biotransformation to toxic, as well as therapeutically active, metabolites involve the initial reduction of the nitro group to stabilized nitro anion radicals (Scheme IX).[67] Under aerobic conditions, the radical can reduce molecular oxygen to form superoxide anion, which can form various toxic oxygen species[68] that have been implicated in the pathogenesis of lung injury caused by nitrofurantoin (Chart I).[57b] In a more anaerobic environment, the nitro anion radical can be reduced further to nitroso, hydroxylamine, and amine metabolites. Although nitroso and hydroxylamine metabolites are often not detected, various products formed during the metabolism of the antibacterial drug nitrofurantoin[69] and the antiprotozoal drug metronidazole[70]

(58) (a) J. R. Mitchell, W. Z. Potter, J. A. Hinson, and D. J. Jollow, *Nature (London)*, **251**, 508 (1974). (b) J. R. Mitchell, W. L. Nelson, W. Z. Potter, H. Sasame, and D. J. Jollow, *J. Pharmacol. Exp. Ther.*, **199**, 41 (1976). (c) P. J. Wirth, C. J. Bettis, and W. L. Nelson, *Mol. Pharmacol.*, **12**, 759 (1975).
(59) R. M. Walker and T. F. McElligott, *J. Pathol.*, **135**, 301 (1981).
(60) K. Wolff, T. B. Fitzpatrick, J. A. Parrish, F. Gschnait, B. Gilchrest, H. Honigsmann, M. A. Pathak, and L. Tannenbaum, *Arch. Dermatol.*, **112**, 943 (1976).
(61) M. J. Ashwood-Smith and E. Grant, *Experientia*, **33**, 384 (1976), and references therein.
(62) (a) S. J. Kolis, T. H. Williams, E. J. Postma, G. J. Sasso, P. N. Confalone, and M. A. Schwartz, *Drug Metab. Dispos.*, **7**, 220 (1979). (b) B. B. Mandula and M. A. Pathak, *Biochem. Pharmacol.*, **28**, 127 (1979).
(63) R. Böhn and G. Zeiger, *Pharmazie*, **35**, 1 (1980).
(64) *FDA Drug Bull.*, **10**, 3, (1980).
(65) C. Oker-Blom, J. Mäkinen, and G. Gothoni, *Toxicol. Lett.*, **6**, 93 (1980).
(66) H. G. Bray, F. M. B. Carpanini, and B. D. Waters, *Xenobiotica*, **1**, 157 (1971).
(67) For a review, see R. P. Mason, ref 2b, Vol. 1, p 151.
(68) J. M. McCord, ref 2b, Vol. 1, p 109.
(69) (a) M. B. Aufrère, B. A. Horner, and M. Vore, *Drug Metab. Dispos.*, **6**, 403 (1978). (b) M. R. Boyd, A. Stiko, and H. A. Sasame, *Biochem. Pharmacol.*, **28**, 601 (1979). (c) F. J. Peterson, R. P. Mason, J. Hovespian, and J. L. Holtzman, *J. Biol. Chem.*, **254**, 4009 (1979).
(70) R. L. Koch, E. J. T. Chrystal, B. B. Beaulieu, Jr., and P. Goldman, *Biochem. Pharmacol.*, **28**, 3611 (1979).

Scheme XI. Postulated Reactive Metabolites of Thiols (a) and Thiono (b) Carbonyl Compounds



implicate these reduced products as intermediates which are possibly toxic.

The 2-nitroimidazole, misonidazole (Chart I), is a chemical radiosensitizing agent by virtue of its high electron affinity.[71] Thus, hypoxic tumor cells can reductively metabolize this drug to produce toxic intermediates similar to those already described. Serious blood dyscrasia, such as aplastic anemia, limit the use of the effective antibiotic chloramphenicol (Chart I). A role for metabolism of the nitro group has not been established as a cause of the toxicity. However, this toxic reaction has not been observed with thiamphenicol, an analogue in which the nitro group has been replaced by a sulfonylmethyl group.[72]

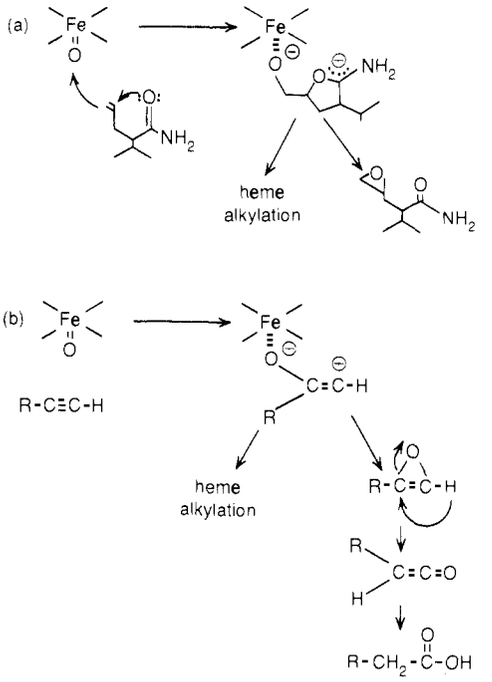
Halogenated Compounds. A separate pathway for the formation of a reactive metabolite of chloramphenicol involves oxidative dehalogenation of the dichloroacetamido group to an oxamyl chloride.[73] The oxamyl chloride either spontaneously hydrolyzes to the corresponding oxamic acid or acylates a lysine residue on cytochrome P-450, which inactivates the enzyme.[74] However, inasmuch as no useful animal model is known for chloramphenicol-induced aplastic anemia, attempts to correlate metabolic activation of chloramphenicol with its toxicity have not been realized.

In contrast, the formation of phosgene has been shown to be responsible for both hepatic and renal toxicity caused by chloroform, a solvent which previously has been used as a general anesthetic and as a preservative–flavor enhancer in pharmaceutical products.[75] Several other inhalation anesthetics (Chart I) are also viscerotoxic, including halothane, fluoroxene, and methoxyflurane. Biotransformation of the anesthetics appears to be required for the development of toxicity, and both oxidative and reductive reactions have been observed (Scheme X).[76]

Mitotane [*o,p'*-DDD, 1-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-2,2-dichloroethane] (Chart I) provides an interesting example of target organ toxicity as a result of enhanced metabolic activation in a particular tissue.[77] Mitotane is employed for the treatment of inoperable adrenocortical carcinoma because of its rather selective adrenocorticolytic activity. In fact, in man and dog the rate of formation of reactive metabolites that covalently bind to tissue proteins is greatest in adrenocortical mitochondria. Although metabolism studies have not been reported to substantiate the hypothesis, investigations with structural analogues indicate that activation is occurring at the C-2 dihalogenated position.[78]

Thiol Derivatives. Thiol and thiono sulfur-containing compounds exhibit various toxic properties, including liver and lung damage, bone-marrow depression, neoplasia, and

Scheme XII. Proposed Mechanism for P-450 Metabolism of Allylisopropylacetamide (a) and Alkynes (b)

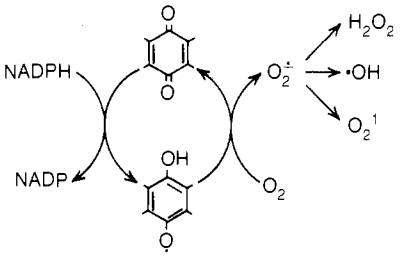


hormonal imbalance.[79] Many of these compounds destroy cytochrome P-450 by suicide inactivation of the hemoprotein. Spironolactone (Chart I), a diuretic and antihypertensive steroid, causes a specific loss of adrenal and testicular microsomal cytochrome P-450 in a reaction that requires oxidation of the deacetylated thiol and binding of the thiol to the apoprotein.[80] A similar sequence of events occurs with the antineoplastic drug 6-thiopurine (Chart I), and evidence was presented for the intermediacy of a highly reactive sulfenic acid metabolite.[81] On the other hand, oxidation of the thiono sulfur drugs methimazole and propylthiouracil (Chart I) is thought to yield electrophilic atomic sulfur (Scheme XI).[79a]

Alkenes and Alkynes. Some drugs that contain alkene and alkyne functional groups have been linked to drug-induced porphyria.[82] Many of these drugs are oxidized by cytochrome P-450 and cause its destruction with concomitant formation of "green pigment" altered heme. Thus, they are suicide, irreversible inhibitors of cytochrome P-450.[83] Initial work on barbiturates showed the importance of an allyl group in causing destruction of cytochrome P-450 by such drugs as allylisopropylacetamide (AIA) and secobarbital (Chart I).[84] Extensive investigations by Ortiz de Montellano and co-workers[83,85] have elucidated the nature of the reactive metabolite that is formed by AIA and its allylation of heme nitrogen (Scheme XIIa).

(71) G. E. Adams, *Br. Med. Bull.*, **29**, 48 (1973).
(72) R. A. Cutler, R. J. Stenger, and C. M. Suter, *J. Am. Chem. Soc.*, **74**, 5475 (1952).
(73) L. R. Pohl, S. D. Nelson, and G. Krishna, *Biochem. Pharmacol.*, **27**, 491 (1978).
(74) J. Halpert, *Biochem. Pharmacol.*, **30**, 875 (1981).
(75) L. R. Pohl, in ref 2b, Vol. 1, p 79.
(76) For a review, see R. W. Vaughan, I. G. Sipes, and B. R. Brown, Jr., *Life Sci.*, **23**, 2447 (1978).
(77) F. Martz and J. A. Straw, *Drug Metab. Dispos.*, **8**, 127 (1980).
(78) F. A. Cobey, I. Taliaferro, and H. B. Haag, *Science*, **123**, 140 (1956).
(79) (a) R. A. Neal, in ref 2b, Vol. 2, p 131. (b) R. H. Menard, D. L. Loriaux, F. C. Bartter, and J. R. Gillette, *Steroids*, **31**, 771 (1978).
(80) R. H. Menard, T. M. Guenther, A. M. Taburet, H. Kon, L. R. Pohl, J. R. Gillette, H. V. Gelboin, and W. F. Trager, *Mol. Pharmacol.*, **16**, 997 (1979).
(81) R. M. Hyslop and I. Jardine, *J. Pharmacol. Exp. Ther.*, **218**, 621 (1981).
(82) F. D. Matteis, *Pharmacol. Rev.*, **19**, 523 (1967).
(83) For a review, see B. Testa and P. Jenner, *Drug Metab. Rev.*, **12**, 1 (1981).
(84) W. Levin, E. Sernatinger, M. Jacobson, and R. Kuntzman, *Science*, **176**, 1341 (1972).
(85) P. R. Ortiz de Montellano, G. S. Yost, B. A. Mico, S. E. Dinizio, M. A. Correira, and H. Kambara, *Arch. Biochem. Biophys.*, **197**, 524 (1979).

Scheme XIII. Proposed Mechanism for the Metabolism of Anthracycline Quinones to Generate Reactive Oxygen Species

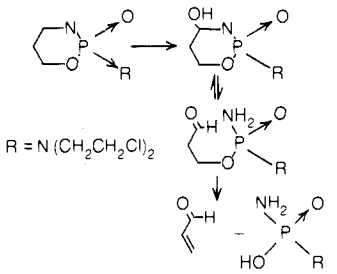


It is noteworthy that the allyl barbiturates (e.g., secobarbital) also have γ-carbonyl oxygen atoms that can stabilize incipient carbonium ions which might be formed at the substituted vinylic carbon atom. However, this structural feature is not required for cytochrome P-450 destruction by several other olefins and ethynyl substrates,[86] and mechanisms involving radical cation formation have been proposed to account for the results.[87] Furthermore, both chemical and metabolic oxidation of the acetylenic π bond, such as would occur in metabolism of the ethynyl sterol norethisterone (Chart I), was found to proceed with a 1,2-shift of the terminal hydrogen, indicating intermediate formation of an oxirene with rearrangement to a ketene (Scheme XIIb).[88] Thus, multiple decomposition pathways are possible for reactive intermediates that are formed from ethinyl sterols.[88b]

Finally, pargyline (Chart I) is a monamine oxidase and aldehyde dehydrogenase inhibitor that is oxidatively N-dealkylated by hepatic cytochrome P-450 to yield a highly reactive, α,β-acetylenic aldehyde, propioaldehyde.[89] Centrilobular hepatic necrosis caused by pargyline in rats has recently been correlated to propioaldehyde formation.[90]

Quinones. Adriamycin and daunorubicin (Chart I) are effective anticancer agents; adriamycin in particular enjoys such widespread use in chemotherapy regimens as to be called the "propranolol of chemotherapy". Unfortunately, the use of these two anthracycline quinones is comprised by potentially fatal cardiac toxicity.[91] Evidence has accumulated[92] which supports the hypothesis that adriamycin is reduced to a transitory semiquinone free radical and that this semiquinone is reoxidized to the quinone with reduction of molecular oxygen to superoxide (Scheme XIII). Subsequent reactions of superoxide to yield the hydroxyl radical may initiate lipid peroxidation and cell damage. The semiquinone also covalently binds to DNA,

Scheme XIV. Mechanism for the Formation of Cytotoxic Metabolites of Cyclophosphamide



a reaction that is thought to be necessary for the antitumor activity of adriamycin.[93]

Another class of quinone anticancer agents is metabolized by reduction to reactive alkylating agents that can cross-link DNA.[94] Mitomycin C (Chart I) is such a bioreductive alkylating agent by virtue of its enzymatic reduction to a hydroquinone and subsequent spontaneous elimination of methanol and the carbamyl group to form a reactive quinone methide metabolite.

Miscellaneous Agents. Cyclophosphamide (Chart I) is the most widely used of the alkylating class of anticancer drugs. The drug requires metabolic activation, presumably by cytochrome P-450, in order to exert its activity.[95] Principle features of the metabolism involve hydroxylation at C-4 which rapidly equilibrates with the acyclic tautomer aldophosphamide (Scheme XIV). An apparent nonenzymatic β-elimination yields the reactive alkylating agent, phosphoramide mustard and acrolein. Like many antitumor agents, the cytotoxic, therapeutically active metabolites are also responsible for several toxic effects, including mutagenicity and carcinogenicity,[96] teratogenicity,[97] and cystitis.[98]

Dacarbazine (Chart I) is a triazenoimidazole anticancer agent that can cause hepatic vascular lesions.[99] This drug is metabolized by oxidative dealkylation to apparently yield the methyldiazonium ion, which alkylates protein and DNA.[100] Thus, metabolism is required for the activation of several anticancer agents to therapeutically active products which are toxic.

Interactions of Reactive Metabolites with Cellular Constituents

Whatever the manifestation of a toxic drug reaction that is mediated by the formation of reactive metabolites, be it direct cellular necrosis or a delayed response such as an immune reaction or carcinogenesis, two primary mechanisms seem to be involved in initiating the chain of reactions that ultimately lead to the observed toxicity: (1)

(86) (a) P. R. Ortiz de Montellano, K. L. Kunze, and B. Mico, *Mol. Pharmacol.*, **18**, 602 (1980). (b) P. R. Ortiz de Montellano and K. L. Kunze, *J. Biol. Chem.*, **255**, 8578 (1980).
(87) P. R. Ortiz de Montellano and K. L. Kunze, *Biochemistry*, **20**, 7266 (1981).
(88) (a) P. R. Ortiz de Montellano and K. L. Kunze, *J. Am. Chem. Soc.*, **102**, 7373 (1980). (b) P. R. Ortiz de Montellano and K. L. Kunze, *Arch. Biochem. Biophys.*, **209**, 710 (1981).
(89) F. N. Shirota, E. G. DeMaster, and H. T. Nagasawa, *J. Med. Chem.*, **22**, 463 (1979).
(90) F. N. Shirota, E. G. DeMaster, H. W. Sumner, and H. T. Nagasawa, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **41**, 1716 (1982).
(91) R. A. Minow, R. S. Benjamin, and J. A. Gottlieb, *Cancer Chemother. Rep.*, **6**, 195 (1975).
(92) (a) K. Handa and S. Sato, *Gann*, **67**, 523 (1976). (b) N. R. Bachur, S. L. Gordon, M. V. Gee, and H. Kon, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 954 (1979). (c) E. G. Minnaugh, M. A. Trush, and T. E. Gram, *Biochem. Pharmacol.*, **30**, 2797 (1981). (d) R. D. Olson, R. C. Boerth, J. G. Gerber, and A. S. Nies, *Life Sci.*, **29**, 1393 (1981).
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(94) (a) H. W. Moore, *Science*, **197**, 527 (1977). (b) K. A. Kennedy, B. A. Teicher, S. Rockwell, and A. C. Sartorelli, *Biochem. Pharmacol.*, **29**, 1 (1980).
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covalent binding of reactive metabolite to tissue macromolecules and (2) reactive metabolite-induced oxidative stress. Holtzman[101] has recently reviewed these two mechanisms and proposed some guidelines for differentiating them. Two important points that should be kept in mind as the two mechanisms are briefly discussed are that the mechanisms are not mutually exclusive and that extrapolation of the results obtained in laboratory animals and in vitro to man is certainly tenuous from a quantitative standpoint and sometimes even from a qualitative standpoint, depending on how good the model is.

Relationship Between Bound Drug Residues and Toxicity. The initial concepts relating covalent binding of reactive metabolites to tissue macromolecules and toxicity were developed by workers who were involved in research on chemical carcinogens.[102] These concepts were expanded to explain acute toxic reactions caused by drugs and other chemicals.[103] Bear in mind that "covalent binding" is an experimental parameter which serves as an index of the formation of highly reactive metabolites that are difficult to measure by other means. Radiolabeled drug is either administered to an animal or used as a substrate for enzyme reactions in vitro. The amount of radiolabel that is retained by various macromolecular preparations (protein, lipid, and/or nucleic acids) is then assayed after exhaustively extracting the preparations with various solvents to remove reversibly bound material. Some of the major problems with this assay technique have been reviewed[104] and include differentiating between reversibly bound and labile covalently bound metabolites and differentiating between bound radiolabel that results from adduct formation and radiolabel that arises from incorporation into macromolecules via the formation of metabolites that are precursors of endogenous substrates. The best way to demonstrate covalent binding is to digest macromolecules and determine structures of amino acid or other adducts. This approach is problematic because it is difficult to ensure that some change has not occurred during the digestion and workup, and in a practical sense such rigorous analysis has only been applied to two of the drugs that have been discussed.[74,100]

However, the covalent binding assay can be a useful tool for the investigation of the role of metabolic activation in initiating a particular toxicity, and some of the approaches that should be applied to establishing such a role have been outlined.[57b,101,104] Most studies have been prospective; that is, some toxic reaction is caused by a drug or chemical. The following questions need to be answered: Is reactive metabolite formation involved? Is a reactive metabolite the ultimate toxin? If the toxicity was first observed in man, then the first problem, and often the most difficult, is to find some good animal model for the toxicity. Although in vitro test systems can provide useful information concerning specific enzymes, enzyme requirements, and mechanisms, they provide little information about time and dose dependencies, dose thresholds and protective mechanisms, and target organ and cell specificity of toxic compounds. In a perspective sense, we might be able to predict that a particular structure would be toxic, and covalent binding experiments in vitro might support that supposition, but the experiment in vivo is necessary to determine how the animal tissue reacts to the insult.

When an animal model for a particular drug-induced toxicity has been developed, we can determine whether the toxicity is mediated through the formation of a reactive metabolite by correlating changes in the amounts of covalent binding with changes in the incidence and severity of toxicity. For example, acetaminophen causes centrilobular hepatic necrosis in man and laboratory animals.[105] Covalent binding of radiolabel that is derived from acetaminophen is also concentrated in the centrilobular regions of the liver that are necrotic, maximal binding preceding necrosis by several hours. Pretreatments of animals with inhibitors of cytochrome P-450, such as piperonyl butoxide and cobaltous chloride, cause parallel decreases in covalent binding and necrosis, whereas pretreatments with inducers of cytochrome P-450 cause either parallel increases or decreases in both parameters, depending on the animal species.

This illustrates an important point that has been thoroughly discussed by Gillette.[106] The severity of necrosis caused by acetaminophen and other toxins is dependent on (A) the proportion of the dose that is converted to reactive metabolite, (B) the proportion of reactive metabolite that becomes covalently bound, (C) the proportion of covalently bound metabolite that is attached to critical macromolecules, and (D) the proportion of (C) that cannot be replaced or repaired that leads to toxicity. Pretreatment of mice with phenobarbital increases the extent of necrosis and the proportion of a dose of acetaminophen that becomes covalently bound by increasing levels of hepatic cytochrome P-450, which thereby increases (A), the proportion of the dose that is converted to reactive metabolite. In marked contrast, phenobarbital pretreatment of hamsters decreases the extent of hepatic necrosis and the proportion of the dose of acetaminophen that becomes covalently bound. Since phenobarbital is known to induce glucuronyltransferase activity as well as cytochrome P-450, it has been proposed that this activity is preferentially increased in the hamster; therefore, (A) is decreased because a larger proportion of the acetaminophen dose is excreted as the glucuronide (refer to Scheme II).

However, with a more complete knowledge of the nature of the reactive metabolite of acetaminophen and reactions that it undergoes, another possible explanation should be considered. The quinone imine is rapidly reduced back to acetaminophen by NADPH–cytochrome P-450 reductase. Phenobarbital also induces this enzyme; therefore, depending on the relative levels of induction of the various enzymes in the mouse vs. hamster, the ratio (B) may decrease, i.e., a decrease in the proportion of reactive metabolite that becomes covalently bound. Thus, knowledge of the various metabolic pathways that are followed by a drug, as well as knowledge of the chemical and biochemical properties of reactive metabolites formed from the drug, allows for a more accurate correlation of covalent binding and toxicity studies. A realization that enzyme inducers and inhibitors are rarely specific also helps in determining the relative importance of toxification and detoxification pathways.

Significantly, no matter what effect treatments have in various species, the extent of covalent binding of radiolabel

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(106) J. R. Gillette, *Biochem. Pharmacol.*, **23**, 2785 and 2927 (1974).

from acetaminophen parallels the magnitude of necrosis. This correlation provides strong evidence that a reactive metabolite of acetaminophen is involved in the toxic reaction, and the hypothesis is essentially proven when the evidence is coupled with results of studies in vitro (see discussion of acetaminophen under Acetanilides).

Is then the reactive metabolite formed from acetaminophen the ultimate toxin, and does its covalent binding to tissue macromolecules cause cell death? Recent studies would suggest that this may not be the case, though the evidence is certainly not compelling. First, various sulfhydryl-containing compounds protect against acetaminophen-induced toxicity even when they are administered to animals after covalent binding of reactive metabolite is maximal.[107] This indicates that covalent binding is not sufficient to cause cell death, but does not necessarily mean that binding is not required, since the sulfhydryl reagents may be protecting the cell by means other than interception of reactive metabolite. Secondly, the regioisomers of acetaminophen, 3-hydroxyacetanilide and 2-hydroxyacetanilide, apparently both deplete glutathione, and they covalently bind to hamster liver as extensively as acetaminophen, but neither is hepatotoxic.[108] A caveat is that these isomers may bind to different tissue macromolecules, thereby decreasing either the proportion of covalently bound metabolite that is attached to critical macromolecules or the proportion bound that might be repaired.

Thus, changes in the incidence or severity of toxicity that is caused by a drug may parallel changes in the covalent binding of reactive metabolites to tissue, but this pathway may or may not be relevant to mediation of the toxicity.[101,106] Inasmuch as the reactive metabolite of acetaminophen is most likely a quinone, it may both bind to tissue nucleophiles and undergo one-electron reduction to a semiquinone free radical, which might lead to cellular oxidative stress. Either, neither, or both of these mechanisms may ultimately lead to cell death.

Relationship Between Oxidative Stress and Toxicity. Oxidative stress as a mechanism of toxicity requires redox cycling of a compound through radical intermediates than can reduce molecular oxygen to superoxide anion.[68] Superoxide anion may then react by loss of an electron of the appropriate spin state to produce the highly reactive singlet oxygen, or the radical anion may be further reduced to hydrogen peroxide and hydroxyl radical. Although mechanisms for the formation of these various species and their further reactions have not been fully elucidated, especially in vivo, it is currently believed that one or more of them is involved in the initiation of membrane lipid peroxidation, which subsequently leads to cell death.[109] Thus, some of the features that distinguish this mechanism are that metabolism in vitro, using drugs that can cause oxidative stress, should produce reactive oxygen species and cause lipid peroxidation, and toxicity in vivo should be enhanced in vitamin E deficient and selenium deficient states.[101] Vitamin E is a known radical scavenger, though it has other effects as well, and selenium is required for the synthesis of most glutathione peroxidases, enzymes that destroy hydrogen peroxide as well as organic peroxides.

The two classes of drugs that appear to cause organ toxic reactions by this mechanism are the nitroaromatics and anthraquinone anticancer agents. There are also a host of other drugs, mostly aromatic amines and catechols (e.g., α-MeDopa), that cause hemolytic reactions, which may be related in some aspects to similar mechanisms. Nitrofurantoin is a drug that induces pulmonary toxicity in rats, and the lesion appears to be caused by oxygen stress.[57b,110] Lethality is greatly enhanced in vitamin E deficient status, especially in combination with a high polyunsaturated fat diet, and vitamin E repletion decreases lethality. In contrast to other furans that covalently bind with high selectivity in the target organ for toxicity, nitrofurantoin was found to bind little in lung tissue compared to other tissues, such as liver or kidney. Investigations in vitro supported the hypothesis that nitrofurantoin could cause toxicity by inducing oxygen stress. In the chick, selenium deficiency was found to enhance toxicity.[101] Thus, at least in these animal models, a distinction between toxic mechanisms seems apparent for nitrofurantoin. However, because of multiple effects that dietary manipulations may produce in an animal, interpretation of the data in this case was facilitated by the additional information obtained from covalent binding studies.

Radical Generation Without Apparent Oxidative Stress as a Mechanism of Toxicity. A few halogenated drugs, in particular halothane, can apparently form free radicals under conditions of reduced oxygen tension that bind to lipid and generate lipid dienes which are precursors of lipid peroxidation.[76] Thus, an animal model system has been developed for halothane-induced centrilobular necrosis that uses phenobarbital pretreatment of rats, followed by anesthesia with halothane in 14% O_2. It is noteworthy that in this model the extent of covalent binding of radiolabeled halothane to protein correlates with the extent of liver damage, but that the protein binding and even the lipid binding may not be responsible for the tissue necrosis. Whether this models what occurs in man has not been adequately investigated, since immune mechanisms seem to be involved.[111] However, as already discussed, metabolic activation of drugs can produce antigenic products, as well as directly acting toxins, and evidence would support this as a likely mechanism in halothane-induced hepatitis.[111b]

A Medicinal Chemist's Approach to Circumventing Drug Toxicity Resulting from Metabolic Activation

When metabolic activation is responsible for drug toxicity, two general approaches can be used to decrease the probability of occurrence of the toxic reaction: (1) molecular modification to block or decrease the formation of toxic metabolites or (2) coadministration of other agents either to promote the formation of nontoxic metabolites or to decrease the toxic response to the reactive metabolite itself. The two approaches will be discussed briefly with reference to the toxic drug reactions that have been outlined.

Alteration of Metabolism by Structural Modification. The most straightforward approach in avoiding toxic effects that are caused by metabolites of chemical agents is to design agents that are not metabolized. This ap-

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proach is also the most difficult, since the extensive structural modifications that would have to be made to most drugs would, no doubt, dramatically alter their therapeutic activity. A more realistic approach would be to use what is known about a particular toxic metabolic reaction to design either an analogue or a prodrug of the therapeutically active agent which circumvents or decreases the rate of formation of the toxic metabolite.[112]

Although the prodrug approach is particularly intriguing because it yields the therapeutically active form of the drug subsequent to metabolism, the approach requires circumvention of toxicity by dispositonal differences. That is, the active form of the drug must be released in a controlled fashion such that concentrations of the reactive metabolite will not exceed toxic levels in tissues that might be affected. Thus, predictability of disposition becomes a major obstacle that has not been overcome with those drugs that cause insidious tissue damage via metabolic activation. However, examples are known in which toxicity due to exaggerated drug action has been effectively controlled using prodrugs.[113] Therefore, the approach is attractive for future investigations. This then leaves us with the analogue approach, which will be considered using several examples.

Acetaminophen is an exceptional example of a drug whose toxic metabolic pathway has been reasonably well defined. Based on the knowledge that the toxic pathway involves oxidation of the drug to a *p*-quinone imine, investigators have published two reports which describe nontoxic analogues of acetaminophen.[114] N-Methylation of acetaminophen would be expected to markedly decrease the rate of formation of a quinone imine inasmuch as the oxidation would have to be preceded by amide N-dealkylation, a known but minor metabolic pathway. *N*-Methylacetaminophen is a nonhepatotoxic analogue of acetaminophen; unfortunately, this minor structural change decreases analgesic activity and increases convulsant activity.[114a] The meta-substituted acetanilide, 3-hydroxyacetanilide, is a nontoxic regioisomer of acetaminophen that cannot form a *p*-quinone imine without first undergoing 4-hydroxylation.[114b] Although this reaction probably occurs, the catechol is considerably less toxic than acetaminophen.[115] The 3-hydroxy isomer and congeners are now being evaluated for analgesic and antipyretic activity.

Of the hydrazines, isoniazid and hydralazine will continue to be used for their respective beneficial therapeutic effects. However, the knowledge that has been gained regarding the pathogenesis of their respective toxic effects will allow patients receiving these drugs to be more readily monitored for possible toxic effects and potential drug interactions. It is possible that some hydrazone derivatives could be used as prodrug forms of these agents if they maintained therapeutic activity and decreased hepatic concentrations. Because safer MAO inhibitors are available, iproniazid has been withdrawn from the market, and knowledge of its metabolism has raised questions in Europe about the continued use of iproclozide.[34] A nontoxic analogue of iproniazid is the *tert*-butyl analogue; however, it also has low MAO activity, suggesting that the N-oxidation process itself may be hindered (S. D. Nelson, unpublished results). Although several analogues of procarbazine have been prepared, none apparently shows any significant improvement in therapeutic index.[116] This may be because the same pathways of metabolism generate both therapeutically active and toxic metabolites, although this has yet to be proven.

The introduction of *N*-acetylprocainamide as a significantly less toxic analogue of procainamide has already been discussed. Thus, the toxicities of some aromatic amines may be decreased by acetylation. However, deacetylation is also a common hydrolytic metabolic pathway for some aryl amides, such as phenacetin, and other aryl amides are toxic via other mechanisms, such as acetaminophen.

Aromatic, heteroaromatic, and nitroaromatic groups are moieties that obviously cannot be replaced in many drug structures. However, the recent cases of liver toxicity caused by ticrynafen would suggest that any studies involving these drug structures be carefully planned to monitor patients until such drugs are proven safe. Furthermore, the propitious use of methyl groups or fluorine on benzenoid aromatics can direct metabolism away from the aromatic ring (e.g., compare the metabolism of benzene and toluene[47]) and thereby decrease the potential for the generation of reactive metabolites. Although substitution of fluorine or the trifluoromethyl group does significantly decrease metabolism of the aromatic ring,[117] this structural modification also significantly decreases the potency of some drugs, e.g., diphenylhydantoin.[117b,c] The apparent beneficial use of the sulfonylmethyl group in place of the nitro group in chloramphenicol has already been discussed.

Enflurane and isoflurane are two relatively new halogenated anesthetics that are metabolized to a significantly lesser extent than other halogenated anesthetics, and they have also been found to be less toxic.[76] The few cases of renal toxicity associated with the use of these agents are apparently related to fluoride release, and deuterium isotope experiments have shown that the loss of fluoride from enflurane is a microsomal oxygenase-mediated process.[118] Deuterium substitution for hydrogen in halogenated anesthetics has created analogues that are considered to be safer because rates of oxidative dehalogenation are slower.[119]

Deuterium-labeled and halogenated analogues of cyclophosphamide have also been prepared in attempts to increase the therapeutic index of this anticancer drug.[95a] Stable isotopes have been used extensively to probe mechanisms of metabolic activation,[120] and substitution

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of deuterium for hydrogen at C-5 of cyclophosphamide slowed the rate of phosphoramide mustard formation but also decreased antitumor activity. Neither *cis*- nor *trans*-5-fluoro- or -chlorocyclophosphamide analogues increased the rate of phosphoramide mustard production or the therapeutic index.

Several analogues of the antitumor drug adriamycin are being evaluated in a search for active compounds with less cardiotoxic potential.[121] Analogues with the least cardiotoxic potential are anthracenediones with redox potentials that are lower than those of the anthracyclines; therefore, they are not reduced as efficiently to their toxic semiquinone radicals.[121b,c]

Thus, armed with a knowledge of the mechanism by which a drug is metabolized to a toxic product, structural analogues can be prepared to block the pathway, slow its rate, and/or provide an alternate pathway for metabolism via processes that do not generate highly reactive products. The major drawback to such an approach is that even minor structural modifications can markedly alter the therapeutic and/or toxicological spectrum of activity inherent to the parent drug structure.

Alteration of Metabolism by Cotreatments. Although not a classical medicinal chemistry approach, a mechanism-based cotreatment regimen is another means of decreasing the frequency of potentially toxic drug reactions. For example, the effective use of sulhydryl-containing compounds to treat acetaminophen overdoses was initiated after the basic steps involved in the mechanism of the toxic reaction were elucidated,[10a] even though the actual mechanism for protection by such agents is unknown.[107] Therefore, it should be possible to coadminister some nontoxic agent with doses of acetaminophen to prevent achievement of toxic levels of reactive metabolites. One possibility that has been suggested is the coadministration of sodium sulfate to maintain adequate levels of tissue sulfate pools necessary for maximal sulfation capacity (a nontoxic metabolic pathway for acetaminophen which becomes rapidly saturated due, apparently, to cosubstrate depletion) and to maintain adequate levels of cysteine for glutathione synthesis.[122] A second suggestion has been the coadministration of ascorbic acid (or a derivative), which probably acts by directly reducing the reactive metabolite.[123] Coadministration of adriamycin with sulfhydryl-containing compounds and ascorbate has also been investigated in animals.[124] Both treatments prevented adriamycin-induced cardiomyopathy in animals. Mixed results have been reported on the cardioprotection afforded by the coadministration of vitamin E and vitamin E plus selenium.[125] All results await verification in man where differences in diet may have a profound influence. A second major disadvantage of cotreatment regimens is that the disposition kinetics of the protective agent must be controlled in order to maintain concentrations of reactive metabolites of the therapeutic drug below toxic levels.

Minimizing the Potential for Toxic Reactions of New Drugs. The most difficult problem is to predict toxic effects of new drug structures with our limited knowledge of metabolic activation as a cause of some drug toxicities. Application of computer-based techniques has been limited and hardly predictive,[126] and only a few papers have examined physicochemical correlates of SAR and the kinds of toxic reactions that have been discussed in this Perspective.[127] Most drugs cannot be readily analyzed by such methods, because they contain multiple sites for metabolism which proceed by different rates in different animals due to absorption, metabolic, and distributional differences.

However, as in any physicochemical process, some step is rate limiting in the development of a toxicological response, and this step is often the formation of the reactive metabolite. A medicinal chemist should have the proper chemical and biochemical perspective to identify those structural features of new drug entities that when oxidized or reduced may lead to the formation of reactive metabolites (see, for example, the correct prediction of a benzyne intermediate from enzymatic oxidation of 1-aminobenzotriazole[128]). With the proper training, the medicinal chemist could also test hypotheses that relate metabolic activation and toxicity by using the chemical, biochemical, and pharmacological–toxicological approaches that have been discussed in this Perspective. Collaborative efforts with individuals specifically trained in some of these other areas is desirable. All individuals involved in such work should strive to relate the chemistry to the biology and to understand the limitations of the various parameters that are used to define toxic pathways (covalent binding, glutathione depletion, isotope effects, etc.). Somehow, with our limited knowledge, we must then be able to make a reasonable judgement as to risk assessment in man and hope that we neither release a dangerous new chemical entity nor, as importantly, abort an effective one.

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