LIVER PARENCHYMAL CELL INJURY

VIII. Lesions of Membranous Cellular Components following Iodoform

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ABSTRACT

Iodoform, a relatively water-insoluble yellow solid, chemically reactive in free-radical reactions, produces early hepatocellular injury qualitatively similar to that of carbon tetrachloride. 2 hr after administration of radioactively labeled iodoform, nonvolatile 14C is preferentially recovered in microsomal lipid and protein. By 30 min microsomal properties are profoundly affected: oxidative demethylation decreases abruptly; increased lipoperoxide decomposition products are detected; and amino acid incorporation into liver protein is depressed. By 1 hr glucose-6-phosphatase is suppressed centrolobularly and increased stainable calcium is present in the midzone. Increased cell sap RNA contents are observed by 2 hr. Morphologically, the biochemical and histochemical changes are associated with progressive dispersion, vacuolation, and degranulation of the granular endoplasmic reticulum. Calcium-associated masses accumulate within the mitochondrial matrix, and mitochondria become progressively pleomorphic. Golgi components dilate and disperse. Membranous components of the cytoplasm of parenchymal cells conglomerate into labyrinthine tubular aggregates. Lipid accumulates in cytoplasmic droplets. Ultimately, centrolobular necrosis ensues. The close cytochemical and morphological similarities between the cellular injury produced in the liver by iodoform and that produced by carbon tetrachloride suggest common pathogenetic mechanisms associated with damage to membranes.

INTRODUCTION

The liver lesion of carbon tetrachloride poisoning is one of the most extensively analyzed model systems of cellular injury (2, 29, 33, 35, 45). The chlorocarbon appears and is concentrated in the liver within minutes following its administration (32, 39). Liver lipids, proteins, and nucleic acids become labeled with the products of its metabolism (38, 39). Increased products of lipoperoxidation accumulate in isolated components of the endoplasmic reticulum (29, 31). Enzymatic functions of the endoplasmic reticulum, including glucose-6-phosphatase, oxidative demethylation, and the ability to synthesize protein, are affected by the end of the 1st hr following poisoning (24, 33, 35, 45, 47). Concomitantly, increased calcium enters midzonal parenchymal cells and then spontaneously disappears by 2 hr (35). With time, lipid accumulates and centrolobular necrosis ensues (25, 29, 33, 36). Although other hepatotoxins, specifically dimethylnitrosamine and thioacetamide, may interfere with protein synthesis early in the course of their lesions, centrolobular necrosis ensues without prior alterations of glucose-6-phosphatase, drug metabolism, or the...
permeability of the plasma membrane to calcium (5, 14, 19, 21, 40).

Among halomethanes, iodoform, a virtually water-insoluble yellow powder (119°C) sparingly soluble in lipids, is at least as reactive as carbon tetrachloride in homolytic cleavage (free radical) reactions (6). This study examines in detail the early morphologic and cytochemical components of the liver lesion of iodoform poisoning. The striking similarities in the toxic effects of the two halomethanes on liver parenchymal cells support a common pathogenetic mechanism and provide a basis for its further elucidation.

MATERIALS AND METHODS

Healthy young male rats (Charles River Breeding Laboratories, Inc., North Wilmington, Mass.), weighing between 150–300 g, were maintained on a diet of Purina Chow and water ad libitum. Iodoform-mineral oil suspensions containing 0.68 g iodoform per ml were prepared by high-speed homogenization.

For morphologic studies, replicate rats were sacrificed at 1/2, 1, 2, 4, and 8 hr following the oral administration by polyethylene stomach tube of a single dose of 2,600 mmole iodoform per 100 g of animal. Control animals were fed 1.5 ml mineral oil per 100 g of animal and sacrificed at 2 hr. Animals were sacrificed by decapitation and exsanguination following a 16 hr fast. The left lateral lobe of the liver was removed immediately and sliced. Cubes of liver were fixed in 0.25M sucrose, and were homogenized in 0.25M sucrose, and were carried out at 0–4°C. Livers of replicate rats were removed, homogenized in 0.25 M sucrose, and were stored in the dark at −16°C until used. All preparations were determined by incubation of cryostat sections with nitroblue tetrazolium blue in appropriate media (23).

Patterns of incorporation of 14C and 125I from iodoform were determined in livers of animals fasted for 16 hr and then fed the appropriately labeled iodoform 2 hr prior to sacrifice. 14C HI3 (New England Nuclear Corporation, Boston, Mass.) and CH125I3 (Radio Chemical Centre, Amersham, England) were stored in the dark at −16°C until used. All preparations were carried out at 0–4°C. Livers of replicate rats were removed, homogenized in 0.25 M sucrose, and the homogenates were fractionated by differential centrifugation into residue, mitochondria, microsomes, and cell sap (17). Liver homogenate and subcellular fractions were chemically fractionated into acid-soluble, lipid, nucleic acid, and protein constituents by a modified Schmidt-Thannhauser procedure (37). Total nucleic acids, DNA, RNA, and protein contents of each fraction were determined (37). Nonvolatile 14C in liver homogenates, subcellular fractions, and their chemical constituents was measured in aliquots, and dried for 1 hr at 80°C. Dried samples were counted at infinite thickness in a thin-window gas-flow counter. 125I contents were determined in a well-type scintillation counter. 14C and 125I standards counted concurrently. Excess potassium or perchlorate present which might interfere with the determination of 14C was removed by precipitation as potassium perchlorate (38).

Functional and chemical properties of microsomes isolated from livers of rats, at times up to 4 hr following intragastric administration of the standard dose of iodoform, were determined. Oxidative demethylation was assayed according to the method of Orrenius and his associates (26). Nicotinamide adenine dinucleotide phosphate (NADPH) -neotetrazolium reductase was determined according to the method of Daliner (7). The glucose-6-phosphatase assay system contained 15 µm glucose-6-phosphate, 30 µm Tris maleate buffer, pH 6.7, 20 µm EDTA, pH 6.7, 250 µm sucrose, and 1 mg microsomes (wet weight) in a final volume of 1.5 ml. After incubation at 37°C for 20 min the reaction was stopped by the addition of an equal volume of 10% TCA, and aliquots of the supernatant were analyzed for inorganic phosphorus according to the method of Fiske and SubbaRow (12).

Conjugated diene contents of microsomal lipids were determined by a modification of the method of Recknagel (30). 1/2 ml aliquots of microsomal suspensions in 0.25 m sucrose containing 300–400 mg microsomes (wet weight) per ml were extracted with 19 volumes 2:1 chloroform:methanol. The supernatant was separated into two phases by the addition of 2.0 ml water according to Folch et al. (13), and the upper phase was discarded. 0.2 ml methanol was added to the lower phase, and the optical density was determined between 210–300 nm by using freshly prepared lower phase as blank (25 ml water to 95 ml 2:1 chloroform:methanol). Peak absorption for diene conjugation products of lipid peroxidation (4) isolated under these conditions shifts from 233 to 240 mµ, in the presence of chloroform. Absorption at the latter wavelength, expressed as optical density units per gram microsomes (wet weight), was used as an indicator of the extent of lipoperoxidation.

14C-glycine incorporation into protein was deter-
mized following the intraperitoneal injection of 25 µc uniformly-labeled 14C-glycine (New England Nuclear Corporation) 30 min prior to sacrifice. Ribosomal, cisternal, and membranous subfractions of microsomes were obtained by first treating microsomes with a final concentration of 0.010 M EDTA (pH 7.4) to remove ribosomes (27 and footnote 1) followed by hypotonic stress of low ionic strength to extract cisternal protein (51). Proteins isolated from the homogenate, microsomes, and microsomal subfractions were dried to constant weight at 104°C, dissolved in a small amount of 0.3 M KOH, and then diluted for counting.

RESULTS

Recovery Patterns of Iodoform Derived 14C and 125I

Nonvolatile 14C and 125I derived from isotopically labeled iodoform are recovered from chemical components of liver homogenates and subcellular fractions 2 hr following poisoning (Table 1). 14C from iodoform preferentially labels proteins and lipids, particularly the protein and lipids of the residue and microsomal fractions (Fig. 1). In contrast, most of the 125I is recovered from the acid-soluble fraction of the cell sap and, to a much lesser extent, from lipids of all fractions (Fig. 1, Table I). Under the conditions used in this study, the bulk of 125I in the acid-soluble fraction is considered to be inorganic iodide (38). The distribution of 125I in chemical constituents of subcellular components following feeding of iodoform differs greatly from that resulting from the addition of the labeled compound to liver homogenates in vitro. In the latter instance, between 80 and 90% of the 125I is recovered in lipid fractions.

Electron Microscopy

Early discernible changes following iodoform involve the endoplasmic reticulum. By 1 hr the ergastoplasm is dispersed, the membranes of the granular endoplasmic reticulum are partially degranulated, and increased numbers of free monomer ribosomes appear in the cytoplasmic matrix (Figs. 4–6, Table II). Cisternae of the granular endoplasmic reticulum may be dilated. These changes are progressive with time, and by 8 hr, in those cells with the most severe degranulation of the endoplasmic reticulum, well-granulated strands are observed only in close approximation to mitochondria (Figs. 7–9).

The extent of degranulation was semiquantitatively estimated in representative electron micrographs. Ribosomes within less than one ribosomal

<table>
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<tr>
<th>Table I</th>
<th>Nonvolatile Radioactivity Recovered in Liver 2 Hr after Oral Feeding of Isotopic Iodoform</th>
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<tbody>
<tr>
<td>Isotope</td>
<td>Distribution of isotope recovered</td>
</tr>
<tr>
<td></td>
<td>Subcellular fractions</td>
</tr>
<tr>
<td></td>
<td>Nonvolatile isotope recovered</td>
</tr>
<tr>
<td>14CH 13 (3)</td>
<td>33</td>
</tr>
<tr>
<td>CH125I 13 (3)</td>
<td>250</td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to number of animals.
diameter of a membrane were considered membrane-bound. Non-membrane-bound ribosomes were considered polysomal if they were within one ribosomal diameter of another ribosome. Free ribosomes were separated from other ribosomes and membranes in two dimensions by a distance greater than 200 Å. In the most severely affected cells decreased numbers of membrane-bound ribosomes and increased numbers of free monomer ribosomes are present in the cytoplasm by 1 hr (Table II). As time following poisoning lengthens, increasing numbers of ribosomes appear free in the cytoplasmic matrix both in the least and in the most severely affected cells (Table II). As a general rule the most severely affected cells are midzonal and centrolobular.

Membranous components of the endoplasmic reticulum decrease in many cells by 8 hr, and large conglomerations of closely packed, interlacing, smooth-surfaced tubules confluent with
FIGURE 2  Light microscopic illustration of alterations in periportal (Figs. 2a, 2b) and centrolobular (Figs. 2c, 2d) cells following poisoning with iodoform. Osmium tetroxide fixation; Epon embedded; 1 μ sections, toluidine blue O. X 1400. 2a. Periportal region in control. 2b. 8 hr following poisoning with iodoform. Some cells (upper left) are diffusely vacuolated. Other cells show increased numbers of lipid vacuoles. Areas devoid of mitochondria are prominent in the centers of cells (arrow). Mitochondria appear more pleomorphic than in controls. 2c. Centrolobular region in control. 2d. 8 hr following poisoning with iodoform. Prominent, diffuse vacuolation is present in some cells, and lipid vacuoles are seen in other cells. The central areas of many cells are again devoid of mitochondria (arrows). Mitochondria appear more pleomorphic than in controls.

Recognizable remnants of both smooth and rough endoplasmic reticulum appear (Figs. 7–9). These conglomerations or labyrinthine tubular aggregates (LTA) differ from smooth endoplasmic reticulum in that their tubular components are smaller, more intricately interwoven, and associated with amorphous electron-opaque deposits (Figs. 7–9).

Striking mitochondrial changes are evident at 1 hr following poisoning. In midzonal parenchymal cells rosette and ring-like calcium-associated electron-opaque granules appear in the mitochondrial matrix immediately adjacent to inner membranes (Figs. 4, 5). Normal matrix granules appear decreased in these mitochondria. Outlines of some mitochondria become markedly
Figure 3  Time course of glucose-6-phosphatase suppression (Figs. 3a-3c) and calcium influx (Figs. 3d-3f) following iodoform. Fresh-frozen cryostat sections, 6 μ thick. X 60. 3a. Glucose-6-phosphatase activity in control liver is uniform throughout. 3b. At 1 hr following iodoform the centrolobular activity is moderately suppressed. 3c. At 8 hr the activity is markedly suppressed except in periportal areas. 3d. Alizarin red-S staining of control liver. Focal areas of increased stainable calcium are not present. 3e. At 1 hr a faint midzonal band of increased staining is prominent. 3f. At 8 hr the mid-zonal band has disappeared and scattered cells in midzonal and centrolobular areas stain intensely for calcium.
FIGURE 4 Parenchymal cell 1 hr after poisoning with iodoform. Small granular calcium-associated, electron-opaque deposits of varying size are present within the mitochondrial matrix. These deposits are close to the internal mitochondrial membranes. An enlarged mitochondrion (M1) contains a pale flocculent matrix. Ribosomes are predominantly membrane associated although, locally, ribosomes appear to lie free within the cytoplasmic matrix. Nucleus and nucleolus appear as in controls. X 30,000.
irregular, and their boundaries scalloped (Fig. 5). Other mitochondria may enlarge slightly and contain a less dense matrix, although normal numbers of cristae mitochondriales persist (Fig. 4, 9). Electron-opaque, intramitochondrial, calcium-associated masses may occasionally be seen within such mitochondria (Fig. 5). The density of the mitochondrial matrix may increase in damaged cells and approach that of microbodies and nearby erythrocytes. In such mitochondria the matrix consists of homogeneously distributed, electron-lucent granules of relatively uniform size (Fig. 9). Mitochondria with less dense, more flocculent matrices of uneven granularity may be seen within the same cell (Fig. 9), and in these cells ribosome-like granules and small dark lipid-like droplets appear (Fig. 9). In cells with marked cisternal dilatation of the endoplasmic reticulum at 8 hr, concentric membranous inclusions appear both within the outer mitochondrial space and within the adjacent cytoplasmic matrix (Fig. 6).

Cell boundaries are well preserved at 1 hr. Occasional focal separations between adjacent cell membranes occur by 8 hr. Microvilli in the perisinusoidal spaces of Disse do not show marked variations, although clubbing and areas devoid of microvilli are occasionally seen (Figs. 6, 7). Vascular changes are not apparent except for the presence of rare fibrin thrombi within sinusoids.

The Golgi apparatus balloon progressively with time and, by 8 hr, the few that are recognized

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FIGURE 6  Cytoplasm of centrolobular liver cell 8 hr after poisoning with iodoform. There is marked dilation of cisternae of endoplasmic reticulum. Intracytoplasmic lipid droplets are abundant. The majority of ribosomes are free in the cytoplasmic matrix as both monomers and polysomes. Mitochondria all have a relatively dense matrix. Concentric membranous bodies (arrows) apparently lie free within the cytoplasmic matrix and also within outer mitochondrial space. Microvilli lining the space of Disse are focally absent. × 27,000. Inset: Membranous inclusion evaginates the outer mitochondrial membrane. × 81,000.
FIGURE 7 Cytoplasm of two midzonal cells 8 hr after poisoning with iodoform. Intracytoplasmic lipid droplets are present in both cells. Tubules consisting of smooth membranes in the upper cell represent smooth endoplasmic reticulum. Well-developed labyrinthine tubular aggregates are present in the lower cell in association with characteristic foci of amorphous increases in density (arrows). Note the pale cytoplasm, obvious free ribosomes, and dilated cisternae of endoplasmic reticulum in the lower cell as compared to the upper cell. Differences in density of mitochondrial matrix exist between the two cells. Microvilli lining the cell-cell interface are clubbed. × 20,000.


**TABLE II**

Estimation of Ribosomal Populations in Cytoplasm of Liver Cells after Poisoning with Iodoform

<table>
<thead>
<tr>
<th>Ribosomes counted</th>
<th>Ribosomal distribution</th>
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<tr>
<td></td>
<td>Membrane bound</td>
</tr>
<tr>
<td></td>
<td>Polyribosomal</td>
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<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Least affected cells</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>226</td>
</tr>
<tr>
<td>1/2 hr.</td>
<td>221</td>
</tr>
<tr>
<td>1 hr.</td>
<td>216</td>
</tr>
<tr>
<td>8 hr.</td>
<td>98</td>
</tr>
<tr>
<td>Most affected cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>237</td>
</tr>
<tr>
<td>1/2 hr.</td>
<td>236</td>
</tr>
<tr>
<td>1 hr.</td>
<td>102</td>
</tr>
<tr>
<td>8 hr.</td>
<td>71</td>
</tr>
</tbody>
</table>

consist of dispersed clusters of dilated vacuoles containing few of the 600-800 Å electron-opaque granules normally present.

Microbodies and lysosomes, present in parenchymal cells of livers of both control and experimental animals, do not increase in number or appear to be morphologically altered during this initial period. Definite changes in nucleoli attributable to iodoform were not recognized.

**Biochemical Functions and Properties of the Endoplasmic Reticulum**

The striking changes in the morphology of the endoplasmic reticulum within 1 hr following iodoform are concomitant with alterations in enzymatic and chemical properties of its microsomal counterpart. Within 1/2 hr following poisoning the ability of microsomes to oxidatively demethylate antipyrine is decreased by nearly one-third, and a significant increase in diene conjugation products of liperoxidation occurs (lipid E240, Table III). 

\[ ^{14}C \text{-glycine incorporation into protein is also decreased to approximately one-half the control values at this time. Decreases in oxidative demethylase activity and } ^{14}C \text{-glycine incorporation are progressive with time, respectively falling to } \frac{1}{2} \text{ and } \frac{1}{2} \text{ their control levels by } 2 \text{ hr (Table III). Although similar amounts of ribosomal, cisternal, and membrane protein are recovered from control and iodoform-poisoned animals, reduction of glycine incorporation is greatest in protein isolated from ribosomes and membranes at 2 hr and is least in cisternal proteins. Lipid peroxidation decreases slightly following its initial } \frac{1}{2} \text{ hr elevation, while increased cell sap RNA contents, a biochemical indicator of degranulation of the rough endoplasmic reticulum, is first noted at 2 hr. In contrast, NADPH-NT-reductase and microsomal protein contents are only slightly altered from normal throughout this early time period (Table III). Glucose-6-phosphatase activity, profoundly affected in the histochemical assay procedure (Fig. 3), is not significantly decreased biochemically until 4 hr following poisoning.}

**DISCUSSION**

Comparison of cellular injury following iodoform with that following other hepatotoxins offers a basis for further elucidation of some basic pathogenetic mechanisms. Recovery of products of iodoform metabolism within liver cell components early in the course of poisoning indicates that this hepatotoxin might be most meaningfully compared with other hepatotoxins which are metabolized (21, 34, 38, 40). The major early morphological and biochemical alterations produced by iodoform reveal a striking similarity to carbon...
Disaggregation of the ergastoplasm and dispersion of the granular endoplasmic reticulum throughout the cytoplasm is the earliest recognizable morphologic change following iodoform. Concomitantly, the membranes are degranulated, free ribosomes appear within the cytoplasmic matrix and, in time, cisternae of the granular endoplasmic reticulum dilate. However, there is striking preservation of the flattened granulated cisternae about mitochondria. Such early dispersal of ergastoplasm and degranulation of endoplasmic reticulum is also a major morphologic feature of poisoning with dimethylnitrosamine (8) and ethionine (1) as well as with carbon tetrachloride (25, 35, 47). This change which results from the feeding of these apparently dissimilar poisons may be but a secondary response to differing primary molecular events. Dimethylnitrosamine methylates nucleic acids and proteins (20, 21). Ethionine produces ATP starvation and, ergo, suppression of synthesis of messenger RNA (11). Cleavage products from the metabolism of carbon tetrachloride may primarily attack the membranes of the cell (40). Iodoform, which is at least as reactive in free-radical reactions as carbon tetrachloride (6), may have a similar mechanism of action.

The early iodoform-induced changes in the endoplasmic reticulum are followed by the formation of labyrinthine tubular aggregates, i.e., conglomerations of closely packed interconnecting smooth-surfaced tubules. Identical structures form following carbon tetrachloride (37). "Nebenkern" observed in livers of rats following dimethylnitrosamine (9) and thioacetamide (42) may represent similar structures. The apparent morphologic differences between labyrinthine tubular aggregates and smooth endoplasmic reticulum suggest that the former may be the result of degenerative changes in the membranes of the endoplasmic reticulum. Indeed, labyrinthine tubular aggregates persist in hepatocytes recovering from carbon tetrachloride injury in which newly synthesized endoplasmic reticulum is also present. 1

The early and transient influx of calcium into the midzone 1 hr following iodoform, preceding by hours the terminal reentry of calcium into the liver lobule, had previously been observed to be unique for carbon tetrachloride poisoning (40). Such an early influx suggests the occurrence of a transient alteration in plasma membrane permeability secondary to direct chemical attack by the toxin. The appearance of calcium-associated electron-opaque intramitochondrial masses at these times following both iodoform and carbon tetrachloride, is interpreted as the result of the physiologic response of mitochondria to increased calcium entry into the cell (10, 28, 35, 37, 48), although primary injury to the membranes of the mitochondria as a participating event cannot be ruled out.

Mitochondrial changes following iodoform also include progressive decrease and increase in mitochondrial matrix density which at times occurs within the same cell. These changes, which are not apparently related to calcium accumulation, may be a reflection of differing respiratory states of the mitochondria (15). Small mitochondria with dense matrices previously designated "pyknotic mitochondria" (49) may be the result of the same processes.

The striking morphologic changes involving the endoplasmic reticulum in the liver lesions of

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1 E. S. Reynolds. Unpublished data.
both iodoform and carbon tetrachloride poisoning are accompanied by marked alterations in the cytochemical properties of the endoplasmic reticulum. The recovery patterns of non-volatile $^{14}$C and $^{125}$I from iodoform in chemical components of subcellular fractions are qualitatively similar to those of isotopic carbon and chlorine following administration of carbon tetrachloride (38, 39). Both agents suppress glucose-6-phosphatase early in the course of poisoning (35, 40). Within 30 min following iodoform, lipoperoxides are increased, oxidative demethylation decreases, and protein synthesis is affected. Similar changes occur following carbon tetrachloride (31, 46, 47). Oddly, following iodoform, increases in cell sap RNA, a biochemical indicator of degranulation of granular endoplasmic reticulum (25, 35, 41, 45, 47), do not occur until after 1 hr. The temporal relationships whereby amino acid incorporation into microsomal protein decreases prior to degranulation of the rough endoplasmic reticulum suggest that protein synthesis fails while the ribosomes are still membrane attached. In sharp contrast to the changes which occur in the endoplasmic reticulum, histochemical analyses of mitochondrial succinic and glutamic dehydrogenase activity show no detectable changes during the early period studied.

Recovery of increased products of lipoperoxidation from microsomes during the time of concentration of the poison by the liver strongly suggests that lipoperoxidation is an important process in the initial cytopathologic lesion (31). Small amounts of diene conjugation products of lipo-

\begin{table}
\centering
\caption{Functions and Properties of the Endoplasmic Reticulum after Poisoning with CHCl₃}
\begin{tabular}{lcccccc}
\hline
 & Microsomal protein content & Oxidative demethylation & NADPH-NT reductase & G-6-phosphatase & Lipid E₀ & $^3$H-glycine incorporation & Cell sap RNA \\
 & mg/\text{g} & mmole CHO/\text{g} \times \text{min} & mmole NTH/\text{g} \times \text{min} & mmole Pi/\text{g} \times \text{min} & \text{cm}²/\text{g} \times \text{min} & \text{cm}²/\text{g} \times \text{min} & \text{m mole/g liv wt} \\
\hline
Control (9) & 74 ± 1 & 0.36 ± 0.02 & 1.8 ± 0.1 & 21 ± 1 & 4.4 ± 0.4 & 1240 ± 170 & 3.2 ± 0.2 \\
1½ hr (5) & 68 ± 3 & 0.26 ± 0.03$^\dagger$ & 1.9 ± 0.2 & 22 ± 1 & 7.6 ± 0.7 & 590 ± 230 & 3.6 ± 0.4 \\
2 hr (7) & 66 ± 3$^\dagger$ & 0.12 ± 0.02$^\ddagger$ & 1.6 ± 0.1 & 17 ± 1 & 5.8 ± 0.4$^\ddagger$ & 200 ± 50$^\ddagger$ & 5.1 ± 0.3$^\ddagger$ \\
\hline
\end{tabular}
\end{table}

$^*$ g microsomes (wet wt.).
$^\dagger$ Numbers in parentheses refer to number of animals.
$^\ddagger P = <0.05.$
$^\ddagger$ $P = <0.005.$

Increased free-radical generation is an attractive hypothesis to explain the subsequent changes in the membranes of the endoplasmic reticulum. Free-radicals react in chain reactions (4, 52), and such reactions in a lipid phase of a membrane could result in partial polymerization of its lipid, protein, and nucleic acid components (52). Destruction of highly unsaturated lipids would also occur (30, 52). Decreased contents of arachidonic and linolenic acid in microsomes following carbon tetrachloride have been postulated to be the result of such reactions (30). Thus, such changes as degranulation of the granular endoplasmic reticulum and the formation of labyrinthine tubular aggregates may be but morphologic manifestations of these underlying chemical events.

The dissociation between carbon and iodine labeling in this study is not incompatible with such a postulated free-radical mechanism. The liberation of iodide ion into the cell sap can result either from the sequestering of an electron by nascent
iodine, or from the subsequent heterolytic cleavage of iodomethyl free radicals.

Concentric membranous inclusions within the outer mitochondrial space of mitochondria with dense matrices in severely injured cells may result from similar degenerative changes in mitochondrial membranes. Mitochondrial enlargement, the formation of cup-shaped mitochondria, and the occurrence of mitochondria with markedly irregular contours seen within 2 hr following carbon tetrachloride (35) as well as following iodoform may be early reflections of these degenerative changes.

The close cytochemical and morphological similarities between the hepatic lesions of carbon tetrachloride and iodoform described in this study are considered to support the hypothesis that both agents have a common pathogenetic mechanism of hepatocellular injury. Consideration of such an hypothesis in view of the known physical and chemical characteristics of both halocarbons suggests that the pathogenetic mechanisms may involve free-radical reactions. Consideration of free-radical induced lipoperoxidation mechanisms in view of changes in membranes common to both toxins suggests that the cellular membranes, specifically those of the endoplasmic reticulum and related organelles, may be primary sites of such an action. Consideration of cellular membranes as the possible primary site of action raises the possibility of conformational change in membrane structure as the initial event which produces the subsequent morphologic and biochemical changes.

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R E F E R E N C E S