Annu. Rev. Pharmacol. Toxicol. 2000. 40:43–65

CHLORINATED METHANES AND LIVER INJURY: Highlights of the Past 50 Years

Gabriel L. Plaa
Département de Pharmacologie, Faculté de Médecine, Université de Montréal, CP 6128, Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7; e-mail: plaag@magellan.umontreal.ca

Key Words carbon tetrachloride, chloroform, ketones, lipid peroxidation, regeneration

■ **Abstract** The chlorinated methanes, particularly carbon tetrachloride and chloroform, are classic models of liver injury and have developed into important experimental hepatoxicants over the past 50 years. Hepatocellular steatosis and necrosis are features of the acute lesion. Mitochondria and the endoplasmic reticulum as target sites are discussed. The sympathetic nervous system, hepatic hemodynamic alterations, and role of free radicals and biotransformation are considered. With carbon tetrachloride, lipid peroxidation and covalent binding to hepatic constituents have been dominant themes over the years. Potentiation of chlorinated methane-induced liver injury by alcohols, aliphatic ketones, ketogenic compounds, and the pesticide chlordecone is discussed. A search for explanations for the potentiation phenomenon has led to the discovery of the role of tissue repair in the overall outcome of liver injury. Some final thoughts about future research are also presented.

INTRODUCTION

My interest in the chlorinated methane hepatotoxicants began during my graduate training at the University of California, San Francisco campus. My master's work dealt with a chemical analytical problem in forensic toxicology. In 1956, immediately after my master's work, however, my mentor, the late Dr. Charles H. Hine, provided me with a graduate student stipend and a very modest research fund to study the halogenated hydrocarbons. So, my PhD dissertation dealt with certain aspects of this subject. Later, during my own academic career, my research programs always involved these agents (1).

According to Drill (2), the hepatotoxic properties of chloroform and carbon tetrachloride were recognized about 100 years ago. These chlorinated methanes are classic models of liver injury and have developed into important experimental hepatotoxicants. Other hepatotoxicants are often compared with these agents.

This describes how knowledge about chlorinated methane-induced liver injury evolved over the past five decades. An exhaustive discussion of the subject is not

presented. Only selected aspects were chosen to highlight some important concepts. Complete coverage of the subject, particularly carbon tetrachloride, prior to 1973 can be found elsewhere (3–6).

EARLY CONCEPTS OF LIVER INJURY

Most of the research interest in chemical-induced liver injury in the early part of the twentieth century focused on the morphological development of the different lesions, based on histological evaluation by light microscopy. The descriptions still serve as the basis of our current understanding of the morphological aspects of liver lesions. The dominant research theme during this period was how dietary conditions or individual dietary components (diets high or low in fats, carbohydrates, or proteins; the presence of choline, methionine, or cystine) could modify the hepatotoxic response (2). By midcentury, scientists turned to explaining various morphological events in terms of altered physiological or biochemical function (7). In the past 50 years, research has largely dealt with mechanisms of action, not only in terms of the target organ itself but in terms of the aggressor toxicant as well.

In 1954, Himsworth (8) published a monograph on liver injury that serves as a wonderful reservoir of the knowledge available at the time. He identified two factors—vascular and nutritional—as playing influential roles in the development of liver injury in its various forms. The so-called vascular factors, believed to reflect circulatory abnormalities, were thought to be responsible for the acute zonal necrotic lesions (centrilobular, periportal, and midzonal) observed in animals following exposure to different hepatotoxicants and a cause of the massive hepatic necrosis seen with other agents. The idea of nutritional factors arose mainly from studies where deficiencies in diets were investigated; the hepatic lesions included were largely chronic, rather than acute, in form. Finally, Himsworth put forth the concept that hepatic necrosis of parenchymal cells could be produced in one of two ways—''by the presence of noxious agents or by the absence of some factor essential to cellular life.''

MITOCHONDRIA AND THE ENDOPLASMIC RETICULUM AS SITES

With the advances made in biochemistry, particularly the isolation and functional characterization of subcellular organelles, researchers began to search for biochemical explanations for the development of liver lesions. The principal model studied was the zonal hepatocellular lesion produced after acute exposure to carbon tetrachloride; the primary pathological events of interest were the accumulation of lipids within the hepatocyte (steatosis) and the appearance of

hepatocellular death (necrosis), two independent events (9). A relatively complete histological, histochemical, and biochemical study by Wahi et al (10) in rats showed that the earliest histological evidence of derangement (necrosis and inflammatory cell infiltration) occurred 6 h after administration of carbon tetrachloride.

In 1956, Christie & Judah (11) proposed that the mechanism of action of carbon tetrachloride was one of altered mitochondrial permeability, leading to loss of essential cofactors and disruption of cellular metabolism. Mitochondrial respiration was depressed 10 h after intoxication of rats with lethal doses; after 15 h, the oxidation of octanoate, pyruvate, citrate, hydroxybutyrate, and malate was markedly reduced. Histologically, however, necrosis began at 5 h, and by 18 h massive necrosis was observed; all animals died within 36 h. This hypothesis received support from Heim et al (12), who found a decrease in coenzyme A content in guinea pig livers treated with lethal doses of carbon tetrachloride. The alterations observed in these studies, however, might well have been a result of the presence of necrotic tissue rather than the cause of the necrosis.

In theory, a biochemical lesion responsible for initiation of such severe lesions should be reflected as a functional change before extensive damage becomes evident histologically. Other investigators, who were interested in mechanisms responsible for steatosis, also looked at mitochondrial function but discounted the effects as causal events. Calvert & Brody (13) were unable to show consistent mitochondrial biochemical changes sooner than 20 h after in vivo haloalkane intoxication; no temporal correlation between the histological findings present at 5 h and the biochemical events was obtained. Recknagel & Anthony (14) observed a lag of 14–20 h between intoxication of the animal and the appearance of mitochondrial changes, whereas increased hepatic lipids were already prominent by 3 h. Both groups showed that the mitochondrial changes could be divorced from the changes in hepatic lipids. Later, the membranes of the endoplasmic reticulum were identified as the site of origin of the triglycerides (5), and triglyceride secretion into plasma was markedly reduced by 2 h (15). Recknagel & Lombardi (16) observed that changes in endoplasmic reticular function (reduced glucose-6-phosphatase activity, increased cytochrome *c* reductase activity) were evident by 2 h after carbon tetrachloride administration, well before the changes seen in mitochondria. Other investigators (5, 6, 9) showed that depressed protein synthesis in the endoplasmic reticulum occurred in rats within 3 h after carbon tetrachloride exposure, accompanied by dispersion of polyribosomes. Also, a rapid decline in liver microsomal cytochrome P450 content was observed. Moore et al (17) showed that the activity of the microsomal calcium pump was markedly reduced 0.5 h after carbon tetrachloride administration in rats. Calcium homeostasis, which involves mitochondrial, endoplasmic reticular, and cytosolic calcium pools, is markedly perturbed after carbon tetrachloride, chloroform, bromotrichloromethane, and 1,1-dichloroethylene intoxication (18–20). Thus, the endoplasmic reticulum appeared as a more likely site of action.

THE SYMPATHETIC NERVOUS SYSTEM AND LIVER INJURY

Altered sinusoidal circulation following carbon tetrachloride intoxication in rats was proposed by several investigators (7). In 1960, Calvert & Brody (21) proposed that carbon tetrachloride exerted its necrotic effect, not by acting on the liver parenchyma directly, but by causing a persistent sympathetic discharge resulting in diminished hepatic blood flow and cellular hypoxia. In contrast to Himsworth (8), who envisioned an action of the hepatotoxicant on hepatic cells and a tissue response leading to mechanical modification of sinusoidal blood flow, the concept of Calvert & Brody centered on the central nervous system as the site of action of carbon tetrachloride. The new provocative hypothesis was based on indirect evidence (no measurements of hepatic blood flow or of tissue hypoxia were performed) using adrenergic- and ganglionic-blocking agents as well as spinal cord transection (21–24) to modify or block the usual hepatotoxic responses to carbon tetrachloride (centrilobular necrosis, lipid accumulation). By far, the best protection was afforded by cervical cordotomy at the level of C-6 or C-7.

Because cervical cordotomy could theoretically affect a number of physiological systems, a series of studies was undertaken in my laboratory to unravel the remarkable protection afforded by this procedure. The possibility of decreased absorption of carbon tetrachloride was eliminated (25). We observed, however, that the rats undergoing cordotomy became poikilothermic. By 10 h after surgical interruption, the rectal temperature of animals transected at C-7 approached that of the room. The severity of the hypothermic response was dependent on the level of the cord transection in a pattern that paralleled the degree of protection afforded by cordotomy. Also, it was shown that if cord-transected (C-6 or C-7) rats were placed in an incubator to maintain normal body temperature, carbon tetrachloride exerted its necrotic effect (26, 27). Furthermore, with animals maintained under hypothermic conditions, one could produce carbon tetrachloride-induced liver necrosis, if the agent was administered three times, every 12 h, and the rats killed 24 h after the last treatment. Finally, hypothermia induced by immersion of normal rats in cold water also resulted in a protective effect comparable to that of cordotomy (27). We showed that the oxygen consumption of cord-sectioned rats maintained at room temperature decreased to 50% of that of normal rats by 1 h and to 30% of that of normal rats by 5 h. Our explanation for the protective effect of cervical cordotomy was that in hypothermia, the metabolic activity of the liver was diminished, and this would reduce the bioactivation of carbon tetrachloride into its hepatotoxic intermediates. We further showed that large infusions of norepinephrine, epinephrine, or mixtures of these catecholamines did not result in lesions similar to those produced by carbon tetrachloride (27). We also found that rats subjected to immunological sympathectomy after birth (injection of antisympathetic nerve growth factor) and later adrenal demedullated were not protected against carbon tetrachloride (28). Thus, these experiments showed that from all

points of view, a vascular role attributed to carbon tetrachloride via release of catecholamines should be rejected as a primary cause of injury.

Regarding steatosis, the Calvert & Brody hypothesis (21) proposed that a persistent sympathetic discharge due to an action of carbon tetrachloride on the central nervous system resulted in an oversupply of fatty acids from adipose tissue to the liver. The events by which carbon tetrachloride causes steatosis are reasonably well understood in terms of pathogenesis and biochemical sequences (5, 29, 30). Generally, the evidence points to a failure of the hepatic triglyceride secretory mechanism as the causal event of major importance in the case of carbon tetrachloride, not enhanced supply of fatty acids from peripheral stores (5). There is agreement that fatty acids must be available from adipose tissue for the liver to synthesize triglycerides, and that interruption of the pituitary-adrenal axis diminishes plasma free fatty acids. This leads to a block in the accumulation of triglycerides. The peripheral stores, however, play a permissive role in this situation, rather than one of initiation.

Although the original sympathetic nervous system explanation for the hepatotoxic action of carbon tetrachloride is no longer tenable, there are acute hepatic hemodynamic consequences following exposure to carbon tetrachloride that justify consideration. Using the isolated perfused rat liver, we demonstrated (31–33) that the circulatory action of carbon tetrachloride was actually biphasic. In these experiments the animals received the haloalkane in vivo; the livers were removed and perfused in vitro at various times after its administration. During the initial phase (1–6 h after treatment), there was a moderate increase in hepatic resistance (evident by perfusate flow/portal pressure curves) that returned to normal by 6 h; this was followed by a more prolonged increase in resistance that persisted for several days (96 h after treatment). The biphasic cycle observed was quite reproducible. To determine the causal relationships involved in the phenomenon, a variety of protective measures were investigated (promethazine, dimethoxy-propyltrimethylammonium chloride, ethylenediaminetetraacetic acid, hypophysectomy, cordotomy, hypothermia), as well as comparisons to the hepatic effects of ethionine (steatosis present but no necrosis) and thioacetamide (necrosis present but no steatosis). These experiments allowed us to conclude that the initial phase (first 6 h) of increased hepatic resistance was due to the accumulation of triglycerides, whereas the later phase (after 6 h) was associated with the appearance of hepatic necrosis.

Although the primary hepatotoxic action of carbon tetrachloride does not involve the catecholamines, adrenoreceptor agonists can affect the progression of the lesion. In mice, epinephrine or norepinephrine administered subcutaneously was shown to potentiate the hepatotoxic properties of a small dose of the chlorinated methane in a dose-related fashion (34). Electrical stimulation of the ventromedial hypothalamus in rats was reported (35) to enhance markedly carbon tetrachloride- or dimethylnitrosamine-induced liver injury, and this effect was attenuated by surgical sympathetic denervation of the liver. The authors suggested that the hypothalamus seemed to be involved in the progression of the lesion, but

attributed the sympathetic effect to one on hepatic metabolism rather than on blood flow. More recently, Roberts and collaborators (36–40) observed that phenylpropylamine and methamphetamine can potentiate carbon tetrachloride- and acetaminophen-induced hepatotoxicity in rodents, but that the temporal aspects of each type of potentiation differ, which suggests that the pathways involved are also different. With carbon tetrachloride, both central and peripheral (hepatic microcirculation) adrenoreceptor components are put forth as possibilities, whereas with acetaminophen the evidence suggests an adrenoreceptor-related effect on liver glutathione (40).

FREE-RADICALS, BIOTRANSFORMATION, AND LIVER INJURY

It is commonly held that in most instances, chemical-induced hepatotoxicity is the result of biochemical disruptions caused by reactive metabolites arising from biotransformation (41–43). The putative chemical species in most cases, however, has not necessarily been identified, but the cascade of events leading to hepatic dysfunction is usually reasonably well described based on in vitro and in vivo studies. Examples where bioactivation becomes the initiating event include such necrogenic hepatotoxicants as carbon tetrachloride, bromotrichloromethane, chloroform, halothane, bromobenzene, acetaminophen, furosemide, isoniazid, thioacetamide, dimethylnitrosamine, allyl formate, and aflatoxin. The bioactivation characteristics of aliphatic organohalogens (including the chlorinated methanes), their detection, and relevance were reviewed by Sipes & Gandolfi (44).

In a seminal article published in 1961, Butler (45) showed that carbon tetrachloride administered to dogs was reduced to chloroform; he postulated the homolytic fission of the carbon-chlorine bond as a possible mechanism, leading to the formation of a free radical as the ultimate toxic moiety. Both Slater (46) and Recknagel (5) proposed, independently, that the putative carbon tetrachloride-derived free radical could attack membranes, leading to peroxidation and resulting in necrosis or steatosis. The trichloromethyl free radical ($\bullet CCl_3$) was eventually identified by spin trapping in rat liver microsomes incubated with carbon tetrachloride and in livers from animals treated with the haloalkane (47). The free radical reacts very rapidly with oxygen to yield a highly reactive trichloromethylperoxy free radical ($\bullet CCl_3O_2$), which is said to be the initiator of lipid peroxidation (47). Furthermore, a carbon dioxide anion radical has been described and its adduct identified in the urine of rats treated with the haloalkane, but its role in the hepatotoxic process, if any, is still not established (48, 49). The biotransformation of carbon tetrachloride occurs in the endoplasmic reticulum and is mediated by cytochrome P450; the principal isoform implicated as the catalyst is CYP2E1, but evidence for CYP2B1/2 exists as well (50–53).

LIPID PEROXIDATION, COVALENT BINDING, AND LIVER INJURY

The carbon tetrachloride-derived free radical(s) can bind irreversibly to hepatic proteins and lipids and can initiate a process of autocatalytic lipid peroxidation by attacking the methylene bridges of unsaturated fatty acid side chains of microsomal lipids. Recknagel & Ghoshal (54) demonstrated that conjugated dienes, typical of peroxidized polyenoic fatty acids, appeared in hepatic microsomal lipids 1.5 h after rats were exposed to nonlethal doses of carbon tetrachloride. The peroxidative process is thought to result in early morphologic alteration of the endoplasmic reticulum, loss of cytochrome P450 activity, loss of glucose-6-phosphatase activity, depressed protein synthesis, loss of the capacity of the liver to form and excrete very-low-density lipoproteins, and eventually cell death (6, 47, 55). Bromotrichloromethane, the bond dissociation energy of which is lower than that of carbon tetrachloride and more reactive to homolytic cleavage (6, 47, 56), is more potent than carbon tetrachloride in terms of hepatotoxicity and lipid peroxidative properties (6, 18, 20, 56). Chloroform, the bond dissociation energy of which is higher than that of bromotrichloromethane or carbon tetrachloride, is also bioactivated by cytochrome P450 but not to a free radical (47); the highly reactive electrophilic metabolite phosgene was demonstrated in phenobarbital-pretreated rats subsequently given chloroform (57, 58).

Lipid peroxidation is not the only process associated with the formation of free radicals after carbon tetrachloride intoxication. The reactive products also bind covalently to hepatic macromolecules; binding to lipids, proteins, and nucleic acids has been demonstrated (59). Binding to cytochrome P450, which leads to its destruction, occurs rapidly in vivo and in some instances can be shown to be independent of lipid peroxidation (60–63). Castro (59) has been a proponent of covalent binding of carbon tetrachloride–derived products as an important element of the hepatotoxic mechanism of this agent. In 1973, Recknagel & Glende (6), as strong supporters of the lipid peroxidation hypothesis, were critical of those advocating a ‘‘toxic metabolite’’–based theory. However, 10 years later, after recognizing the difficulties brought on by some of the artificial conditions used in vitro for following lipid peroxidation, Recknagel et al (18) also put lipid peroxidation into perspective; they pointed out that the covalent binding of carbon tetrachloride–derived products could provoke secondary mechanisms that finally resulted in important pathological consequences.

Lipid peroxidation, however, need not always appear after exposure to hepatotoxicants, even if reactive metabolites are formed (64, 65). 1,1-Dichloroethylene, trichloroethylene, ethylene dibromide, dimethylnitrosamine, and thioacetamide serve as examples. Klaassen & Plaa (66) found no evidence of the presence of conjugated dienes (a sensitive in vivo indicator of lipid peroxidation) after administration of chloroform in rats with dosages that resulted in steatosis and necrosis; depression of hepatic glucose-6-phosphatase activity (associated with peroxida-

tion in the endoplasmic reticulum) was also absent. Brown et al (67) found that rats pretreated with phenobarbital, but not untreated animals, produce conjugated dienes during chloroform exposure; depression of glucose-6-phosphatase activity was also reported to occur after chloroform only in phenobarbital-pretreated rats (68). Because chloroform-induced liver injury is more severe in phenobarbital-pretreated rats, the possibility exists that the initial lesion induced by chloroform in these animals is merely aggravated by the additional appearance of lipid peroxidation. It is interesting to note that Wang et al (69) recently compared the time courses of carbon tetrachloride and chloroform hepatotoxic responses and found that cellular degeneration and necrosis appear sooner following carbon tetrachloride intoxication in rats. Previously, we had established dose-response curves for hepatotoxicity with several haloalkanes (70–72) and demonstrated in mice and dogs that the potency of carbon tetrachloride as an hepatotoxicant was much greater than chloroform. Perhaps the presence of lipid peroxidation with carbon tetrachloride accounts for the differences in potency between these two chlorinated methanes. These findings and others cast doubt on the general applicability of lipid peroxidation as a mechanism of action for hepatotoxicants (73, 74).

Normal cellular metabolism itself can lead to reactive oxygen species (superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl radical), and all cells contain defense systems to prevent or limit damage; glutathione is the major element, but α-tocopherol and ascorbic acid play important roles (75). An imbalance between prooxidants and antioxidants is known as oxidative stress; redox cycling can cause oxidative stress in cells. Calcium-induced permeability transition of the mitochondrial inner membrane may initiate cell death in oxidative stress; morphological and functional changes in mitochondria are features of oxidative stress-induced cell injury (76–78). It is now established that nonparenchymal cells can be involved in oxidative stress leading to hepatotoxicity (79). Reactive oxygen intermediates are generated by macrophages, as well as by endothelial cells and stellate cells (Ito cells), but under physiological conditions, cellular antioxidants normally present prevent the intermediates from producing cytotoxicity. Enhanced formation of oxygen intermediates has been demonstrated with carbon tetrachloride, galactosamine, and 1,2-dichlorobenzene. With the latter agent, recent evidence indicates that Kupffer cell–derived oxygen species are largely responsible for lipid peroxidation (80) Also, Kupffer cell activation and inflammatory cells have been implicated in the potentiation of carbon tetrachloride liver injury by retinol (81, 82).

POTENTIATION OF LIVER INJURY

The potentiation of liver injury caused by one agent because of the simultaneous or sequential exposure to another chemical is not a recent discovery. Anecdotal clinical evidence and experimental laboratory evidence of interactions between ethanol and carbon tetrachloride or chloroform appeared in the literature before

1930 (see 2, 3), but experiments designed to explain this interesting phenomenon did not appear until much later. With the development of the pentobarbital sleeping time assay for quantifying liver injury (70), an experimental tool was available to assess this phenomenon in rodents. In 1962 Kutob & Plaa (83) demonstrated that administration of a nonlethal dose of ethanol to mice prior to their subsequent exposure to a small dose of chloroform resulted in potentiation of the haloalkane-induced liver injury. Later these findings were extended to carbon tetrachloride in experiments where elevations in plasma aminotransferase activity to quantify liver injury were employed (71, 72, 84). As an explanation for the potentiation of chloroform toxicity, we proposed that the elevation in hepatic triglycerides resulting from the ethanol pretreatment might cause enhanced hepatic retention of chloroform, thus increasing the hepatic internal ''dose'' of toxicant. Some evidence supporting the hypothesis was presented (83), but the issue was never investigated in depth and remains unresolved.

Aliphatic alcohols other than ethanol can enhance the hepatotoxic properties of carbon tetrachloride (85). We studied the potentiating characteristics of isopropanol (86–90) and showed that the potency of isopropanol exceeds that of ethanol; the severity of the hepatotoxic response is also more extensive with isopropanol potentiation. Isopropanol is rapidly biotransformed to acetone; comprehensive dose-effect and time-effect studies (87, 91), as well as various scenarios of altered metabolism, demonstrated that acetone, the major metabolite of isopropanol, is responsible for the potentiating properties of isopropanol. Finally, it was postulated that the isopropanol-carbon tetrachloride interaction observed in rodents might present itself in humans during occupational exposures (90). Later two industrial accidents did occur, one in the United States (92) and the other in Taiwan (93); they mimicked the potentiation phenomenon we first observed in rodents.

Other aliphatic ketones have been shown to potentiate the hepatotoxic properties of carbon tetrachloride and chloroform. These include the following: 2-butanone (methyl ethyl ketone), 2-pentanone (methyl propyl ketone), 2-hexanone [methyl *n*-butyl ketone (MnBK)], 2,5-hexanedione (metabolite of MnBK), 4-methyl-2-pentanone [methyl isobutyl ketone (MiBK)], 1-hydroxy-4-methyl-2-pentanone (metabolite of MiBK), and 2-heptanone (methyl amyl ketone) (94–99). Also, certain chemicals are biotransformed to ketones (''ketogenic'' chemicals), like *n*-hexane, 2-butanol, or 4-methyl-3-pentanol, and are effective potentiators (100–103). The metabolic ketosis produced by 1,3-butanediol (biotransformed to β-hydroxybutyrate) is responsible for the potentiating properties of this agent, as an excellent correlation exists between the plasma concentrations of β-hydroxybutyrate and the severity of the potentiation (101). Furthermore, the potentiating properties of this ketone body probably accounts for the potentiation of carbon tetrachloride liver injury observed in acute alloxan- or streptozotocin-induced diabetic rats (104–107), as well as the differences in chloroform toxicity observed in fed and fasting rats (108).

Carbon tetrachloride and chloroform are not the only chlorinated hydrocarbon solvents whose hepatotoxic properties are enhanced by ketones. The others consist of 1,1,2-trichloroethane, 1,1-dichlorethylene, bromoform, bromodichloromethane, and dibromochloromethane, but not 1,1,1-trichloroethane, 1,1,2,2,-tetrachloroethane, trichloroethylene, or tetrachloroethylene (103, 109–111). There is a strong suggestion that weak hepatotoxic chlorinated alkanes are not converted into potent hepatotoxicants by a previous exposure to ketones. With the brominated methane derivatives, however, this conclusion does not appear to be applicable because potent hepatotoxic combinations are produced by ketone potentiation (103, 109, 111).

Pessayre et al (112) showed that trichloroethylene can aggravate the hepatotoxic response to carbon tetrachloride in rats and that mixtures of these two agents are more potent hepatotoxicants than either given singly; the interaction was confirmed by others (113–115). Acetone potentiates the hepatotoxicity of trichloroethylene-carbon tetrachloride mixtures and has variable effects on the hepatotoxic effects of other chlorinated hydrocarbon mixtures composed of chloroform, carbon tetrachloride, 1,1,1-trichloroethane, 1,1,2-trichloroethane, tetrachloroethylene, 1,1,2,2-tetrachloroethane, or 1,1-dichloroethylene (113, 116). Furthermore, multiple exposures of acetone to rats receiving concurrently repetitive administrations of carbon tetrachloride were shown to enhance the appearance of liver fibrosis (117). Thus, the potentiation of halogenated hydrocarbon hepatotoxicants by ketones and ketogenic substances is extensive and covers both acute and chronic aspects of the injury.

Chlordecone (Kepone), a cyclic organochlorine pesticide containing a carbonyl group, is a remarkable potentiator of chlorinated methane liver injury, in contrast to its nonketonic analog mirex. We were the first to describe the potentiating properties of chlordecone on chloroform hepatotoxicity in mice (118) and continued to study the chlordecone-chloroform combination later in rats. Shortly thereafter, Mehendale and his colleagues published their first chlordecone-potentiation experiments with carbon tetrachloride in rats (119); later they demonstrated that bromotrichloromethane hepatotoxicity was also potentiated (120). Mehendale's group has published extensively on the carbon tetrachloride potentiation model and has made some important observations, including the role of tissue repair on the overall outcome of the potentiation (121). The acute effects of chlordecone on chloroform toxicity persist because the agent is poorly metabolized and is very lipophilic (122, 123); a threshold chlordecone liver concentration appears to exist. The potentiation of chloroform can still be elicited 20 days after exposure to a single dose of chlordecone, coinciding with the presence of enhanced covalent binding of chloroform-derived reactive metabolites and persistent chlordecone liver residues (122).

Regarding mechanisms involved in the potentiations observed with ethanol, isopropanol, 1,3-butanediol, various aliphatic ketones, and chlordecone, increased production of haloalkane-derived reactive metabolites (via cytochrome P450) is certainly of major importance (51, 100, 122, 124–132). The induction of CYP2E1 by ethanol and acetone is well established. Methyl *n*-alkyl ketones were shown

to induce CYP2E1 and CYP2B1/2 (133) and chlordecone was shown to induce CYP2B1/2 (134–136). Experiments where the irreversible (covalent) binding of chloroform-derived or carbon tetrachloride–derived radioactivity to liver constituents (usually microsomal proteins or lipids) was followed in vivo or in vitro have consistently shown increased binding in treatment regimens with the various potentiators. One exception is a study reported by Davis & Mehendale (137) with chlordecone, where enhanced covalent binding of carbon tetrachloride–derived radiolabel was not observed; in this experiment, however, the dosage of chlordecone employed (5 mg/kg) was below the threshold dose of chlordecone (10 mg/kg) determined by Plaa et al (123). Later, Britton et al (131) used 15 mg of chlordecone/kg and demonstrated a 67% increase in cytochrome P450 content and an increase in the covalent binding of carbon tetrachloride–derived radioactivity to microsomal protein and lipids in vivo. In another study from Mehendale's group (138), the authors report that increased covalent binding after chlordecone was not found, but on this occasion the authors apparently measured radiolabel bound to total liver proteins. All things considered, enhanced bioactivation of the haloalkane hepatotoxicant (likely due to induction of cytochrome P450) appears to be the major mechanism of action.

Nevertheless, there are indications that other mechanisms may also be involved in these potentiations. With isopropanol potentiation, mitochondrial and lysosomal damage following carbon tetrachloride appears more severe than that produced by a larger dose of carbon tetrachloride given alone (139). The lesion observed in animals treated with chlordecone and carbon tetrachloride or chloroform differs from that seen with carbon tetrachloride or chloroform given alone (118, 119). More severe hepatobiliary dysfunction (possibly due to altered membranes) was reported with the combination of chlordecone-carbon tetrachloride (119, 140). Lysosomal fragility to osmotic stress in vitro was enhanced when the hepatic organelles were obtained from rats treated in vivo with the combination of chlordecone-chloroform or acetone-chloroform (130). In the same study, morphological evaluation suggested mitochondria respond differently to chloroform in chlordecone- or 2-hexanone–pretreated animals compared with vehicle-pretreated rats; the mitochondria appeared to have reached a terminal stage of damage earlier than the cell in general. Finally, Mehendale and his colleagues (121, 141–143) postulate that in chlordecone-potentiated carbon tetrachloride hepatotoxicity, early tissue repair processes are markedly disrupted; the greater severity of the lesion observed and its consequences appear due to the absence of this protective mechanism. Thus it is clear that a complete explanation for the potentiation phenomenon remains unresolved.

TISSUE REPAIR AND RECOVERY

Although various aspects of chemical-induced liver injury have been studied for over 50 years, interest generally has focused on the early initiating events leading to hepatocellular dysfunction, rather than on the later recovery phase of the lesion.

Searching for the ''biochemical lesion'' has dominated research in this area. However, repair is an important component of the lesion. Hepatocellular regeneration begins within 6 h after administration of a small dose of carbon tetrachloride in rats; yet the centrilobular necrosis is just becoming evident (144, 145). With the combination of carbon tetrachloride and several potentiating agents (*n*-hexane, 2-hexanone, 2,5-hexanedione, isopropanol, and acetone), recovery time was assessed using biochemical indices (activities of serum enzymes) and morphological patterns (quantitative histology) of liver injury; appropriate dose-response curves were established from the percentage of animals affected (146). Recovery time was shown to be related to the maximal severity of the lesion, regardless of the potentiating combination. Although pretreatment with the potentiator resulted in an enhanced hepatotoxic response from a small dose of carbon tetrachloride, the dose-response curve for the enhanced response was no different than that produced by a larger, but equitoxic, dose of the haloalkane given alone. These data were interpreted as indicating that the five potentiators did not alter the temporal progression of carbon tetrachloride-induced liver injury.

Mehendale and his collaborators have performed an extensive series of experiments to assess the role of tissue repair in potentiated liver injury (142, 147–150). The studies originated from the observation that chlordecone-potentiated carbon tetrachloride hepatotoxicity in rats was quantitatively quite remarkable and resulted in greatly enhanced lethality when compared with the results obtained in animals not pretreated with the pesticide. Normally, two tissue repair processes are observed after exposure to a small dose of the haloalkane (121, 142); the early phase regeneration (EPR) response (arrested G_2 hepatocytes activated to proceed through mitosis) occurs quickly (peaks at about 6 h) and is followed (at about 24 h) by the secondary phase regeneration (SPR) response (hepatocytes mobilized from G_0/G_1 to proceed through mitosis). During chlordecone potentiation of carbon tetrachloride liver injury, EPR is thought to be eliminated and SPR decreased; thus the progression of the severe injury is facilitated and leads to lethality. There is evidence that induction of EPR may accelerate SPR. It is interesting to note that a large dose of carbon tetrachloride given alone also results in a regeneration response similar to that obtained with chlordecone and a small dose of haloalkane. Experiments performed with colchicine, partial hepatectomy, carbon tetrachloride autoprotection, nutritional factors, and different animal species have provided data consistent with the purported roles attributed to EPR, SPR, and liver injury (121, 142, 143).

The role of tissue repair has been assessed with other hepatotoxicants (143). The data indicate that thioacetamide, *o*-dichlorobenzene, and trichloroethylene when given alone affect hepatic tissue regeneration in a manner not unlike that observed with carbon tetrachloride. Increased lethality, however, was not observed with isopropanol- or ethanol-potentiated carbon tetrachloride–induced liver injury (151, 152). Mehendale (141) and Soni & Mehendale (143) have proposed a two-stage model for chemical-induced hepatotoxicity. Stage one would involve initiation and infliction of injury; stage two would lead to recovery or

progression to massive injury, depending on the effects of the toxicant on cellular regeneration (enhanced regeneration would lead to recovery; inhibition would lead to massive injury). Although various aspects of the repair-recovery process are still hypothetical and speculative, the concept itself is thought-provoking and certainly an important contribution to the understanding of chemical-induced liver injury. It will be interesting to see how it evolves with time.

SOME FINAL THOUGHTS

The amount of knowledge acquired over the past 50 years about the liver injury produced by chlorinated methanes, particularly carbon tetrachloride, is truly remarkable. One can wonder, however, what might have happened if chloroform, instead of carbon tetrachloride, had been the gold standard for studying the biochemistry of chemical-induced acute necrogenic liver injury. Certainly the free-radical picture and the phenomenon of lipid peroxidation as we know it today might have been very different because each one seems to play a different role with chloroform. Acquired knowledge about carbon tetrachloride has had a great influence on research approaches designed to understand other types of chemical-induced hepatotoxicity. In drug-induced liver disease, the acetaminophen and halothane models, however, have now attained their own distinct identities. Also, the chlorinated methanes are not very useful for understanding the idiosyncratic liver injury that may occur in an unpredictable fashion with some therapeutic agents.

The steatosis observed after the acute administration of chlorinated methanes is largely attributed to a failure of the triglyceride secretory mechanism of the hepatocyte. Yet, the possible effects of these agents on the more recently described molecular events involved in triglyceride secretion (153–156) remain to be investigated. This area of research should be brought up-to-date, in line with more current concepts.

Unfortunately, the perception of altered mitochondria as only a late event in the temporal development of the liver injury produced by the chlorinated methanes might have contributed to an apparently lessened interest in the role of this organelle in other forms of chemical-induced liver injury. Yet, the importance of mitochondrial function in oxidative stress-related aspects of hepatotoxicity is now evident. Also, early mitochondrial dysfunction has been proposed as an important element in bromobenzene toxicity (157), and inhibition of mitochondrial β-oxidation has been associated with the microvesicular steatosis observed in rats following valproic acid intoxication (158). This biochemical lesion is now considered of major importance in other forms of liver injury in humans and animals (159, 160). It would be worthwhile that in the future, mitochondrial function be revisited even for chlorinated methane–induced liver injury, possibly as a contributory lesion.

Despite all the advances made with chemical-induced liver injury, we still cannot establish which of the changes observed lead to cell death and which are secondary disturbances. We know what can be done to a liver cell and yet not destroy it. Judah's words published in 1970 (9) are still appropriate 30 years later: ''Necrosis is a histologist's conception. The dead cell is recognized by changes that are the consequences of cell death. . . . These signs take some time to develop, hence one is ignorant of the precise moment of cell death.'' It is likely that the fervent search for the ''biochemical lesion'' pursued over the past 40 years has distorted our ability to recognize what the necrotic process actually represents. In this regard, the two-stage model of toxicity proposed by Mehendale (141) from chlordecone-carbon tetrachloride interactions is a novel way of looking at chemical-induced liver injury. Cohen & Khairallah (161) discussed an analogous situation with acetaminophen hepatotoxicity (a field largely influenced by prior experience acquired with carbon tetrachloride). They came to the conclusion that multiple independent insults to cells may be involved in toxicity and proposed the concept of a multistage process as being appropriate for acetaminophen; collectively, a number of cellular events set in motion and perpetuate the processes that determine outcome. Such ideas should become stimulating influences and should be pursued in future hepatotoxic research.

The discovery by Mehendale and his colleagues (121, 142, 143) of the consequences of early-phase–regeneration and secondary-phase–regeneration tissue repair processes, and their interactions, on the outcome of carbon tetrachloride–induced hepatotoxicity (with or without chlordecone potentiation) is an exciting and intriguing development. It expands our conception of the overall process of liver injury in a significant manner. The fact that elements of the processes are applicable to other hepatotoxicants appears to be quantifiable and follows dose-dependent criteria (including the appearance of a threshold) contributes greatly to their importance. The pursuit of the biochemical and molecular aspects of these phenomena in much greater depth, including genetic expression, should undoubtedly have a marked influence on our better understanding of chemical-induced hepatotoxicity. The next 15 years or so should be really interesting.

LITERATURE CITED

1. Plaa GL. 1997. A four-decade adventure in experimental liver injury. *Drug Metab. Rev.* 29:1–37
2. Drill VA. 1952. Hepatotoxic agents: mechanism of action and dietary interelationship. *Pharmacol. Rev.* 4:1–42
3. von Oettingen WF. 1955. The Halogenated Aliphatic, Olefinic, Cyclic, Aromatic, and Aliphatic-Aromatic Hydrocarbons Including the Halogenated Insecticides, Their Toxicity and Potential Dangers. Public Health Serv. Publ. no. 414. Washington, DC: US Gov. Print. Off. 430 pp.
4. Popper H, Schaffner F. 1957. *Liver: Structure and Function.* New York: McGraw-Hill. 777 pp.
5. Recknagel RO. 1967. Carbon tetrachloride hepatotoxicity. *Pharmacol. Rev.* 19: 145–208
6. Recknagel RO, Glende EA Jr. 1973. Car-

bon tetrachloride hepatotoxicity: an example of lethal cleavage. *CRC Crit. Rev. Toxicol.* 2:263–97
7. Stoner HB, Magee PN. 1957. Experimental studies on toxic liver injury. *Br. Med. Bull.* 13:102–7
8. Himsworth HP. 1954. *The Liver and Its Diseases.* Cambridge, MA: Harvard Univ. Press. 222 pp.
9. Judah JD, McLean AEM, McLean EK. 1970. Biochemical mechanisms of liver injury. *Am. J. Med.* 49:609–16
10. Wahi PN, Tandon HD, Bharadwai TP. 1955. Acute carbon tetrachloride hepatic injury, parts I and II. *Acta Pathol. Microbiol. Scand.* 37:305–15
11. Christie GS, Judah JD. 1954. Mechanism of action of carbon tetrachloride on liver cells. *Proc. R. Soc. London Ser. B* 142: 241–57
12. Heim F, Leuschner F, Ott A. 1956. Der Einfluss von Tetrachlorkohlenstoff auf die Ferment- und Co-enzym-A Aktivität der Leber. *Arch. exp. Pathol. Pharmakol.* 229:360–65
13. Calvert DN, Brody TM. 1958. Biochemical alterations of liver function by halogenated hydrocarbons. I. In vitro and in vivo changes and their modification by ethylenediamine tetracetate. *J. Pharmacol. Exp. Ther.* 124:273–81
14. Recknagel RO, Anthony DD. 1959. Biochemical changes in carbon tetrachloride fatty liver: separation of fatty changes from mitochondrial degeneration. *J. Biol. Chem.* 234:1052–59
15. Recknagel RO, Lombardi B, Schotz MC. 1960. New insight into pathogenesis of carbon tetrachloride fat infiltration. *Proc. Soc. Exp. Biol. Med.* 104:608–10
16. Recknagel RO, Lombardi B. 1961. Studies of biochemical changes in subcellular particles of rat liver and their relationship to a new hypothesis regarding the pathogenesis of carbon tetrachloride fat accumulation. *J. Biol. Chem.* 236:564–69
17. Moore L, Davenport GR, Landon EJ. 1976. Calcium uptake of a rat liver microsomal subcellular fraction in response to in vivo administration of carbon tetrachloride. *J. Biol. Chem.* 251: 1197–201
18. Recknagel RO, Glende EA Jr, Waller RL, Lowrey K. 1982. Lipid peroxidation: biochemistry, measurement, and significance in liver cell injury. See Ref. 162, pp. 213–41
19. Plaa GL, Hewitt WR 1989. Detection and evaluation of chemically induced liver injury. In *Principles and Methods of Toxicology,* ed. AW Hayes, pp. 599–628. New York: Raven. 2nd ed.
20. Comporti M. 1998. Lipid peroxidation as a mediator of chemical-induced hepatocyte death. See Ref. 163, pp. 221–57
21. Calvert DN, Brody TM. 1960. Role of the sympathetic nervous system in CCl_4 hepatotoxicity. *Am. J. Physiol.* 198:669–76
22. Brody TM, Calvert DN. 1960. Release of catechol amines from the adrenal medulla by CCl_4. *Am. J. Physiol.* 198: 682–85
23. Brody TM, Calvert DN, Schneider AF. 1961. Alterations of carbon tetrachloride-induced pathologic changes in rat by spinal transection, adrenalectomy, and adrenergic blocking agents. *J. Pharmacol. Exp. Ther.* 131:341–45
24. Stern PH, Brody TM. 1963. Catecholamine excretion following carbon tetrachloride administration. *J. Pharmacol. Exp. Ther.* 141:65–73
25. Larson RE, Plaa GL, Crews LM. 1964. Effect of spinal cord transection on carbon tetrachloride hepatotoxicity. *Toxicol. Appl. Pharmacol.* 6:154–62
26. Larson RE, Plaa GL. 1963. Spinal cord transection and CCl_4 toxicity. *Experientia* 19:604–6
27. Larson RE, Plaa GL. 1965. A correlation of the effects of cervical cordotomy, hypothermia, and catecholamines on carbon tetrachloride-induced hepatic necrosis. *J. Pharmacol. Exp. Ther.* 147:103–11
28. Larson RE, Plaa GL, Brody MJ. 1964. Immunological sympathectomy and

CCl_4 hepatotoxicity. *Proc. Soc. Exp. Biol. Med.* 116:557–60
29. Plaa GL, Larson RE. 1964. CCl_4-induced liver damage. *Arch. Environ. Health* 9:536–43
30. Lombardi B. 1966. Considerations on the pathogenesis of fatty liver. *Lab. Invest.* 15:1–20
31. Rice AJ, Roberts RJ, Plaa GL. 1967. The effect of carbon tetrachloride, administered in vivo, on the hemodynamics of the isolated perfused rat liver. *Toxicol. Appl. Pharmacol.* 11:422–31
32. Rice AJ, Plaa GL. 1968. Effect of hypophysectomy and spinal cord transection on carbon tetrachloride-induced changes in the hemodynamics of the isolated perfused rat liver. *Toxicol. Appl. Pharmacol.* 12:194–201
33. Rice AJ, Plaa GL. 1969. The role of triglyceride accumulation and of necrosis in the hemodynamic responses of the isolated perfused rat liver after administration of carbon tetrachloride. *Toxicol. Appl. Pharmacol.* 14:151–62
34. Schwetz BA, Plaa GL. 1969. Catecholamine potentiation of carbon tetrachloride-induced hepatotoxicity in mice. *Toxicol. Appl. Pharmacol.* 14:495–509
35. Iwai M, Shimazu T. 1988. Effects of ventromedial and lateral hypothalamic stimulation on chemically-induced liver injury in rats. *Life Sci.* 42:1833–40
36. Roberts SM, Harbison RD, Seng JE, James RC. 1991. Potentiation of carbon tetrachloride hepatotoxicity by phenylpropanolamine. *Toxicol. Appl. Pharmacol.* 111:175–88
37. Roberts SM, Harbison RD, James RC. 1994. Methamphetamine potentiation of carbon tetrachloride hepatotoxicity in mice. *J. Pharmacol. Exp. Ther.* 271: 1051–57
38. Roberts SM, Harbison RD, James RC. 1995. Mechanistic studies on the potentiation of carbon tetrachloride hepatotoxicity by methamphetamine. *Toxicology* 97:49–57
39. Roberts SM, Harbison RD, Westhouse RA, James RC. 1995. Exacerbation of carbon tetrachloride-induced liver injury in the rat by methamphetamine. *Toxicol. Lett.* 76:77–83
40. Roberts SM, DeMott RP, James RC. 1997. Adrenergic modulation of hepatotoxicity. *Drug Metab. Rev.* 29:329–53
41. Plaa GL, Hewitt WR. 1982. Biotransformation products and cholestasis. In *Progess in Liver Diseases*, ed. H Popper, F Schaffner, pp. 179–94. New York: Grune & Stratton
42. Plaa GL. 1991. Toxic responses of the liver. In *Casarett and Doull's Toxicology*, ed. MO Amdur, J Doull, CD Klaassen, pp. 334–53. New York: Pergamon. 4th ed.
43. Plaa GL. 1997. Free-radical-mediated liver injury. See Ref. 164, pp. 175–84
44. Sipes IG, Gandolfi AJ. 1982. Bioactivation of aliphatic organohalogens: formation, detection, and relevance. See Ref. 162, pp. 181–212
45. Butler TC. 1961. Reduction of carbon tetrachloride in vivo and reduction of carbon tetrachloride and chloroform in vitro by tissues and tissue constituents. *J. Pharmacol. Exp. Ther.* 134:311–19
46. Slater TF. 1966. Necrogenic action of carbon tetrachloride in the rat: a speculative mechanism based on activation. *Nature* 209:36–40
47. Cheeseman KH, Albano EF, Tomasi A, Slater TF. 1985. Biochemical studies on the metabolic activity of halogenated alkanes. *Environ. Health Perspect.* 64: 85–101
48. Connor HD, Thurman RG, Galizi MD, Mason RP. 1986. The formation of a novel free radical metabolite from CCl_4 in the perfused rat liver and in vivo. *J. Biol. Chem.* 261:4542–48
49. LaCagnin LB, Connor HD, Mason RP, Thurmond RD. 1988. The carbon dioxide anion radical adduct in the perfused rat liver: relationship to halocarbon-

induced toxicity. *Mol. Pharmacol.* 33: 351–57
50. Raucy JL, Kraner JC, Lasker JM. 1993. Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit. Rev. Toxicol.* 23:1–20
51. Raymond P, Plaa GL. 1995. Ketone potentiation of haloalkane-induced hepato- and nephrotoxicity. II. Implication of monooxygenases. *J. Toxicol. Environ. Health* 46:317–28
52. Kim SG, Chung HC, Cho JY. 1996. Molecular mechanism for alkyl sulfide-modulated carbon tetrachloride-induced hepatotoxicity: the role of cytochrome P450 2E1, P450 2B and glutathione-S-transferase expression. *J. Pharmacol. Exp. Ther.* 277:1058–66
53. Gruebele A, Zawaski K, Kaplan D, Novak RF. 1996. Cytochrome P4502E1- and cytochrome P4501B1/2B2-catalyzed carbon tetrachloride metabolism: effects on signal transduction as demonstrated by altered immediate-early (c-Fos and c-Jun) gene expression and nuclear AP-1 and NF-kappa B transcription factor levels. *Drug Metab. Disp.* 24:15–22
54. Recknagel RO, Ghoshal AK. 1966. Lipid peroxidation as a vector in carbon tetrachloride hepatotoxicity. *Lab. Invest.* 15:132–48
55. Comporti M. 1985. Lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.* 53:599–623
56. Koch RR, Glende EA Jr, Recknagel RO. 1974. Hepatotoxicity of bromotrichloromethane—bond dissociation energy and lipoperoxidation. *Biochem. Pharmacol.* 23:2907–15
57. Mansuy D, Beaune P, Cresteil T, Lange M, Leroux JP. 1977. Evidence for phosgene formation during liver microsomal oxidation of chloroform. *Biochem. Biophys. Res. Commun.* 79:513–17
58. Pohl LR, Bhooshan B, Whittaker NF, Krishna G. 1977. Phosgene: a metabolite of chloroform. *Biochem. Biophys. Res. Commun.* 79:684–91
59. Castro JA. 1984. Mechanistical studies and prevention of free radical cell injury. In *IUPHAR 9th Int. Congr. Pharmacol., Proc.*, ed. W Paton, J Mitchell, P Turner, 2:243–50. London: Macmillan
60. Manno M, DeMatteis F, King IJ. 1988. The mechanism of the suicidal, reductive inactivation of microsomal cytochrome P-450 by carbon tetrachloride. *Biochem. Pharmacol.* 37:1981–90
61. Tierney DJ, Haas AL, Koop DR. 1992. Degradation of cytochrome P450 2E1: selective loss after labilization of the enzyme. *Arch. Biochem. Biophys.* 293: 9–16
62. Moody DE. 1992. Effect of phenobarbital treatment on carbon tetrachloride-mediated cytochrome P-450 loss and diene conjugate formation. *Toxicol. Lett.* 61:213–24
63. Fujii K. 1997. Preventive effect of isoflurane on destruction of cytochrome P450 during reductive dehalogenation of carbon tetrachloride in guinea-pig microsomes. *Drug Metab. Drug Interact.* 14: 99–107
64. Plaa GL, Witschi HP. 1976. Chemicals, drugs, and lipid peroxidation. *Annu. Rev. Pharmacol. Toxicol.* 16:125–41
65. Cluet J-L, Boisset M, Boudene C. 1986. Effect of pretreatment with cimetidine or phenobarbital on lipoperoxidation in carbon tetrachloride- and trichloroethylene-dosed rats. *Toxicology* 38:91–102
66. Klaassen CD, Plaa GL. 1969. Comparison of the biochemical alterations elicited in livers from rats treated with carbon tetrachloride, chloroform, 1,1,2-trichlorethane and 1,1,1-trichloroethane. *Biochem. Pharmacol.* 18:2019–27
67. Brown BB Jr, Sipes IG, Sagalyn AM. 1974. Mechanisms of acute hepatic toxicity: chloroform, halothane, and glutathione. *Anesthesiology* 41:554–61
68. Lavigne JG, Marchand C. 1974. The role of metabolism in chloroform hepatotoxicity. *Toxicol. Appl. Pharmacol.* 29:312–26

69. Wang PV, Kaneko T, Tsukada H, Nakano M, Nakajima T, Sato A. 1997. Time courses of hepatic injuries induced by chloroform and by carbon tetrachloride: comparison of biochemical and histopathological changes. *Arch. Toxicol.* 71: 638–45
70. Plaa GL, Evans EA, Hine CH. 1958. Relative hepatotoxicity of seven halogenated hydrocarbons. *J. Pharmacol. Exp. Ther.* 123:224–29
71. Klaassen CD, Plaa GL. 1966. The relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. *Toxicol. Appl. Pharmacol.* 9:139–51
72. Klaassen CD, Plaa GL. 1967. The relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. *Toxicol. Appl. Pharmacol.* 10:119–31
73. Anders MW. 1988. Bioactivation mechanisms and hepatocellular damage. See Ref. 165, pp. 389–400
74. Popper H. 1988. Hepatocellular degeneration and death. See Ref. 165, pp. 1087–103
75. Liebler DC, Reed DJ. 1997. Free-radical defense and repair mechanisms. See Ref. 164, pp. 141–71
76. Farber JL. 1990. The role of calcium ions in toxic cell injury. *Environ. Health Perspect.* 84:107–11
77. Rosser BG, Gores GJ. 1995. Liver cell necrosis: cellular mechanisms and clinical implications. *Gastroenterology* 108: 252–75
78. Reed DJ. 1998. Evaluation of chemical-induced oxidative stress as a mechanism of hepatocyte death. See Ref. 163, pp. 187–220
79. Laskin DL, Gardner CR. 1998. The role of nonparenchymal cells and inflammatory macrophages in hepatotoxicity. See Ref. 163, pp. 297–320
80. Hoglen NC, Younis HS, Hartley DP, Gunawardhana L, Lantz RC, Sipes IG. 1998. 1,2-Dichlorobenzene-induced lipid peroxidation in male Fischer 344 rats is Kupffer cell dependent. *Toxicol. Sci.* 45: 376–85
81. Hooser SB, Rosengren RJ, Hill DA, Mobley SA, Sipes IG. 1994. Vitamin A modulation of xenobiotic-induced hepatotoxicity in rodents. *Environ. Health Perspect.* 102(Suppl. 9):39–43
82. Badger DA, Sauer JM, Hoglen NC, Jolley CS, Sipes IG. 1996. The role of inflammatory cells and cytochrome P450 in the potentiation of CCl_4-induced liver injury by a single dose of retinol. *Toxicol. Appl. Pharmacol.* 141:507–19
83. Kutob SD, Plaa GL. 1962. The effect of acute ethanol intoxication on chloroform-induced liver damage. *J. Pharmacol. Exp. Ther.* 135:245–52
84. Strubelt O, Obermeier F, Siegers CP. 1978. The influence of ethanol pretreatment on the effects of nine hepatotoxic agents. *Acta Pharmacol. Toxicol.* 43: 211–18
85. Cornish HH, Adefuin J. 1967. Potentiation of carbon tetrachloride toxicity by aliphatic alcohols. *Arch. Environ. Health* 14:237–40
86. Traiger GJ, Plaa GL. 1971. Differences in the potentiation of carbon tetrachloride in rats by ethanol and isopropanol pretreatment. *Toxicol. Appl. Pharmacol.* 20:105–12
87. Traiger GJ, Plaa GL. 1972. Relationship of alcohol metabolism to the potentiation of CCl_4 hepatotoxicity induced by aliphatic alcohols. *J. Pharmacol. Exp. Ther.* 183:481–88
88. Traiger GJ, Plaa GL. 1973. Effect of isopropanol on CCl_4-induced changes in perfused rat liver hemodynamics. *Arch. Int. Pharmacodyn. Thér.* 202:102–5
89. Traiger GJ, Plaa GL. 1973. Effect of aminotriazole on isopropanol- and acetone-induced potentiation of CCl_4 hepatotoxicity. *Can. J. Physiol. Pharmacol.* 51:291–96
90. Traiger GJ, Plaa GL. 1974. Chlorinated hydrocarbon toxicity: potentiation by

isopropyl alcohol and acetone. *Arch. Environ. Health* 28:276–78
91. Plaa GL, Traiger GJ. 1973. Mechanism of potentiation of CCl_4-induced hepatotoxicity. In *Pharmacology and the Future of Man—Proc. 5th Congr. Pharmacol.*, ed. T Loomis, 2:100–13. Basel: Karger
92. Folland DS, Schaffner W, Grinn HE, Crofford OB, McMurray DR. 1976. Carbon tetrachloride toxicity potentiated by isopropyl alcohol. *J. Am. Med. Assoc.* 236:1853–56
93. Deng JG, Wang JD, Shih TS, Lan FL. 1987. Outbreak of carbon tetrachloride poisoning in a color printing factory related to the use of isopropyl alcohol and an air conditioning system in Taiwan. *Am. J. Ind. Med.* 12:11–19
94. Traiger GJ, Bruckner JV. 1976. The participation of 2-butanone in 2-butanol-induced potentiation of carbon tetrachloride hepatotoxicity. *J. Pharmacol. Exp. Ther.* 196:493–500
95. Hewitt WR, Miyajima H, Côté MG, Plaa GL. 1980. Acute alteration of chloroform-induced hepato- and nephrotoxicity by n-hexane, methyl n-butyl ketone, and 2,5-hexandione. *Toxicol. Appl. Pharmacol.* 53:230–48
96. Hewitt WR, Brown EM, Plaa GL. 1983. Relationship between the carbon skeleton length of ketonic solvents and potentiation of chloroform-induced hepatotoxicity in rats. *Toxicol. Lett.* 16: 297–304
97. Raisbeck MF, Brown EM, Hewitt WR. 1986. Renal and hepatic interactions between 2-hexanone and carbon tetrachloride in F-344 rats. *Toxicol. Lett.* 31: 15–21
98. Vézina M, Kobusch AB, du Souich P, Greselin E, Plaa GL. 1990. Potentiation of chloroform-induced hepatotoxicity by methyl isobutyl ketone and two metabolites. *Can. J. Physiol. Pharmacol.* 68: 1055–61
99. Raymond P, Plaa GL. 1995. Ketone potentiation of haloalkane-induced hepato- and nephrotoxicity. I. Dose-response relationships. *J. Toxicol. Environ. Health* 45:465–80
100. Hewitt WR, Miyajima H, Côté MG, Hewitt LA, Cianflone DJ, Plaa GL. 1982. Dose-response relationships in 1,3-butanediol-induced potentiation of carbon tetrachloride toxicity. *Toxicol. Appl. Pharmacol.* 64:529–40
101. Pilon D, Brodeur J, Plaa GL. 1986. 1,3-Butanediol-induced increases in ketone bodies and potentiation of CCl_4 hepatotoxicity. *Toxicology* 40:165–80
102. Pilon D, Charbonneau M, Brodeur J, Plaa GL. 1986. Metabolites and ketone body production following methyl n-butyl ketone exposure as possible indices of MnBK potentiation of carbon tetrachloride hepatotoxicity. *Toxicol. Appl. Pharmacol.* 85:49–59
103. Plaa GL. 1988. Experimental evaluation of haloalkanes and liver injury. *Fundam. Appl. Toxicol.* 10:563–70
104. Hanasono GK, Côté MG, Plaa GL. 1975. Potentiation of carbon tetrachloride-induced hepatotoxicity in alloxan- or streptozotocin-diabetic rats. *J. Pharmacol. Exp. Ther.* 192:592–604
105. Hanasono GK, Witschi HP, Plaa GL. 1975. Potentiation of the hepatotoxic responses to chemicals in alloxan-diabetic rats. *Proc. Soc. Exp. Biol. Med.* 149:903–7
106. Villarruel M, Fernández G, de Ferreyra EC, de Fenos OM, Castro JA. 1982. Studies on the mechanism of alloxan-diabetes potentiation of carbon tetrachloride-induced liver necrosis. *Br. J. Exp. Pathol.* 63:388–93
107. Watkins JB, Sanders RA, Beck LV. 1988. The effect of long-term streptozotocin-induced diabetes on the hepatotoxicity of bromobenzene and carbon tetrachloride and hepatic biotransformation in rats. *Toxicol. Appl. Pharmacol.* 93:329–38
108. Wang PV, Kaneko T, Sato A, Charbonneau M, Plaa GL. 1995. Dose- and route-dependent alteration of metabolism and toxicity of chloroform in fed and fasting

rats. *Toxicol. Appl. Pharmacol.* 135:19–26
109. Plaa GL, Hewitt WR. 1982. Potentiation of liver and kidney injury by ketones and ketogenic substances. In *Advances in Pharmacology and Therapeutics II*, ed. H Yoshida, Y Hagihara, S Ebashi, 5:65–75. Oxford, UK: Pergamon
110. MacDonald JR, Gandolfi AJ, Sipes IG. 1982. Acetone potentiation of 1,1,2-trichloroethane hepatotoxicity. *Toxicol. Lett.* 13:57–69
111. Hewitt WR, Brown EM, Plaa GL. 1983. Acetone-induced potentiation of trihalomethane toxicity in male rats. *Toxicol. Lett.* 16:385–96
112. Pessayre D, Cobert B, Descatoire V, Degott C, Babany G, et al. 1982. Hepatotoxicity of trichloroethylene-carbon tetrachloride mixtures in rats. *Gastroenterology* 83:761–72
113. Charbonneau M, Oleskevich A, Brodeur J, Plaa GL. 1986. Acetone potentiation of rat liver injury induced by trichloroethylene-carbon tetrachloride mixtures. *Fundam. Appl. Toxicol.* 6:654–61
114. Borzelleca JR, O'Hara TM, Gennings C, Granger RH, Sheppard MA, Condie LW Jr. 1990. Interactions of water contaminants. I. Plasma enzyme activity and response surface methodology following gavage administration of CCl_4 and $CHCl_3$ or TCE singly and in combination in the rat. *Fundam. Appl. Toxicol.* 14: 477–90
115. Steup DR, Wiersma D, McMillan DA, Sipes IG. 1991. Pretreatment with drinking water solutions containing trichloroethylene or chloroform enhances the hepatotoxicity of carbon tetrachloride in Fischer 344 rats. *Fundam. Appl. Toxicol.* 16:798–809
116. Charbonneau M, Greselin E, Brodeur J, Plaa GL. 1991. Influence of acetone on the severity of the liver injury induced by haloalkane mixtures. *Can. J. Physiol. Pharmacol.* 69:1901–7
117. Charbonneau M, Tuchweber B, Plaa GL. 1986. Acetone potentiation of chronic liver injury induced by repetitive administration of carbon tetrachloride. *Hepatology* 6:694–700
118. Hewitt WR, Miyajima H, Côté MG, Plaa GL. 1979. Acute alteration of chloroform-induced hepato- and nephrotoxicity by mirex and Kepone. *Toxicol. Appl. Pharmacol.* 48:509–27
119. Curtis LR, Williams WL, Mehendale HM. 1979. Potentiation of the hepatotoxicity of carbon tetrachloride following preexposure to chlordecone (Kepone) in the male rat. *Toxicol. Appl. Pharmacol.* 51:283–93
120. Agarwal AK, Mehendale HM. 1982. Potentiation of bromotrichloromethane hepatotoxicity and lethality by chlordecone preexposure in the rat. *Fundam. Appl. Toxicol.* 2:161–67
121. Calabrese EJ, Mehendale HM. 1996. A review of the role of tissue repair as an adaptive strategy: why low doses are often non-toxic and why high doses can be fatal. *Food Chem. Toxicol.* 34:301–11
122. Hewitt LA, Caillé G, Plaa GL. 1986. Temporal relationships between biotransformation, detoxication and chlordecone potentiation of chloroform-induced hepatotoxicity. *Can. J. Physiol. Pharmacol.* 64:477–82
123. Plaa GL, Caillé G, Vézina M, Iijima M, Côté MG. 1987. Chloroform interaction with chlordecone and mirex: correlation between biochemical and histological indices of toxicity and quantitative tissue levels. *Fundam. Appl. Toxicol.* 9:198–207
124. Sipes IG, Stripp B, Krishna G, Maling HM, Gillette JR. 1973. Enhanced hepatic microsomal activity to pretreatment of rats with acetone or isopropanol. *Proc. Soc. Exp Biol. Med.* 142:237–40
125. Maling HM, Stripp B, Sipes IG, Highman B, Saul W, Williams MA. 1975. Enhanced hepatotoxicity of carbon tetrachloride, thioacetamide, and dimethylnitrosamine by pretreatment of rats with

ethanol and some comparisons with potentiation by isopropanol. *Toxicol. Appl. Pharmacol.* 33:291–308
126. Cianflone DJ, Hewitt WR, Villeneuve DC, Plaa GL. 1980. Role of biotransformation in the alterations of chloroform hepatotoxicity produced by Kepone and mirex. *Toxicol. Appl. Pharmacol.* 53: 140–49
127. Branchflower RV, Pohl LR. 1981. Investigation of the mechanism of the potentiation of chloroform-induced hepatotoxicity and nephrotoxicity by methyl n-butyl ketone. *Toxicol. Appl. Pharmacol.* 61: 407–13
128. Hewitt LA, Hewitt WR, Plaa GL. 1983. Fractional hepatic localization of $^{14}CHCl_3$ in mice and rats treated with chlordecone or mirex. *Fundam. Appl. Toxicol.* 3:489–95
129. Hewitt LA, Valiquette C, Plaa GL. 1987. The role of biotransformation-detoxication in acetone-, 2-butanone-, and 2-hexanone-potentiated chloroform-induced hepatotoxicity. *Can. J. Physiol. Pharmacol.* 65:2313–18
130. Hewitt LA, Palmason C, Masson S, Plaa GL. 1990. Evidence for the involvement of organelles in the mechanism of ketone-potentiated chloroform-induced hepatotoxicity. *Liver* 10:35–48
131. Britton RS, Dolak JA, Glende EA Jr, Recknagel RO. 1987. Potentiation of carbon tetrachloride hepatotoxicity by chlordecone: dose-response relationships and increased covalent binding in vivo. *J. Biochem. Toxicol.* 2:43–55
132. Wang PV, Kaneko T, Tsukada H, Nakano M, Sato A. 1997. Dose- and route-dependent alterations in metabolism and toxicity of chemical compounds in ethanol-treated rats: difference between highly (chloroform) and poorly (carbon tetrachloride) metabolized hepatotoxic compounds. *Toxicol. Appl. Pharmacol.* 142:13–21
133. Imaoka S, Funae Y. 1991. Induction of cytochrome P450 isozymes in rat liver by methyl n-alkyl ketones and n-alkylbenzenes. Effects of hydrophobicity of inducers on inducibility of cytochrome P450. *Biochem. Pharmacol.* 42(Suppl.): S143–50
134. Lewandowski M, Levi P, Hodgson E. 1989. Induction of cytochrome P-450 isozymes by mirex and chlordecone. *J. Biochem. Toxicol.* 4:195–99
135. Kocarek TA, Schuetz EG, Guzelian PS. 1991. Selective induction of cytochrome P450e by Kepone (chlordecone) in primary cultures of adult rat hepatocytes. *Mol. Pharmacol.* 40:203–10
136. Kocarek TA, Schuetz EG, Guzelian PS. 1994. Regulation of cytochrome P450 2B1/2 mRNAs by Kepone (chlordecone) and potent estrogens in primary cultures of adult rat hepatocytes on Matrigel. *Toxicol. Lett.* 71:183–96
137. Davis ME, Mehendale HM. 1980. Functional and biochemical correlates of chlordecone exposure and its enhancement of CCl_4 hepatotoxicity. *Toxicology* 15:91–103
138. Young RA, Mehendale HM. 1989. Carbon tetrachloride metabolism in partially hepatectomized and sham-operated rats pre-exposed to chlordecone (Kepone). *J. Biochem. Toxicol.* 4:211–19
139. Côté MG, Traiger GJ, Plaa GL. 1974. Effect of isopropanol-induced potentiation of carbon tetrachloride on rat hepatic ultrastructure. *Toxicol. Appl. Pharmacol.* 30:14–25
140. Curtis LR, Mehendale HM. 1981. Hepatobiliary dysfunction and inhibition of adenosine triphosphatase activity of bile canaliculi-enriched fractions following in vivo mirex, photomirex, and chlordecone exposures. *Toxicol. Appl. Pharmacol.* 61:429–40
141. Mehendale HM. 1991. Role of hepatocellular regeneration and hepatolobular healing in the final outcome of liver injury—a two-stage model of toxicity. *Biochem. Pharmacol.* 42:1155–62
142. Mehendale HM. 1994. Mechanism of the

interactive amplification of halomethane hepatotoxicity and lethality by other chemicals. In *Toxicology of Chemical Mixtures*, ed. RSH Yang, pp. 299–334. San Diego: Academic
143. Soni MG, Mehendale HM. 1998. Role of tissue repair in toxicologic interactions among hepatotoxic organics. *Environ. Health Perspect.* 106(Suppl. 6):1307–17
144. Lockard VG, Mehendale HM, O'Neal RM. 1983. Chlordecone-induced potentiation of carbon tetrachloride hepatotoxicity: a light and electron microscopic study. *Exp. Mol. Pathol.* 39:230–45
145. Lockard VG, Mehendale HM, O'Neal RM. 1983. Chlordecone-induced potentiation of carbon tetrachloride hepatotoxicity: a morphometric and biochemical study. *Exp. Mol. Pathol.* 39:246–56
146. Charbonneau M, Iijima M, Côté MG, Plaa GL. 1985. Temporal analysis of rat liver injury following potentiation of carbon tetrachloride hepatotoxicity with ketonic or ketogenic compounds. *Toxicology* 35:95–112
147. Bell AN, Young RA, Lockard VG, Mehendale HM. 1988. Protection of chlordecone-potentiated carbon tetrachloride hepatotoxicity and lethality by partial hepatectomy. *Arch. Toxicol.* 61:392–405
148. Mehendale HM, Purushotam KR, Lockard VG. 1989. The time course of liver injury and [^{3}H]thymidine incorporation in chlordecone-potentiated $CHCl_3$ hepatotoxicity. *Exp. Mol. Pathol.* 51:31–47
149. Mehendale HM, Thakore KN, Rao CV. 1994. Autoprotection: stimulated tissue repair permits recovery from injury. *J. Biochem. Toxicol.* 9:131–39
150. Soni MG, Mehendale HM. 1991. Protection from chlordecone-amplified carbon tetrachloride toxicity by cyanidanol: regeneration studies. *Toxicol. Appl. Pharmacol.* 108:58–66
151. Ray SD, Mehendale HM. 1990. Potentiation of CCl_4 and $CHCl_3$ hepatotoxicity and lethality by various alcohols. *Fundam. Appl. Toxicol.* 15:429–40
152. Rao PS, Dalu A, Kulkarni SG, Mehendale HM. 1996. Stimulated tissue repair prevents lethality in isopropanol-induced potentiation of carbon tetrachloride hepatotoxicity. *Toxicol. Appl. Pharmacol.* 140:235–44
153. Vance JE, Vance DE. 1990. Lipoprotein assembly and secretion by hepatocytes. *Annu. Rev. Nutr.* 10:337–56
154. Rusinol A, Verkade H, Vance JE. 1990. Assembly of rat hepatic very low density lipoproteins in the endoplasmic reticulum. *J. Biol. Chem.* 268:3555–62
155. Kuipers F, Jong MC, Lin Y, Eck M, Havinga R, et al. 1997. Impaired secretion of very low density lipoprotein-triglycerides by apolipoprotein E-deficient mouse hepatocytes. *J. Clin. Invest.* 100:2915–22
156. Yamauchi T, Iwai M, Kobayashi N, Shimazu T. 1998. Noradrenaline and ATP decrease the secretion of triglyceride and apoprotein B from perfused rat liver. *Eur. J. Physiol.* 435:368–74
157. Maellaro E, Del Bello B, Casini AF, Comporti M, Ceccarelli D, et al. 1990. Early mitochondrial disfunction in bromobenzene treated mice: a possible factor of liver injury. *Biochem. Pharmacol.* 40:1491–97
158. Tang W, Borel AG, Fujimiya T, Abbott FS. 1995. Fluorinated analogues as mechanistic probes in valproic acid hepatotoxicity: hepatic microvesicular steatosis and glutathione status. *Chem. Res. Toxicol.* 8:671–82
159. Fromenty B, Pessayre D. 1995. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacol. Ther.* 67:101–54
160. Tennant BC, Baldwin BH, Graham LA, Ascenzi MA, Hornbuckle WE, et al. 1998. Antiviral activity and toxicity of fialuridine in the woodchuck model of hepatitis B virus infection. *Hepatology* 28:179–91
161. Cohen SD, Khairallah A. 1997. Selective protein arylation and acetaminophen-

induced hepatotoxicity. *Drug Metab. Rev.* 29:59–77
162. Plaa GL, Hewitt WR, eds. 1982. *Toxicology of the Liver.* New York: Raven
163. Plaa GL, Hewitt WR, eds. 1998. *Toxicology of the Liver.* Washington, DC: Taylor & Francis. 2nd ed.
164. Wallace KB, ed. 1997. *Free Radical Toxicology.* Washington, DC: Taylor & Francis
165. Arias IM, Jakoby WB, Popper H, Schachter D, Shafritzs DA, eds. 1988. *The Liver: Biology and Pathobiology.* New York: Raven. 2nd ed.