Chlorinated Triphenyl-Induced Extensions of the Hepatic Endoplasmic Reticulum[1] (36313)

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Chlorinated aromatic compounds are widespread ecological pollutants frequently detected as residues in the food supply and tissues of birds, fish, and mammals (1–4), including man (5). Potential hazards of the compounds are suggested by alteration of hepatic metabolism (6), production of neural disorders (7), alteration of reproduction (8), and simulation of estrogenic action (9) following their ingestion. Since the liver has a primary role in the metabolism of foreign compounds, the hepatic effects were evaluated biochemically and ultrastructurally following the ingestion of a commercial highly chlorinated triphenyl[2] (CT). This compound is advantageous for evaluation of effects of chlorinated aromatic hydrocarbons since the structure is devoid of additional chlorinated ethane, oxyacetate, thio, or other groups found in insecticides or herbicides such as DDT,[3] 2-4,D,[4] or chlorbenside. Hepatocytes of rats fed CT consistently contained numerous multilayered concentric membrane arrays (CMA) and proliferated smooth endoplasmic reticulum (ER). Associated with the modified membrane structures were alterations in enzyme activity and biochemical composition of the microsomes.

Materials and Methods. Male Sprague-Dawley rats (initially weighing 100 g) were fed a diet containing 1% CT for 3 weeks; the control group received a similar diet without the additive. At regular intervals rats were deprived of food for 24 hr and subsequently killed by exsanguination. Liver tissue obtained for electron microscopy was fixed in Veronal-acetate buffered osmium tetroxide, dehydrated in ethanol, cleared in propylene oxide, and embedded in an Epon–Araldite mixture. Sections were cut and stained with toluidine blue for light microscopic evaluation; and thinner sections were cut and stained with uranyl acetate for electron microscopy. The remaining portions of the livers were perfused (through the portal vein) with isotonic KCl, weighed, and homogenized at 0° with 2 vol of the salt solution. Microsomes were isolated by centrifugation at 100,000*g* for 90 min from the postmitochondrial supernatant, which was obtained by centrifugation at 9000*g* for 20 min; washed by homogenization with KCl and recentrifuged; resuspended in KCl equal to 3 vol of the original liver material; and immediately assayed for enzyme activity. Conditions and product reactions for aromatic hydroxylase, nitroreductase, and *N*-demethylase, employing substrate concentrations of 100 m*M* aniline, 3 m*M* *p*-nitrobenzoic acid, and 6 m*M* aminopyrine, respectively, were modifications of reported procedures (10). Esterase activity was determined by the rate of formation of nitrophenol in a 3.5 m*M* *p*-nitrophenylacetate incubate buffered to pH 6.5. Glucose-6-phosphatase activity was determined by incubation of 40 m*M* glucose-6-phosphate at pH 6.5 and measurement of phosphate (11). RNA concentration was determined (12) on, and lipid was extracted (13) from, samples that had been frozen at −80°. Phospholipid concentration was determined by digestion of the lipid extract and determination of inorganic phosphate (11). Cholesterol was separated from the lipid extract on a silicic acid

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[2] Aroclor 5460, Monsanto Chemical Company, St. Louis, MO.

[3] 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane.

[4] (2,4-Dichlorophenoxy)acetic acid.

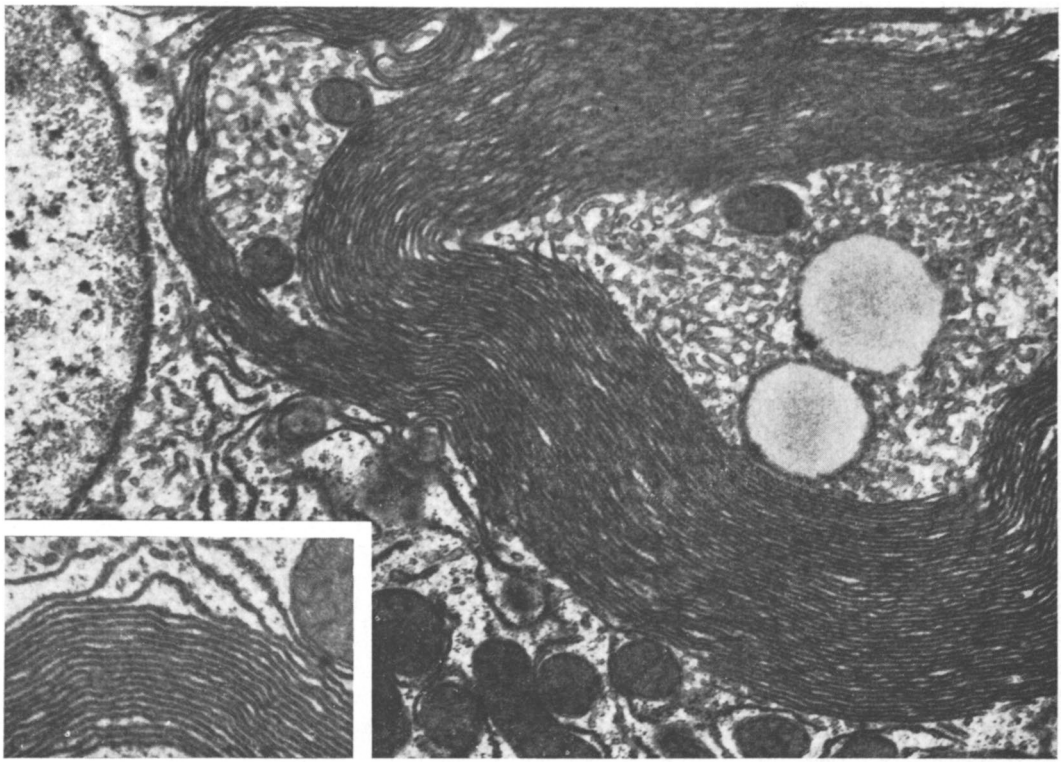

FIG. 1. Concentric membrane array within a liver cell of a rat given chlorinated triphenyl for 3 weeks and a higher magnification of the array. Note the proximity of the concentric array to lipid droplets, the laminar arrangement of paired membranes, and the ribosomes attached to the outer membrane lamina (18,600×; inset, 22,600×).

column (14) and quantified by a reported procedure (15). Protein determination (16) on alkaline-washed microsomal material (17) provided estimation of membrane protein in the microsomal suspensions, which is subsequently referred to as microsomal protein. Data were analyzed using the *t* test of significance.

Results. The rats readily ate the diet and attained 83% the weight of the controls at 3 weeks. Ingestion of CT produced marked hypertrophy of the liver; however, extrahepatic effects were not apparent. At 1 week, the relative liver weight was 197% that of the control parameter; and at 3 weeks, the experimental value was 288% that of the corresponding control value. After 5 days, CMA were occasionally observed within the hepatocytes; and after 3 weeks, 60% of the hepatocyte profiles throughout the liver lobules of all experimental animals contained CMA (Fig. 1). These structures were composed of multilayers of smooth paired membranes, similar in structure to long flattened cisternae, that frequently circumscribed lipid droplets, smooth ER, and mitochondria. Ribosomes were associated with the outer membranes of these arrays.

The biochemical alterations of the microsomes at 21 days are summarized in Table I. Increased microsomal protein per gram of liver and a decrease in RNA per milligram of microsomal protein (RNA/protein) quantitatively reflected the hypertrophic agranular membrane system depicted electron microscopically. Compositional changes of the membranous material were indicated by the reduction in cholesterol/protein and slight increase in phospholipid/protein. The increase in esterase activity was parallel to that of protein; whereas increases in *N*-demethylation and nitroreduction were greater than the

TABLE I. Hepatic Alterations Following Ingestion of Chlorinated Triphenyl for 21 Days. The values show the means ± the standard deviations.

Analysis	Control	Experimental
Liver wt (g/100 g of body wt)	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 PO_4/15 min)[c]	3.98[a] ± 0.51	1.08 ± 0.08
Esterase (μmol *p*-nitrophenol/min)[c]	9.75 ± 3.00	10.3 ± 1.21
Aromatic hydroxylase (mμmol *p*-aminophenol/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 *p*-aminobenzoate/hr)[c]	32.6[b] ± 6.8	45.4 ± 6.2

[a] Difference between mean and following mean is significant: $p < .001$; [b] $p < .01$.
[c] Per mg of microsomal protein.

increase in membranous material. Specific activities of glucose-6-phosphatase and aromatic hydroxylase were reduced.

Discussion. Hepatic biochemical changes induced by CT represent proliferative alterations which result in increased capacity of hepatic function. An increase in each enzymatic and compositional parameter investigated is evident when the nearly 3-fold liver hypertrophy and more than doubled quantity of microsomal protein per gram of liver is considered.

Proliferation of ER in response to CT administration results in the formation of new membranes which are distinct in morphology, enzyme content, and composition. Investigation of these membrane characteristics offers additional information about the heterogeneous nature and metabolic capabilities of the ER. Other investigators have demonstrated that microsomal subfractions are distinguished on the basis of morphology (18), differing sensitivity to cations (18), enzyme distribution (19), cytochrome distribution (20), incorporation of lipid precursors (17), neutral lipid content (17), and response to phenobarbital (21). Phospholipid–protein relationships for smooth and rough subfractions of microsomes from control rat livers are similar and cholesterol/protein is higher in smooth microsomes than in the rough variety; furthermore, these lipid relationships are maintained in the subfractions of microsomes recovered from the proliferated ER following phenobarbital administration to the rat (17). Total microsomes isolated from rats fed CT are characterized by a moderate increase in phospholipid/protein, a significant decrease in cholesterol/protein, and variable changes in specific enzyme activity. Thus the proliferation of ER is more complex than a nonspecific increase in membranous material. The neogenesis of membranes represents preferential synthesis, increased incorporation, or decreased degradation of phospholipid; a reduction in synthesis, decrease in incorporation, or increase in loss of cholesterol; and an altered rate of synthesis or degradation of enzymes.

The biochemically demonstrated proliferation of membranous material induced by CT is morphologically represented by increased vesicular smooth ER and formation of CMA. Hypertrophic vesicular smooth ER is associated with increased enzyme activity (22), unaltered phospholipid/protein (17), and increased cholesterol/protein (17) of the microsomes. Thus membranes with low choles-

terol and high phospholipid levels are likely representative of CMA.

Structures similar to CMA have been observed in experimental hepatomas (23) and following the administration of numerous compounds including DDT (24), chlorodibenzodioxin (25), phenobarbital (26), thiohydantoin (27), triparanol (28), aflatoxin (29), ethionine (30), carbon tetrachloride (31), and amino azo dyes (32). Morphologic studies on the sequential events leading to their formation resulted in the conclusion that the arrays originate from the rough ER (25). Other investigators have described structures similar in appearance to CMA as a proliferative development of the smooth ER (29, 30, 32). However, the indicated lower cholesterol and higher phospholipid levels for the CMA suggest the structures are a distinct entity of the ER.

Certain functional properties are in agreement with the biochemical composition of the new membranes. Consistent with increased proportion of phospholipid within the membrane structure is the proposal that the proliferated membrane system provides a site for localization of the hydrophobic CT. The phospholipid may enhance the suitability of the membrane for sequestration of the foreign material or for approximation of the compound to the enzymes of the membrane. The proximity of the CMA to the lipid droplets, also a probable location of the lipid soluble triphenyl, provides accessibility of the lipid component of the membrane of this structure to the compound. Cholesterol within the membrane structure has been related to rigidity of the membrane by its ability to condense the unsaturated fatty acids of the phospholipids (33). 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 of the phospholipids.

The study of total microsomes isolated from livers of rats fed CT have demonstrated an association of increased phospholipid and decreased cholesterol levels with the morphologic appearance of proliferated vesicular smooth ER and CMA. The nature of this relationship, and particularly the function of the CMA, must be clarified by separation of the CMA as a distinct microsomal entity from the smooth and rough fractions and analysis of all fractions for phospholipid, cholesterol, protein, and enzymatic activity.

Summary. Ingestion of a highly chlorinated triphenyl by rats induces liver hypertrophy with proliferation of the vesicular smooth endoplasmic reticulum and formation of large concentric membrane arrays within the cytoplasm of the hepatocytes. At 21 days these changes result in increased hepatic function. Associated with the intracellular membrane alterations are increased phospholipid/protein, decreased cholesterol/protein, and altered specific enzyme activities of the microsomes. It is proposed that the phospholipid and cholesterol levels of the new membranes are compatible with an altered structure for localization of the foreign lipid soluble compound.

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