Chlorinated Aromatic Hydrocarbon Induced Modifications of the Hepatic Endoplasmic Reticulum: Concentric Membrane Arrays*

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A number of commercial compounds having chlorinated aromatic hydrocarbon structures are used extensively for medical(3), industrial(4) and agricultural(5) purposes. Quantities of certain parent chlorinated aromatic hydrocarbons and metabolites have been repeatedly detected as residues in the food supply and tissues of birds, fish, and mammals(6-9) including man(10). Chlorinated diphenyl-p-dioxins have been identified as contaminants of many commercial products, such as certain edible fats, herbicides and disinfectants. It is suggested that the compound results from the conjugation of commercial chlorinated phenols(11). Polychlorinated polyphenyls are among the most abundant chlorinated hydrocarbon global pollutants. Their contamination of the environment is a result of the widespread commercial use of these compounds for their properties of insulation, adhesiveness, thermoplasticity, chemical inertness, and insolubility.

Following this investigation of sequential biochemical and ultrastructural alterations within the liver produced by three chlorinated aromatic hydrocarbons — chlorinated diphenyl-p-dioxin, polychlorinated biphenyls (PCBs), and a highly chlorinated triphenyl (PCT)—certain similarities of biochemical and ultrastructural effects of the compounds were observed. The livers of rats fed the chlorinated aromatic hydrocarbons consistently contained numerous multi-layered concentric membrane arrays, proliferated smooth endoplasmic reticulum (ER), and altered microsomal enzyme activity.

Materials and Methods

Separate groups of male Sprague-Dawley rats initially weighing 100 grams were fed diets containing the following chlorinated aromatic hydrocarbons: 1% PCTs (Aroclor 5460), 0.02% PCBs (Aroclor 1254), and 0.002% chlorinated diphenyl-p-dioxin, respectively. At regular intervals the rats were deprived of food for 24 hours and sacrificed by exsanguination. The livers were weighed and portions were removed for electron microscopy. This tissue was immediately placed in veronal acetate buffered osmium tetroxide and cut into small cubes. Following one and one half hours of fixation, the tissues were dehydrated in a graded series of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Sections were cut at a thickness of 0.5–0.8 microns for light microscopic evaluation, and thinner sections at a thickness of approximately 60 millimicrons were cut and stained with uranyl acetate for electron microscopic examination. The remaining portions of the livers were perfused through the portal vein with isotonic KCl, weighed, and homogenized at 0° with two volumes of the salt solution. Microsomes were isolated from the post mitochondrial supernatant obtained by centrifugation at 9,000 × G for 20 minutes, washed by homogenization with KCl and recentrifuged, resuspended in KCl equal to three volumes of the original liver material, and immediately assayed for the activities of aromatic

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hydroxylase(12), nitroreductase(12), N-demethylase(12), nitrophenyl acetate hydrolysis (esterase) (13), and glucose-6-phosphatase(14). RNA(15), phospholipid(16), and cholesterol(16) concentrations were determined from samples that had been frozen at −80°. Protein determination(17) on alkaline-washed(18), microsomal material provided estimation of membrane protein in the microsomal suspensions, which is subsequently referred to as microsomal protein.

Results

The rats from the dioxin and PCT groups readily ate the experimental diets and attained at least 80% of the weight of the controls at three weeks. Hypertrophy of the liver, varying from a moderate enlargement, following chlorinated diphenyl-p-dioxin ingestion, to an increase in the relative liver weight of 8 grams per hundred grams body weight, following PCB and PCT ingestion, was observed. Neurological side effects, including tremors, convulsions, or sedation, were not observed. The rats from the dioxin and PCT group remained on the diet for four weeks without apparent extra-hepatic effects other than the small decrease in weight gained. The rats from the PCB group did not gain weight and were sacrificed at 8 days.

The ultrastructural alterations within the hepatocytes of rats from the three experimental groups were similar and will be considered collectively. The changes were primarily limited to the endoplasmic reticulum and consistently affected all experimental animals. The hepatocytes contained numerous multi-layered, concentric membrane arrays and proliferated smooth ER. Preceding the formation of the concentric arrays, the parallel cisternae of the rough ER, representative of the control hepatocytes (Fig. 1) were reorganized into sinuous patterns that encompassed lipid droplets, mitochondria and other organelles (Fig. 2). Annular profiles consisting of paired membranes with ribosomes attached to the

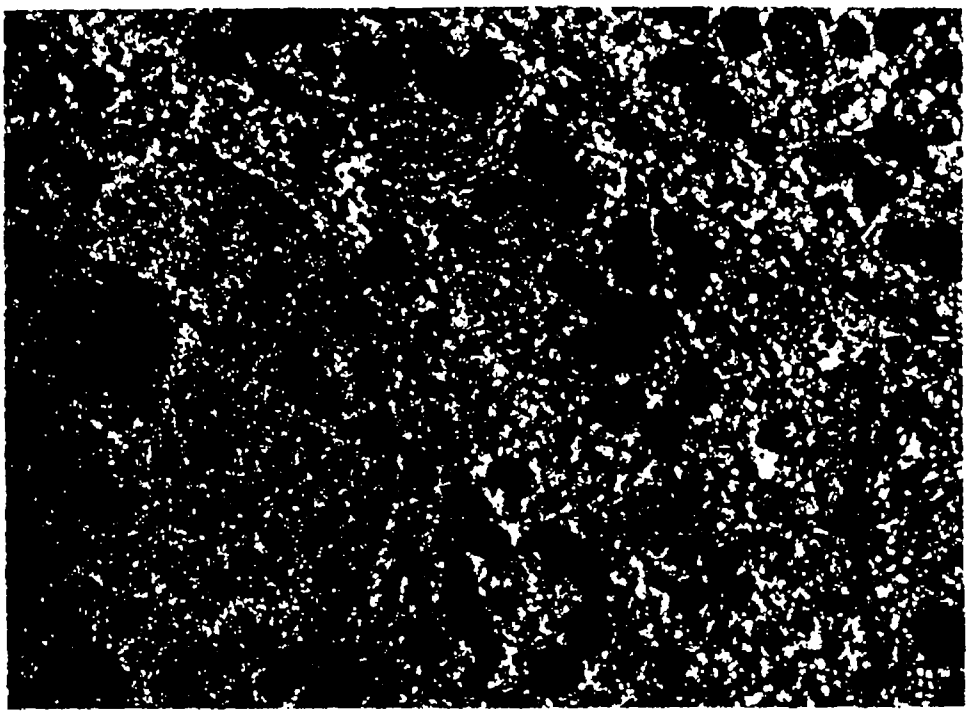

FIGURE 1. Elements of the rough endoplasmic reticulum are characteristically arranged in parallel lamellae in hepatocytes of rats fed a control diet (× 8,620).

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FIGURE 2. Following the ingestion of a chlorinated aromatic hydrocarbon, the parallel membranes are reorganized into sinuous cisternae that encompass lipid droplets, mitochondria, and other organelles (following ingestion of chlorinated diphenyl-p-dioxin; × 15,500).

central surface of the inner membrane and the peripheral surface of the outer membrane were also prevalent (Figs. 3 & 4). Shortly after the development of the annular formations, concentric annuli composed of variable numbers of degranulated paired membranes were observed (Fig. 5). These structures composed of multi-layers of membranes were also evident following light microscopic evaluation of the toluidine blue-stained sections. Within three weeks, each experimental group developed multi-layered membrane arrays composed of several units of concentric annuli (Fig. 6). Lamellar profiles of paired membranes resembling flattened cisternae partially circumscribed the

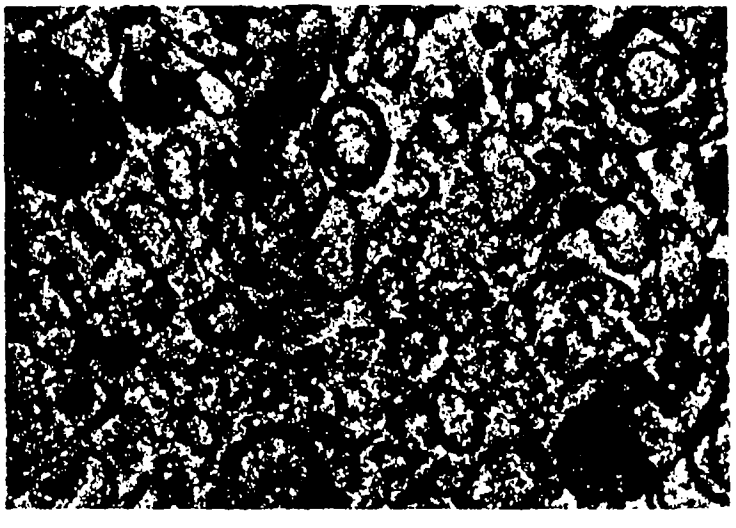

FIGURE 3. Membranes of the endoplasmic reticulum are also represented as annular profiles in hepatocytes of the experimental rats (following ingestion of chlorinated diphenyl-p-dioxin; × 24,300).

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FIGURE 4. The concentric annuli with attached ribosomes are apparently derived from the rough endoplasmic reticulum (following ingestion of chlorinated diphenyl-p-dioxin; ×35,600).

annular units. The outer membrane pairs of the lamellae were continuous with elements of the smooth ER and rough ER. Ribosomes were occasionally attached to the surfaces of the outer membrane pairs of the multi-layered arrays. The concentric membrane arrays increased in size and complexity in direct relationship to the time the rats remained on the experimental diets.

Associated with the modified membrane structures were alterations in enzyme activity and biochemical composition of the microsomal fraction of these cells (Table 1). Following the ingestion of PCTs for 21 days, there was an increase in microsomal protein per gram of liver. Compositional changes of the membrane material were indicated by the reduction in cholesterol to protein ratio and slight increase in phospholipid to protein ratio. The increase in esterase activity was parallel to that of protein, whereas, increases in N-demethylation and nitroreduction were greater than the increase in membrane material. Specific activities of glucose-6-phosphatase and aromatic hydroxylase were reduced. The enzymatic changes following PCB ingestion were parallel to those of the PCT group.

Discussion

Ingestion of the chlorinated aromatic hydrocarbons by rats induces liver hypertrophy with proliferation of the ER and formation of large concentric membrane arrays within the cytoplasm of the hepatic cells. These changes measured after 21 days PCT ingestion result in increased hepatic function. An increase in total activity of each enzyme investigated is evident when the nearly three-fold liver hypertrophy and more than doubled quantity of microsomal protein per gram liver is considered. Associated with the enzymatic alterations are an increased ratio of phospholipid to protein and a decreased ratio of cholesterol to protein. Increased enzyme activity and proliferated smooth ER are commonly produced by a

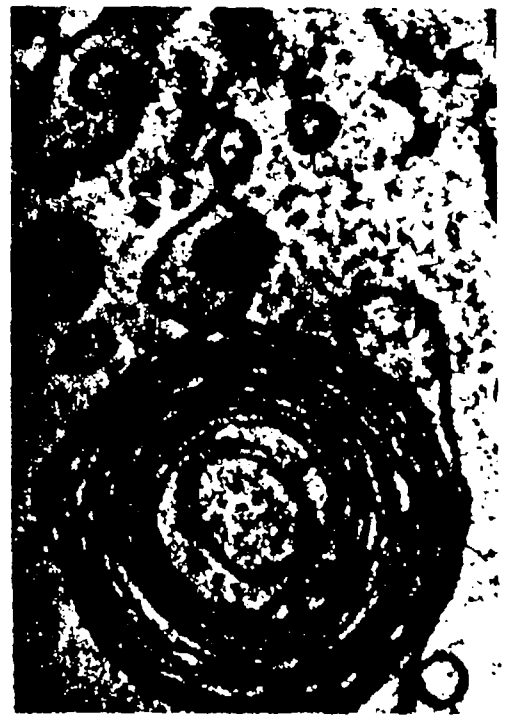

FIGURE 5. Larger concentric membrane arrays consist of numerous distinct rings of paired membranes (following ingestion of chlorinated diphenyl-p-dioxin; ×30,300).

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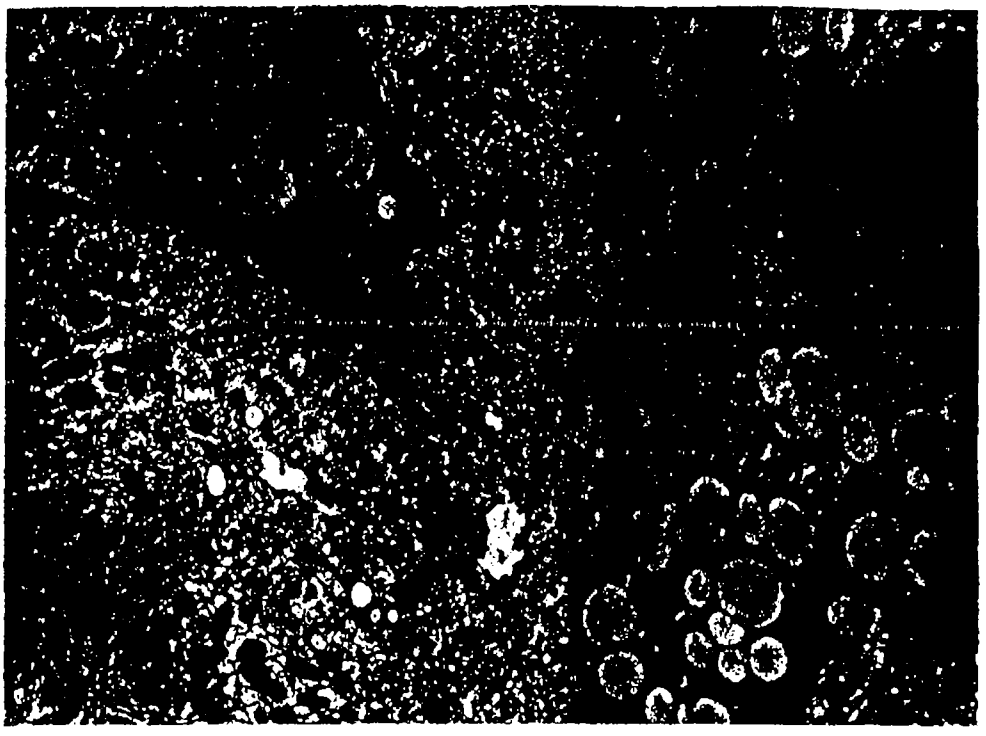

FIGURE 6. Following ingestion of polychlorinated triphenyl for three weeks extensive concentric membrane arrays develop within the hepatocytes. The membrane arrays encompass lipid droplets, mitochondria, and other cellular organelles (× 3,700).

wide variety of agents including phenobarbital; however, the phospholipid/protein are unaltered and cholesterol/protein increased(18). Thus the membranes low in cholesterol and high in phospholipid composition, which are characteristic of chlorinated triphenyl induced microsomes, likely represent the concentric membrane arrays.

Structures similar to concentric membrane arrays have been observed following the administration of numerous other compounds including phenobarbital(19), thiohydantoin(20), triparanol(21), aflatoxin(22), ethionine(23), carbon tetrachloride(24), and amino azo dyes(25). An evaluation of the sequential changes that occurred following consumption of the chlorinated aromatic hydrocarbons suggests that arrays of concentric paired membranes originate from cisternal elements of the ER. Modification of the rough ER included reorganization of parallel cisternae into sinuous cisternae and concentric annular profiles of paired membranes. The concentric annuli are similar to the compositional units of the multilayered membrane arrays. This similarity suggests that the reorganized cisternae of the rough ER are incorporated into the arrays of the concentric paired membranes. Continuity of these arrays with the rough and smooth ER further support a postulate of a rough ER derivation.

Enzymatic and compositional changes of the microsomes suggest the modification of the ER may be correlated to several probable functions. Consistent with the increased proportion of phospholipids within the membrane structure is the proposal that the proliferated membranes provide a site for storage, for isolation, or for contact with membrane enzymes of the hydrophobic chlorinated aromatic hydrocarbon. The increased proportion of phospholipid enhances the suitability of the membrane to sequester the foreign material. The proximity of the concentric

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Table 1. Hepatic alterations in rats fed chlorinated triphenyl for 21 days.

	Cont.	Exp.
Liver weight (g/100 g body weight)	3.00[a]±0.28	8.63±0.79
Microsomal protein (mg/g liver)	20.1[a]±0.5	54.5±4.1
RNA (μg)[c]	180[a]±33	63.7±4.3
Phospholipid (μg)[c]	455[b]±24	508±30
Cholesterol (μg)[c]	44.0[a]±8.9	17.9±1.5
Glucose-6-phosphatase (μmol PO4/15 min)[c]	3.98[a]±0.51	1.08±0.08
Esterase (μmol ρ-nitrophenol/min.)[c]	9.75±3.0	10.3±1.21
Aromatic hydroxylase (mμmol ρ-amino-phenol/30 min.)[c]	30.2[a]±6.2	6.06±0.29
N-demethylase (mμmol formaldehyde/ 30 min.)[c]	103[a]±19	166±7
Nitroreductase (mμmol ρ-amino-benzoate/hr.)[c]	32.6[b]±6.8	45.4±6.2

[a] Difference between mean and following mean is significant ($p < .001$).

[b] Difference between mean and following mean is significant ($p < .01$).

[c] per mg protein.

microsomal membrane arrays to lipid droplets, also a probable location of the lipid soluble compound, provides accessibility of the lipid component of the membrane to the compound. Hydroxylation activity present within the microsomes converts a lipid soluble hydrophobic compound into a form which has the potential to bind with activated glucuronide (uridine diphosphate glucuronic acid) to form a glucosiduronate. The resulting water soluble conjugate could then be excreted through the biliary or urinary system. Although the specific activity of this enzyme is reduced, the overall activity within the liver is substantially increased over the control value. Cholesterol within the membrane structure has been related to the rigidity of the membrane by its ability to condense the unsaturated fatty acids of the phospholipids(26). Lack of cholesterol suggests a less stable structure or, alternatively, regions available for insertion of other compounds, such as the chlorinated aromatic compounds among the fatty acids and the phospholipids. Thus the enzymatic and compositional alterations of the newly formed membranes suggest a membrane suited for localizing the chlorinated aromatic hydrocarbon for possible sequestration of metabolites for excretion.

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