Annu. Rev. Pharmacol. Toxicol. 1990. 30:169–95

COVALENT AND NONCOVALENT INTERACTIONS IN ACUTE LETHAL CELL INJURY CAUSED BY CHEMICALS

Sidney D. Nelson and Paul G. Pearson

Department of Medicinal Chemistry, University of Washington, Seattle, Washington 98195

KEY WORDS: immune-mediated toxicity, lipid peroxidation, oxidative stress

INTRODUCTION

Several chemicals, including some drugs, cause cell injury by mechanisms that are poorly characterized. Although a few drugs are toxins that act directly, most require metabolic activation to form the ultimate toxic species. The concept of metabolic activation was first described by the Millers (1) in a review of their pioneering work on aminoazo dyes and 2-acetylaminofluorene as procarcinogens. Brodie et al (2) expanded on this concept to include a role for metabolic activation in acute cell injury. Since that time several reviews (3–12) and books (13–15) have discussed the role of metabolic activation in the pathogenesis of a variety of toxicities.

Although a substantial body of information is available on the metabolic reactions that produce toxic metabolites and on the chemical nature of these metabolites, considerably less is known about how ultimate toxic species interact with cellular constituents and how the interactions cause cell injury. Reactions of toxic metabolites may result in covalent bond formation between the metabolite and a target molecule, or they may alter (usually by oxidation or reduction) the target molecule without formation of a covalent bond. Some of the more likely targets of these interactions are listed in Table 1.

0362-1642/90/0415-0169$02.00

Table 1 Possible interactions of reactive metabolites

Covalent interactions with critical macromolecules
1. DNA
2. Peptides and/or proteins
3. Lipids
4. Carbohydrates
Noncovalent interactions
1. Alterations of GSH/GSSG, NAD(P)/NAD(P)H
2. Generation of reactive oxygen species
3. Lipid peroxidation
4. Intercalation and complexation

The major target molecule for initiation of chemical carcinogenesis is most likely DNA; and since the structures of several DNA-reactive metabolite adducts have been characterized, knowledge of how the alteration in structure initiates carcinogenesis is progressing. For example, a specific G→A transition induced by methylation of O^6 of deoxyguanosine in the Ha-ras-1 oncogene has identified the oncogene as a target for *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis by this agent in rats (16).

In contrast, the target molecules that interact with reactive metabolites to produce acute lethal cell injury have not been well characterized. The issue is further complicated by the fact that some reactive metabolites may indirectly cause cell injury by acting as haptens and by forming immunogens (17–19). This review discusses what is known about covalent and noncovalent interactions of reactive metabolites with cellular constituents and highlights some of the controversy surrounding the relative importance of the role that these interactions may play in acute lethal cell injury.

COVALENT INTERACTIONS

Although several chemicals are metabolized to reactive intermediates that form covalent bonds with tissue macromolecules, the relationship between covalent binding and tissue injury is not clear. In fact, in only a few cases is it known that covalent bond formation between a chemical and a particular macromolecule causes acute lethal cell injury. One of the major hepatotoxins from the mushroom *Amanita phalloides,* phalloidin, binds to a form of actin that inhibits its depolymerization, whereas the other major toxin, α-aminitin, binds to RNA-polymerase B and inhibits messenger RNA formation and thereby, protein synthesis (20). Another example is the cholinesterase in-

hibitor, paraoxon, which inhibits acetylcholinesterase activity at the motor end-plate of muscle fibers, after which, necrosis of skeletal muscle ensues (21).

Thus, in general, covalent binding is an experimental parameter and serves as an index of the exposure of a tissue to reactive metabolites that are difficult to measure by other means. With some limiting assumptions, Gillette (22) has formulated covalent binding as a measure of the area under the curve of a reactive metabolite in a tissue. This is dependent on (*a*) the proportion of the dose of the chemical that is converted to reactive metabolite, (*b*) the proportion of reactive metabolite that becomes covalently bound, and (*c*) the proportion of the bound metabolite that is not lost from the tissue due to either secretion or repair or both.

The measurement of covalently bound chemicals to macromolecules is not inherently difficult and relies on the use of either radiochemical or immunochemical techniques to detect and quantitate bound adducts. Each method has advantages and disadvantages. The major disadvantage of the radiochemical method is lack of differentiation of bound chemical and/or metabolites vs incorporation of radiolabel into macromolecules via decomposition of the chemical into precursors of endogenous compounds. This assay technique has been well reviewed (23). The major disadvantage of immunoassay techniques is that they may not quantitate all adducts that are formed from a chemical because of epitope selectivity.

More rigorous approaches to characterization of covalently bound adducts usually have employed macromolecular digestion and analysis of small peptide, amino acid, fatty acid, and nucleoside (or pyrimidine/purine base) adducts by mass spectrometric techniques. Such an approach is inherently difficult because of the low levels of adducts found within large biological matrices that are available for structural elucidation. However, the increased sensitivity that has accompanied advances in technology in both isolation and mass spectrometry has made such characterization possible. The use of these methods to study mechanisms of chemical-induced toxicities (24), to monitor exposure to genotoxic agents (25), and to assess the transfer of bound residues through the food chain (26) have been discussed. Selected examples are discussed later in this chapter.

Of course, the more important objectives are to determine which adducts are involved in the pathogenesis of toxicity, and what the function of the adducted proteins are. It is clear that all adducts are not involved in pathogenetic mechanisms, which is no real surprise because it has been known for several years that particular adducts of DNA (e.g. O^6-alkylguanines) are significantly more damaging than others (27).

Unfortunately, correlations of particular amino acid adduct levels on pro-

teins with toxicity do not exist, as evidenced by the fact that reactive metabolites of chloramphenicol (28) and thioacetamide (29) both covalently bind to lysine residues, but only thioacetamide is hepatotoxic. Moreover, covalent interactions of chemicals with some proteins may not cause lethal cell injury. For example, several drugs and chemicals are suicide inactivators of cytochromes P-450, and reactive metabolites of these compounds bind covalently to the prosthetic heme group and/or to the apoprotein (30). Although some of these agents can cause porphyria, they do not cause acute lethal cell injury (31).

It also is likely that different reactive metabolites of bromobenzene bind to different proteins, some of which are more critical for cell viability than others, because some metabolites such as *p*-bromophenol bind covalently to proteins in liver and kidney but are not toxic to either organ (32). Furthermore, *o*-bromotoluene binds more extensively to hepatic proteins than bromobenzene, but it is less hepatotoxic (33). As a final example in this regard, naphthalene binds more extensively to nontarget tissue proteins (liver and kidney), than to target tissue proteins (lung) in some species (34). Because naphthalene is oxidized at different rates in the various tissues to different naphthalene oxide enantiomers (35), it will be of interest to compare the toxicities of these enantiomers.

Some reports have appeared on the alkylation and arylation of individual proteins by reactive metabolites (36–44), and binding is not totally random. With the breakthroughs in the development of antibodies to covalently bound metabolites and in molecular biological techniques to characterize proteins, major advances in the isolation and identification of target proteins have occurred. Human antiendoplasmic reticulum antibodies have been detected in the sera of patients exposed to the hepatotoxic drugs—tienilic acid (45) and halothane (46). These antibodies are directed against a human liver cytochrome P-450 and a liver carboxylesterase, respectively. A second halothane-induced neoantigen recently has been identified as a trifluoroacetylated protein disulfide isomerase (47). Although it is not known if the covalent modifications to these proteins have anything to do with the pathogenesis of hepatic necrosis caused by either tienilic acid or halothane, it is the first time any target proteins have been thoroughly characterized.

Finally, the possibility should be considered that reversible covalent bonds of chemicals to macromolecules are involved in some toxic mechanisms, as glutathione and proteins are known to react reversibly with some reactive metabolites (48, 49). Glutathione conjugates of allylisothiocyanate and benzylisothiocyanate exist in equilibrium with the parent isothiocyanate, and their cytotoxicity to isolated hepatocytes is mediated via the release of free isothiocyanate. Although this process may initially yield a detoxication product, it has been proposed that reversible conjugate formation with glu-

tathione may play a role in the interorgan translocation of allyl- and benzylisothiocyanates (50).

In a similar manner, following inhalation exposure of rodents to [^{14}C] methylisocyanate a rapid uptake and systemic distribution of ^{14}C to extrapulmonary organs occurred (51). The form of ^{14}C distributed in blood and tissues has not been identified, but speculation centers upon the reversible reaction of alkylisocyanates with pulmonary glutathione as a transport process (51, 52). This phenomena may account for the significant levels of covalent binding to hepatic proteins following administration of methylisocyanate to rodents (53). By analogy, *S*-(*N*-methylcarbamoyl)glutathione, a major metabolite of the investigational antitumor agent and hepatotoxin *N*-methylformamide is believed to be formed from metabolically generated methylisocyanate (54). This conjugate is related structurally to the reactive glutathione and cysteine conjugates of allyl- and benzylisothiocyanate (50), and in principle the conjugate may spontaneously release methylisocyanate; in antitumor tests *S*-(*N*-methylcarbamoyl)-glutathione possesses 100-fold greater cytotoxic activity against TLX5 lymphoma cells than does its metabolic precursor *N*-methylformamide (55, 56).

Other examples of reversible reactions with GSH and proteins have been reported for a reactive open chain acrylonitrile derivative of furazolidine (49), and adducts formed upon the interaction of formaldehyde with glutathione are in labile equilibrium with each other (57). Depending on the environment where the metabolite is reformed, these reversible adducts may, or may not, represent toxication processes. Examples of these and other reactions of reactive metabolites to form covalent bonds with tissue macromolecules are presented in the last part of this chapter.

NONCOVALENT INTERACTIONS

Chemicals that lead either directly or indirectly to the generation of reactive oxygen species may cause oxidative stress in cells with progression to acute lethal cell injury. Oxidative stress has been defined as a disturbance in the prooxidant-antioxidant balance in cells in favor of the prooxidant state (58). It should be made clear that some reactions of chemicals that cause oxidative stress may involve covalent interactions. However, the ultimate insult results not from the covalent interaction per se, but from subsequent alterations in the redox state of the cell. These alterations may involve marked shifts in ratios of GSH/GSSG, NAD(P)/NAD(P)H, protein thiols/disulfides, and the status of other antioxidants such as Vitamin E. How processes elicited by these alterations, such as lipid peroxidation and changes in calcium homeostasis, lead to cell death is a question that is being investigated in several laboratories and has been the subject of other reviews (59–65).

Several targets have been implicated in the pathogenesis of tissue injury caused by oxidative stress. The enzyme glutathione reductase has been well characterized, and inhibition of this enzyme by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) has been localized by X-ray crystallography to a specific active site cysteinyl residue which is carbamoylated by BCNU (66). Other enzymes that contain thiol groups which undergo reversible oxidation-reduction reactions are important in metabolic regulation and may be targets for chemical toxins that cause oxidative stress (67). For example, the exposure of some cells to hydrogen peroxide inactivates glyceraldehyde-3-phosphate dehydrogenase, apparently by oxidation of two active-site cysteinyl thiols to a disulfide (68, 69). This results in a marked decrease in intracellular ATP levels. Hydrogen peroxide also causes DNA damage in cells, which activates the nuclear enzyme, poly(ADP-ribose)polymerase (70). Because this enzyme utilizes NAD, cellular NAD is depleted, which also results in ATP depletion. However, ATP depletion may not be the major mechanism of cell toxicity because inhibitors of the polymerase do not block ATP depletion but do inhibit NAD depletion and cell death (71). Interestingly, the oxidant activity is not required to activate the polymerase, inasmuch as the DNA-methylating agent, dimethyl sulphate, also can cause cytotoxicity via this mechanism (71). Thus, both covalent and noncovalent interactions may result in similar mechanisms of pathogenesis.

Thiol oxidation has been implicated in oxidant stress caused by organic peroxides, such as *t*-butylhydroperoxide, as well as by other oxidizing agents such as diamide and some quinones (72–75). Membrane Ca^{2+} ATPases have been suggested to be important targets for these agents, based on studies that demonstrate rapid inhibition of Ca^{2+} ATPase activity in plasma (76, 77) and reticular (78, 79) membranes, which is in part reversible by thiols. A few other enzymes whose activity relies on thiols also may be important targets for these compounds. They include the mitochondrial NAD(P) transhydrogenase (80) and F_0 of ATP synthase (81), and cytosolic thiol transferase (thiol-disulfide oxidoreductase) (82). Cytoskeletal proteins that contain functional thiol groups such as actin (83), and other as yet uncharacterized proteins (84), are additional targets for *S*-thiolation reactions that may be important in the pathogenesis of acute cell injury mediated by oxidative reactions. However, it should be stressed that covalent interactions with these same targets also may be important for toxicity (85), and that reactions with thiol moieties may not be as important with some of these proteins, such as the NAD(P) transhydrogenase, as is oxidative depletion of pyridine nucleotides (86).

Finally, lipid peroxidation may be initiated either by reactive oxygen species generated by reactive metabolites of chemicals, or by the addition of reactive metabolite radicals to unsaturated lipids (87). Thus, both noncovalent and covalent interactions may be involved as an initiating event, depending on

the initiator and probably on oxygen concentrations as well. Although unsaturated lipids are primary targets in lipid peroxidation, other targets may be more important in the development of lethal cell injury (88). Whether it is changes in membrane permeability caused by the lipid peroxidation event itself, reactions of the initiating radicals with proteins, or reactions of products of lipid peroxidation with proteins that leads to the ultimate injury is not known. Therefore, it is not known to what extent covalent and noncovalent interactions are involved in the process, or to what extent lipid peroxidation contributes to many chemical-induced toxicities (89).

COVALENT AND NONCOVALENT INTERACTIONS OF SPECIFIC COMPOUNDS

The remainder of this chapter focuses on specific compounds to illustrate the following possible situations:

1. Covalent interactions are directly responsible for acute lethal cell injury (*n*-hexane).
2. Covalent interactions are indirectly responsible via antigen and immunogen formation (halothane).
3. Noncovalent interactions are primarily responsible for acute lethal cell injury (carbon tetrachloride).
4. Both covalent and noncovalent interactions contribute to acute lethal cell injury (acetaminophen).

Although the focus is on the few examples mentioned, other compounds that seem to fall into a particular category are tabulated and in some cases briefly described.

COVALENT INTERACTIONS ARE DIRECTLY RESPONSIBLE

N-Hexane Neurotoxicity

The mechanism of neurotoxicity caused by *n*-hexane is probably the most completely characterized mechanism of cell injury caused by reactive metabolites. *n*-hexane is oxidized to the γ-diketone, 2,5-hexanedione, which causes well-defined peripheral neuropathy in rats and humans, as well as testicular dysfunction (90). Several lines of evidence support the view that 2,5-hexanedione and other γ-diketones form pyroles with protein lysyl ε-amino groups of axonal cytoskeletal proteins (91–94). Additional evidence suggests

that a subsequent oxidation of the pyrrole ring leads to a cross-linking reaction of the neurofilaments (95, 96). A similar reaction of 2,5-hexanedione with tubulin in testicular cells is thought to cause testicular damage (97, 98). Specific cytoskeletal proteins have recently been identified as targets of 2,5-hexanedione and other neurotoxins such as acrylamide (99, 100). However, it is not known why these agents show such striking target-organ selectivity.

Other Compounds

Table 2 contains a list of several other compounds which form reactive metabolites that covalently bind to target organ tissue, and whose toxicity is thought to result from the covalent interaction. In a few cases, such as bromobenzene (101) and allyl alcohol (102, 103), lipid peroxidation also has been proposed as a mechanism of toxicity. It is probably true that more than one mechanism may contribute to the toxicity caused by any compound. However, the weight of the evidence in the case of bromobenzene and allyl alcohol supports a mechanism that primarily involves a covalent interaction of reactive metabolites with hepatocyte proteins. In the case of bromobenzene, fairly compelling arguments have been published (12, 104) that question the conclusions of the only study (101) on a role for lipid peroxidation. In the case of allyl alcohol, recent studies show that lipid peroxidation may not be a critical event in toxicity caused by this agent because peroxidation can be prevented without affecting cytotoxicity (105, 106).

It should be stressed that for most of the compounds listed in Table 2 structures of the reactive metabolites are not known, and for none of the compounds other than *n*-hexane have adduct structures or target macromolecules been identified. However, progress is being made, and some recent studies with valproic acid suggest that an unsaturated metabolite may act like the known hepatotoxins, 4-pentenoic acid and methylenecyclopropylacetic acid, by covalently binding to and inhibiting 3-ketoacyl-CoA thiolase, an enzyme of the mitochondrial fatty acid β-oxidation pathway (107).

COVALENT INTERACTIONS LEAD TO IMMUNOGEN FORMATION

A basic tenet of the hapten hypothesis of drug hypersensitivity is that the drug binds covalently to a macromolecule, such as a protein, and that the drug-protein conjugate is recognized as an immunogen (17–19, 108). Various kinds of immune complexes may then form between the antigen and antibodies directed against it to cause tissue damage. In general, there is little information on (*a*) why some drugs (and/or their metabolites) form im-

Table 2 Covalent and noncovalent interactions in the expression of acute lethal cell injury by foreign compounds.

Compound	Reference(s)
Covalent Interactions	
Acetaminophen	41, 42, 147–155, 188
Acrylamide	100
Acetylamimofluorene	1
Adriamycin (doxorubicin)	67, 192
Allyl alcohol	105, 106
Amineptine	193
Benzene	194–198
Benzo[a]pyrene	36, 37
Bromobenzene	12, 32, 104, 199, 200
Carbon tetrachloride	120, 121
Chloroform	201, 202
Chlorotrifluoroethylene	203, 204
Cortisol	205
1,2-dibromoethane	206–208
S-(1,2-dichlorovinyl)-L-cysteine	209
Diethylstilboestrol	210, 211
Furans	212
2-Methylfuran,3-methylfuran	213, 214
4-Ipomeanol	215–217
2-(*N*-ethyl-carbamoylhydroxymethyl)furan	218
Furosemide	219, 220
Halothane	46, 47, 109, 112
Hexachlorobutadiene	221, 222
n-Hexane (γ-diketones)	90–99
Hydrazines	223, 224
Acetylhydrazine	225
Isoniazid	224, 226, 227
Iproniazid	224, 226, 228
Isaxonine	231
3-Methylindole	232–235
N-Methylformamide	230, 236
Mitomycin C	238
1-Naphthol	239
Naphthalene (2-Methylnaphthalene)	34, 35
Paraoxon	21
Phalloidin	20
Pyrrolizidine alkaloids	
Senniconine, seneciphylline	240
Quinones	145
2-Bromohydroquinone	241
Hydroquinone	242
Naphthoquinones	146
Procainamide	19
Carbon Disulfide	244–246
Thioacetamide	29, 247

Table 2 (*continued*)

Compound	Reference(s)
Trichloroethylene (and related compounds)	248, 250
Valproic Acid	107
Zomepirac	118, 119
Reversible Covalent Interactions	
Allylisothiocyanate	50
Benzylisothiocyanate	48, 50
Furazolidine	48, 49
Methylisocyanate	51–55
Noncovalent Interactions	
Acetaminophen	147, 153–166, 188
Adriamycin	124, 252–255
Allyl alcohol	102, 103
Bleomycin	252, 255–257
Bromobenzene	101
Carbon tetrachloride	88, 125–134, 243
Diquat	140–142
Hydrogen peroxide	68–70
Hydroxyalkenals	136–139, 229–230
Nitrofurantoin	252–254
Nitroimidazoles	252–254
Paraquat	123, 140, 141, 253, 258
Quinones	253, 254
Menadione	74, 76, 79, 83, 85, 259
Dopamine (6-hydroxydopamine)	251
Mitomycin C	252
Covalent Interaction with Prosthetic Heme, but with No Acute Toxicity	
Alkynes, Alkenes	30, 31, 260
Covalent Interaction Results in an Immunogenic Response	
Halothane	43–47, 113, 114
Zomepirac	118, 119
Procainamide	19, 115, 261–263
Tienilic acid	45

munogens and others do not, (*b*) what processes lead to neoantigen formation, and (*c*) which tissue macromolecules are involved.

Halothane Hepatitis

The most thoroughly characterized drug hypersensitivity reaction that leads to acute lethal cell injury is the hepatotoxicity caused by the general anesthetic drug, halothane. Halothane is both oxidized and reduced by cytochrome(s)

P-450 to reactive metabolites (109, 110). Oxidation yields trifluoroacetyl halide, which binds covalently to hepatic proteins (111, 112). Two independent lines of evidence implicate the oxidative pathway in antigen formation. First, increasing oxygen tension enhances hepatotoxicity and neoantigen production in rabbits exposed to halothane (113). Secondly, deuterated halothane is less hepatotoxic in rats than halothane and produces lesser amounts of neoantigens that contain the covalently bound trifluoroacetyl group (44). Sera from patients with severe hepatic necrosis caused by halothane contain antibodies that recognize at least five distinct neoantigens that contain the trifluoroacetyl group, whereas sera from patients exposed to halothane who do not develop hepatitis, or who have viral hepatitis, do not contain these antibodies (43, 44, 114). The halothane antibodies render hepatocytes that contain halothane neoantigens susceptible to cytotoxic killing by lymphocytes (114). Overall the results constitute the first example wherein a specific covalent interaction of drug metabolite-protein neoantigens is implicated in lethal cell injury associated with a drug hypersensitivity reaction.

Other Compounds

Other drugs and chemicals are thought to cause immune related toxicities (17), but only a few of these cause acute lethal cell injury. Table 2 lists those drugs whose mechanisms of cell damage have been associated with immunogen formation. Several of these are metabolized to arylamines (or arylhydrazines) that may be oxidized to reactive arylhydroxylamines and/or arylnitroso compounds that can covalently bind to nucleophilic sites on macromolecules (19, 115). Several others are nonsteroidal antiinflammatory drugs, some of which have been removed from the market because they have caused adverse reactions, including renal cellular damage (116, 117). A common structural feature of these drugs is their arylalkanoic acid moieties which are metabolized to glucuronides. The glucuronides of some of these drugs have been shown to react with proteins (118, 119) and therefore may be immunogenic, although this has yet to be demonstrated.

NONCOVALENT INTERACTIONS ARE PRIMARILY RESPONSIBLE

For most compounds listed in Table 2 under the heading of noncovalent interactions, covalent interactions with tissue macromolecules also have been described. For example, reactive metabolites of carbon tetrachloride bind to proteins and lipids (40, 120, 121) and cause lipid peroxidation (122) as early events in the cytotoxic process. However, in most cases free radical intermediates are formed that either abstract a hydrogen from cellular components or reduce molecular oxygen to oxyradicals that cause lipid peroxida-

tion. The latter reaction will prevail when the redox potential of the radical couple is less than that of the $O_2/O_2^{\cdot-}$ couple, especially in cells with relatively high oxygen tension. Radicals derived from the 1-electron reduction of paraquat (123) and adriamycin (124) can cause such redox cycling and lipid peroxidation. Other compounds, such as the quinone, menadione, can cause oxidative stress apparently via redox cycling without causing a significant extent of lipid peroxidation (74). A few of these examples are discussed in more detail below.

Carbon Tetrachloride Hepatotoxicity

Reductive metabolism to the trichloromethyl radical by cytochrome P-450 is the first step in the pathogenesis of hepatotoxicity caused by carbon tetrachloride (125, 126), and both the trichloromethyl radical (127) and lipid radicals generated from it or from oxygenated trichloromethyl radicals have been detected in vitro and in vivo in rats (128). Because either a lack of oxygen or high oxygen partial pressures decrease lipid peroxidation, cytotoxicity, and electrophilic chlorine generation, it is believed that the trichloromethylperoxy radical plays a major role in the initiation of lipid peroxidation (129), and that lipid peroxidation initiates acute lethal cell injury (130). It would have been useful to know the effect of changing pO_2 on covalent binding of radiolabeled carbon tetrachloride in the hepatocyte system.

Lipid peroxidation induced by carbon tetrachloride appears to cause several biochemical changes in the cell that may ultimately lead to cell death. One early event is loss of the hepatocyte endoplasmic reticular calcium pump activity (131, 132). It also has been found that hepatocyte plasma membranes have a marked increase in permeability (133). Both changes may lead to a sustained rise in intracellular Ca^{2+} that has been associated with cell death (134).

The products of lipid peroxidation which are responsible for the ultimate damage to the cell are unknown. Several lipid hydroperoxides (133, 135) and their reduced products, such as 4-hydroxynonenal (136), are cytotoxic. Attention has been focused on the 4-hydroxyunsaturated aldehydes because they are major products of carbon tetrachloride-induced lipid peroxidation (137) and inhibit several cellular enzymes such as protein kinase C (138), as well as react with DNA (139). If these reactions are important in the pathogenesis of acute lethal cell injury caused by agents that cause lipid peroxidation, they represent a covalent insult as a result of initial noncovalent interactions.

Other Compounds

Most of the compounds listed in Table 2 under the heading of noncovalent interactions cause oxidant stress in cells. The bipyridyl compounds, paraquat and diquat, cause acute lethal cell injury to lung tissue and liver tissue (123,

140). The metabolic reduction products are radical cations that can cause redox cycling. Mechanisms that involve both cellular thiol oxidation and lipid peroxidation as a result of the redox cycling may be important in the pathogenesis of acute lethal cell injury (123, 140–142).

Menadione, and some other quinones with appropriate redox potentials, can also redox cycle and cause oxidant stress as already discussed (74, 76, 79, 83, 85). However, it should be kept in mind that most quinones are not only oxidants, but also are potent electrophiles that covalently react with nucleophilic groups (143), particularly with soft nucleophiles on peptides and proteins. The role that these covalent interactions play in the cytotoxic mechanism depends on the physicochemical characteristics of the quinone. Moreover, some structurally complex quinones, such as adriamycin, may be metabolized in parts of the molecule that are distant from the quinone structure, and possibly yield products that cause toxicity by mechanisms distinct from those attributed to the quinone structure (144). Additional examples of these kinds of interactions are discussed in the next section of this chapter.

Covalent and Noncovalent Interactions are Important

Quinones are a class of compounds with the ability to both arylate tissue macromolecules and to cause oxidative stress (143–146). Quinones and their related quinone imines are soft electrophiles that react rapidly with soft nucleophiles, such as the thiolate anion to form covalent addition compounds. Quinones also are oxidants that can be reduced by biological reductants, such as glutathione and pyridine nucleotides. If one-electron reduced semiquinones are formed, they may redox cycle by reducing molecular oxygen, a reaction that depends on the redox potential of the semiquinone/hydroquinone couple vs the O_2/O_2-couple. Thus, in principal, quinones can disrupt normal cellular biochemistry by both covalent and noncovalent interactions. Although the physicochemical factors that determine the extent of covalent/noncovalent reactions in cells have not been studied in detail, quinones and quinone imines have been found to cause cytotoxicity by either one or both reactions, depending on their structure (85, 143, 147). Some of the difficulties in determining the relative importance of covalent and noncovalent interactions in quinone-mediated acute lethal cell injury are discussed in the examples below.

Acetaminophen

Acetaminophen is a widely used analgesic and antipyretic that can cause hepatocellular necrosis when administered in high doses. Seminal work in the early 1970s established a role for cytochrome P-450 metabolism (148) and GSH depletion (149) in the pathogenesis of acetaminophen's toxicity. These

studies also showed a strong correlation between the extent of covalent binding of a reactive metabolite of acetaminophen to proteins in cells and extent of lethal cell injury (150, 151). A cytochrome P-450 oxidative metabolite of acetaminophen, *N*-acetyl-*p*-benzoquinone imine (NAPQI), appears to be primarily responsible for the binding and toxicity (41, 42, 152–155).

Several studies have questioned the role of covalent binding in cell injury caused by acetaminophen. Some compounds decrease cytotoxic effects caused by acetaminophen, but have little effect on covalent binding (156–159). The results of these studies can be accommodated by a mechanism that involves the ability of NAPQI to serve as an oxidant. In fact, NAPQI is a good chemical oxidant with a standard reduction potential, $E^0 = 0.978 \pm 0.001$ V (160), and a one-electron reduction potential, $E^1{}_7 = 0.707 \pm 0.010$V (161).

NAPQI also has been found to be a good oxidant in biological systems in which it oxidizes cellular thiols and pyridine nucleotides (153, 162–165). These oxidations may be involved in the pathogenesis of cytotoxicity caused by acetaminophen (159, 165), and indirect support for (166) and against (167) such a mechanism of oxidant stress in hepatotoxicity caused by acetaminophen has been published.

Redox cycling of NAPQI is another mechanism that has been proposed for acute lethal cell injury caused by acetaminophen (168). Such a mechanism would require the generation of superoxide anion, a reaction that has not been observed with NAPQI (154, 169, 170). Reduction of oxygen to superoxide by radical products of NAPQI would not be predicted to occur because of the relatively high redox potential of the semiquinone imine radical (161). However, in some cases lipid peroxidation has been observed and postulated as a mechanism for cell death caused by acetaminophen (163–174). It has been postulated that superoxide anion may be generated from the acetaminophen radical via a coupled reaction wherein one-electron oxidation of thiols yields thiyl radicals that can reduce molecular oxygen (161). Another possibility in vivo is that oxy radicals are generated by invasive blood cells, such as activated macrophages (175) and/or neutrophils (176), in response to initial tissue damage. Such a mechanism is consistent with the results of studies indicating that lipid peroxidation may play a role in acetaminophen hepatotoxicity, but can be dissociated from the hepatotoxic response (177–179).

Results of studies with analogs of acetaminophen also indicate that both covalent and noncovalent interactions may be important in the pathogenesis of acute lethal cell injury. Regioisomers of acetaminophen, 2'- and 3'-hydroxyacetanilide, bind covalently to hepatic proteins without causing hepatotoxicity (180, 181). Moreover, reactive metabolites of 3'-hydroxyacetanilide form glutathione conjugates like those of acetaminophen (184). A conclusion

from these studies might be that covalent binding does not play a role in hepatotoxicity caused by acetaminophen. However, recent investigations have shown that the subcellular distribution of covalent binding of acetaminophen is different than that of 3'-hydroxyacetanilide (185). Reactive metabolites of acetaminophen were found to bind more extensively to mitochondrial proteins than did reactive metabolites of 3'-hydroxyacetanilide. Although this could be the reason for the difference in toxicity between the two isomers, acetaminophen also caused more extensive oxidation of mitochondrial GSH to GSSG. Therefore, until we know which proteins are altered by acetaminophen treatment, we will not know whether the binding or the oxidant stress caused by reactive metabolites of acetaminophen is more important in mediating lethal cell injury.

Investigations with dimethylated analogs of acetaminophen have provided conflicting results. The initial studies on chemical reactivity and toxicity of 3,5-dimethylacetaminophen and 2,6-dimethylacetaminophen provided evidence that the former compound was more hepatotoxic in rats and mice (186). Other studies then showed that the quinone imine oxidative metabolite of 3,5-dimethylacetaminophen oxidized cellular thiols to a greater extent, but covalently bound to hepatocyte proteins to a lesser extent than did the quinone imine of 2,6-dimethylacetaminophen (147, 187, 188). These results would appear to implicate a major role for oxidative stress in toxicity by these hydroxyacetanilides. However, in contrast to the initial results in vivo, it was found that the 2,6-dimethylated isomer was more cytotoxic (147). This result was consistent with the results of studies reported by another group who found that 3,5-dimethyl-acetaminophen was not hepatotoxic in mice (189). Inconsistent with this result is the finding that 3,5-dimethylacetaminophen is more cytotoxic to cultured mouse hepatocytes than either acetaminophen or 2,6-dimethylacetaminophen, and the cytotoxicity correlated with protein thiol oxidation rates (190). Results of the investigations could be further complicated by the fact that *N*-deacetylation of the quinone imine of 3,5-dimethylacetaminophen may yield a highly toxic arylating agent (191).

Overall, the results of both chemical and biochemical investigations support the contention that covalent and noncovalent interactions play major roles in the pathogenesis of acute lethal cell injury caused by the major reactive metabolite of acetaminophen, NAPQI. Until target proteins are identified and characterized, we will not know if one or the other interaction, or both, are critical events in initiating cell death.

CONCLUSION

Mechanisms of acute lethal cell injury caused by chemicals are a likely consequence of alterations in several intracellular and extracellular biochem-

ical processes. Until the sequences of events that lead to cell death are more clearly understood, the roles of covalent and noncovalent interactions of chemicals and their reactive metabolites with tissue macromolecules in the mediation of cell injury will remain speculative. Whether several simultaneous covalent and/or noncovalent alterations are required to initiate the sequence of events that lead to cell death is also not clear. However, in a few cases target macromolecules for covalent interactions have been identified, and evidence has been mounting for the role of covalent and/or noncovalent reactions of other chemicals with cellular ion transport and structural proteins in the sequence of events that lead to cell death. With the recent advances in both physicochemical and immunochemical techniques to identify and purify macromolecules, and advances in structural-functional characterization of proteins by both classical physicochemical and newer molecular biological techniques, the roles of covalent and noncovalent interactions in lethal cell injury are amenable to investigation at the molecular level.

Literature Cited

1. Miller, E. C., Miller, J. A. 1966. Mechanisms of chemical carcinogenesis: Nature of proximate carcinogens and interactions with macromolecules. *Pharmacol. Rev.* 18:805–38
2. Brodie, B. B., Reid, W. D., Cho, A. K., Sipes, G., Krishna, G., Gillette, J. R. 1971. Possible mechanism of liver necrosis caused by aromatic organic compounds. *Proc. Natl. Acad. Sci. USA* 68:160–64
3. Mitchell, J. R., Jollow, D. J. 1975. Metabolic activation of drugs to toxic substances. *Gastroenterology* 68:392–410
4. Nelson, S. D., Boyd, M. R., Mitchell, J. R. 1977. Role of metabolic activation in chemical-induced tissue injury. In *Drug Metabolism Concepts,* pp. 155–85. Washington, DC: Am. Chem. Soc.
5. Boyd, M. R. 1980. Biochemical mechanisms of chemical-induced lung injury: role of metabolic activation. *Crit. Rev. Toxicol.* 7:101–76
6. Nelson, S. D. 1982. Metabolic activation and drug toxicity. *J. Med. Chem.* 25:753–65
7. Cummings, S. W., Prough, R. A. 1983. Metabolic formation of toxic metabolites. In *Biological Basis of Detoxication,* ed. J. Caldwell, W. B. Jakoby, pp. 1–30. New York: Academic
8. Rush, G. F., Smith, J. H., Newton, J. F., Hook, J. B. 1984. Chemically induced nephrotoxicity: Role of metabolic activation. *Crit. Rev. Toxicol.* 13:99–160
9. Guengerich, F. P., Liebler, D. C. 1985. Enzymatic activation of chemicals to toxic metabolites. *Crit. Rev. Toxicol.* 14:259–308
10. Nelson, S. D., Harvison, P. J. 1987. Roles of cytochrome P-450 in chemically induced cytotoxicity. In *Mammalian Cytochromes P-450,* ed. F. P. Guengerich, pp. 19–80. Boca Raton, Fla: CRC
11. Kadulbar, F. F., Hammons, G. J. 1987. The role of cytochrome P-450 in the metabolism of chemical carcinogens. See Ref. 10, pp. 81–130
12. Monks, T. J., Lau, S. S. 1988. Reactive intermediates and their toxicological significance. *Toxicology* 52:1–53
13. Mitchell, J. R., Horning, M. G., eds. 1984. *Drug Metabolism and Drug Toxicity.* New York: Raven
14. Anders, M. W., ed. 1985. *Bioactivation of Foreign Compounds.* Orlando: Academic
15. Kocsis, J. J., Jollow, D. J., Witmer, C. M., Nelson, J. O., Snyder, R., eds. 1986. *Biological Reactive Intermediates III.* New York: Plenum
16. Zarble, H., Sukumer, S., Arthur, A. V., Martin-Zanca, D., Barbacid, M. 1985. Direct mutagenesis of Ha-*ras*-1 oncogenes by *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis in rats. *Nature* 315:382–84
17. Park, B. K., Coleman, J. W., Kitteringham, N. R. 1987. Drug disposition and hypersensitivity. *Biochem. Pharmacol.* 36:581–90
18. Pohl, L. R., Satoh, H., Christ, D. D.,

Kenna, J. G. 1988. The immunlogic and metabolic basis of drug hypersensitivities. *Annu. Rev. Pharmacol. Toxicol.* 18:367–87
19. Uetrecht, J. P. 1989. Idiosyncratic drug reactions: Possible role of reactive metabolites generated by leukocytes. *Pharm. Res.* 6:265–73
20. Frimmer, M. 1987. What we have learned from phalloidin. *Toxicol. Lett.* 35:169–82
21. Wecker, L., Kianta, T., Dettbarn, W. D. 1978. Relationship between acetylcholinesterase inhibition and the development of myopathy. *J. Pharmacol. Exp. Ther.* 206:97–104
22. Gillette, J. R. 1987. Significance of covalent binding of chemically reactive metabolites of foreign compounds to proteins and lipids. See Ref. 15, pp. 63–82
23. Gillette, J. R., Pohl, L. R. 1977. A prospective on covalent binding and toxicity. *J. Toxicol. Environ. Health* 2:849–71
24. Burlingame, A. L., Straub, K., Baillie, T. A. 1983. Mass spectrometric studies on the molecular basis of xenobiotic-induced toxicities. *Mass Spectrom. Rev.* 2:331–87
25. Farmer, P. B., Neumann, H.-G., Henschler, D. 1987. Estimation of exposure of man to substances reacting covalently with macromolecules. *Arch. Toxicol.* 60:251–60
26. Lu, A. Y. H., Miwa, G. T., Wislocki, P. G. 1988. Toxicological significance of covalently bound drug residues. *Rev. Biochem. Toxicol.* 9:1–27
27. Pegg, A. E. 1983. Alkylation and subsequent repair of DNA after exposure to dimethylnitrosamine and related carinogens. *Rev. Biochem. Toxicol.* 5:83–133
28. Halpert, J. 1981. Covalent modification of lysine during the suicide inactivation of rat liver cytochrome P-450 by chloramphenicol. *Biochem. Pharmacol.* 30:875–81
29. Dyroff, M. C., Neal, R. A. 1981. Identification of the major protein adduct formed in rat liver after thioacetamide administration. *Cancer Res.* 41:3430–35
30. Ortiz de Montellano, P. R., Correia, M. A. 1983. Suicidal destruction of cytochrome P-450 during oxidative metabolism. *Annu. Rev. Pharmacol. Toxicol.* 23:481–503
31. Marks, G. S., McCluskey, S. A., Mackie, J. E., Riddick, D. S., James, C. A. 1988. Disruption of hepatic heme biosynthesis after interaction of xenobiotics with cytochrome P-450. *FASEB J.* 2:2774–83
32. Monks, T. J., Hinson, J. A., Gillette, J. R. 1982. Bromobenzene and *p*-bromophenol toxicity and covalent binding *in vivo*. *Life Sci.* 30:841–48
33. Wiley, R. A., Hanzlik, R. P., Gillesse, T. 1979. Effect of substituents on *in vitro* metabolism and covalent binding of substituted bromobenzenes. *Toxicol. Appl. Pharmacol.* 49:249–55
34. O'Brien, K. A. F., Smith, L., Cohen, G. M. 1985. Differences in napthalene-induced toxicity in the mouse and rat. *Chem.-Biol. Interact.* 55:109–22
35. Buckpitt, A. R., Castagnoli, N. Jr., Nelson, S. D., Jones, A. D., Bahnson, L. S. 1987. Stereoselectivity of napthalene epoxidation by mouse, rat, and hamster pulmonary, hepatic, and renal microsomal enzymes. *Drug Metab. Dispos.* 15:491–98
36. Tunek, A., Schelin, C., Jergil, B. 1979. Microsomal target proteins of metabolically activated aromatic hydrocarbons. *Chem.-Biol. Interact.* 27:133–44
37. Schelin, C., Tunek, A., Jernstrom, B., Jergil, B. 1980. Irreversible binding of isolated benzo[a]pyrene metabolites to specific rat liver microsomal proteins. *Mol. Pharmacol.* 18:529–35
38. Sun, J. D., Dent, J. G. 1980. A new method for measuring covalent binding of chemicals to cellular macromolecules. *Chem.-Biol. Interact.* 32:41–61
39. Anderson, N. L., Giere, F. A., Nance, S. L., Gemmell, M. A., Tollaksen, S. L., Anderson, N. G. 1987. Effects of toxic agents at the protein level: Quantitative measurement of 213 mouse liver proteins following xenobiotic treatment. *Fundam. Appl. Toxicol.* 8:39–50
40. Vittozzi, L., Nastainczyle, W. 1987. Binding of reactive metabolites of CCl_4 to specific microsomal proteins. *Biochem. Pharmacol.* 36:1401–6
41. Bartalone, J. B., Birge, R. B., Sparks, K., Cohen, S. D., Khairallah, E. A. 1988. Immunochemical analysis of acetaminophen covalent binding to proteins. *Biochem. Pharmacol.* 37:4763–74
42. Pumford, N. R., Hinson, J. A., Potter, D. W., Rowland, K. L., Benson, R. W., Roberts, D. W. 1989. Immunochemical quantitation of 3-(cysteinyl-S-yl)acetaminophen adducts in serum and liver proteins of acetaminophen-treated mice. *J. Pharmacol. Exp. Ther.* 248:190–96
43. Kenna, J. G., Neuberger, J., Williams, R. 1987. Identification by immunoblotting of three halothane-induced liver mi-

crosomal polypeptide antigens recognized by antibodies in sera from patients with halothane-associated hepatitis. *J. Pharmacol. Exp. Ther.* 242:733–40

44. Kenna, J. G., Satoh, H., Christ, D. D., Pohl, L. R. 1988. Metabolic basis for a drug hypersensitivity: Antibodies in sera from patients with halothane hepatitis recognize liver neoantigens that contain the trifluoroacetyl group derived from halothane. *J. Pharmacol. Exp. Ther.* 245:1103–9
45. Beaune, P., Dansette, P. M., Mansuy, D., Kiffel, L., Finck, M., et al. 1987. Human anti-endoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis are directed against a human liver cytochrome P-450 that hydroxylates the drug. *Proc. Natl. Acad. Sci. USA* 84:551–55
46. Satoh, H., Martin, B. M., Schulick, A. H., Christ, D. D., Kenna, J. G., Pohl, L. R. 1989. Human anti-endoplasmic reticulum antibodies in sera of patients with halothane-induced hepatitis are directed against a trifluoracetylated carboxylesterase. *Proc. Natl. Acad. Sci. USA* 86:322–26
47. Martin, J. L., Kenna, J. G., Martin, B. M., Pohl, L. R. 1989. Trifluoroacetylated protein disulfide isomerase is a halothane-induced neoantigen. *Toxicologist* 9:5 (Abstr.)
48. van Bladeren, P. J. 1988. Formation of toxic metabolites from drugs and other xenobiotics by glutathione conjugation. *Trends Pharmacol. Sci.* 9:295–99
49. Vroomen, L. H. M., Berghmans, M. C. J., Groten, J. P., Koeman, J. H., van Bladeren, P. J. 1988. Reversible interaction of a reactive intermediate derived from furazolidone with glutathione and protein. *Toxicol. Appl. Pharmacol.* 95: 53–60
50. Bruggeman, I. M., Temmink, J. H. M., van Bladeran, P. J. 1986. Glutathione- and cysteine-mediated cytotoxicity of allyl and benzyl isothiocyanate: *Toxicol. Appl. Pharmacol.* 83:349–59
51. Ferguson, J. S., Kennedy, A. L., Stock, M. F., Brown, W. E., Alarie, Y. 1988. Uptake and distribution of ^{14}C during and following exposure to [^{14}C]methylisocyanate. *Toxicol. Appl. Pharmacol.* 94:104–17
52. Brown, W. E., Green, A. E., Cedel, T. E., Cairns, J. 1987. Biochemistry of protein-isocyanate interactions: A comparison of the effects of aryl vs alkyl isocyanates. *Environ. Health Perspect.* 72:5–11
53. Bhattacharya, B. K., Sharma, S. K., Jaiswail, D. K. 1988. *In vivo* binding of [^{14}C]methylisocyanate to various tissue proteins. *Biochem. Pharmacol.* 37: 2489–93
54. Threadgill, M. D., Axworthy, D. B., Baillie, T. A., Farmer, P. B., Farrow, K. C., et al. 1987. The metabolism of *N*-methylformamide in mice: Primary kinetic deuterium isotope effect and identification of *S*-(*N*-methylcarbamoyl)glutathione as a metabolite. *J. Pharmacol. Exp. Ther.* 242:312–19
55. Han, D.-H., Pearson, P. G., Baillie, T. A., Dayal, R., Tsang, L. H., et al. 1989. Chemical synthesis and cytotoxic properties of *N*-alkylcarbamic acid thioesters, metabolites of hepatotoxic formamides. *Chem. Res. Toxicol.* In press
56. Bill, C. A., Gescher, A., Hickman, J. A. 1988. Effects of *N*-methylformamide on the growth, cell cycle, and glutathione status of murine TLX5 lymphoma cells. *Cancer Res.* 48:3389–93
57. Naylor, S., Mason, R. P., Sanders, J. K., Williams, D. H., Moneti, G. 1988. Formaldehyde adducts of glutathione. *Biochem. J.* 249:573–79
58. Sies, H. 1985. Oxidative stress: Introductory remarks. In *Oxidative Stress,* ed. H. Sies, pp. 1–8. London: Academic
59. Trump, B. G., Berezesky, I. K. 1984. Role of sodium and calcium regulation in toxic cell injury. See Ref. 13, pp. 261–300
60. Mitchell, J. R., Smith, C. V., Lauterburg, D. H., Hughes, H., Corcoran, G. B., Horning, E. C. 1984. Reactive metabolites and the pathophysiology of acute lethal cell injury. See Ref. 13, pp. 301–19
61. Rasmussen, H. 1984. Cellular calcium homeostasis and cell injury. In *Mechanisms of Hepatocyte Injury and Death,* ed. D. Keppler, H. Popper, L. Bianchi, W. Reutter, pp. 59–70. Hingham, Mass.: MTP
62. Farber, J. L. 1984. The role of calcium in liver cell death. See Ref. 61, pp. 111–18
63. Bellomo, G., Orrenius, S. 1985. Altered thiol and calcium homeostasis in oxidative hepatocellular injury. *Hepatology* 5:876–82
64. Richter, C., Frei, B. 1988. Ca^{2+} release from mitochondria induced by prooxidants. *Free Radic. Biol. Med.* 4:365–75
65. Pascoe, G. A., Reed, D. J. 1989. Cell calcium, vitamin E, and the thiol redox system in cytotoxicity. *Free Radic. Biol. Med.* 6:209–24
66. Karplus, P. A., Krauth-Siegel, R. L., Schirmer, R. H., Schulz, G. E. 1988.

Inhibition of glutathione reductase by the nitrosourea drugs 1,3-bis(2-chloroethyl)-1-nitrosourea and 1-(2-chloroethyl)-3-(2-hydroxyethyl)-1-nitrosourea. *Eur. J. Biochem.* 171:193–98

67. Ziegler, D. M. 1985. Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. *Annu. Rev. Biochem.* 54:305–29
68. Brodie, A. E., Reed, D. J. 1987. Reversible oxidation of glyceraldehyde 3-phosphate dehydrogenase thiols in human lung carcinoma cells by hydrogen peroxide. *Biochem. Biophys. Res. Commun.* 148:120–25
69. Hyslop, P. A., Hinshaw, D. B., Halsey, W. A., Schraufstatter, I. U., Sauerheber, R. D., et al. 1988. Mechanisms of oxidant-mediated cell injury. *J. Biol. Chem.* 263:1665–75
70. Schraufstatter, I. U., Hyslop, P. A., Hinshaw, D. B., Spragg, R. G., Sklar, L. A., Cochrane, C. G. 1986. Hydrogen peroxide-induced injury of cells and its prevention by inhibitors of poly (ADP-ribose) polymerase. *Proc. Natl. Acad. Sci. USA* 83:4908–12
71. Stubberfield, C. R., Cohen, G. M. 1988. NAD^+ depletion and cytotoxicity in isolated hepatocytes. *Biochem. Pharmacol.* 37:3967–74
72. Jones, D. P., Thor, H., Smith, M. T., Jewell, S. A., Orrenius, S. 1983. Inhibition of ATP-dependent microsomal Ca^{2+} sequestration during oxidative stress and its prevention by glutathione. *J. Biol. Chem.* 258:6390–93
73. Kosower, N. S., Song, K.-R., Kosower, E. M. 1969. Glutathione IV. Intracellular oxidation and membrane injury. *Biochim. Biophys. Acta* 192:23–28
74. DiMonte, D., Bellomo, G., Thor, H., Nicotera, P., Orrenius, S. 1984. Menadione-induced cytotoxicity is associated with protein thiol oxidation and alteration in intracellular Ca^{2+} homeostasis. *Arch. Biochem. Biophys.* 235:343–50
75. Fabregat, I., Satrustegui, J., Machado, A. 1984. Interaction with protein SH groups could be involved in adriamycin cardiotoxicity. *Biochem. Med.* 32:289–95
76. Nicotera, P., Moore, M., Mirabelli, F., Bellomo, G., Orrenius, S. 1985. Inhibition of hepatocyte plasma membrane Ca^{2+}-ATPase activity by menadione metabolism and its restoration by thiols. *FEBS Lett.* 181:149–53
77. Hebbel, R. P., Shalev, O., Foker, W., Rank, B. H. 1986. Inhibition of erythrocyte Ca^{2+}-ATPase by activated oxygen through thiol- and lipid-dependent mechanisms. *Biochim. Biophys. Acta* 862:8–16
78. Scherer, N. M., Deamer, D. W. 1986. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulhydryl groups in the Ca^{2+}-ATPase. *Arch. Biochem. Biophys.* 216:589–601
79. Floreani, M., Carpenedo, F. 1989. Inhibition of cardiac sarcoplasmic reticulum Ca^{2+}-ATPase activity by menadione. *Arch. Biochem. Biophys.* 270: 33–41
80. Persson, B., Rydstrom, J. 1987. Evidence for a role of a vicinal dithiol in catalysis and proton pumping in mitochondrial nicotinamide nucleotide transhydrogenase. *Biochem. Biophys. Res. Commun.* 142:537–78
81. Yagi, T., Hatefi, Y. 1987. Thiols in oxidative phosphorylation: thiols in the F_o of ATP synthase essential for ATPase activity. *Arch. Biochem. Biophys.* 254:102–9
82. Gan, Z.-R., Wells, W. W. 1987. Identification and reactivity of the catalytic site of pig liver thioltransferase. *J. Biol. Chem.* 262:6704–7
83. Mirabelli, F., Salis, A., Marinoni, V., Finardi, G., Bellomo, G., et al. 1988. Menadione-induced bleb formation in hepatocytes is associated with the oxidation of thiol groups in actin. *Arch. Biochem. Biophys.* 264:261–69
84. Rokutan, K., Thomas, J. A., Sies, H. 1989. Specific S-thiolation of a 30-kDa cytosolic protein from rat liver under oxidative stress. *Eur. J. Biochem.* 179: 233–39
85. Thor, H., Miracelli, F., Salis, A., Cohen, G. M., Bellomo, G., Orrenius, S. 1988. Alterations in hepatocyte cytoskeleton caused by redox cycling and alkylating quinones. *Arch. Biochem. Biophys.* 266:397–407
86. Vercesi, A. E. 1987. The participation of NADP, the transmembrane potential and the energy-linked NAD(P) transhydrogenase in the process of Ca^{2+} efflux from rat liver mitochondria. *Arch. Biochem. Biophys.* 252:171–78
87. Kappus, H. 1987. A survey of chemicals inducing lipid peroxidation in biological systems. *Chem. Phys. Lipids* 45:105–15
88. Recknagel, R. O. 1983. A new direction in the study of carbon tetrachloride toxicity. *Life Sci.* 33:401–8
89. Poli, G., Albano, E., Diazani, M. U. 1987. The role of lipid peroxidation in liver damage. *Chem. Phys. Lipids* 45:117–42
90. Spencer, P. S., Schaumburg, H. H., Sabri, M. I., Veronesi, B. 1980. The enlarging view of hexacarbon neuro-

toxicity. *CRC Crit. Rev. Toxicol.* 7: 279–356
91. DeCaprio, A. P., O'Neill, E. A. 1985. Alterations in rat axonal cytoskeletal proteins induced by *in vitro* and *in vivo* 2,5-hexandione exposure. *Toxicol. Appl. Pharmacol.* 78:235–47
92. Sayre, L. M., Autilio-Gambetti, L., Gambetti, P. 1985. Pathogenesis of experimental giant neurofilamentors axonopathies: a unified hypothesis based on chemical modification of neurofilaments. *Brain Res. Rev.* 10:69–83
93. Genter, M. B., Szakal-Quin, G., Anderson, C. W., Anthony, D. C., Graham, D. G. 1987. Evidence that pyrrole formation is a pathogenic step in γ-diketone neuropathy. *Toxicol. Appl. Pharmacol.* 87:351–62
94. DeCaprio, A. P., Briggs, R. G., Jackowski, S. J., Kim, J. C. S. 1988. Comparative neurotoxicity and pyrrole-forming potential of 2,5-hexanedione and perdeuterio-2,5-hexanedione in the rat. *Toxicol. Appl. Pharmacol.* 92:75–85
95. Graham, D., Szakal-Quin, G., Priest, J. W., Anthony, D. C. 1984. *In vitro* evidence that covalent crosslinking of neurofilaments occurs in γ-diketone neuropathy. *Proc. Natl. Acad. Sci. USA* 81:4979–82
96. Rosenberg, C. K., Anthony, D. C., Szakal-Quin, G., Genter, M. B., Graham, D. G. 1987. Hyperbaric oxygen accelerates the neurotoxocity of 2,5-hexanedione. *Toxicol. Appl. Pharmacol.* 87:374–79
97. Boekelheide, K. 1987. 2,5-Hexanedione alters microtubule assembly. I. Testicular atrophy, not nervous system toxicity correlates with enhanced tubulin polymerization. *Toxicol. Appl. Pharmacol.* 88:370–82
98. Boekelheide, K. 1988. Rat testis during 2,5-hexanedione intoxidation and recovery. II. Dynamics of pyrrole reactivity, tubulin content and microtubule assembly. *Toxicol. Appl. Pharmacol.* 92:28–33
99. Abou-Donia, M. B., Lapadula, D. M., Suwita, E. 1988. Cytoskeletal proteins as targets for organophosphorus compound and aliphatic hexacarbon-induced neurotoxicity. *Toxicology* 49:469–77
100. Sager, P. R., Matheson, D. W. 1988. Mechanisms of neurotoxicity related to selective disruption of microtubules and intermediate filaments. *Toxicology* 49: 479–92
101. Casini, A., Giorli, M., Hyland, R. J., Serroni, A., Gilfor, D., Farber, J. L. 1982. Mechanisms of cell injury in the killing of cultured hepatocytes by bromobenzene. *J. Biol. Chem.* 257:6721–28
102. Badr, M. Z., Belinsky, S. A., Kauffman, K. C., Thurman, R. G. 1986. Mechanism of hepatotoxicity to periportal regions of the liver lobule due to allyl alcohol: Role of oxygen and lipid peroxidation. *J. Pharmacol. Exp. Ther.* 238:1138–42
103. Jaeschke, H., Kleinwaechter, C., Wendel, A. 1987. The role of acrolein in allyl alcohol-induced lipid peroxidation and liver cell damage in mice. *Biochem. Pharmacol.* 36:51–57
104. Smith, C. V., Lauterburg, B. H., Mitchell, J. R. 1985. Covalent binding and acute lethal injury *in vivo:* How has the original hypothesis survived a decade of critical examination? In *Drug Metabolism and Disposition: Considerations in Clinical Pharmacology*, ed. G. R. Wilkinson, M. D. Rawlins, pp. 161–81. Lancaster: MTP
105. Dogterom, P., Nagelkerke, J. F., van Steveninck, J., Mulder, G. J. 1988. Inhibition of lipid peroxidation by disulfiram and diethylthiocarbamate does not prevent hepatotoxin-induced cell death in isolated hepatocytes. *Chem.-Biol. Interact.* 66:251–65
106. Hormann, V. A., Moore, D. R., Rikans, L. E. 1989. Relative contributions of protein sulfydryl loss and lipid peroxidation to allyl alcohol-induced cytotoxicity in isolated rat hepatocytes. *Toxicol. Appl. Pharmacol.* 98:375–84
107. Baillie, T. A. 1988. Metabolic activation of valproic acid and drug-mediated hepatotoxicity. Role of the terminal olefin, 2-*n*-propyl-4-pentenoic acid. *Chem. Res. Toxicol.* 1:195–99
108. de Weck, A. L. 1983. Immunopathological mechanisms and clinical aspects of allergic reactions to drugs. In *Allergic Reactions to Drugs*, ed. A. L. de Weck, H. Bundgaard, pp. 75–133. Berlin: Springer-Verlag
109. van Dyke, R. A., Gandolfi, A. J. 1974. Studies on irreversible binding of radioactivity from [^{14}C]-halothane to rat hepatic microsomal lipids and proteins. *Drug Metab. Dispos.* 2:469–76
110. Uehleke, H., Werner, T., Greim, H., Kraimer, M. 1977. Metabolic activation of halothane and tests *in vitro* for mutagenicity. *Xenobiotica* 7:393–400
111. Karashima, D., Hirokata, Y., Shigematsu, A., Furukawa, T. 1977. The *in vitro* metabolism of halothane (2-bromo-2-chloro-1,1,1-trifluorethane) by hepatic microsomal cytochrome P-450. *J. Pharmacol. Exp. Ther.* 203:409–16

112. Gandolfi, A. J., White, R. D., Sipes, I. G., Pohl, L. R. 1980. Bioactivation and covalent binding of halothane *in vitro:* Studies with [^{3}H]- and [^{14}C]-halothane. *J. Pharmacol. Exp. Ther.* 214:721–25
113. Neuberger, J., Mieli-Vergani, G., Tredger, J. M., Davis, M., Williams, R. 1981. Oxidative metabolism of halothane in the production of altered hepatocyte membrane antigens in acute halothane-induced necrosis. *Gut* 22: 669–72
114. Vergani, D., Mieli-Vergani, G., Alberti, A., Neuberger, J., Eddleston, A. L. W. F., Williams, R. 1980. Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. *New Engl. J. Med.* 303:66–71
115. Utrecht, J. P. 1988. Mechanism of drug-induced lupus. *Chem. Res. Toxicol.* 1:133–43
116. Bakke, O. M., Wardell, W. M., Lasagne, L. 1984. Drug discontinuations in the United Kingdom and the United States, 1964 to 1983: Issues of safety. *Clin. Pharmacol. Ther.* 35:559–67
117. Clive, D. M., Stoff, J. F. 1984. Renal syndromes associated with non-steroidal anti-inflammatory drugs. *New Engl. J. Med.* 310:563–72
118. Smith, P. C., McDonagh, A. F., Benet, L. Z. 1986. Irreversible binding of zomepirac to plasma protein *in vitro* and *in vivo*. *J. Clin. Invest.* 77:934–39
119. Benet, L. Z., Spahn, H. 1988. Acyl migration and covalent binding of drug glucuronides-potential toxicity mediators. In *Cellular and Molecular Aspects of Glucuronidation,* ed. G. Siest, S. Magdalou, B. Burchell, pp. 261–69. London: John Libbey Eurotext
120. Rao, K. S., Recknagel, R. O. 1969. Early incorporation of carbon-labeled carbon tetrachloride into rat liver particulate lipids and proteins. *Exp. Mol. Pathol.* 10:219–28
121. Trudell, J. R., Boesterling, B., Trevor, A. J. 1982. Reductive metabolism of carbon tetrachloride by human cytochromes P-450 reconstituted in phospholipid vesicles; mass spectral identification of trichloromethyl radical bound to dioleoyl phosphatidyl choline. *Proc. Natl. Acad. Sci. USA* 79:2678–82
122. Rao, K. S., Recknagel, R. O. 1968. Early onset of lipoperoxidation in rat liver after carbon tetrachloride administration. *Exp. Mol. Pathol.* 9:271–78
123. Smith, P., Heath, D. 1976. Paraquat. *CRC Crit. Rev. Toxicol.* 4:411–45
124. Olson, R. D., Boerth, R. C., Gerber, J. G., Nies, A. S. 1981. Mechanism of adriamycin cardiotoxicity: evidence for oxidative stress. *Life Sci.* 29:1393–1401
125. Slater, T. F. 1981. Activation of carbon tetrachloride: chemical principles and biological significance. In *Free Radicals, Lipid Peroxidation and Cancer,* ed. D. C. H. McBain, T. F. Slater, pp. 243–70. London: Academic
126. Wolf, C. R., Harrelson, W. G., Nastainczyk, W. M., Philpot, R. M., Kalyanaraman, B., Mason, R. P. 1980. Metabolism of carbon tetrachloride in hepatic microsomes and reconstituted monooxygenase systems and its relationship to lipid peroxidation. *Mol. Pharmacol.* 18:553–58
127. Lai, E. K., McCay, P. B., Noguchi, T., Fong, K.-L. 1979. *In vivo* spin-trapping of trichloromethyl radicals formed from CCl_4. *Biochem. Pharmacol.* 28:2231–35
128. McCay, P. B., Lai, E. K., Poyer, J. L., DuBose, C. M., Janzen, E. G. 1984. Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. *J. Biol. Chem.* 259:2135–43
129. Mico, B. A., Pohl, L. L. 1983. Reductive oxygenation of carbon tetrachloride: trichloromethylperoxyl radical as a possible intermediate in the conversion of carbon tetrachloride to electrophilic chlorine. *Arch. Biochem. Biophys.* 225: 596–609
130. DeGroot, H., Littauer, A., Hugh-Wissemann, D., Wissemann, P., Noll, T. 1988. Lipid peroxidation and cell viability in isolated hepatocytes in a redesigned Oxystat system: evaluation of the hypothesis that lipid peroxidation, preferentially induced at low oxygen partial pressures, is decisive for CCl_4 liver cell injury. *Arch. Biochem. Biophys.* 264:591–99
131. Moore, L., Davenport, G. R., Landon, E. J. 1976. Calcium uptake of a rat liver microsomal subcellular fraction in response to *in vivo* administration of CCl_4. *J. Biol. Chem.* 251:1197–1201
132. Lowrey, K., Glende, E. A., Recknagel, R. O. 1981. Destruction of liver microsomal calcium pump activity by carbon tetrachloride and bromotrichloromethane. *Biochem. Pharmacol.* 30:135–40
133. Tsokos-Kuhn, J. O., Smith, C. V., Mitchell, J. R., Tate, C. A., Entman, M. L. 1986. Evidence for increased membrane permeability of plasmalemma vesicles from livers of phenobarbital induced CCl_4-intoxicated rats. *Mol. Pharmacol.* 30:444–51
134. Long, R. M., Moore, L. 1987. Cytosol-

ic calcium after carbon tetrachloride, 1,1-dichloroethylene, and phenylephrine exposure. *Biochem. Pharmacol.* 36: 1215–21

135. Corongiu, F. P., Poli, G., Dianzani, M. U., Cheeseman, K. H., Slater, T. F. 1986. Lipid peroxidation and molecular damage to polyunsaturated fatty acids in rat liver. Recognition of two classes of hydroperoxides formed under conditions *in vivo*. *Chem.-Biol. Interact.* 59:147–55
136. Benedetti, A., Comparti, M., Esterbauer, H. 1980. Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. *Biochim. Biophys. Acta* 620:281–96
137. Poli, G., Dianzani, M. U., Cheeseman, K. H., Slater, T. F., Lang, J., Esterbauer, H. 1985. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride or ADP-iron in isolated rat hepatocytes and rat liver microsomal suspensions. *Biochem. J.* 227: 629–38
138. Poli, G., Albano, E., Dianzani, M. U., Melloni, E., Pontremoli, S., et al. 1988. Carbon tetrachloride-induced inhibition of protein kinase C in isolated rat hepatocytes. *Biochem. Biophys. Res. Commun.* 153:591–97
139. Sodum, R. S., Chung, F.-L. 1988. 1,N^2-Etheno-deoxyguanosine as a potential marker for DNA adduct formation by trans-4-hydroxy-2-nonenal. *Cancer Res.* 48:320–23
140. Burk, R. J., Lawrence, R. A., Lane, J. M. 1980. Liver necrosis and lipid peroxidation in the rat as a result of paraquat and diquat administration. *J. Clin. Invest.* 65:1024–31
141. Sandy, M. S., Moldeus, P., Ross, D., Smith, M. T. 1986. Role of redox cycling and lipid peroxidation in bipyridyl herbicide cytotoxicity. *Biochem. Pharmacol.* 35:3095–101
142. Smith, C. V. 1987. Evidence for participation of lipid peroxidation and iron in diquat-induced hepatic necrosis *in vivo*. *Mol. Pharmacol.* 32:417–22
143. Rossi, L., Moore, G. A., Orrenius, S., O'Brien, P. J. 1986. Quinone toxicity in hepatocytes without oxidative stress. *Arch. Biochem. Biophys.* 251:25–35
144. Olson, R. D., Mushlin, P. S., Brenner, D. E., Fleischer, S., Cusack, B. J., et al. 1988. Doxorubicin cardiotoxicity may be caused by its metabolite, doxorubicinol. *Proc. Natl. Acad. Sci. USA* 85:3585–89
145. Irons, R. D., Sawahata, T. 1985. Phenols, catechols, and quinones. See Ref. 14, pp. 259–81
146. Miller, M. G., Rodgers, A., Cohen, G. M. 1986. Mechanism of toxicity of naphthoquinones to isolated hepatocytes. *Biochem. Pharmacol.* 35:1177–84
147. Rundgren, M., Porubek, D. J., Harvison, P. J., Cotgreave, I. A., Moldeus, P., Nelson, S. D. 1988. Comparative cytotoxic effects of *N*-acetyl-*p*-benzoquinone imine and two dimethylated analogues. *Mol. Pharmacol.* 34:566–72
148. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Davis, D. C., Gillette, J. R., Brodie, B. B. 1973. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Ther.* 187:185–94
149. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R., Brodie, B. B. 1973. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.* 187:211–17
150. Jollow, D. J., Mitchell, J. R., Potter, W. Z., Davis, D. C., Gillette, J. R., Brodie, B. B. 1973. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. *J. Pharmacol. Exp. Ther.* 187:195–202
151. Potter, W. Z., Davis, D. C., Mitchell, J. R., Jollow, D. J., Gillette, J. R., Brodie, B. B. 1973. Acetaminophen-induced hepatic necrosis. III. Cytochrome P-450-mediated covalent binding *in vitro*. *J. Pharmacol. Exp. Ther.* 187:203–10
152. Hinson, J. A. 1980. Biochemical toxicology of acetaminophen. *Rev. Biochem. Toxicol.* 2:103–29
153. Dahlin, D. C., Miwa, G. T., Lu, A. Y. H., Nelson, S. D. 1984. *N*-acetyl-*p*-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. *Proc. Natl. Acad. Sci. USA* 81:1327–31
154. Holme, J. A., Dahlin, D. C., Nelson, S. D., Dybing, E. D. 1984. Cytotoxic effects of *N*-acetyl-*p*-benzoquinone imine, a common arylating intermediate of paracetamol and *N*-hydroxy paracetamol. *Biochem. Pharmacol.* 33:401–6
155. Hoffman, K.-J., Streeter, A. J., Axworthy, D. B., Baillie, T. A. 1985. Identification of the major covalent adduct formed *in vitro* and *in vivo* between acetaminophen and mouse liver proteins. *Mol. Pharmacol.* 27:566–73
156. Labadorios, D., David, M., Portmann, B., Williams, R. 1977. Paracetamol-induced hepatic necrosis in the mouse:

Relationship between covalent binding, hepatic glutathione depletion and the protective effect of alpha-mercaptopropionyl glycine. *Biochem. Pharmacol.* 26:31–35

157. DeValia, J. L., Ogilvie, R. C., McLean, A. E. M. 1982. Dissociation of cell death from covalent binding of paracetamol by flavones in a hepatocyte system. *Biochem. Pharmacol.* 31:3745–49
158. Albano, E., Rundgren, M., Harvison, P. J., Nelson, S. D., Moldeus, P. 1985. Mechanisms of *N*-acetyl-*p*-benzoquinone imine cytotoxicity. *Mol. Pharmacol.* 28:306–11
159. Tee, L. B. G., Boobis, A. R., Hugett, A. C., Davies, D. S. 1986. Reversal of acetaminophen toxicity in isolated hamster hepatocytes by dithiothreitol. *Toxicol. Appl. Pharmacol.* 83:294–314
160. Novak, M., Pelecanou, M., Pollack, L. 1986. Hydrolysis of the model carcinogen *N*-(pivaloyloxy)-4-methoxyacetanilide: Involvement of *N*-acetyl-*p*-benzoquinonc imine. *J. Am. Chem. Soc.* 108:112–20
161. Bisby, R. H., Tabassum, N. 1988. Properties of the radicals formed by one-electron oxidation of acetaminophen-a pulse radiolysis study. *Biochem. Pharmacol.* 37:2731–38
162. Blair, I. A., Boobis, A. R., Davies, D. J., Cresp, T. M. 1980. Paracetamol oxidation: synthesis and reactivity of *N*-acetyl-*p*-bcnzoquinonc imine. *Tetrahedron Lett.* 21:4947–50
163. Potter, D. W., Hinson, J. A. 1986. Reactions of *N*-acetyl-*p*-benzoquinone imine with reduced glutathione, acetaminophen, and NADPH. *Mol. Pharmacol.* 30:33–41
164. Coles, B., Wilson, I., Wardman, P., Hinson, J. A., Nelson, S. D., Ketterer, B. 1988. The spontaneous and enzymatic rcaction of *N*-acetyl-*p*-bcnzoquinonc imine with glutathione: A stopped-flow kinetic study. *Arch. Biochem. Biophys.* 264:253–60
165. Moore, M., Thor, H., Moore, G., Nelson, S. D., Moldeus, P., Orrcnius, S. 1985. The toxicity of acetaminophen and *N*-acetyl-*p*-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic Ca^{2+}. *J. Biol. Chem.* 260:13035–40
166. Kyle, M. E., Nakae, D., Serroni, A., Farber, J. L. 1988. 1,3-(2-chloroethyl)-1-nitrosourea potentiates the toxicity of acetaminophen both in phenobarbital-induced rat and in hepatocytes cultured from such animals. *Mol. Pharmacol.* 84:584–89
167. Smith, C. V., Mitchell, J. R. 1985. Acetaminophen hepatotoxicity *in vivo* is not accompanied by oxidant stress. *Biochem. Biophys. Res. Commun.* 133:329–36
168. Rosen, G. M., Singletary, W. V. Jr., Rauckman, E. J., Killenberg, P. G. 1983. Acetaminophen hepatotoxicity. An alternative mechanism. *Biochem. Pharmacol.* 32:2053–59
169. Powis, G., Svingen, B. A., Dahlin, D. C., Nelson, S. D. 1984. Enzymatic and non-enzymatic reduction of *N*-acetyl-*p*-benzoquinone imine and some properties of the *N*-acetyl-*p*-benzoquinone imine radical. *Biochem. Pharmacol.* 33:2367–70
170. Fischer, V., West, P. R., Nelson, S. D., Harvison, P. J., Mason, R. P. 1985. Formation of 4-aminophenoxyl free radical from the acetaminophen mctabolite *N*-acetyl-*p*-benzoquinone imine. *J. Biol. Chem.* 260:11446–50
171. Wendel, A., Feuerstein, S., Konz, K.-H. 1979. Acute paracetamol intoxication of starved micc leads to lipid peroxidation *in vivo*. *Biochem. Pharmacol.* 28: 2051–55
172. Fairhurst, S., Barber, D. J., Clark, B., Horton, A. A. 1982. Studies on paracetamol-induced lipid peroxidation. *Toxicology* 23:249–59
173. Albano, E., Poli, G., Charpotto, E., Biasi, F., Dianzani, M. U. 1983. Paracetamol-stimulated lipid peroxidation in isolated rat and mouse hepatocytes. *Chem.-Biol. Interact.* 47:249–63
174. Kyle, M. E., Miccadei, S., Nakae, D., Farber, J. L. 1987. Superoxide dismutase and catalase protect cultured hepatocytes from the cytotoxicity of acetaminophen. *Biochem. Biophys. Res. Commun.* 149:889–96
175. Laskin, D. L., Pilaro, A. M. 1986. Potential role of activated macrophages in acctaminophen hepatotoxicity. *Toxicol. Appl. Pharmacol.* 86:204–15
176. Jaeschke, H., Mitchell, J. R. 1989. Neutrophil accumulation exacerbates acetaminophen-induced liver injury. *FASEB J.* 3:A920
177. Burk, R., Lane, J. M. 1979. Ethane production and liver necrosis in rats after administration of drugs and other chemicals. *Toxicol. Appl. Pharmacol.* 50:467–78
178. Younes, M., Siegers, C.-P. 1985. The role of iron in the paracetamol- and CCl_4-induced lipid peroxidation and heptatoxicity. *Chem.-Biol. Interact.* 55:327–34
179. Younes, M., Cornelius, S., Siegers, C.-P. 1986. Ferrous ion supported *in vivo* lipid peroxidation induced by paraceta-

mol—its relation to hepatotoxicity. *Res. Commun. Chem. Pathol. Pharmacol.* 51:89–99

180. Roberts, S. A., Jollow, D. J. 1979. Acetaminophen structure-toxicity studies: *in vivo* covalent binding of a non-hepatotoxic analog, 3'-hydroxyacetanilide. *Fed. Proc.* 38:426
181. Roberts, S. A., Jollow, D. J. 1979. Acetaminophen structure-toxicity studies: lack of liver necrosis after 2'-hydroxyacetanilide. *Pharmacologist* 21:220
182. Nelson, E. B. 1980. The pharmacology and toxicology of meta-substituted acetanilide. I. Acute toxicity of 3'-hydroxyacetanilide in mice. *Res. Commun. Chem. Pathol. Pharmacol.* 28: 447–56
183. Streeter, A. J., Bjorge, S. M., Axworthy, D. B., Nelson, S. D., Baillie, T. A. 1984. The microsomal metabolism and site of covalent binding to protein of 3'-hydroxyacetanilide, a non-hepatotoxic positional isomer of acetaminophen. *Drug Metab. Dispos.* 12:565–76
184. Rashed, M. S., Nelson, S. D. 1989. Characterization of glutathione conjugates of reactive metabolites of 3'-hydroxyacetanilide, a nonhepatotoxic positional isomer of acetaminophen. *Chem. Res. Toxicol.* 2:41–45
185. Tirmenstein, M. A., Nelson, S. D. 1989. Subcellular binding and effects on calcium homeostasis produced by acetaminophen and a nonhepatotoxic regioisomer, 3'-hydroxyacetanilide, in mouse liver. *J. Biol. Chem.* 264:9814–19
186. Fernando, C. R., Calder, I. C., Ham, K. N. 1980. Studies on the mechanism of toxicity of acetaminophen. Synthesis and reactions of *N*-acetyl-2,6-dimethyl- and *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imines. *J. Med. Chem.* 23:1153–58
187. Rosen, G. M., Rauckman, E. J., Ellington, S. P., Dahlin, D. C., Christie, J. C., Nelson, S. D. 1984. Reduction and glutathione conjugation reactions of *N*-acetyl-*p*-benzoquinone imine and two dimethylated analogues. *Mol. Pharmacol.* 25:151–57
188. Porubek, D. J., Rundgren, M., Harvison, P. J., Nelson, S. D., Moldeus, P. 1987. Investigation of mechanisms of acetaminophen toxicity in isolated rat hepatocytes with the acetaminophen analogues 3,5-dimethylacetaminophen and 2,6-dimethylacetaminophen. *Mol. Pharmacol.* 31:647–53
189. van de Straat, R., deVries, J., Groot, E. J., Zijl, R., Vermeulen, N. P. E. 1987. Paracetamol, 3-monoalkyl- and 3,5-dialkyl derivatives: Comparison of their hepatotoxicity in mice. *Toxicol. Appl. Pharmacol.* 89:183–89
190. Birge, R. B., Bartalone, J. B., Nishanian, E. V., Bruno, M. K., Mangold, J. B., et al. 1988. Dissociation of covalent binding from the oxidative effects of acetaminophen. *Biochem. Pharmacol.* 37:3383–93
191. Rossi, L., McGirr, L. G., Silva, J., O'Brien, P. J. 1988. The metabolism of *N*-acetyl-3,5-dimethyl-*p*-benzoquinone imine in isolated hepatocytes involves *N*-deacetylation. *Mol. Pharmacol.* 34: 674–81
192. Scheulen, M. E., Kappus, H. 1982. Metabolic activation of adriamycin by NADPH-cytochrome P-450 reductase, rat liver and heart microsomes and covalent protein binding of metabolites. In *Biological Reactive Intermediates IIA*, ed. R. Snyder, D. V. Parke, J. J. Kocsis, D. J. Jollow, C. G. Gibson, C. M. Witmer. pp. 471–85. New York: Plenum
193. Geneve, J., Larrey, D., Letteron, P., Descatoire, V., Tinel, M., et al. 1987. Metabolic activation of the tricyclic antidepressant amineptine I. Cytochrome P-450-mediated *in vitro* covalent binding. *Biochem. Pharmacol.* 36:323–29
194. Artellinoi, G., Grilli, S., Calaeci, A., Mazzulo, M., Prodi, G. 1985. *In vivo* and *in vitro* binding of benzene to nucleic acids and proteins of various rat and mouse organs. *Cancer Lett.* 28:159–68
195. Smart, R. C., Zannoni, V. G. 1985. Effect of ascorbate on covalent binding of benzene and phenol metabolites to isolated tissues preparations. *Toxicol. Appl. Pharmacol.* 77:334–43
196. Tunek, A., Platt, K. L., Przybilski, M., Oesch, F. 1980. Multistep metabolic activation of benzene. Effect of superoxide dismutase on covalent binding to microsomal macromolecules and identification of glutathione conjugates using high-pressure liquid chromatography and field desorptiom mass spectrometry. *Chem.-Biol Interact.* 33:1–17
197. Lutz, W. K., Schlatter, C. H. 1977. Mechanism of the carcinogenic action of benzene: Irreversible binding to rat liver DNA. *Chem.-Biol Interact.* 18:241–45
198. Snyder, R., Lee, E. W., Kocsis, J. J. 1978. Binding of labeled benzene metabolites to mouse liver bone and marrow. *Res. Commun. Chem. Pathol. Pharmacol.* 20:191–94
199. Lau, S. S., Abrams, G. D., Zannoni, V. G. 1980. Metabolic activation and de-

toxication of bromobenzene leading to cytotoxicity. *J. Pharmacol. Exp. Ther.* 214:703–8

200. Weller, P. E., Narasimhan, N., Buben, J. A., Hanzlik, R. P. 1988. *In vitro* metabolism and covalent binding among ortho-substituted bromobenzenes of varying hepatotoxicity. *Drug Metab. Dispos.* 16:232–37
201. Anders, M. W., Pohl, L. L. 1985. Halogenated alkanes. See Ref. 14, pp. 283–315
202. Ilett, K. F., Reid, W. D., Sipes, I. G., Krishna, G. 1973. Chloroform toxicity in mice. Correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecules. *Exp. Mol. Pathol.* 19:215–29
203. Buckley, L. A., Clayton, J. W., Nagle, R. B., Gandolfi, A. J. 1982. Chlorotrifluoroethylene nephrotoxicity in rats: A subacute study. *Fundam. Appl. Toxicol.* 2:181–86
204. Dohn, D. R., Anders, M. W. 1982. The enzymatic reaction of Chlorotrifluoroethylene with glutathione. *Biochem Biophys. Res. Commun.* 109:1339–45
205. Bucala, R., Fishman, J., Cerami, A. 1982. Formation of covalent adducts between cortisol and 16 α-hydroxyestrone and protein: possible role in the pathogenesis of cortisol toxicity and systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* 79:3320–24
206. Hill, D. L., Shih, T. W., Johnston, T. P., Struck, R. F. 1978. Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. *Cancer Res.* 38:2438–42
207. Van Bladeran, P. J., Breimer, D. D., van Huijgervoort, J. A. C. T. M., Vermuelen, N. P. E., van der Gen, A. 1981. The metabolic formation of *N*-acetyl-S-2-hydroxyethyl-L-cysteine from tetradeutero-1,2-dibromoethane: relative importance of oxidation and glutathione conjugation *in vivo*. *Biochem. Pharmacol.* 30:2499–502
208. Ozawa, N., Guengerich, F. P. 1983. Evidence for the formation of an S-[2-(N^7-guanyl)ethyl]glutathione adduct in glutathione mediated binding of the carcinogen 1,2-dibromoethane to DNA. *Proc. Natl. Acad. Sci. USA* 80:5266–77
209. Darnerud, P. A., Brandt, I., Vernon, F. J., Bakke, J. E. 1988. S-(1,2-dichloro-[^{14}C]-vinyl)-L-cysteine (DCVC) in mouse kidney: Correlation between tissue-binding and toxicity. *Toxicol. Appl. Pharmacol.* 95:423–34
210. Lutz, W. K., Jaggi, W., Schlatter, C. 1982. Covalent binding of diethylstilbestrol to DNA in rat and hamster liver and kidney. *Chem.-Biol. Interact.* 42:251–57
211. Haaf, H., Metzler, M. 1985. Covalent binding of diethylstilbestrol to microsomal protein *in vitro* correlates with the organotropism of its carcinogenicity. *Carcinogenesis* 6:659–60
212. Burka, L. T., Boyd, M. R. 1985. Furans. See Ref. 14, pp. 243–57
213. Ravindranath, V., Burka, L. T., Boyd, M. R. 1984. Reactive metabolites from the bioactivation of toxic furans. *Science* 224:884–86
214. Ravindranath, V., McMenamin, M. G., Dees, J. H., Boyd, M. R. 1986. 2-methylfuran toxicity in rats. Role of metabolic activation *in vivo*. *Toxicol. Appl. Pharmacol.* 85:78–91
215. Boyd, M. R., Sasame, H. A., Franklin, R. B. 1980. Comparison of ratios of covalent binding to total metabolism of the pulmonary toxin, 4-ipomeanol, *in vitro* in pulmonary and hepatic microsomes, and the effects of pretreatments with phenobarbital or 3-methylcholanthrene. *Biochem. Biophys. Res. Commun.* 93:1167–72
216. Buckpitt, A. R., Statham, C. N., Boyd, M. R. 1982. *In vivo* studies on the target tissue metabolism, covalent binding, glutathione depletion, and toxicity of 4-ipomeanol in birds, species deficient in pulmonary enzymes for metabolic activation. *Toxicol. Appl. Pharmacol.* 65:38–52
217. Dutcher, J. S., Boyd, M. R. 1979. Species and strain differences in target organ alkylation and toxicity by 4-ipomeanol. Predictive value of covalent binding in studies of target organ toxicities by reactive metabolites. *Biochem. Pharmacol.* 28:3367–72
218. Guengerich, F. P. 1977. Studies on the activation of a model furan compound-toxicity and covalent binding of 2-(*N*-ethylcarbamoyl - hydroxymethyl)furan. *Biochem. Pharmacol.* 26:1909–15
219. Mitchell, J. R., Nelson, W. L., Potter, W. Z., Sasame, H. A., Jollow, D. J. 1976. Metabolic activation of furosemide to a chemically reactive, hepatotoxic intermediate. *J. Pharmacol. Exp. Ther.* 199:41–52
220. Wirth, P. J., Bettis, C. J., Nelson, W. L. 1975. Microsomal metabolism of furosemide: Evidence for the nature of the reactive intermediate involved in covalent binding. *Mol. Pharmacol.* 12:759–68
221. Wolf, C. R., Berry, P. N., Nash, J. A., Green, T., Lock, E. A. 1984. Role of microsomal and cytosolic glutathione S-transferases in the conjugation of hex-

achloro-1,3-butadiene and its possible reference to nephrotoxicity. *J. Pharmacol. Exp. Ther.* 228:202–8

222. Nash, J. A., King, L. J., Lock, E. A., Green, T. 1984. The metabolism and disposition of hexachloro-1,3-butadiene in the rat and its relevance to nephrotoxicity. *Toxicol. Appl. Pharmacol.* 73:124–37
223. Prough, R. A., Moloney, S. J. 1985. Hydrazines. See Ref. 14, pp. 433–47
224. Timbrell, J. A. 1979. The role of metabolism in the hepatotoxicity of isoniazid and iproniazid. *Drug Metab. Rev.* 10:125–47
225. Woodward, K. N., Timbrell, J. A. 1984. Acetylhydrazine hepatotoxicity: the role of covalent binding. *Toxicology* 30:65–74
226. Nelson, S. D., Mitchell, J. R., Timbrell, J. A., Snodgrass, W. R., Corcoran, G. B. 1976. Isoniazid and iproniazid: Activation of metabolites to toxic intermediates in man and rat. *Science* 193:901–3
227. Timbrell, J. A., Mitchell, J. R., Snodgrass, W. R., Nelson, S. D. 1980. Isoniazid hepatotoxicity: The relationship between covalent binding and metabolism *in vivo*. *J. Pharmacol. Exp. Ther.* 213:364–69
228. Nelson, S. D., Mitchell, J. R., Snodgrass, W. R., Timbrell, J. A. 1978. Hepatotoxicity and metabolism of iproniazid and hydrazine. *J. Pharmacol. Exp. Ther.* 206:574–85
229. Cadenas, E., Mueller, A., Brigelius, R., Esterbauer, H., Sies, H. 1983. Effects of 4-hydroxynonenal on isolated hepatocytes. Studies on chemiluminescence response, alkane production and glutathione status. *Biochem. J.* 214:479–87
230. Cadenas, E., Mueller, A., Brigelius, R., Sies, H., Lang, J., Esterbauer, H. 1984. Low-level chemiluminescence, alkane production and glutathione depletion in isolated hepatocytes caused by a diffusible product of lipid peroxidation, 4-hydroxynonenal. In *Oxygen Radicals*, ed. W. Bors, M. Saran, D. Tait, pp. 317–24. Berlin: de Gruyter
231. Fouin-Fortunet, H., Letteron, P., Tinel, M., Degott, C., Flejou, J. F., Pessayre, D. 1984. Mechanism for isaxonine hepatitis. II. Protective role of glutathione and toxicological studies in mice. *J. Pharmacol. Exp. Ther.* 229: 851–58
232. Nocerini, M. R., Carlson, J. R., Breeze, R. G. 1983. Effect of glutathione status on covalent binding and pneumotoxicity of 3-methylindole in goats. *Life Sci.* 32:449–58
233. Bray, T. M., Carlson, J. R., Nocerini, M. R. 1984. *In vitro* covalent binding of 3-[^{14}C]methylindole metabolites in goat tissues *Proc. Soc. Exp. Biol. Med.* 176:48–53
234. Nocerini, M. R., Yost, G. S., Carlson, J. R., Liberato, D. J., Breeze, R. G. 1985. Structure of the glutathione adduct of activated 3-methylindole indicates that an imine methide is the electrophilic intermediate. *Drug Metab. Disp.* 13: 690–94
235. Nocerini, M. R., Carlson, J. R., Yost, G. S. 1985. Glutathione adduct formation with microsomally activated metabolites of the pulmonary alkylating and cytotoxic agent, 3-methylindole. *Toxicol. Appl. Pharmacol.* 81:75–84
236. Pearson, P. G., Gescher, A., Harpur, E. S. 1987. Hepatotoxicity of *N*-methylformamide in mice. 1. Relationship with glutathione status. *Biochem. Pharmacol.* 36:381–84
237. Pearson, P. G., Gescher, A., Harpur, E. S., Threadgill, M. D. 1987. Hepatotoxicity of *N*-methylformamide in mice. 2. Covalent binding of metabolites of [^{14}C]-labelled *N*-methylformamide to hepatic proteins. *Biochem. Pharmacol.* 36:385–90
238. Tomaz, M., Lipman, R., Chowdary, D., Pawlak, J., Verdine, G. L., Nakanishi, K. 1987. Isolation and structure of a covalent cross-link adduct between Mitomycin C and DNA. *Science* 235:1204–8
239. D'Arcy Doherty, M., Cohen, G. M., Smith, M. T. 1984. Mechanisms of toxic injury to isolated hepatocytes by 1-naphthol. *Biochem. Pharmacol.* 33: 543–49
240. Mattocks, A. R. 1973. Mechanisms of pyrrolizidine alkaloid toxicity. In *Pharmacology and the Future of Man*, ed. G. H. Acheson, 2:114–23. Basel, Switzerland: Karger
241. Schnellman, R. G., Monks, T. J., Lazaro, M. J., Lau, S. S. 1989. 2-bromohydroquinone-induced toxicity to rabbit renal proximal tubules: The role of biotransformation, glutathione, and covalent binding. *Toxicol. Appl. Pharmacol.* 99:19–27
242. Jowa, L., Winkle, S., Kalf, G. F., Witz, G., Snyder, R. 1986. Deoxyguanosine adducts formed from benzoquinone and hydroquinone. See Ref. 15, pp. 223–35
243. Waller, R. L., Glende, E. A., Recknagel, R. O. 1983. Carbon tetrachloride and bromotrichloromethane toxicity. Dual role of covalent binding of metabolic cleavage products and lipid

peroxidation in depression of microsomal calcium sequestration. *Biochem. Pharmacol.* 32:1613–17
244. Jarvisalo, J., Savolainen, H., Elovaara, E., Vainio, H. 1977. The *in vivo* toxicity of CS_2 to liver microsomes: Binding of labelled CS_2 and changes of the microsomal enzyme activities, *Acta Pharmacol. Toxicol.* 40:329–36
245. Dalvi, R. R., Poore, R. E., Neal, R. A. 1974. Studies on the metabolism of carbon disulfide by rat liver microsomes. *Life Sci.* 14:1785–96
246. Catagnani, G. L., Neal, R. A. 1975. Evidence for the formation of a protein bound hydrosulfide resulting from microsomal mixed function oxidase catalyzed desulfuration of carbon disulfide. *Biochem. Biophys. Res. Commun.* 65: 629–36
247. Porter, W. R., Gudzinowicz, M. J., Neal, R. A. 1979. Thioacetamide induced hepatic necrosis. II. Pharmacokinetics of thioacetamide and thioacetamide-S-oxide in the rat. *J. Pharmacol. Exp. Ther.* 208:386–91
248. Forkert, P. G., Birch, D. W. 1989. Pulmonary toxicity of trichloroethylene in mice. Covalent binding and morphological manifestations. *Drug. Metab. Dispos.* 17:106–13
249. Forkert, P. G., Stringer, V., Racz, W. J. 1986. Effects of administration of metabolic inducers and inhibitors on pulmonary toxicity and covalent binding by 1,1-dichloroethylene in CD-1 mice. *Exp. Mol. Pathol.* 45:44–58
250. Forkert, P. G., Stringer, V., Troughton, K. M. 1986. Pulmonary toxicity of 1,1-dichloroethylene: correlation of early changes with covalent binding. *Can. J. Physiol. Pharmacol.* 64:112–21
251. Graham, D. G., Tiffany, S. M., Bell, W. R., Gutknecht, W. F. 1978. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells *in vitro*. *Mol. Pharmacol.* 14:644–53
252. Kappus, H. 1986. Overview of enzyme systems involved in bio-reduction of drugs and in redox cycling. *Biochem. Pharmacol.* 35:1–6
253. Trush, M. A., Mimnaugh, E. G., Gram, T. E. 1982. Activation of pharmacological agents to radical intermediates: Implications for the role of free radicals in drug action and toxicity. *Biochem. Pharmacol.* 31:3335–46
254. Kappus, H., Sies, H. 1981. Toxic drug effects associated with oxygen metabolism: Redox cycling and lipid peroxidation. *Experentia* 37:1233–41
255. Favaudon, V. 1982. On the mechanism of reductive activation in the mode of action of some anti-cancer drugs. *Biochimie* 64:457–75
256. Kilkuskie, R. E., Macdonald, T. L., Hecht, S. D. 1984. Bleomycin may be activated for DNA cleavage by NADPH-cytochrome P-450 reductase. *Biochemistry* 23:6165–71
257. Mahmutoglo, I., Kappus, H. 1985. Oxy radical formation during redox cycling of bleomycin-iron (III) complex by NADPH cytochrome P-450 reductase. *Biochem. Pharmacol.* 34:3091–94
258. Bus, J. S., Aust, S. D., Gibson, J. E. 1975. Lipid peroxidation a possible mechanism of paraquat toxicity. *Res. Commun. Chem. Pathol. Pharmacol.* 11:31–38
259. Di Monte, D., Ross, D., Bellomo, G., Eklow, L., Orrenius, S. 1984. Alteration in intracellular thiol homeostasis during the metabolism of menadione by isolated rat hepatocytes. *Arch. Biochem. Biophys.* 235:334–42
260. Osawa, Y., Pohl, L. L. 1989. Covalent binding of the prosthetic heme to protein: A potential mechanism for the suicide inactivation or activation of hemoproteins. *Chem. Res. Toxicol.* 2: 131–41
261. Freeman, W. R., Uetrecht, J. P., Woosley, R. L., Oates, J. A., Harbison, R. L. 1981. Covalent binding of procainamide *in vitro* and *in vivo* to hepatic protein in mice. *Drug Metab. Dispos.* 9:188–92
262. Uetrecht, J. P., Sweetman, B. J., Woosley, R. L., Oates, J. A. 1984. Metabolism of procainamide to a hydroxylamine by rat and human hepatic microsomes. *J. Pharmacol. Exp. Ther.* 232:420–25
263. Uetrecht, J. P. 1985. Reactivity and possible significance of hydroxylamine and nitroso metabolites of procainamide. *J. Pharmacol. Exp. Ther.* 232:1064–69