Halothane Hepatotoxicity:

Enhancement by Polychlorinated Biphenyl Pretreatment

Edward S. Reynolds, M.D.,* and Mary Treinen Moslen, M.S.†

Pretreatment of rats with the potent mixed-function oxidase system inducer, Aroclor 1254 (150 μmol/kg for seven days), provides a model system for the study of halothane hepatotoxicity. Within two hours of the end of anesthesia (0.85 per cent for five hours), values of the serum transaminases (SCOT and SGPT) were increased to above normal and morphologic injury was recognized in centrilobular parenchymal cells of animals pretreated with Aroclor 1254. Necrosis of the centrilobular hepatocytes was prominent within 24 hours. Ultrastructural examination of hepatic tissue at intervals after anesthesia indicated that the injury primarily involved the endoplasmic reticulum (ER), progressing from dispersion of the rough ER to vacuolization of the rough ER and coalescence of the smooth components of this membranous system into tubular aggregates. These changes were preceded by selective deactivation of the mixed-function oxidase components, cytochrome P-450 and zoxazolamine hydroxylase, and early transient increases in conjugated dienes of lipids. Although pretreatment with Aroclor 1254 potentiated halothane hepatotoxicity, neither covalent binding of labeled halothane to liver proteins nor excretion of water-soluble labeled metabolites in urine was enhanced 24 hours after intraperitoneal administration of a low non-hepatotoxic or high hepatotoxic dose of 14C-labeled halothane (10 and 10,000 μmol/kg, respectively). These findings suggest that the toxic pathways(s) for the biotransformation of halothane do not involve increased production of water-soluble or protein-bound metabolites. (Key words: Anesthetics, volatile, halothane; Biotransformation (drug), enzyme induction; Pharmacology; Aroclor 1254; Biotransformation (drug), phenobarbital; Liver, hepatotoxicity; Liver, glutathione; Liver, microsomes; Proteins, covalent binding; Metabolism, cytochrome P-450.)

PRETREATMENT OF RATS WITH AROCLOR 1254 PROVIDES AN ANIMAL SYSTEM IN WHICH EXTENSIVE CENTRILOBULAR NECROSIS OF THE LIVER CONSISTENTLY FOLLOWS HALOTHANE ANESTHESIA, UNLIKE OTHER ANIMAL SYSTEMS WHERE INJURY FOLLOWING EXPOSURE(S) TO HALOTHANE IS ONLY FOCAL OR INCONSISTENT.1-3 AROCLOR 1254 IS A POTENT INDUCER OF CERTAIN COMPONENTS OF THE HEPATIC MIXED-FUNCTION OXIDASE SYSTEM,4 THE ENZYME SYSTEM CONSIDERED PRIMARILY RESPONSIBLE FOR THE METABOLISM OF HALOTHANE.5,6

In prior studies5,7 we examined the effects of phenobarbital pretreatment on the hepatotoxicity and metabolism of halothane. In our experience,8 the inductive effects of Aroclor 1254 pretreatment on the mixed-function oxidase system differed from those of phenobarbital: Aroclor 1254 induced a P-450 species with a peak absorbance at 448 nanometers, and strikingly enhanced arene hydrocarbon hydroxylation activities without enhancing amino pyrine N-demethylation. In phenobarbital-pretreated animals, halothane anesthesia (0.85 per cent for five hours) caused focal necrosis, transient increases of lipid-conjugated dienes in microsomes, and persistent loss of cytochrome P-450. Metabolic studies in vivo indicated that urinary excretion of 14C-halothane metabolites but not its covalent binding to liver constituents was enhanced by phenobarbital pretreatment.

This study was designed to characterize the progressive structural and functional aberrations caused by halothane anesthesia in rats pretreated with Aroclor 1254, and to determine whether hepatic injury is associated with enhanced excretion and covalent binding of halothane metabolites in vivo.

Materials and Methods

Male Sprague-Dawley rats (200 g) from Charles River were housed in wire-floored cages over granulated clay and allowed free access to food and water. Mixed-function oxidase enzymes were induced by giving the rats an oral dose of Aroclor 1254 (150 μmol/kg) or phenobarbital (400 μmol/kg) every morning for seven days. Control animals received the administrative vehicle (water with 0.1 per cent of the solubilizing agent Tween 80). Previously we used this multiple-dose procedure to compare in uniformly handled rats the differential inductive effects of six chemicals, including phenobarbital, Aroclor 1254, and 3-methylcholanthrene.8 On the eighth day after an overnight fast, the rats were exposed to room air or to halothane, 0.85 per cent in air for five hours in an inhalation chamber, previously described.3 Animals were sacrificed at various times before, during and after halothane administration to obtain material for morphologic evaluation or biochemical analyses.

For the study of morphologic changes a small piece of the right anterior lobe of the liver of every rat exposed to halothane was excised immediately after sacrifice and preserved in formaldehyde for histologic evaluation. Liver tissues
of the experimental controls (room air-exposed rats) of all three pretreatment groups were similarly prepared for histologic evaluation. Liver tissues of room air-exposed (experimental controls) or halothane-exposed rats previously pretreated with Aroclor 1254 were prepared for ultrastructural examination using the following technique: At selected intervals after halothane administration the rats were anesthetized with pentobarbital intraperitoneally and their livers fixed in situ by perfusion via the portal vein of cacodylate-buffered glutaraldehyde, 1 per cent (pH 7.4), which was warmed to 37°C. Following fixation, the liver was removed, sliced thinly, and cubed; the cubes were postfixed in osmium tetroxide and uranyl acetate, then embedded in Epon.

For the study of biochemical changes, rats were decapitated and blood was collected for measurement of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) using Sigma Chemical Co. reagent kits. Livers were perfused and homogenized with ice-cold 0.25 M sucrose. Portions of homogenates were reserved for glutathione determination with Ellman's reagent. Microsomes were isolated and enzyme components of individual animals were assayed on the day of sacrifice as previously described. Microsomal lipid conjugated diene contents and lipid phosphorus were measured according to established techniques.

Metabolic studies were made in rats pretreated with vehicle or Aroclor 1254 and fasted overnight. 4H-1 Halaethane (2.8 mCi/mmol) was administered intraperitoneally in tracer (10 μmol/kg) or anesthetic (10,000 μmol/kg, diluted 1:1,000 with unlabeled halothane) doses. The anesthetic dose produced narcosis in all animals and caused necrosis of the free margins of the liver, peritoneal exudation, and mild ascites. After receiving the labeled halothane, each rat was placed in an individual plastic metabolism cage, provided water ad libitum, and after five hours, given two pieces of rat chow. At 24 hours the rats were sacrificed and livers were fractionated by differential centrifugation. The label excreted in the urine and contained in hepatic subcellular fractions and chemical constituents was quantitated by established techniques.

Statistical relationships were calculated by t test using a Wang 600 programmable calculator.

**Results**

**Hepatic Injury**

Serum transaminase values of the rats pretreated with Aroclor 1254 increased within two hours of termination of halothane exposure and were threefold higher than those of controls 19 hours after halothane anesthesia (table 1). Serum transaminase values of rats pretreated with the administrative vehicle or phenobarbital were not increased by halothane anesthesia. Histologic evaluation of liver tissue obtained 19 hours after halothane anesthesia revealed widespread vacuolization of centrilobular and midzonal parenchyma throughout the liver with rather prominent necrosis of centrilobular hepatocytes in all rats pretreated with Aroclor 1254. Focal hepatic necrosis in all animals pretreated with phenobarbital, and no injury in the animals pretreated with the administrative vehicle. Both phenobarbital and Aroclor 1254 pretreatments caused marked proliferation of the smooth endoplasmic reticulum, but neither treatment alone caused recognizable hepatocellular injury.

**Table 1. Serum Transaminase Values* of Rats Pretreated with the Administrative Vehicle,† Phenobarbital, or Aroclor 1254 before Exposure to Room Air or Halothane Anesthesia (0.85 Per Cent for Five Hours)**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Exposure</th>
<th>Time1 Sacrificed</th>
<th>SCOT Karmen Units</th>
<th>SGPT Karmen Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Air</td>
<td>19 hours</td>
<td>179 ± 9</td>
<td>43 ± 3</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>19 hours</td>
<td>184 ± 11</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Air</td>
<td>2 hours</td>
<td>163 ± 2</td>
<td>35 ± 7</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>2 hours</td>
<td>159 ± 17</td>
<td>36 ± 7</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>19 hours</td>
<td>172 ± 11</td>
<td>46 ± 3</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>19 hours</td>
<td>184 ± 12</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>Air</td>
<td>0 hours</td>
<td>153 ± 18</td>
<td>40 ± 2</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>0 hours</td>
<td>149 ± 21</td>
<td>41 ± 5</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>2 hours</td>
<td>374 ± 152§</td>
<td>129 ± 68§</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>6 hours</td>
<td>344 ± 32§</td>
<td>101 ± 11§</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>19 hours</td>
<td>211 ± 20</td>
<td>45 ± 3</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>19 hours</td>
<td>603 ± 128§</td>
<td>126 ± 24§</td>
</tr>
</tbody>
</table>

* Means ± SEM.
† Water containing Tween 80, 0.1 per cent.
‡ Time after exposure.
§ P < 0.05; difference between halothane and respective air-exposure groups.
The progression of light-microscopic changes in hepatic architecture in rats pretreated with Aroclor 1254 and anesthetized with halothane is illustrated in figure 1. By the end of five hours of anesthetic exposure there is cytoplasmic pallor of centrolobular parenchyma, a change that corresponds to dispersion of the rough endoplasmic reticulum. Six hours later the centrolobular parenchyma is diffusely vacuolated and foci of necrosis are apparent. By 19 hours these pathologic changes involving centrolobular parenchyma are accentuated. In contrast, glycogen has returned to the periportal parenchyma.

Examination of the changes in centrolobular cells by electron microscopy confirms vacuolization of the rough endoplasmic reticulum and aggregation of the smooth endoplasmic reticulum by the sixth hour following exposure to halothane (fig. 2). The aggregates of smooth endoplasmic reticulum contain regions where material of increased electron opacity appears applied to the cytoplasmic surfaces of membrane profiles at points of greatest constriction in tubular diameters (fig. 3). These changes are similar to those found in the livers of rats exposed to carbon tetrachloride, and may represent areas of membrane collapse.19

**Biochemical Changes**

The capacity of the microsomal mixed-function oxidase system to hydroxylate oxazolamine was decreased threefold at the end of halothane anesthesia (table 2). No other mixed-function oxidase activity measured was affected. Diminished contents of cytochrome P-450 were apparent by one hour after the onset of halothane administration (table 3). Conjugated diene contents of microsomal lipid, which doubled after one hour of anesthesia, returned to normal by the end of the anesthesia period, while hepatic glutathione contents of these Aroclor 1254-treated rats were not decreased one, five or seven hours after the onset of halothane administration (fig. 4).

**14C-HALOTHANE EXCRETION AND BINDING**

Rats pretreated with the administrative vehicle and those pretreated with Aroclor 1254 excreted similar amounts of radiolabeled metabolites of halothane in urine during the first 24-hour period following either a high or a low dose of 14C-halothane (table 4). Similar quantities of label were recovered from liver homogenates and protein. Slightly higher contents of label were recovered in the microsomal fraction of the Aroclor 1254-pretreated rats given the high dose of halothane. The label recovered in the protein subfraction is considered covalently bound, since it was not removed by the extensive extraction procedure, while the label recovered in the homogenate and microsomes is nonvolatile.7 More detailed studies of 14C-halothane binding were not attempted.
Fig. 2. Portion of cytoplasm of centrilobular parenchymal cells six hours following five hours of halothane anesthesia in a rat pretreated with Aroclor 1254. Nucleus to left. The rough endoplasmic reticulum is vacuolated, while the smooth forms tubular aggregates. Note prominent outlines of tubules with extra thick electron-opaque walls in tubular aggregates (arrows). Mitochondria appear normal. (×10,000).

Discussion

Halothane-induced hepatic injury in the rat pretreated with Aroclor 1254 appears to involve primarily the endoplasmic reticulum. These changes include structural disorganization of its macro-molecular constituents, early selective deactivation of cytochrome P-450 and zoxazolamine hydroxylase.
Fig. 3. Higher-power view of a portion of tubular tangle of smooth endoplasmic reticulum six hours following halothane anesthesia. Some tubular walls are thickened by the appearance of electron-opaque material on their cytoplasmic matrix surfaces (arrows). Lumens of these tubules seem narrowed compared with those of adjacent "thin-walled" tubular profiles. Numerous monomer and dimer ribosomes are present in the interstices between tubular profiles (×110,000).

(two enzymatic components of this organelle), and an early, but transient, increase in the conjugated dienes of microsomal lipid. Sipes and Brown found Aroclor 1254 pretreatment enhanced the in-vitro covalent binding of 14C-halothane metabolites to microsomal macromolecules, particularly lipids. However, in this study in vivo using both a tracer dose and an anesthetic dose of 14C-halothane, we found Aroclor 1254 pretreatment did not increase the amount of 14C-metabolite excreted in the urine or that recovered in liver homogenate or protein at 24 hours, while the label recovered in the microsomal fraction was slightly higher after the anesthetic dose.

Biotransformation of halothane in vitro is considered dependent upon the terminal oxidase of the mixed-function oxidase system, cytochrome P-450, and is enhanced by chemical induction of this enzyme system. The interaction between cytochrome P-450 and a substrate is considered to occur in a sequence of steps. First, the substrate binds to the oxidized cytochrome P-450 molecule; second, this complex is reduced by an electron transferred via flavoproteins from NADPH; third, activated oxygen joins the reduced P-450 substrate complex; fourth, a second electron transferred from either NADPH or NADH reduces the triple complex, which then decomposes to oxidized products, H2O and oxidized P-450. Figure 5 schematizes this reaction with halothane

<table>
<thead>
<tr>
<th>Time after Onset</th>
<th>Oxidative N-Demethylation</th>
<th>Arne Hydrocarbon Hydroxylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane Exposure</td>
<td>Antipyrine (nmol/mg Prot-Min)</td>
<td>Ethylmorphine (nmol/mg Prot-Min)</td>
</tr>
<tr>
<td>Air control (n = 8)</td>
<td>13.3 ± 0.9</td>
<td>59 ± 5</td>
</tr>
<tr>
<td>1 hour (n = 4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5 hours (n = 8)</td>
<td>13.9 ± 1.2</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>7 hours (n = 3)</td>
<td>13.7 ± 1.5</td>
<td>55 ± 8</td>
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</tbody>
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* Means ± SEM.
† P < .001 compared with air control.

<table>
<thead>
<tr>
<th>Time after Onset</th>
<th>Cytochrome P-450</th>
<th>Cytochrome b</th>
<th>NADPH Cytochrome c Reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane Exposure</td>
<td>(nmol/mg Prot)</td>
<td>(nmol/mg Prot)</td>
<td>(nmol/mg Prot-Min)</td>
</tr>
<tr>
<td>Air control (n = 8)</td>
<td>2.66 ± .03</td>
<td>1.29 ± .03</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>1 hour (n = 4)</td>
<td>2.11 ± .07</td>
<td>1.30 ± .02</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>5 hours (n = 8)</td>
<td>2.01 ± .07</td>
<td>1.26 ± .06</td>
<td>72 ± 4</td>
</tr>
<tr>
<td>7 hours (n = 3)</td>
<td>1.94 ± .03</td>
<td>1.16 ± .03</td>
<td>75 ± 3</td>
</tr>
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</table>

* Means ± SEM.
† P < .001 compared with air control.
as a substrate and shows possible intermediates, reactions with cellular constituents, and identified metabolites.

Conditions that alter the multimolecular mixed-function oxidase system, such as depletion of cofactors, or the induction, repression, inhibition or topographic rearrangement of various macromolecular components, change halothane metabolic patterns and may result in accelerated production of reactive intermediates capable of toxic interactions with cell components. If toxification occurs at rates in excess of the detoxification reaction, uncoupling toxification from detoxification reactions, potentially toxic metabolites are shifted into injury-producing pathways. For example, we have found that pheno- barbital pretreatment, which increases cytochrome P-450 content, nearly halves the exhalation of $^{14}$CF$_3$CHBrCl-derived $^{14}$C label, and doubles the excretion of label in the urine but does not increase or change the pattern of label incorporation in liver organelles or chemical constituents at two or 24 hours.\textsuperscript{7} In addition, pretreatment with carbon tetrachloride, which causes cytochrome P-450 deactivation and endoplasmic reticulum injury, decreases the total amount of halothane-derived $^{14}$C metabolite excreted in the urine and recovered in the liver without changing the hepatic pattern of label incorporation.\textsuperscript{7} In-vitro anaerobic conditions enhance halothane defluorination and covalent binding of $^{14}$C-halothane-labeled metabolites to cellular constituents, particularly lipids.\textsuperscript{13,14} Pretreatments that increase cytochrome P-450 content further enhance the in-vitro defluorination and covalent binding of halothane under hypoxic conditions.\textsuperscript{13} Van Dyke and colleagues have attributed this increased binding under hypoxic conditions to the generation of reactive intermediates by reductive attack on the halothane molecule.\textsuperscript{13-15}

Electron transfer from NADPH to the halothane P-450 complex could result in activation of the halothane molecule to a "bound" free radical species intermediate, such as CF$_3$CHCl, with release of Br$^-$ (Species A of fig. 5) or CF$_3$CBrCl + H$. Subsequent interaction of the reduced complex with activated oxygen could yield oxidized intermediates (Species I, J and K), interact with phosphatidy- lethanolamine$^{16}$ (Species M), or be further oxidized to the major urinary metabolite, trifluoroacetic acid (Species L). Alternatively, if not rapidly oxidized, the radical intermediate might dissociate from the cytochrome complex and react with nearby cellular constituents$^{17}$ (Species B). Dehydro- fluorination of halothane to CF$_3$ = CBrCl$^{16}$ (Species D), a highly reactive molecule, could result in its covalent binding to lipids (Species E), glutathione (Species F), or protein (Species H). Mono-oxidation of the CF$_3$ = CBrCl species at P-450 would yield a reactive epoxide species also capable of covalent binding. Identification of bromochlorodifluoroethyl mercapturic acid (Species G) in human urine after exposure to halothane suggests the formation of such an intermediate.$^{16}$ Other evidence for the biologically unusual breaking of the C-F bond is provided by the increased serum fluoride levels in hypoxic animals exposed to halothane$^{14}$ and the exhalation of $^{14}$CO$_2$ by animals given halothane labeled in the 1 position, i.e., $^{14}$CF$_3$CHBrCl.$^{5,18}$

Table 4. $^{14}$C-1 Halothane Metabolites* at 24 Hours in Urines and Livers of Rats Pretreated with the Administrative Vehicle or Aroclor 1254

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>$^{14}$C-Halothane Administered Intraperitoneally (μmol/kg)</th>
<th>$^{14}$C Excreted in Urine (μmol/kg)</th>
<th>$^{14}$C Recovered in Liver Homogenates (μmol/kg)</th>
<th>$^{14}$C Bound to Liver Protein (μmol/kg)</th>
<th>$^{14}$C Recovered in Liver Microsomes (μmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 (n = 4)</td>
<td>0.71 ± 0.05</td>
<td>0.17 ± 0.01</td>
<td>0.043 ± 0.002</td>
<td>0.044 ± 0.008</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>10 (n = 4)</td>
<td>0.85 ± 0.04</td>
<td>0.17 ± 0.01</td>
<td>0.039 ± 0.002</td>
<td>0.043 ± 0.003</td>
</tr>
<tr>
<td>Vehicle</td>
<td>10,000 (n = 6)</td>
<td>572 ± 30</td>
<td>221 ± 34</td>
<td>56 ± 10</td>
<td>44 ± 7</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>10,000 (n = 5)</td>
<td>632 ± 31</td>
<td>285 ± 34</td>
<td>61 ± 8</td>
<td>67 ± 5†</td>
</tr>
</tbody>
</table>

* Means ± SEM.
† P < .05 compared with vehicle-pretreated group receiving the same dose of $^{14}$C-halothane.
**Fig. 5.** Schematization of the interaction of halothane with cytochrome P-450 (left), showing possible intermediates formed by reduction (electron capture) (Species A and D) and oxidation (Species I or J). Interaction of the halothane free radical with an unsaturated lipid could result in either addition across a double bond (Species B) or hydrogen abstraction (Species C). Rearrangement of the cis,cis-unconjugated acid (upper right) after hydrogen abstraction would yield a cis,trans-conjugated fatty acid. Note the halothane species binding to phospholipids is shown containing chloride atom as indicated by Van Dyke and Gandolfi. Postulated intermediates and metabolites are in brackets. Oxidation pathway adapted from Cohen et al.

$^{14}$CO$_2$ exhalation in both phenobarbital-induced and non-induced control animals is initially log-linear, but after several hours the pattern shifts in phenobarbital-pretreated rats to gradually declining or nearly constant exhalation, possibly due to continuing breakdown of primary metabolites or bound species by oxidative defluorination.

In order to cause hepatic injury, halothane must
alter functional or structural components of the hepatocytes. Intermediates of both oxidative and reductive metabolic pathways could interact with cellular constituents. Injury due to a generalized massive attack by reactive electrophilic intermediates is inconsistent with the relatively constant levels of the soluble antioxidant glutathione measured during and after injury-producing exposure to halothane. Furthermore, Uhleke found that addition of glutathione to microsomal activation systems caused only a slight decrease in $^{14}$C-halothane binding to proteins.

It should be pointed out that little $^{14}$C-halothane binds to cellular constituents. Uhleke et al. reported that even under conditions enhancing halothane binding in vitro, phenobarbital pretreatment and anaerobic incubation, only 2 mmol $^{14}$C-halothane label were bound/mg protein in 30 minutes, while under comparable conditions more than twice as much $^{14}$C-labeled carbon tetrachloride was incorporated. Approximately 5 per cent of a tracer dose (10 μmol/kg) of $^{14}$C-1 halothane label is recovered in the liver of a phenobarbital-pretreated animal two hours after intraperitoneal injection, and approximately 40 per cent of this label is acid-soluble and presumed to be trifluoroacetic acid. With increasing doses of halothane, proportionally lower percentages of the total administered halothane label are recovered in the liver.

Investigations of the nature of the recoverable $^{14}$C-halothane in liver constituents indicated halothane label preferentially binds to hepatic endoplasmic reticulum. Label incorporation into lipid is transient, peaking about two hours after administration, thereafter sharply declining, with little label recoverable in hepatic lipids at 12 hours. In contrast, label incorporation into protein occurs at a slower rate, peaking at around six hours and thereafter slowly decreasing at a rate approximating the normal turnover time of hepatic proteins. Oxidized intermediates of halothane are preferentially incorporated into proteins, as indicated by the hepatic binding of trifluoroethanol (which also has trifluoroacetalddehyde [Species K] as an oxidized intermediate) only to hepatic protein constituents, in a pattern similar to that found 12 hours after administration of $^{14}$C-halothane in vivo. According to this study, neither covalent binding of oxidized halothane intermediates to proteins nor the pathways leading to production of soluble, excretable metabolites appear critical to the hepatotoxicity of halothane.

Reductive pathways of halothane biotransformation via free radicals could result in disruption of the endoplasmic reticulum membranes through interactions with the polyunsaturated fatty-acid tails of membrane phospholipids. The initial reaction would be either addition across a double bond (Species B of fig. 5) or abstraction of a labile hydrogen from a methylene bridge carbon with the production of a volatile product such as CF₃CCH₄ (Species C). Either reaction is a free radical chain propagation step that radicalizes the fatty acid and must be followed by addition of an oxygen molecule with subsequent peroxidative decomposition, polymerization of the radicalized fatty acid with another polyunsaturated fatty acid, or the annihilation of the fatty-acid radical through the addition of a second free radical. Peroxidative decomposition, polymerization, and branch chain halogenations of the phospholipid tail would alter the physical-chemical properties of the membrane interior. Rearrangement of the radical lipid conjugate after hydrogen abstraction yields a conjugated diene with distinctive absorption properties. Peroxidative decomposition of membrane lipids yields measurable byproducts. Increased contents of lipid-conjugated dienes have been measured in induced animals during halothane anesthesia.

Wood et al. have measured both increased lipid conjugated dienes and malonaldehyde, a peroxidative byproduct, after in vitro exposure of microsomes to halothane in the presence of an NADPH-generating system and oxygen. Interestingly, when this microsomal system was exposed to halothane at low oxygen tension, the extent of diene formation correlated with halothane-derived $^{14}$C label incorporation and both label incorporation and the diene spectrum were stable during subsequent exposure to oxygen. This stability could be explained by the addition of a radical halothane species to the previously radicalized (by hydrogen abstraction or radical addition) fatty acid in a classic free radical chain termination process.

Unfortunately for those striving to document or quantitate haloalkane-induced membrane injury via reactive free radical intermediates in vivo, both covalent labeling to lipids and enhanced conjugated diene spectra are transient phenomena whether induced by halothane or the more extensively studied carbon tetrachloride. The early but persistent deactivation of cytochrome P-450 found in this study in Aroclor 1254-pretreated rats and in prior studies only in phenobarbital-pretreated rats could be secondary to peroxidative membrane alteration or due to direct attack by a reactive halothane species. That only exposure of induced animals to halothane anesthesia results in deactivation of mixed-function oxidase components and increased conjugated diene suggests that enzyme induction leads to altered, either uncoupled or accelerated, production of potentially toxic intermediates.

Although this study does not provide direct evidence that the hepatotoxicity of halothane is related to its metabolism, it does indicate that the toxicity of halothane is not related to increased protein binding or its oxidation to soluble excretable...
metabolites. The significance of binding to lipids will be difficult to judge because of its transient nature. However, metabolic studies of the more stable products of reductive pathways, such as volatile dehalogenated species or fluoride ion, should clarify this long-sought relationship.

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References