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Damage to Hepatic Cellular Membranes by Chlorinated Olefins with Emphasis on Synergism and Antagonism

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The fundamental reactivity or stability of the chloroethylene molecules affects their hepatotoxic potential. Extent and symmetry of the chlorine substitution, which alters electron delocalization, charge polarization, and solubility, affect biologic response. The most nonsymmetrically depolarized chloroethylene, 1,1-dichloroethylene (1,1-DCE) is the most hepatotoxic and causes a unique pattern of hepatocellular injury involving mitochondria, plasma membranes, and chromatin. The injury caused by the other chloroethylenes examined appears to profoundly affect the structural integrity of the endoplasmic reticulum with toxic potential in the order: trichloroethylene (TRI) > vinyl chloride (VCM) > perchloroethylene (PER). Pretreatments which increased cytochrome P-450 contents, thus presumably augmenting metabolic activation to a reactive intermediate such as an epoxide, enhanced or were synergistic to the hepatotoxic potential of TRI, VCM and PER but were protective or antagonistic to 1,1-DCE hepatotoxicity. Biologic response to 1,1-DCE may be expressed by a different metabolic pathway. Glutathione appears to be involved in the biologic response to all nonsymmetric chloroethylenes and to act as an antagonist against injury. Marked differences in the patterns of injury and the biologic responses suggest that more than one mechanism is involved in the production of injury by chloroethylenes.

Introduction

Our studies of the acute hepatotoxicity of the chloroethylenes have shown not only certain similarities in their biologic effects, but marked differences in both relative toxicity and in the pattern of hepatocellular injury. These marked differences suggest that the chloroethylenes produce injury by more than one mechanism. The relative reactivity of these molecules is affected by the extent to which their electron distribution is altered by chlorine substitution. Two effects are involved. First, the more electronegative halogens attract electrons. Second, the orbital of one of the lone pairs of electrons of the chlorine atom overlaps with the pi electrons of the carbon–carbon double bond. This interaction involves a partial loss of electrons by the chlorine atom and a gain by the double bond as illustrated by the small curved arrows in Figure 1 (*1*). The result is that the carbon–chlorine bond acquires some double bond character and the molecule become polarized as shown for vinyl chloride (VCM). When two chlorine atoms are attached to one carbon of the double bond, as with 1,1-dichloroethylene (1,1-DCE), the net electron delocalization is enhanced. A more polarized molecule results. On the other hand, symmetrical attachment of the chlorines to both carbons of the double bond leads to a more uniform electron delocalization, as illustrated by the four resonance forms of perchloroethylene (PER), resulting in a more stable molecule. For trichloroethylene (TRI), electron delocalization would be less extreme than for 1,1-DCE but more marked than for PER and somewhat similar to VCM. Increasing chlorine substitution also alters the biologic response to this series of chloroethylenes by increasing the lipid solubility and by depressing the volatility or "exhalability."

Our semiquantitative analysis of these factors (molecular reactivity/stability, lipid solubility and volatility) indicates an order of toxicity: 1,1-DCE

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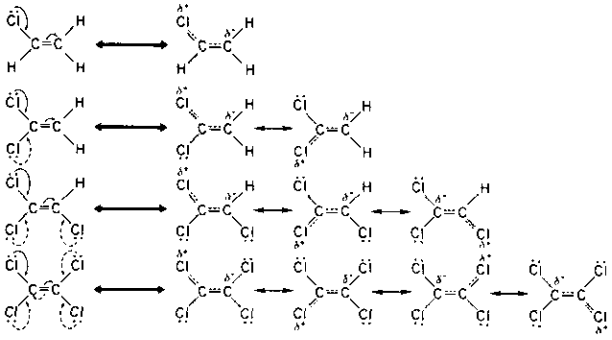

FIGURE 1. Substitution of chlorine atoms for hydrogen atoms affects the electron distribution of chloroethylene molecules. Electrons are attracted to the more electro-negative halogen atom. Another factor must also be considered for the orbital (position in space) of one of the lone pairs of electrons of the chlorine atom overlaps with the orbital of the pi electrons of the carbon to carbon double bond. The small curved arrows illustrate this phenomenon as movement of the electrons associated with the chlorine to the carbon to chlorine bond coupled with movement of electrons in the double bond towards the other carbon. This shift of electrons causes a polarization of the molecule as indicated by the partial charges. The chlorine to carbon bond has some double bond character as indicated by the dashed bonding lines. Symmetry and extent of chlorine substitution which alter both the degree of molecular polarization and of electron delocalization affect the molecular stability/reactivity. Principles of the chloroethylene electron delocalization and description of resulting molecular polarization from Musgrave (*1*).

>> TRI > VCM > PER. Fortunately, this ranking corresponds to our experimental assessment of the acute hepatotoxicity potential of these four chloroethylenes.

We approached the study of the biochemical mechanism(s) responsible for the toxicity of the chlorinated olefins by pretreating animals with chemicals which modify components of the liver mixed-function oxidase system, the enzyme system most likely to be involved in the initial biotransformation of lipid-soluble xenobiotics. We hoped that determination of factors which were synergistic or enhancing of the hepatotoxic potential would indicate the mechanism of activation, while determination of factors which were antagonistic or protective against the hepatotoxic course would clarify the nature of the reactive molecular species. Our working hypothesis was that the hepatotoxic potential of these chloroethylenes would be modulated by pretreatments which nonuniformly enhance and/or uncouple the enzymatic components involved in biotransformation of these chloroethylenes to excretable products via reactive intermediates such as epoxides or aldehydes. Powell (*2*) first suggested an epoxide intermediate for the metabolism of TRI in 1945. Liebman and colleagues (*3–5*) had already demonstrated that the liver mixed-function oxidase system is involved in the initial biotransformation of TRI, and that pretreatment with phenobarbital (PBT) causes alterations in the rate and route of TRI metabolism. Carlson (*6*) reported that pretreatment with PBT or 3-methylcholanthrene (3-MC) exacerbates the hepatotoxicity of TRI.

Methods

Our basic experimental procedure utilizes a 7-day gavage pretreatment of 200 g male Sprague Dawley rats with one of six agents: 400 μmole/kg isomolar doses of PBT, 3-MC, hexachlorobenzene (HCB), spironolactone (SNL) or pregnenolone-16α-carbonitrile (PCN) or 150 μmole/kg of Aroclor 1254. Control animals are given the administrative vehicle (5 ml/kg of 0.1% Tween 80). The animals are then fasted overnight and on the morning of day 8 sacrificed for determination of microsomal enzyme components or exposed to one of the chloroethylenes. Similarly pretreated "controls" are exposed only to room air. Details of pretreatment conditions, microsomal enzyme component assays, other chemical analyses, chloroethylene exposure techniques, histologic examination and methods to quantitate liver injury using serum transaminase activities or liver metal contents have been described (*7, 8*). Chloroethylene exposures were: VCM (5% × 6 hr), 1,1-DCE (0.02% × 4 hr), TRI (1% × 2 hr) and PER (7.5 mmole/kg, PO). Microsomal enzyme components assayed included: cytochrome P-450 and b_5, cytochrome c reductase by NADPH and NADH, oxidative *N*-demethylation of dimethylaminoantipyrine and ethylmorphine, glucose 6-phosphatase, and aryl hydrocarbon hydroxylation of zoxazolamine and 3,4-benzpyrene. The six "agents" chosen to modify the liver mixed function oxidase system gave six different patterns of enzyme induction (*7, 8*).

Results of Parallel Studies

Our working hypothesis proved rational. The acute hepatotoxicity of these four chloroethylenes was modulated to different extents in the animals pretreated with the six inducing agents as compared to the control animals given the administrative vehicle. To determine if induction of a specific mixed function oxidase system component was associated with modulation of chloroethylene-induced liver injury, we compared mean enzyme component levels (assayed at times compatible with onset of chloroethylene administration) with mean serum transaminase levels of similarly pretreated animals sacrificed after chloroethylene exposure. There was

a striking correlation between mean cytochrome P-450 contents and mean serum glutamic oxaloacetic transaminase (SGOT) activities 24 hr after TRI exposure (significance < 1%, df = 5, $r = 0.95$) by linear regression analysis or the power regression analysis shown in Figure 2 (bottom) (8). There was a less perfect correlation (significance ~5%, df = 5, $r = 0.73$) between mean rates of reduction of cytochrome P-450 by NADPH (measured as NADPH-cytochrome c reductase) and SGOT 24 hr after TRI. No other relationship between a mixed function oxidase component and SGOT after TRI exposure was apparent. In this study we also found that the extent of SGOT elevation in individual animals correlated to prolongation of anesthesia recovery time after TRI exposure and to enhanced urinary excretion of trichlorinated metabolites. We concluded that the relative degree of TRI-induced liver injury related directly to cytochrome P-450 content at the time of exposure and to the extent of TRI biotransformation.

Since PER was considered to be a more stable molecule and thus to have a lesser potential for metabolic activation to a reactive species, we looked for augmentation of PER-induced injury only in animals pretreated with PBT or Aroclor 1254, the most potent inducers of cytochrome P-450. While urinary recoveries of total trichlorinated products were approximately 5- and 7-fold greater in the PBT- and Aroclor 1254-pretreated animals compared to the vehicle-pretreated "control" animals, only in Aroclor 1254-pretreated rats did we find evidence of PER-induced liver injury in terms of SGOT elevation and focal histologic necrosis (8).

As shown at the top of Figure 2, pretreatments which were potent inducers of cytochrome P-450 also enhanced the hepatotoxicity of VCM as reflected by elevated activities of serum alanine α-ketoglutarate transaminase (SAKT) 24 hr after VCM exposure (7). This relationship was significant at ~1% level.

Pretreatment with inducers of cytochrome P-450 had a diametrically opposite effect on the response of animals to 1,1-DCE exposure (Fig. 2, middle) (7). Essentially, as mean cytochrome P-450 content increases, the hepatotoxic effect of 1,1-DCE decreases as reflected by SAKT activities, histologic appearance, and liver metal contents of animals sacrificed 6 hr after onset of 1,1-DCE. Again there were correlations of lesser significance (~5%) between mean rates of reduction of cytochrome P-450 by NADPH and the mean SAKT activities after exposure to VCM or 1,1-DCE. Attempts to correlate other mixed function oxidase components with SAKT activities after VCM or 1,1-DCE exposure

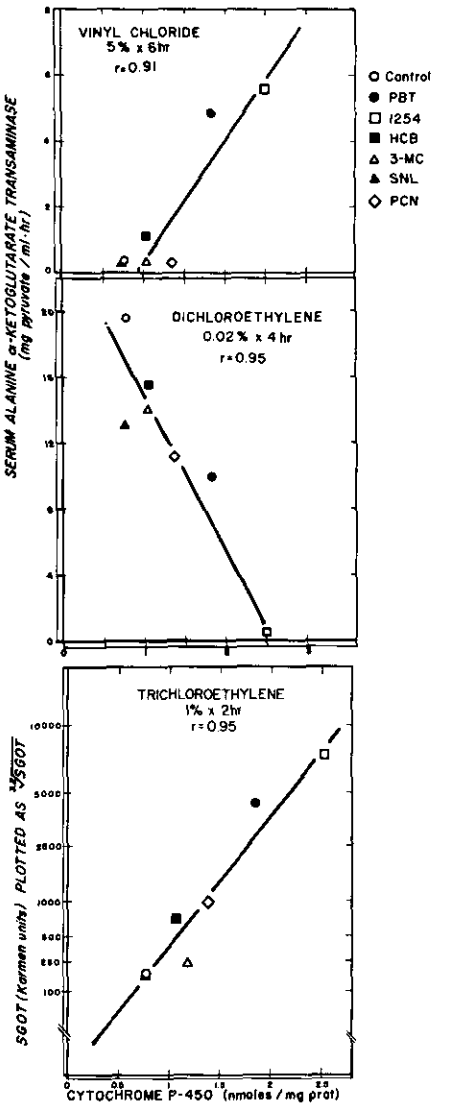

FIGURE 2. Correlations between mean liver microsomal cytochrome P-450 contents at the beginning of chloroethylene exposure and the mean serum enzyme transaminase activities after chloroethylene exposure in groups of animals pretreated with one of six inducers of mixed function oxidase components plus the "control" animals pretreated with the administrative vehicle: (O) control; (●) PBT; (□) Aroclor 1254; (■) HCB; (△) 3-MC; (▲) SNL; (◇) PCN. Top correlation is between increasing cytochrome P-450 contents and elevated SAKT activities 24 hr after onset of VCM exposure (significant at the 1% level, df = 5). Middle correlation is between decreasing cytochrome P-450 contents and elevated SAKT activities 6 hr after onset of 1,1-DCE exposure (significant at the 1% level, df = 5). Bottom correlation between increasing cytochrome P-450 contents and elevated SGOT activities 24 hr after onset of TRI exposure is a power relationship such that within the experimental range each 2-fold increase in cytochrome P-450 corresponds approximately to a 10-fold increase in SGOT (significant at < 1% level, df = 5). Data for the VCM and 1,1-DCE correlations from Reynolds et al. (7). Data for the TRI correlation from Moslen et al. (8)

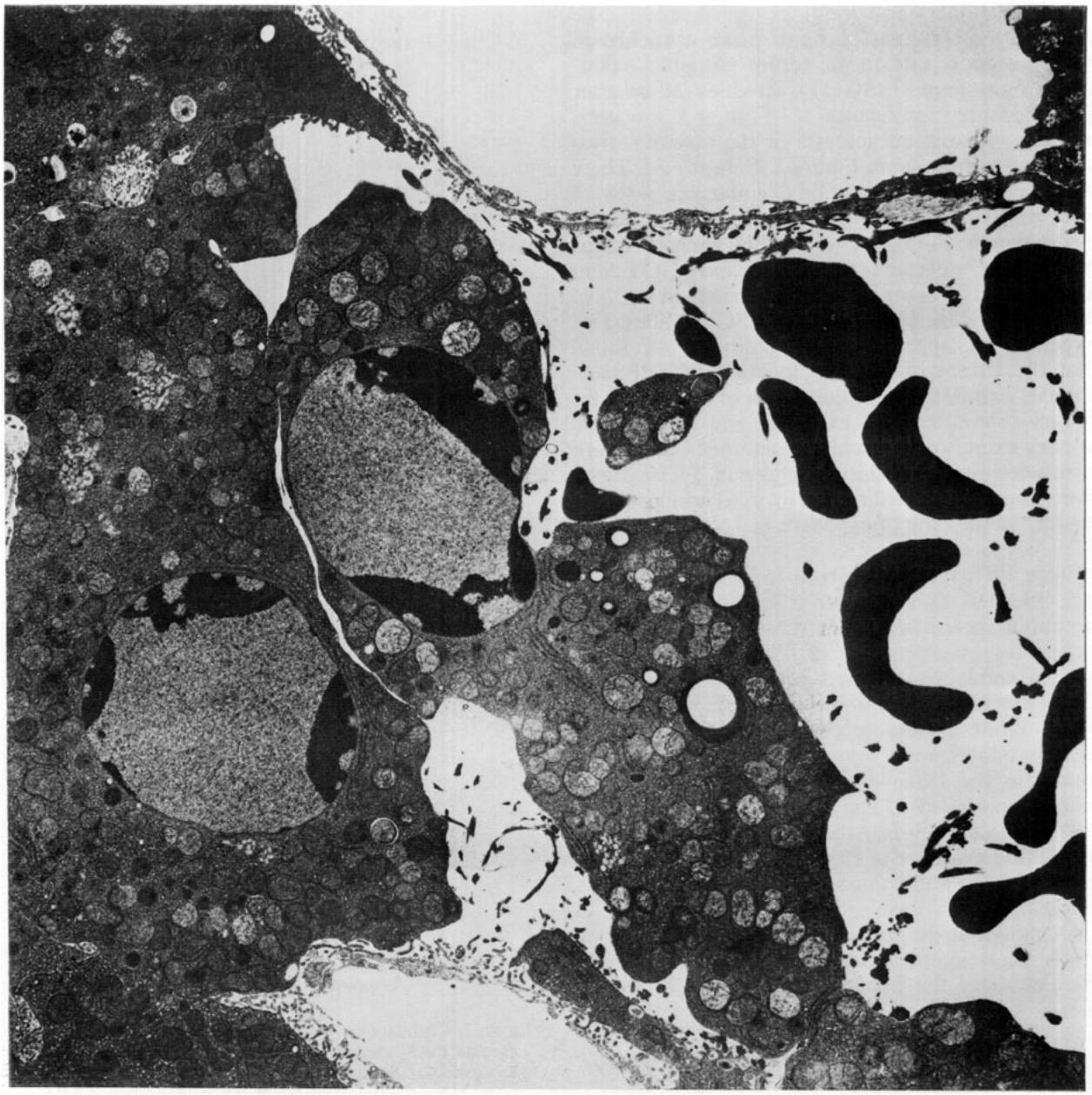

FIGURE 3. Midzonal parenchymal cells 2 hr after the onset of exposure to 0.02% DCE. Cell borders are retracted from sinusoidal walls at upper left and center right. Space formed contains cytoplasmic protrusions, material resembling fibrin and erthyrocytes. Cytoplasm of retracted cells focally contains swollen mitochondria and clusters of small vacuoles in the regions of the golgi apparatus. Strands of rough endoplasmic reticulum are intact. Nuclei show striking segregation of chromatin towards the margins of the nuclear envelope. Bile canaliculi appear intact. × 6,000.

resulted in non-significant scattergrams. It should be pointed out that the amount of 1,1-DCE (0.02% × 4 hr) to which the animals were exposed was much less than that of TRI (1% × 2 hr), which was in turn much less than the amount of VCM (5% × 6 hr) administered. Our experimental studies with VCM, 1,1-DCE, TRI, and PER as well as the comparative microsomal enzyme component analyses were essentially concurrent using overlapping series of similarly pretreated animals.

Differences in Acute Hepatic Injury

Because of the differences in the basic molecular stability/reactivity of the four chloroethylenes as

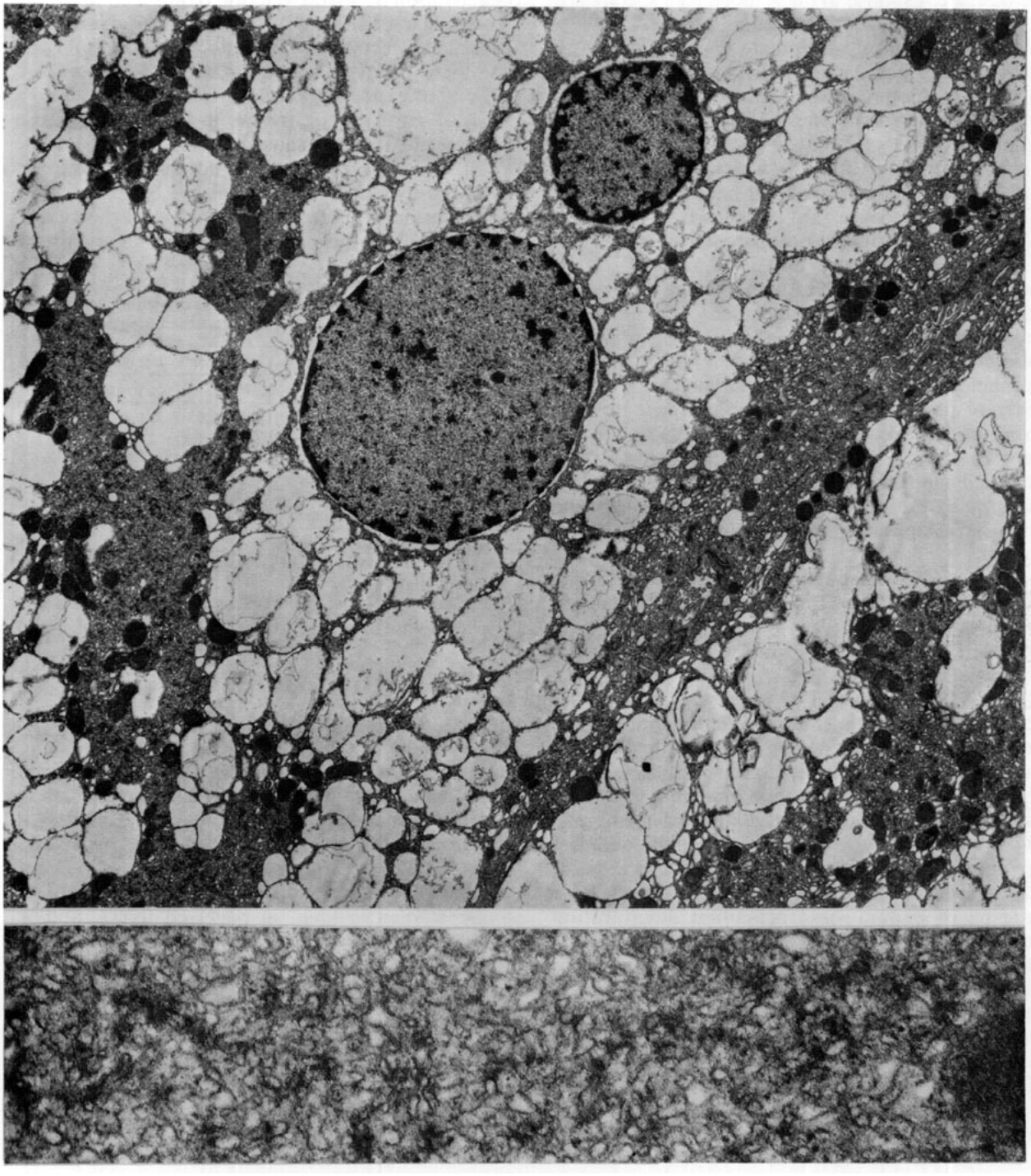

FIGURE 4. Portion of centrolobular liver cells of PBT pretreated animals 8 hr after onset of exposure to TRI (1%, 2 hr). Rough endoplasmic reticulum in perinuclear regions of parenchymal cells is vacuolated and smooth is condensed into tangled tubular masses flecked with areas of increased electron opacity ("labyrinthine" tubular aggregates). × 20,000. Inset on bottom. Higher power of tangled tubular masses. Tubular diameters show greatest narrowing in areas of increased electron opacity. Radioopaque material appears applied on outer surfaces of tubular profiles. × 120,000.

illustrated in Figure 1, variations were expected in the biologic response to these molecules specifically in the extent of biotransformation, type and stability of intermediate(s), and ultimate excreted products. Such factors could contribute to the differences found in the nature of the hepatocellular injury.

Hepatic injury following 1,1-DCE (0.02% × 4 hr) occurs abruptly and first appears as a prominent midzonal stripe of necrosis which rapidly evolves into hemorrhagic centrolobular necrosis by the end of the 4-hr exposure (*7*). As shown in Figure 3, parenchymal cell injury is apparent 2 hr after onset of 1,1-DCE exposure and is characterized by retraction of cell borders with the formation of a pericellular "lacunae" which may contain cytoplasmic projections, red blood cells and fibrin. Nuclear changes in such cells are striking with loss of perinucleolar chromatin, and clumping and coalescence of perinuclear chromatin into cresentric deposits of electron-opaque material against the nuclear envelope. Mitochondria in the cytoplasm of such cells appear swollen and outer mitochondrial membranes are ruptured. In contrast, rough and smooth endoplasmic reticulum appear relatively normal.

Hepatocellular structural derangement following TRI exposure (1% × 2 hr) in PBT-pretreated animals presents in a different form than 1,1-DCE with increased cytoplasmic disorder, random dispersion of organelles including ergastroplasm and degranulation and vacuolization of rough endoplasmic reticulum (*9*). Smooth endoplasmic reticulum then coalesces into tubular aggregates. By 8 hr after the onset of TRI exposure, coalescent tangles of smooth endoplasmic reticulum membranes contain electron-opaque regions suggestive of membrane collapse (Fig. 4). Vacuolization of rough endoplasmic reticulum is also found in PBT and Aroclor 1254 animals after PER administration. Similar patterns of endoplasmic reticulum denaturation were found in PBT and Aroclor 1254 pretreated rats after VCM exposure, and in animals exposed to other halogenated hydrocarbons including carbon tetrachloride and halothane (*10*). The enhanced hepatotoxic potential of carbon tetrachloride and of halothane in animals pretreated with inducers of the mixed function oxidase system is considered related to enhanced rates of their activation to reactive intermediates (possibly free radicals) by components of this system (*10, 11*).

Carbon tetrachloride causes selective deactivation of specific mixed function oxidase system components (*12*). It is not clear whether this occurs as a direct consequence of molecular attack by the reactive intermediate or as a consequence of lipid peroxidation of the membrane (*10*). We have found that exposure to VCM or TRI results in selective deactivation of mixed function oxidase components including cytochrome P-450 (*13, 14*). Table 1 compares the effects of exposure to 1,1-DCE, TRI and halothane on cytochrome P-450 and b_5 contents at the end of exposure. While TRI exposure caused a loss of both cytochrome P-450 and b_5, and halothane exposure caused a loss of cytochrome P-450, 1,1-DCE exposure did not diminish contents of either cytochrome P-450 or b_5. Note that each combination of pretreatment and halocarbon exposure shown in this table resulted in injury to the liver.

Table 1. Contents of rat liver microsomal cytochromes P-450 and b_5 at end of chlorinated hydrocarbon exposure.

		Cytochrome, nmole/mg protein[a]	
Pretreatment	Exposure	P-450	b_5
None	Air	0.76 ± 0.03	1.19 ± 0.04
None	1,1-DCE[b]	0.73 ± 0.08	1.22 ± 0.08
PBT	Air	2.50 ± 0.06	1.64 ± 0.10
PBT	TRI[c]	1.87 ± 0.11[d]	1.29 ± 0.06[e]
Aroclor 1254	Air	2.66 ± 0.03	1.29 ± 0.06
Aroclor 1254	Halothane[f]	2.00 ± 0.07[d]	1.26 ± 0.06

[a]Means ± SEM, for three or more animals per group.
[b]0.02% 1,1-dichloroethylene, 4 hr.
[c]1% trichloroethylene, 2 hr.
[d]$p < 0.001$ compared to similarly pretreated animals exposed to air.
[e]$p < 0.05$ compared to similarly pretreated animals exposed to air.
[f]0.85% halothane, 5 hr.

Similarities in Biologic Response

Because the chloroethylenes are each members of the same chemical family, certain similarities in biologic response were expected and found. All of the chlorinated ethylenes (including *cis-* and *trans*-1,2-DCE) have been reported to be metabolized by isolated perfused livers (*15*). Metabolic studies *in vivo*, in perfused livers, and/or *in vitro* have shown that the biotransformation of each chloroethylene results in the production of a relatively stable oxidized metabolite such as an acid or alcohol (*3, 15–17*). The nature of the oxidized metabolites formed via the biotransformation of each of the chlorinated ethylenes with chlorine(s) attached to both carbons is compatible with the rearrangement of an epoxide (oxirane) intermediate involving chlorine migration (*15, 16*). VCM, 1,1-DEC, TRI, and PER are all capable of being activated by liver preparations (or perhaps are naturally sufficiently reactive) to bind to hepatocellular macromolecules (*15, 18–20*). *In vitro* covalent binding of radiolabeled VCM or TRI in microsomal activation systems can be minimized by addition of inhibitors of the mixed function oxidase system (*20, 21*).

Further evidence that the chlorinated ethylenes can be activated to reactive molecules are the comparative mutagenic tests of Greim et al. (*22*). Each of the nonsymmetric chlorinated ethylenes (VCM, 1,1-DCE, and TRI) was found to be activated to a bacterial mutagen by NADPH-dependent microsomal generating systems. In contrast, no mutagenic activity was detected for the symmetrically chlorinated ethylenes, *cis*- and *trans*-1,2-DCE, and PER. The mutagenicity of nonsymmetric chloroethylenes, such as VCM, appears related to electrophilic metabolites, since VCM metabolites, chloroethylene oxide, 2-chloroacetaldehyde, and 2-chloroethanol—but not chloroacetic acid—are mutagenic (*23*).

VCM, 1,1-DCE, and TRI are classified as carcinogens: VCM exposure in man and experimental animals is associated with angiosarcoma (*24, 25*). 1,1-DCE exposure results in kidney tumors in mice (*26*), and TRI feeding leads to hepatic tumors in mice (*27*).

Reduced glutathione (GSH) is involved in some way in the biologic response to VCM, 1,1-DCE, and TRI. This relationship was first noted for 1,1-DCE by Jaeger et al. (*28*), who found that fasting and other treatments which deplete hepatic GSH enhance the hepatotoxicity of 1,1-DCE. TRI also causes more extensive liver injury in fasted-PBT pretreated animals than in fed-PBT pretreated animals (*14*). We have examined the relationship of 1,1-DCE-induced depletion of hepatic GSH to the manifestation of early liver injury by quantifiable compositional parameters such as changes in hepatic metal contents. As shown in Figure 5, liver GSH contents rapidly plummet during the first 2 hr of 1,1-DCE exposure. Concomitant with this drop, Na contents rise. Striking increases in liver Ca content follow the increases in liver Na at times when GSH contents slowly rebound. In contrast, K, Mg, and Zn contents decrease moderately in concert.

Interestingly enough, liver metals have also proved to be sensitive indicators of the progressive hepatocellular derangement caused by TRI. As shown in Figure 6, the pattern of metal change is different than with 1,1-DCE, with an initial loss in liver Ca during TRI exposure, followed at later times by the typical metal imbalances, specifically Na and Ca influx and K loss, found after the administration of other hepatotoxins such as CCl_4 (*12*). As shown in Figure 7, we have found that in PBT animals which are vulnerable to the hepatotoxicity of TRI, liver GSH contents are progressively depleted during TRI exposure, then rise above normal levels (*14*). However, TRI exposure to vehicle-pretreated animals does not result in

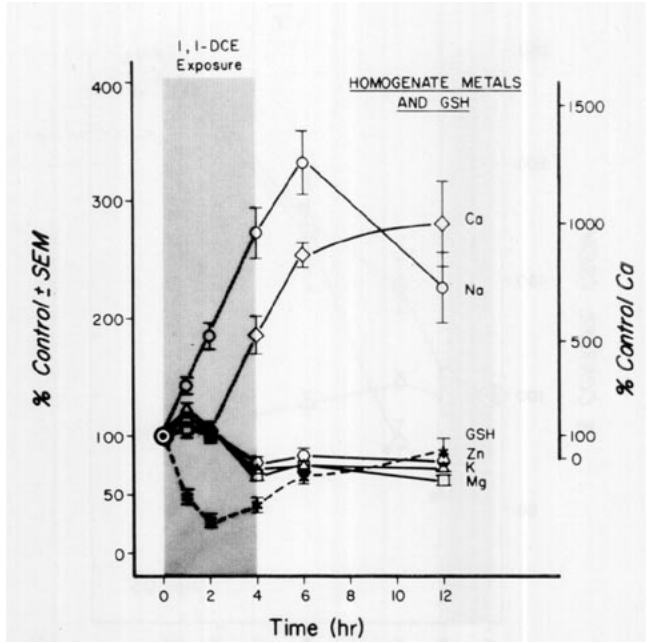

FIGURE 5. Alterations in liver metal and GSH contents of nonpretreated fasted rats during and after exposure to 1,1-DCE (0.02%, 4 hr) expressed as percentage of control values obtained from similarly pretreated animals sacrificed at equivalent times during the 12 hr experimental period: (○) Na; (△) K; (□) Mg; (○) Zn; (◇) Ca; (*) GSH. During the first 2 hr of 1,1-DCE exposure GSH contents rapidly plummet concomitant with an increase in Na content. By the end of 1,1-DCE exposure Ca, K, Mg, and Zn contents deviated significantly ($p < 0.05$) from the control values. The dramatic influx in Ca continued to 12 hr, Na contents peaked at 6 hr, and the decrease in Zn, K, and Zn reached a plateau in concert after the 4 hr. GSH contents were clearly being replenished as the injury, reflected by the marked metal imbalance, became manifest.

detectable liver injury and does not deplete liver GSH contents. Nevertheless, liver GSH contents progressively rise following exposure. GSH depletion in TRI-exposed, PBT-pretreated rats is particularly marked in the microsomal fraction, the organelle which appears profoundly affected in the hepatotoxic course of TRI. VCM exposure has also been associated with progressive depression of hepatic contents of GSH (*29*).

In vitro covalent binding of ^{14}C-VCM or TRI to cellular macromolecules by microsomal generating systems is diminished by the addition of GSH and approximately doubled by the addition of trichloropropene oxide (*20, 30*). It should be pointed out that while trichloropropene oxide is a potent inhibitor of epoxide hydrase *in vitro*, it also depletes hepatic GSH *in vitro* (*31*).

Figure 8 shows schematically the probable and potential pathways of TRI biotransformation. Activation of TRI via a NADPH–P-450 system leads to an epoxide which can rearrange to an aldehyde and

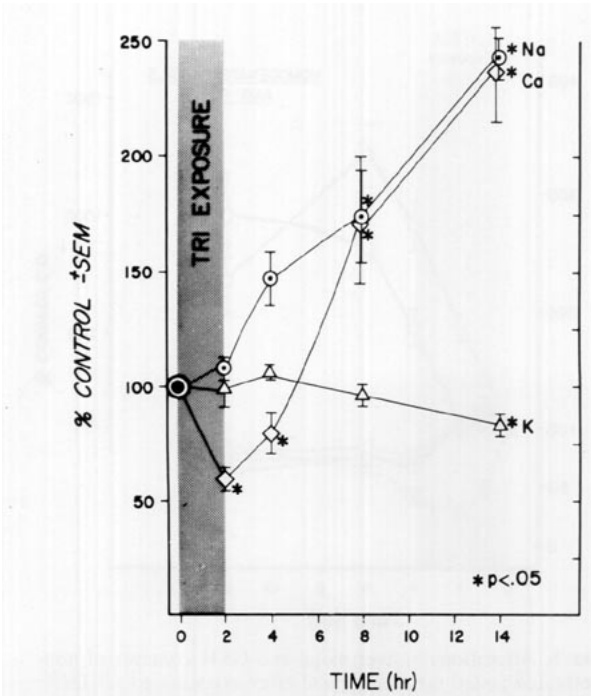

FIGURE 6. Alterations in liver metal contents of PBT-pretreated animals exposed to TRI (1%, 2 hr) expressed as percentage of control values obtained from similarly pretreated animals exposed to room air and sacrificed at equivalent times during the 14 hr experimental period. Ca contents are diminished nearly half by the end of exposure. The metal imbalance at 8 and 14 hr, influx of Na and Ca coupled with loss of K, is similar to that found after administration of other hepatotoxins such as CCl_4 (*12*). Data from Reynolds and Moslen (*9*).

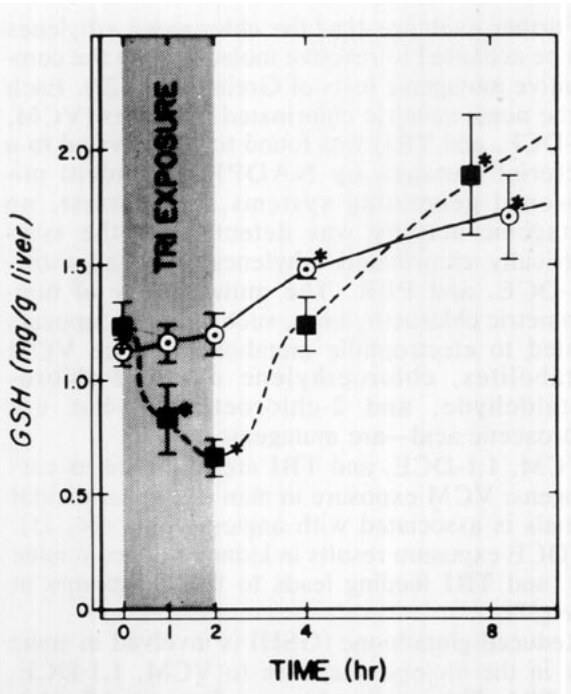

FIGURE 7. Alterations in liver GSH contents of (■) PBT-pretreated and (●) vehicle-pretreated animals during and after TRI exposure (1%, 2 hr). The GSH content of the PBT-pretreated animals is progressively depressed to almost half the pre-exposure levels by the end of TRI exposure and then rebounds. GSH contents of vehicle pretreated animals are essentially constant during exposure, then rise to above pre-exposure levels. Asterisks (*) denote statistically significant points ($p < 0.05$). Data from Moslen et al. (*14*).

be hydrolyzed and transformed by other enzyme systems to trichloroacetic acid or to trichloroethanol, which is subsequently conjugated with glucuronide to form the major urinary metabolite. Fernańdez et al. (*32*) concluded from the low recovery of TRI and its metabolites in the breath and urine of human subjects that "other metabolites exist, or that eventually other means of elimination occur." Our findings on the alterations of hepatic GSH contents during and after TRI exposure indicate some involvement of GSH in TRI biotransformation (*14*), perhaps via GSH transferases.

Other members of the chloroethylene family may be biotransformed more extensively by minor metabolic routes. For example, trichloroacetic acid is the major metabolite of PER, presumably formed via epoxidation followed by a chloride shift producing an acid chloride which would be rapidly hydrolyzed to trichloroacetic acid (*16*). Formation of the major urine-excreted metabolites of VCM; i.e., thiodiglycollic acid and cysteine conjugates, was reported to involve GSH by Green and Hathway (*33*). Bolt et al. (*18*) have suggested that the coupling of reactive VCM metabolites to glutathione may be viewed as an alternate metabolic pathway which prevents binding to cellular macromolecules, and may have particular significance in preventing the reactions with nucleic acids. Watanabe et al. (*34*) have recovered 9–13% of the given dose of ^{14}C-VCM as exhaled $^{14}CO_2$. It is not clear at what point, or which intermediate, in the biotransformation of chloroethylenes leads to the breakdown of the chloroethylene carbon to carbon bond.

Essentially the biologic response to the chloroethylenes must be considered as a series of steps as shown in Figure 9. Biotransformation requires uptake into the cell and into specific organelles, interaction with an enzyme system, metabolic activation and transformation, perhaps subsequent rearrangements or enzymatic conjugations, and ultimately excretion. If epoxidation is the essential reaction in the biotransformation of a chloroethylene to a hepatotoxin, then any pretreatment or condition (such as induction of P-450) which promotes or enhances this epoxidation step (K_8) could be considered "synergistic" to the hepatotoxic effect. Similarly synergistic is any pre-

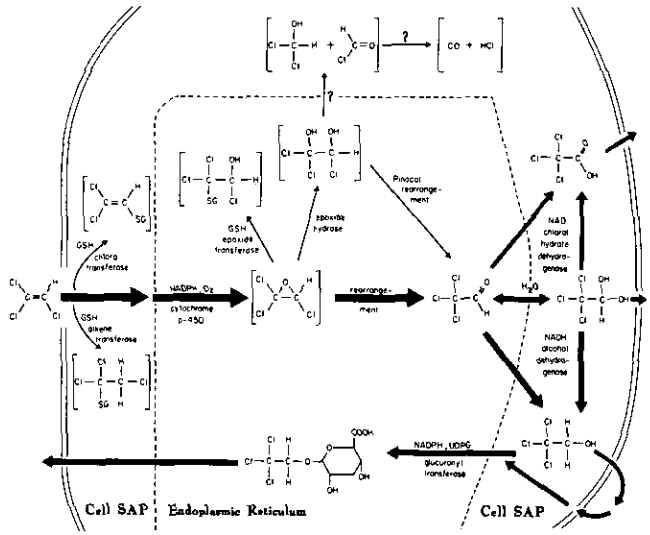

FIGURE 8. Biotransformation of trichloroethylene is assumed to occur chiefly along the metabolic route indicated by the larger arrows. NADPH dependent oxidation by cytochrome P-450 produces an epoxide as the primary metabolite which rearranges by chloride migration to form trichloroacetaldehyde as the major secondary metabolite. Trichloroacetaldehyde is converted to a tertiary generation of metabolites through hydration, enzymatic oxidation, and enzymatic reduction to an alcohol. Trichloroethanol subsequently is conjugated to a glucuronide, the major urinary metabolite. Smaller arrows indicate possible minor metabolic routes such as GSH epoxide transfer which may account for the apparent involvement of GSH in the biotransformation of TRI (*14*). Other members of the chloroethylene family may be metabolized to a greater extent by minor metabolic routes due to the comparative stability/instability of the respective chloroethylene molecule or its intermediates (*16, 33, 34*).

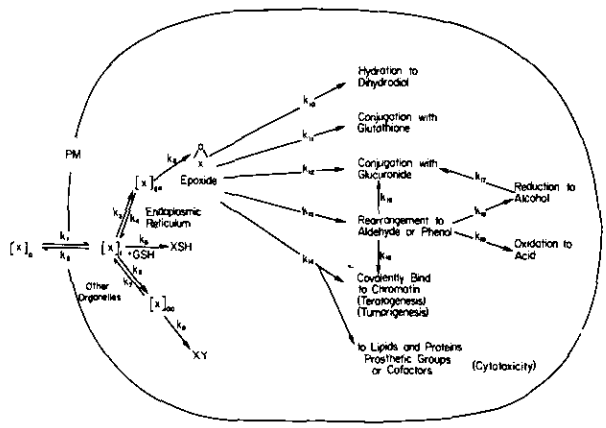

FIGURE 9. Kinetic flow diagram of the cellular response to a xenobiotic. Xenobiotics must first pass through the cell plasma membrane (PM) by a reversible process (k_1, k_2) then interact with functional components of the endoplasmic reticulum (k_3, k_4), with glutathione (GSH) in the cell sap (k_5) leading to a glutathione conjugate, or with other organelles (k_6, k_7) leading (k_9) to a product XY. Interaction with the mixed function oxidase system of the endoplasmic reticulum (k_8) is assumed to lead to generation of a reactive epoxide capable of interaction with vital cell components (k_{11}). Hydration (k_{10}), conjugation (k_{11}, k_{12}) and rearrangement (k_{13}) transform the epoxide intermediate presumably to less toxic compounds. However compounds formed subsequently, such as aldehydes, may also be capable of toxic interaction with cell components (k_{16}) until further converted to acids (k_{19}), alcohols (k_{18}), or glucuronide conjugates (k_{15}, k_{17}). Induction of the pathway (k_8) which generates the toxic epoxide species and conditions which reduce the efficiency of pathways which detoxify the reactive species (k_{10}, k_{11}, k_{12}, k_{15}, k_{18}, k_{19}) would be considered synergistic to hepatotoxicity, since the result is an enhancement of the hepatotoxic action (k_{11}). Conditions which promote biotransformation by other enzymatic processes, such as k_6, or maintain adequate GSH (k_5) contents would be considered antagonistic, since the result is a diminution of hepatotoxic action.

treatment or condition hampering the processes, such as glucuronidation (k_{12}) or GSH conjugation (k_{11}), which transform reactive intermediate(s) to less toxic species. In contrast, conditions or pretreatments "antagonistic" to the hepatotoxic effect would be those which increase the concentration (or the capacity to renew supplies) of endogenous protective species such as glutathione (k_5) which "defend" cellular components against "attack" by reactive molecular species.

Obviously the fundamental reactivity/stability of the molecule has an influence on its biologic course. VCM, TRI, and PER apparently require metabolic activation before their hepatotoxic potential can be expressed. 1,1-DCE, in contrast, because of the extensive electron depolarization due to the attachment of 2 chlorines on one carbon (Fig. 1), may react spontaneously with endogenous electrophiles of the cell such as iron in the cytochromes of electron transport systems of mitochondria and other organelles. GSH may play a vital antagonistic role in the biologic response to 1,1-DCE by protecting vulnerable macromolecules against attack by this highly depolarized molecule.

Delocalization of electrons of 1,1-DCE away from the chlorines may activate the molecule towards a nucleophilic attack of such a nature that a chlorine is replaced by an attacking nucleophile (*1*). Epoxidation of 1,1-DCE may be a relatively unimportant metabolic route or in fact may not occur. Bonse et al. (*15*) reported a high uptake of 1,1-DCE by isolated perfused rat liver but did not determine the metabolites formed, although they were able to identify di- and trichlorinated acid and/or alcohol metabolites of 1,2-*cis*- and *trans*-dichloroethylene, TRI and PER. Bonse et al. (*15*) were able to synthesize the epoxide (oxirane) of all chlorinated ethylenes except 1,1-DCE. Leibman and Ortiz (*17*) have identified monochloroacetic acid as a product of the biotransformation of 1,1-DCE by rat liver 9000*g* supernatant fractions and an NADPH generating system. We have detected monochloroacetic acid but not dichloroacetic acid in the urine

of animals exposed to 1,1-DCE (Reynolds et al., unpublished observation).

Is it possible that monochloroacetic acid is produced from 1,1-DCE not via cytochrome P-450 generated epoxide, but via a dechlorinating system requiring cytochrome P-450, NADPH, O_2 and perhaps GSH? Van Dyke and colleagues (*35, 36*) have demonstrated a microsomal dechlorinating system that is inducible by PBT, preferentially dechlorinates compounds with more than one chlorine attached to a carbon, produces oxidized metabolites including acids and alcohols, and can be reconstituted with cytochrome P-450, NADPH cytochrome c reductase, and lecithin. Multiple cytochrome P-450 species with varying substrate specificities, markedly different catalytic properties, and preferential inducibility are known (*37*). Pretreatments which induce cytochrome P-450 may be antagonistic to the hepatotoxicity of 1,1-DCE (Fig. 2, center) because the essential reaction of the cytochrome P-450 system on 1,1-DCE is a detoxifying dechlorination, not a toxifying epoxidation as for VCM, TRI, and PER.

This report describes experimental studies conducted over several years which involved at various stages the active collaboration of Drs. Rudolph J. Jaeger, Sandor Szabo, and Paul J. Boor. We would also like to thank Elizabeth A. Gasch, Regina Bean, Richard J. Treinen, and Theresa Paolini Kingsley for their skillful assistance.

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