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Model Studies in Cytochrome P-450-Mediated Toxicity of Halogenated Compounds: Radical Processes Involving Iron Porphyrins

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Haloalkane toxicity originates from attack on biological targets by reactive intermediates derived from haloalkane metabolism by a hemoprotein, cytochrome P-450. Carbon-centered radicals and their peroxyl derivatives are most likely involved. The reactions of iron porphyrin—a model for cytochrome P-450—with various carbon-centered and peroxyl radicals generated by pulse radiolysis are examined. Competition between iron porphyrin and unsaturated fatty acids for attack by peroxyl radicals is pointed out. These kinetic data are used to derive a model for toxicity of haloalkanes with particular attention to carbon tetrachloride and halothane. The importance of local oxygen concentration and structural arrangement of fatty acids around cytochrome P-450 is emphasized.

Introduction

Halogenated alkanes (e.g. carbon tetrachloride, chloroform) are widely used in industry as solvents, components of refrigerants, aerosol propellants, fire extinguishers (chlorinated and fluorinated alkanes), in medicine as anesthetic agents (e.g. halothane, $CF_3CHClBr$), and in agriculture (*1*). All are toxic to some degree and represent a severe risk to people directly exposed. Also, as contaminants of the environment, these compounds represent a general hazard to health (*1*).

The major toxic effects of halogenated alkanes are not exerted through their physical properties but rather as a consequence of biochemical activation and interactions of metabolites with cellular components (*2–6*). The metabolism includes oxidative and/or reductive pathways. Carbon tetrachloride is oxidized to phosgene ($COCl_2$) (*7,8*) and carbon dioxide (*9*) and reduced to chloroform (*10,11*) and hexachloroethane (*11*). The enzymic degradation of halothane leads to trifluoroacetic acid (CF_3COOH) (*12*), chlorotrifluoroethane (CF_3CH_2Cl) (*13*) and chlorodifluoroethylene (CF_2CHCl) (*13*). Chloroform is essentially metabolized to phosgene, a toxic molecule which binds to proteins (*14,15*). However, these final metabolites do not appear to be responsible for acute toxicity which more likely involves reactive, short-lived intermediates. Conclusive evidence supports the idea that, among them, radicals derived from haloalkanes are the most important. With the use of spin-trapping techniques, the $\cdot CCl_3$ and $CF_3\dot{C}HCl$ radicals have been detected in the course of *in vivo* and *in vitro* metabolism of carbon tetrachloride (*16*) and halothane (*17*), respectively. Subsequently, under aerobic conditions, the peroxyl radicals $CCl_3\dot{O}_2$ and $CF_3CHCl\dot{O}_2$ are expected to be formed (*18,19*). They are much more reactive than the parent species (*20,21*) and are supposed to abstract hydrogen atom from unsaturated fatty acids leading, via chain reactions, to severe lipid peroxidation (*3–6*). Oxidative metabolism of carbon tetrachloride (but not halothane) also leads to the formation of electrophilic chlorine reacting with biological targets (*21,22*).

The biochemical activation of halogenated alkanes involves a microsomal hemoprotein, cytochrome P-450, which normally hydroxylates lipophilic substances (*23*). In Figure 1, an enzymic cycle is shown that includes the current knowledge on the hydroxylation mechanism. It can also account for the activation of haloalkanes. The substrate is written in the general form RR'CHX to illustrate possible reactions at C-H or C-X bonds (of course, the first possibility does not apply to carbon tetrachloride). Substrate binding occurs first (step a). It is accompanied by typical spectral changes, transition to high spin state and increase of the redox potential of the ferric cytochrome P-450. Electron transfer (b) and oxygen binding (c) follow. After a second reduction step (d), the oxygen molecule is split with the postulated formation of an oxenoid intermediate (e). In-

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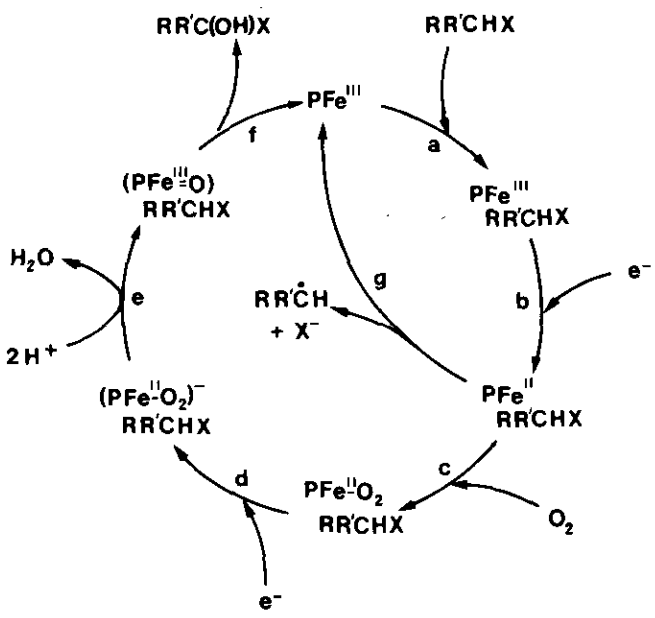

FIGURE 1. Cytochrome P-450 enzymic cycle.

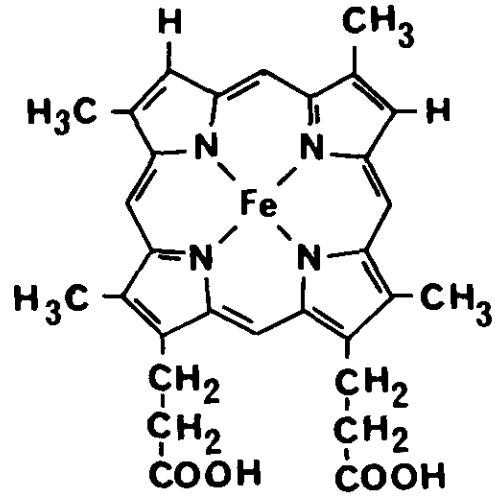

FIGURE 2. Iron deuteroporphyrin IX.

sertion of oxygen leads to the hydroxylated substrate which is then released (f). Halogenated alkanes behave as other substrates as far as steps a and b are concerned. So, carbon tetrachloride (*24*) and halothane (*24*) are bound by ferric cytochrome P-450. An alternative pathway is possible after reduction of the iron: direct electron transfer to the halogenated alkane leading to the alkyl radical (reductolysis) and regeneration of ferric cytochrome P-450 (step g). The feasibility of this reaction is supported by numerous experiments on biological systems as reported in this issue and also by studies on iron (II) porphyrins as models of cytochrome P-450 (*25–27*). In this paper we will be interested in the fate of radicals formed via step (g). As they are produced at the heart of cytochrome P-450, the following questions arise: do these radicals (or derived radicals) react with the hemoprotein and what are the reaction products? How can the radicals escape the hemoprotein environment and react with other biological targets? We will examine the possibility of reaction of radicals at the iron porphyrin locus by using iron deuteroporphyrin as a model of cytochrome P-450. The structure of this compound, very similar to the cytochrome P-450 prosthetic group, is shown in Figure 2. Reaction of some radicals with fatty acids and cholesterol as biological targets will also be discussed. All these reactions have been investigated by using pulse radiolysis methods which are briefly described below.

Radical Production in Solution by Radiolysis

The interaction of high-energy ionizing radiations (X, γ, β rays) with matter is nonspecific. As a consequence, radiolysis of moderately concentrated solutions essentially affects the solvent. By the use of appropriate scavengers, the primary products of solvent radiolysis can be sequentially converted to the desired species. These species can be produced at appreciable concentrations by a short pulse of radiation (usually less than 100 nsec) delivered by a linear accelerator or other high-energy electron generators (*28*). Their reactions with the solute of interest are then followed by appropriate fast detection techniques (*29*). Because of their characteristic intense spectra, porphyrins are particularly suited to study by absorption spectroscopy.

The main species, present a few nanoseconds after irradiation of aqueous 2-propanol solutions, are solvated electrons (e_s^-) and the reducing α-hydroxyisopropyl radicals, $(CH_3)_2\dot{C}OH$. The latter are formed by radiolysis of 2-propanol and also by scavenging of hydrogen atoms (H·) and hydroxyl radical (OH·) produced by water radiolysis. Solvated electrons originate from radiolysis of both solvents. These processes are summarized in Eqs. (1)–(3):

$$H_2O \rightsquigarrow e_s^-,\ OH\cdot,\ H\cdot \tag{1}$$

$$(CH_3)_2CHOH \rightsquigarrow e_s^-,\ (CH_3)_2\dot{C}OH \tag{2}$$

$$OH\cdot,\ H\cdot + (CH_3)_2CHOH \rightarrow (CH_3)_2\dot{C}OH + H_2O,\ H_2 \tag{3}$$

The fate of the $(CH_3)_2\dot{C}OH$ radicals depends on pH and on the presence of added solutes. In alkaline solutions, they deprotonate leading to the more reducing form $(CH_3)_2CO^{\dot{-}}$ ($pK_a = 12.2$) (*30*). Both forms may react with halogenated alkanes via electron transfer:

$$RX + (CH_3)_2\dot{C}OH \rightarrow R\cdot + X^- + (CH_3)_2CO + H^+ \tag{4}$$

$$RX + (CH_3)_2CO^{\dot{-}} \rightarrow R\cdot + X^- + (CH_3)_2CO \tag{5}$$

The anionic form of the radical is the most reactive. Rate constants for reactions involving various haloalkanes have been determined by using competition kinetics (*31,32*). Some results are given in Table 1. It can be noted that only the most reducing $(CH_3)_2\dot{C}O^-$ radical gives rise to a detectable reaction with some haloalkanes.

In most cases, advantage is taken in scavenging solvated electrons with acetone (*37*) which lead to the $(CH_3)_2\dot{C}O^-$ radicals which are "de facto" the only species produced by radiolysis of alkaline solutions. In the same way, $(CH_3)_2\dot{C}OH$ radicals are the only species formed in neutral or acidic medium. Alternatively, e_s^- reacts with haloalkanes according to (*37*)

$$RX + e_s^- \rightarrow R\cdot + X^- \quad (6)$$

with $k \approx 10^9 - 10^{10-1}$ sec^{-1}.

It follows from values reported in Table 1 that pulse irradiation of aqueous 2-propanol solutions containing 0.2–0.3 M CCl_4, $CF_3CHClBr$, or CH_3I leads within a few hundred nanoseconds to the quantitative formation of $\dot{C}Cl_3$, $CF_3\dot{C}HCl$, or $\dot{C}H_3$ radicals, respectively (only alkaline solutions are considered in the latter case). It may be noted that both theoretical calculations (*38*) and analysis (*39*) confirm that bromide is the halogen removed in the case of halothane.

In the presence of oxygen the radicals described above react to form the peroxyl radicals (*18,19*) $CCl_3O_2^\cdot$, $CF_3CHClO_2^\cdot$, $CH_3O_2^\cdot$ with rate constants on the order of 10^9 M^{-1} sec^{-1}. In aerated solutions of aqueous 2-propanol which contain ca. 10^{-3} M oxygen, these reactions proceed within a few microseconds. So, it must be emphasized that even in the latter system, which appears more complex, the primary radiolytic species are quantitatively converted to the radical of interest.

Reactions of Iron Porphyrins with Alkyl Radicals

The reactions of iron(III) porphyrins with $\dot{C}Cl_3$, $CF_3\dot{C}HCl$, or $\dot{C}H_3$ radicals are easily investigated by pulse irradiating the previously mentioned deaerated porphyrin solutions containing a large excess of the appropriate halogenated alkane. Results reported in Table 1 show that $\dot{C}Cl_3$ (*35*) and $CF_3\dot{C}HCl$ (*32*) radicals react very slowly, if at all, with the ferric complex (the uncertainty on the lower limit of the reaction rate is inherent in the short life of the radicals which decay via self-reaction). The reactivity of alkyl radicals towards ferric porphyrins drastically depends on the nature of carbon substituents. So, the reaction with the methyl radical is fast. The product is an iron–methyl complex. It decays over a few hundred microseconds, probably undergoing hydrolysis leading to the loss of the less toxic methanol molecule (*31*).

When the haloalkane concentration is lowered enough, it does not compete efficiently with the iron(III) porphyrin for reaction with the $(CH_3)_2\dot{C}OH$ or $(CH_3)_2\dot{C}O^-$ radicals. With appropriate choice of concentrations, half the reducing radicals may be used to produce iron(II) porphyrin according to:

$$PFeIII + (CH_3)_2\dot{C}OH \rightarrow PFeII + (CH_3)_2CO + H^+ \quad (7a)$$

$$PFeIII + (CH_3)_2CO^{\dot{-}} \rightarrow PFeII + (CH_3)_2CO \quad (7b)$$

with $k = 3.7 \times 10^8$ and 9×10^8 M^{-1} sec^{-1}, respectively (*31,35*). The remaining radicals are converted to the alkyl radicals as above. Pulse irradiation of such systems thus produces equal concentrations of iron(II) porphyrins and alkyl radicals, and their mutual reactions can be easily followed when the radicals are not scavenged by the excess iron(III) porphyrin. This method was applied to the study of $\cdot CCl_3$ (*35*) and $CF_3\dot{C}HCl$ (*32*) radical reactions with iron(II) porphyrins. In the case of the methyl radicals (*31*), the iron(II) porphyrin was chemically prepared, and the methyl radical was produced from electron scavenging by methyl chloride. As shown in Table 1, all the alkyl radicals studied react with iron(II) porphyrin at nearly diffusion-controlled rates. The reaction products were identified as complexes with the alkyl group bound to the iron. The methyl derivative was found to be fairly stable in anaerobic conditions and

Table 1. Reaction rate constants.

Medium[a]	Reactants	Products	k, M^{-1} sec^{-1}	Reference
A	$CCl_4 + (CH_3)_2\dot{C}OH$	$^\cdot CCl_3$	$1-7 \times 10^8$	(*33,34,35*)
B	$CCl_4 + (CH_3)_2\dot{C}O^-$	$^\cdot CCl_3$	8×10^8	(*36*)
C	$CF_3CHClBr + (CH_3)_2\dot{C}OH$	$CF_3\dot{C}HCl$	5×10^7	(*32*)
B	$CF_3CHClBr + (CH_3)_2\dot{C}O^-$	$CF_3\dot{C}HCl$	5.8×10^8	(*32*)
A	$CH_3I + (CH_3)_2\dot{C}OH$	—	$< 10^6$	(*36*)
B	$CH_3I + (CH_3)_2\dot{C}O^-$	$^\cdot CH_3$	1.1×10^8	(*31*)
A	$PFe^{III} + {}^\cdot CCl_3$	—	$< 10^6$	(*35*)
B,C	$PFe^{III} + CF_3\dot{C}HCl$	—	$< 10^6$	(*32*)
B	$PFe^{III} + \dot{C}H_3$	$PFe^{IV}CH_3$	2.3×10^9	(*31*)
A	$PFe^{II} + \dot{C}Cl_3$	$PFe^{III}CCl_3$	2×10^9	(*35*)
B,C	$PFe^{II} + CF_3\dot{C}HCl$	$PFe^{III}CH(CF_3)Cl$	$7-14 \times 10^9$	(*32*)
A	$PFe^{II} + \dot{C}H_3$	$PFe^{III}CH_3$	3.9×10^9	(*31*)

[a] Media: A = buffered 2-propanol water mixture (1/1, pH ≅ 7); B = alkaline 2-propanol water mixture (NaOH = 0.05 N); C = acidic 2-propanol water mixture ($HClO_4 = 6 \times 10^{-3}$ N).

it was characterized spectroscopically. The complexes formed with $\cdot CCl_3$ and $CF_3\dot{C}HCl$ were not stable enough to allow full characterization, but they are expected to have a sufficient lifetime to be considered as possible intermediates in the metabolism of the corresponding haloalkanes.

Reactions of Iron(III) Porphyrins with Peroxyl Radicals

As outlined above, alkyl radicals formed through reaction of ferrous cytochrome P-450 with haloalkanes are likely to react with oxygen to yield peroxyl radicals. Ferric cytochrome P-450 might back react with these new intermediates. The significance of this process was examined by considering the reactivity of iron(III) deuteroporphyrin as representative of that of cytochrome P-450.

Pulse radiolysis studies showed that the peroxyl radicals $CCl_3\dot{O}_2$, $CF_3CHClO_2^{\cdot}$, $CHCl_2O_2^{\cdot}$, $CH_2ClO_2^{\cdot}$, $CH_3O_2^{\cdot}$ react with iron(III) porphyrin (*40*) with rates ranging between 6×10^7 and 2.6×10^8 M^{-1} sec^{-1} (see Table 2). The spectrum of the porphyrin species produced in the reaction was found to be independent of the nature of the peroxyl radicals. On the other hand, the reactivity of the peroxyl radicals decreases in the following order:

$$CCl_3O_2^{\cdot} > CHCl_2O_2^{\cdot} > CH_2ClO_2^{\cdot} > CF_3CHClO_2^{\cdot} >> CH_3O_2^{\cdot}$$

indicating dependence on the ionization potentials expected for these species. Also, the rate constant for the reaction of $CCl_3O_2^{\cdot}$ radical with the ferric porphyrin was drastically lowered when experiments were performed in a less polar medium (neat 2-propanol or neat carbon tetrachloride). All these features were taken as indicative of the electron transfer reaction (*40*)

$$PFe^{III} + RO_2^{\cdot} \rightarrow [PFe^{III}]^+ + RO_2^- \quad (8)$$

where R stands for a alkyl or haloalkyl group derived from a haloalkane. Oxidized iron porphyrin $[PFe^{III}]^+$ species, which are probably better formulated as porphyrin π-cation radicals rather than iron(IV) species, have been prepared chemically and electrochemically and are well characterized spectroscopically. They can be reversibly reduced to the starting PFe^{III} state (*43*).

Another interesting case of an oxidizing intermediate is provided by the $(CH_3)_2C(OH)O_2^{\cdot}$ radical which is formed by pulse radiolysis of aerated aqueous 2-propanol solutions containing acetone but no haloalkane. In this particular case, the porphyrin oxidation strongly depends on pH. Kinetic analysis of this system led to the scheme shown in eq. (9), (*44*).

$$(CH_3)_2C(OH)O_2^{\cdot} + PFe^{III} \rightarrow [(CH_3)_2C(OH)O_2^{\delta-}\text{—}Fe^{III}P^{\delta+}]$$

$$\xrightarrow{H^+} (CH_3)_2C(OH)O_2H + [PFe^{III}]^+ \qquad (CH_3)_2C(OH)O_2^- + [PFe^{III}]^+ \quad (9)$$

Equation (9) shows the formation of an intermediate complex leading to the oxidized $[PFe^{III}]^+$ form either via dependent or acid-independent pathways. No acid-dependent reaction was found in the case of the related $CH_2(OH)O_2^{\cdot}$ radical. These data show how local acid concentrations, which can be provided by some amino acid residues in proteins, can affect the reactions of some peroxyl radicals.

Reactions of Peroxyl Radicals with Fatty Acids and Iron(III) Porphyrins

The abstraction of hydrogen from unsaturated fatty acids by peroxyl radicals is generally thought to be the key event in the peroxidation of lipids (*3–6*). This abstraction was recently investigated by means of pulse radiolysis and found to be not very fast (*45*). The results reviewed above suggest that back reaction of cytochrome P-450 with the peroxyl radicals will compete with their reaction with lipids. This problem is examined now.

The characteristic absorption spectrum of $[PFe^{III}]^+$ (ϵ = 8000 M^{-1} cm^{-1} at 655 nm in acidic solutions) (*40*) was used as an internal marker for investigating the competition between iron(III) porphyrin and various fatty acids or cholesterol for reactions with peroxyl radicals derived from carbon tetrachloride or halothane (*41*). Upon addition of fatty acid or cholesterol (LH), the growth profile of $[PFe^{III}]^+$ changed to two components instead of a single one observed in the absence of fatty acid. The first component exhibited decrease of both half-life and amplitude as functions of fatty acid concentration which was accounted for the reaction competing with oxidation of PFe^{III}:

$$RO_2^{\cdot} + LH \rightarrow RO_2H + L^{\cdot} \quad (10)$$

The kinetics of the second unexpected component were independent of the fatty acid concentration but dependent on the porphyrin concentration. The final concentration of $[PFe^{III}]^+$ formed was independent of concentration of any competitors. This behavior suggests that the second component may be assigned (*41*) to subsequent oxidation of PFe^{III} by peroxyl radicals formed by the rapid reaction of fatty acids radicals with oxygen:

$$L^{\cdot} + O_2 \rightarrow LO_2^{\cdot} \quad (11)$$

$$PFe^{III} + LO_2^{\cdot} \rightarrow [PFe^{III}]^+ + LO_2^- \quad (12)$$

The rate constants of reactions derived from kinetic analysis are reported in Table 2. They show a selectivity of $CCl_3O_2^{\cdot}$ or $CF_3CHClO_2^{\cdot}$ radicals toward the various unsaturated fatty acids with a progression linked to the number of doubly allylic hydrogens present; this has also been reported for other hydrogen-abstracting radicals (*46*). It is also noted that the rate of hydrogen abstraction depends strongly on the substituents of the

Table 2. Rate constants for reactions of peroxyl radicals in acidic 2-propanol–water mixtures ($HClO_4$ = 0.1 M).

Reactants	Rate constant, M^{-1} sec^{-1} [a]				
	PFe^{III}	Oleic acid	Linoleic acid	Linolenic acid	Cholesterol
$CCl_3\dot{O}_2$	2.5×10^{8} [b]	4×10^{5}	5×10^{5}	11×10^{5}	6×10^{5}
$CF_3CHCl\dot{O}_2$	9×10^{7} [c]	2×10^{4}	8×10^{4}	3×10^{5}	5×10^{4}
$(CH_3)_2C(OH)\dot{O}_2$	2.7×10^{7} [d]	$< 3 \times 10^{3}$	$\simeq \times 10^{3}$	—	—
$L\dot{O}_2$	$3-4 \times 10^{7}$ [e]	$\simeq 10^{2}$ [f]	$\simeq 10^{2}$ [f]	$\simeq 10^{2}$ [f]	

[a] Data from Brault et al. (*41*) unless otherwise noted.
[b] Data of Brault and Neta (*40*).
[c] Reactivity of the $CF_3CHCl\dot{O}_2$ radical slightly depends on pH; rate constants for reaction in other solutions are given elsewhere (*40*).
[d] Reactivity of the $(CH_3)_2C(OH)\dot{O}_2$ radical strongly depends on pH; the value applies only to $[H^+]$ = 0.1 M.
[e] Same values obtained with all three fatty acids used.
[f] Data of Bateman (*42*).

peroxyl radical (compare the reactivity of $CCl_3O_2^{\bullet}$., $CF_3CHClO_2^{\bullet}$, and $LO_2^{\bullet}$). By contrast, rate constants for the oxidation of PFe^{III} span less than an order of magnitude.

Model for Halocarbon Toxicity: A Kinetic Approach

The number of intermediate species identified in the course of haloalkane metabolism under particular conditions is quite large. The probability that these intermediates play an actual role is determined by their formation rate and their reactivities towards biological targets. Thus, in addition to trapping techniques that allow identification of intermediates, a kinetic approach appears to be necessary.

In Figure 3, a scheme is presented accounting for reactions discussed in this paper with particular emphasis on the role of cytochrome P-450. A crucial step in the metabolism of haloalkanes is their reduction to yield alkyl (or haloalkyl) radicals. The rate of this reaction is governed by the strength of the carbon bond which depends on the nature of the halogen to be released and on the number of electrophilic substituents attached to the carbon. Thus, iron(II) porphyrins react about 10000 times faster with carbon tetrachloride than with chloroform (*27*). An intermediate value is obtained for halothane. According to the relative rates of reaction of ferrous cytochrome P-450 with the haloalkane and with oxygen, the metabolism will proceed either via the hydroxylation pathway or via reductolysis of a carbon halogen bond. Chloroform and carbon tetrachloride appear to be examples of these two mechanisms, respectively. Trichloromethanol formed through chloroform hydroxylation is then expected to decompose to the well-identified metabolite, phosgene, and to hydrochloric acid (*14,15*). Halothane might belong to an intermediate class (*47*). As it is not quickly reduced, hydroxylation of its C-H bond followed by hydrolysis of the $CF_3C(OH)ClBr$ intermediate could account for the formation of trifluoroacetic acid. However, as inferred from trapping experiments, the reductive pathway is also important. Theoretical calculations suggest that one C-Cl bond could be split in the reaction of carbon tetrachloride with the oxene complex of cytochrome P-450 (*48*). However, because ferrous cytochrome P-450 is expected to reduce carbon tetrachloride much faster than it binds oxygen, this reaction is not likely to occur. Other experimental results provide arguments against this hypothesis (*22*).

When formed, the alkyl radicals may either back-react with the iron(III) cytochrome P-450, or attack proximal targets, or diffuse and/or react with oxygen. The data of Table 1 show that, with the exception of the methyl radical, no fast back-reaction is expected at the ferric porphyrin locus. On the other hand, reactions of the $^{\bullet}CCl_3$ or $CF_3\dot{C}HCl$ radicals with ferrous cytochrome P-450 would be fast. Further reduction of a carbon–halogen bond might lead to the $:CCl_2$ or $CF_3\ddot{C}H_2$ carbenes (*26,35*). The $:CCl_2$ carbene has been detected by trapping techniques (*49*) but in very small amounts, suggesting a marginal process. In keeping with the last observation, it must be pointed out that reduction of ferric cytochrome P-450 to the ferrous state is very slow (*23*) compared to the lifetime of radicals. Thus, scavenging of $\cdot CCl_3$ or $CF_3\dot{C}HCl$ radicals by ferric or ferrous cytochrome P-450 appears as unlikely. On the other hand, these radicals are expected to bind to double bonds of some lateral chains of proteins. CCl_3 and CF_3CHCl adducts to phospholipids have been detected (*50,51*).

The formation of peroxyl radicals will depend on local oxygen concentration and diffusion of reactants. If oxygen concentration is high enough, the haloalkyl radicals will be converted to the peroxyl form before they escape from the cytochrome pocket and oxidation of the ferric cytochrome P-450 would occur. It is not clear if this process would correspond to detoxification or not. Indeed, the porphyrin cation radical formed could react with proximal amino acid residues or undergo further degradation. This might account for bleaching of cytochrome P-450 observed upon aerobic metabolism of carbon tetrachloride (*5,6,52,53*). Alternatively, cytochrome P-450 reductase provides a repair pathway through back reduction of the hemoprotein to the ferric state. The peroxyl anion formed on reaction (*8*) is expected to protonate. The hydroperoxide might then decompose, leading to the more reactive alkoxyl radicals or to some of the products of haloalkane oxidative me-

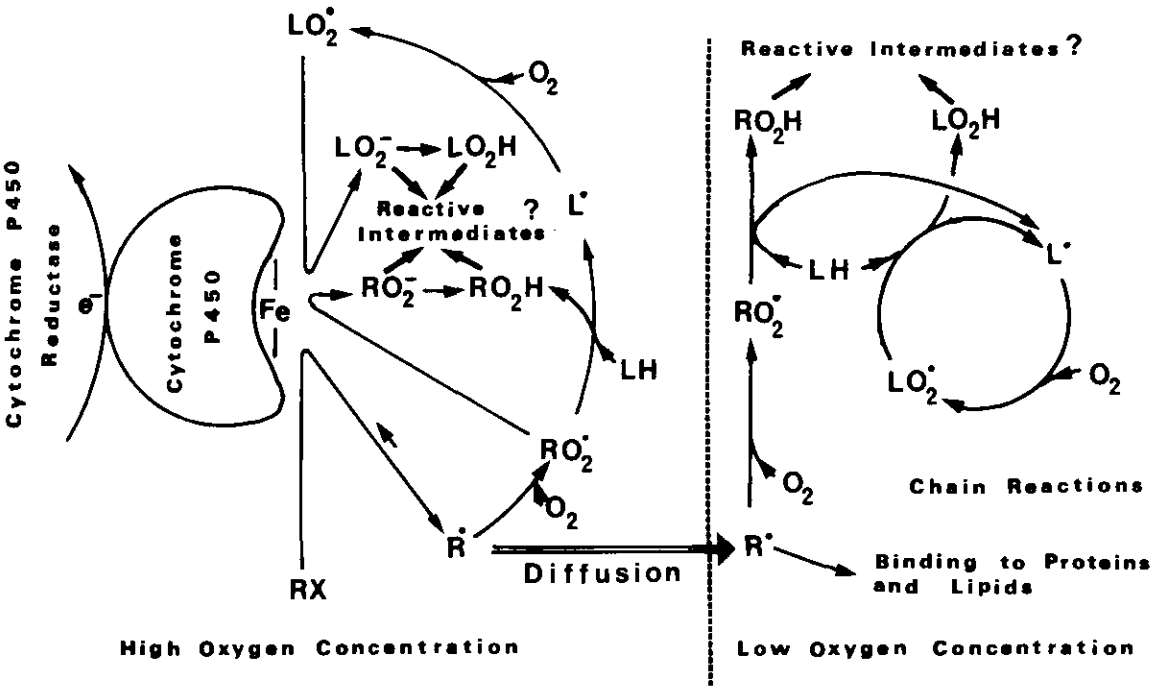

FIGURE 3. Radical reactions in haloalkane toxicity.

tabolism. Further studies are needed to clarify the fate of hydroperoxide derived from carbon tetrachloride or halothane.

At low oxygen concentrations, the haloalkyl radical is likely to diffuse out of the cytochrome pocket before reacting with oxygen. In this case, attack by either the haloalkyl radical or its peroxyl derivative would occur at more remote targets, such as unsaturated fatty acids of the microsomal membrane. This view agrees with results of De Groot and Noll (*54*) who found that peroxidation of lipids induced by halothane drastically depends on oxygen with a maximum value at low concentration (1 mm Hg oxygen).

It must be pointed out that peroxyl radicals formed from fatty acids are also potent oxidants of cytochrome. Actually, the rate constant for the reaction of $LO_2^{\cdot}$ radicals with our porphyrin model, 3-4 $\times$ 10^7 M^{-1} sec^{-1}, is much greater than the rate constant for the lipid propagation reaction:

$$LO_2^{\cdot} + LH \rightarrow LO_2H + L^{\cdot} \quad (12)$$

which was assumed (*44*) to be about 1 $\times$ 10^2 M^{-1} sec^{-1}. Owing to geometric constraints, the peroxidation chain is still believed to develop in the quite rigid structure where the fatty acid molecules are embedded and are not supposed to quickly diffuse. Some fatty acid peroxyl radicals, however, may also back-react with the cytochrome. In particular, this process could be important for fatty acid molecules which, because they are necessary to the enzyme activity, lie very near the iron porphyrin of the hemoprotein. This process could account for cytochrome P-450 destruction which accompany halocarbon metabolism. The peroxyl radicals may also decompose to alkoxy radicals which are expected to be better H-abstracting and good oxidizing species. So, depending on oxygen tension, different pattern of radicals can be produced. Actually, carbon-centered ($L^{\cdot}$) as well as oxygen-centered radicals LO· and/or $LO_2^{\cdot}$) derived from fatty acids have been detected (*55*).

Conclusion and Perspectives

Studies on the reactions of various radiolytically generated radicals with iron porphyrin and fatty acids have made it possible to derive a model for haloalkanes metabolism based on kinetic data. The crucial role of oxygen is outlined. The overall mechanism is probably more complex (*56*). Information on the fate of some intermediates is still lacking. The micro-environment provided by the hemoprotein to its prosthetic group could orientate the reactions of some intermediates. Cage effects, local acid concentrations, etc., might be important parameters. More refined models are under study to take these problems into account.

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