

Adenofibrosis in the Rat Liver

With Persistence of Polychlorinated Biphenyls in Adipose Tissue

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Fifty male Sherman strain rats were fed 500 ppm of a polychlorinated biphenyl (PCB) (Aroclor 1254) for six months. Five each were killed zero, one, two, three, four, six, eight, and ten months after exposure to Aroclor had ceased. The livers of these rats were examined by light and electron microscopy. Liver lesions persisted although exposure to PCBs ceased.

Ten months after exposure ceased, 1,192 ppm PCBs were still present in the rats' adipose tissue and 22.65 ppm in the rat livers.

Aroclor patterns found in the tissues by electron capture gas chromatography differed from patterns of dietary Aroclors. Mass spectral analysis of liver and adipose tissue revealed three major Aroclor components with masses of 324, 358, and 392. These contained isotopic clusters indicative of the presence of C₁, C₁, and C₁, respectively.

In a previously reported study¹ Sherman strain rats were fed polychlorinated biphenyls (PCBs) (Aroclor 1254 and Aroclor 1260). The rats developed characteristic morphological changes in the liver. These changes consisted of hypertrophy of the liver cells, a brown pigment in Kupffer cells, lipid accumulation in the cytoplasm of hepatocytes and, at the higher dietary levels, adenofibrosis. Because of the wide distribution of PCBs in the environment² and

their persistence, the hepatic lesion was studied further. The present study was undertaken to see whether, once exposure to PCBs was stopped, the morphological changes produced in the liver might disappear.

Materials and Methods

A total of 50 male weanling, specific-pathogen-free, random-bred Sherman strain rats were tagged individually and group-caged, ten rats per cage. They were given Aroclor 1254 in their diet for six months. The dose of 500 ppm, which is equivalent to an average daily intake of 36.4 mg/kg body weight,¹ was chosen because in the previous study all ten exposed male rats developed adenofibrosis within a period of eight months. The Aroclor was mixed into ground laboratory chow as previously described.¹ After six months of

Fig 1.—Area of adenofibrosis in liver section consisting primarily of ducts surrounded by flattened epithelium. Ducts are surrounded by little fibrosis (hematoxylin-eosin, original magnification $\times 150$).



Fig 2.—Rat liver section showing cluster of small columnar cells resembling pancreatic tissue adjacent to blood vessel. Hepatocytes surrounding lesion are vacuolated (hematoxylin-eosin, original magnification $\times 175$).

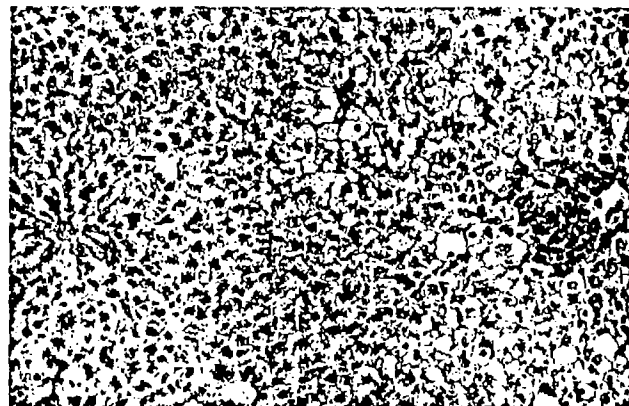


Fig 3.—Section magnification $\times 5$

Fig 4.—Portion citrate-uranyl acetate

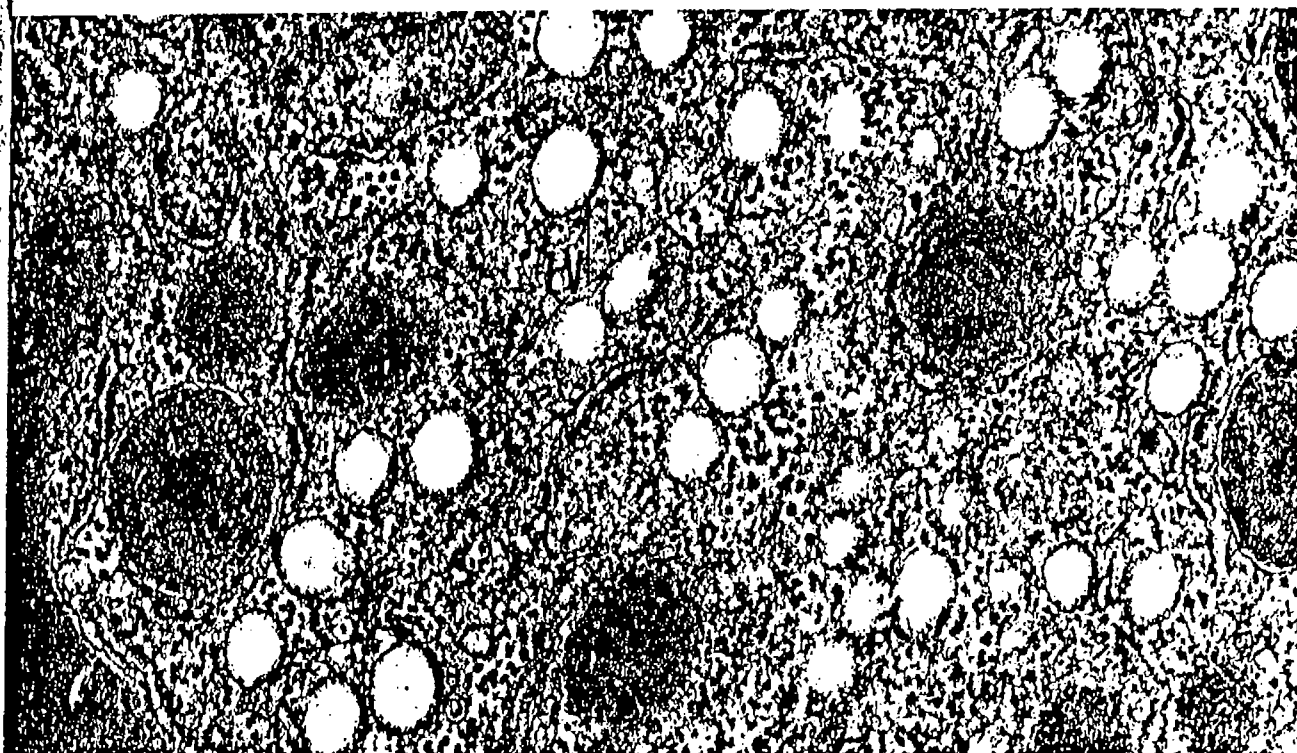


Fig 3.—Section of hepatic tissue in latter part of recovery phase illustrating numerous small lipid vacuoles (LV) (lead citrate-uranyl acetate, original magnification $\times 51,300$).

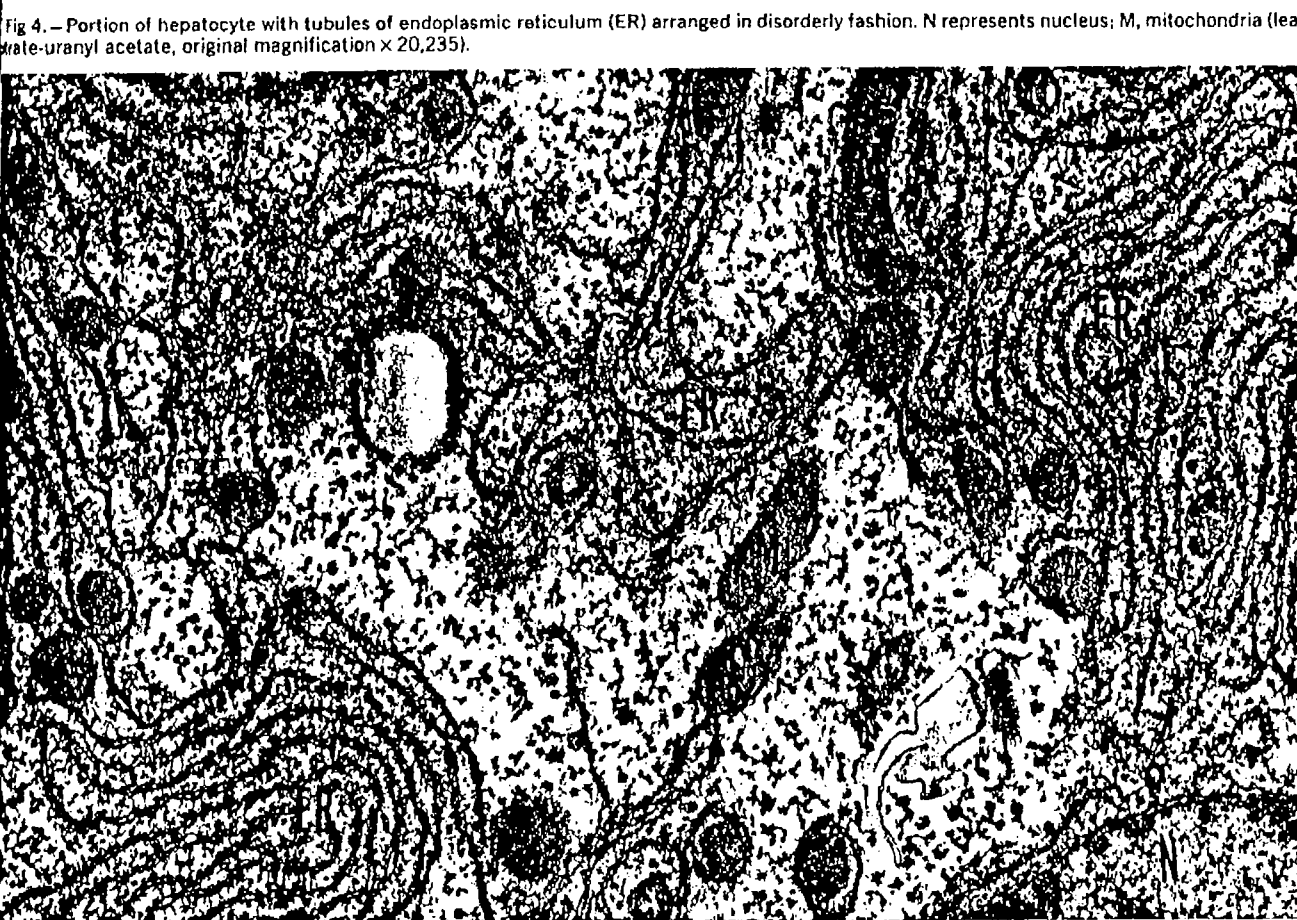


Fig 4.—Portion of hepatocyte with tubules of endoplasmic reticulum (ER) arranged in disorderly fashion. N represents nucleus; M, mitochondria (lead citrate-uranyl acetate, original magnification $\times 20,235$).

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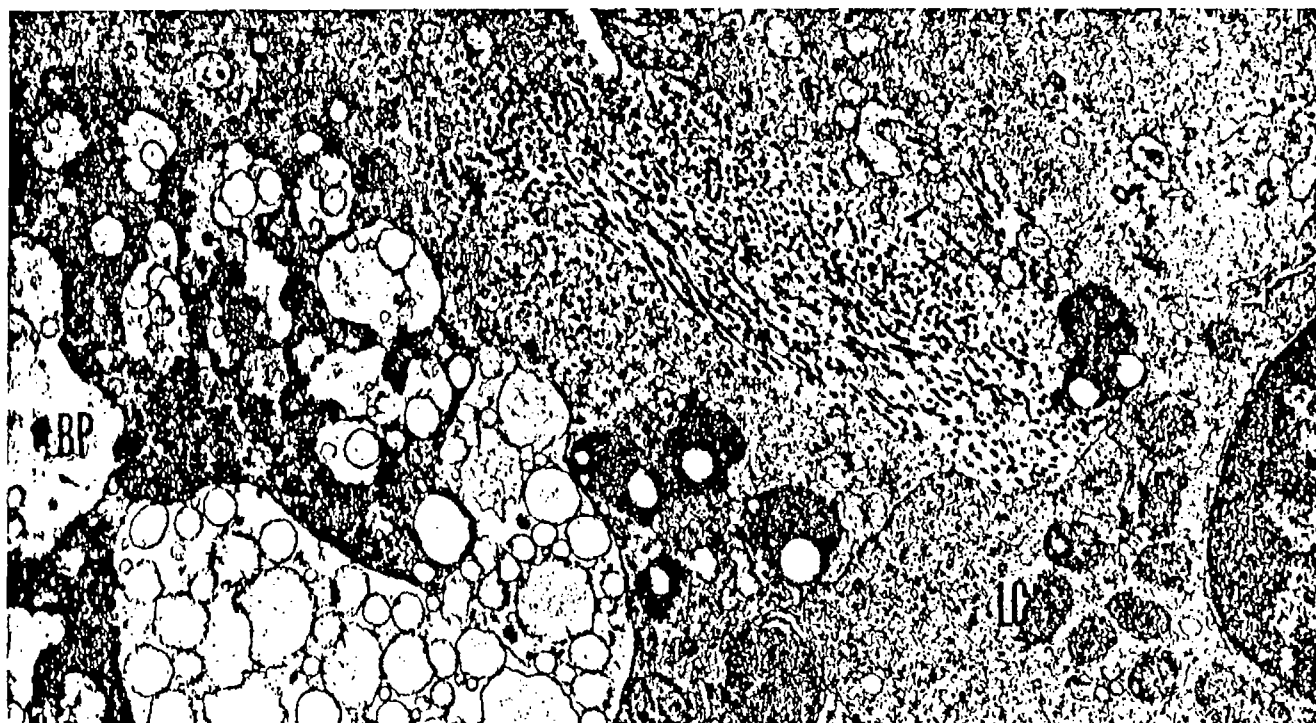


Fig 5. - Border of area of adenofibrosis in liver. Note round clusters of dark granules intermixed with web-like clusters of vacuoles. These formations illustrate appearance of brown pigment (BP). C represents collagen; LC, liver cell (lead citrate-uranyl acetate, original magnification $\times 20,235$).

receiving the experimental diet, the rats received no more Aroclor and were fed plain laboratory chow. At this time five rats were killed. Additional killings of five randomly selected rats were made one, two, three, four, six, eight, and ten months after exposure to PCB had been discontinued. At autopsy the livers were removed, weighed, and the tissue fixed in phosphate-buffered 5% glutaraldehyde for three hours for electron microscopic examination, post-fixed in 1% osmium tetroxide, embedded in maraglass, sectioned with a glass knife, and stained with lead citrate and uranyl acetate. Tissue for the light microscope was fixed in 4% buffered formaldehyde solution and stained with hematoxylin and eosin. Frozen tissue of selected formalin-fixed hepatic tissue was cut on the cryostat and stained with oil red O to demonstrate lipids. At the time of the last sacrifice, ten months after exposure to PCB-containing diets had been discontinued, adipose tissue and liver from seven previously exposed rats and the adipose tissue and liver from two rats of the same age that had been fed only plain chow were analyzed for the presence of PCBs by electron capture (EC) gas chromatography according to methods described by Curley et al.²

In order to determine which Aroclor constituents were of such chemical stability that they remained at a measurable

residual level ten months after dietary intake had ceased, adipose and hepatic composites were analyzed by gas chromatography-mass spectrometry. The conditions for mass spectral analysis were as follows: the mass spectrometer was interfaced with a gas chromatograph and equipped with a mass marker accurate within ± 0.3 mass units. The gas chromatographic column temperature was 220 C; flash heater, 235 C; glass-coiled column, 3.05 m $\times$ 0.64 mm; 1.5% OV-17/1.95% QF-1 on 60/80 mesh chromosorb "W" (high performance, acid washed, dimethyldichlorosilane treated), helium pressure; 0.84 kg/sq cm; flow rate (rotameter setting 3), 45 ml/min; separator temperature, 225 C; source energy, 70 eV; accelerating voltage 3.5 kV; trap current 60 μ amp; box current 50 μ amp, and leak current 8 μ amp.

Results

During the course of the experiment three rats died. The livers of the rats that were killed at the time exposure to Aroclor 1254 was discontinued were enlarged, with average weights of 6.32% of the body weight. When the experimental diets had been discontinued for four months, the liver made up only 3.6% of the body weight, and after eight months recovery the average weight

amounted to 3.34% of the average body weight. The fraction of the body weight for the liver in control adult male rats ranges from 2.44% to 2.59%.¹

Forty livers were studied microscopically. The hepatocytes were enlarged in 39 livers and all 40 livers showed either vacuolated or foamy cytoplasm because of increased lipid accumulation. Cytoplasmic inclusions similar to those described previously⁴ were observed in the livers of 24 rats. Adenofibrosis, which in many instances was very extensive, was present in 36 of the 40 livers, and a brown pigment was noted in the Kupffer cells and other macrophages in 25 livers. Of the four rats that did not develop adenofibrosis, one each was killed two and six months after the poisoned diets were discontinued. The other two rats without adenofibrosis were killed ten months after onset of exposure. The adenofibrosis ranged in size from grayish-white lesions measuring 0.5 to 1 cm to areas of 5 cm with a pitted surface. Quite often the lesion was multicentric. The adenofibrosis¹ is illustrated in Fig 1 and 2. In this study the appearance of

Fig 6. - Gas chromatogram of Aroclor 1254 extract of adipose tissue.

the lesion is extensive lesions of glandular cells that are rounded and contain larger lipid droplets and connective tissue sheath.

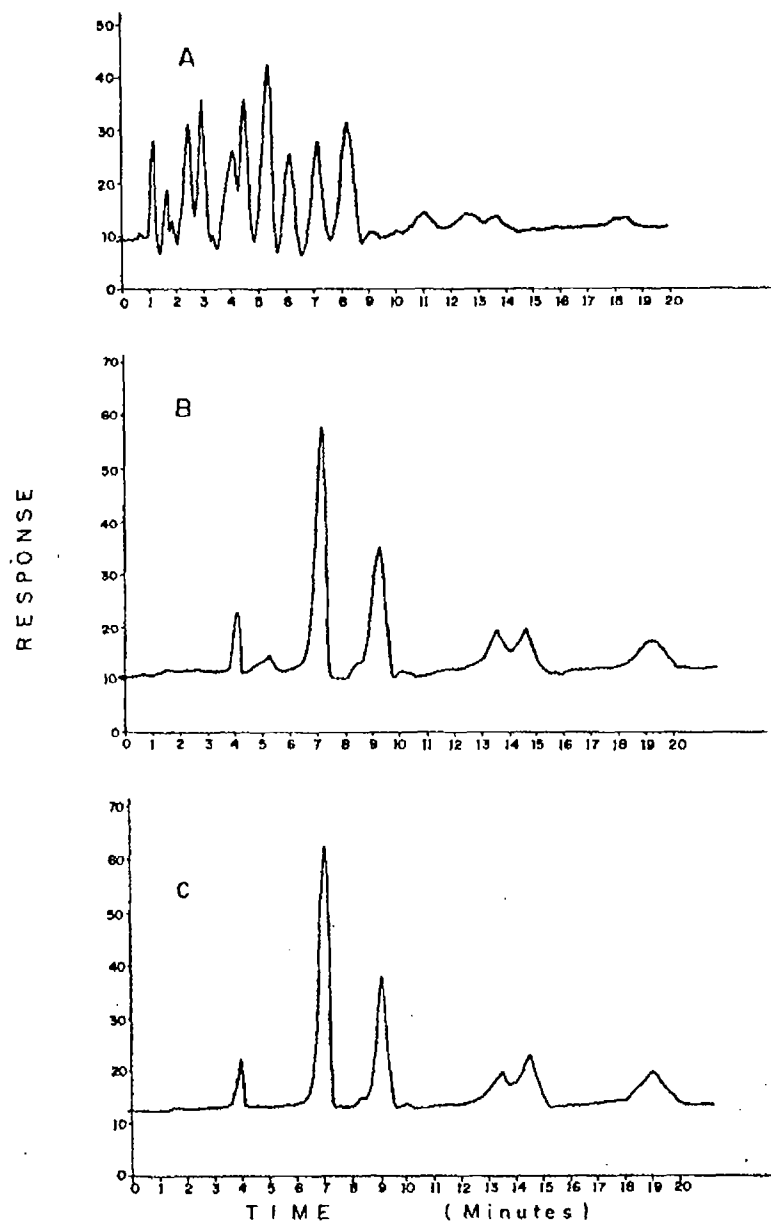


Fig 6.—Gas chromatographic traces of Aroclor 1254 standard (A), hepatic tissue extract (B), and extract of adipose tissue (C). Response expressed as percentages, with 100% = 1.0 mv.

the lesion, particularly when it was extensive, varied a great deal. Small lesions consisted predominantly of glandular pale-staining epithelial cells that formed ducts and were surrounded by very little fibrosis. The larger lesions had extensive fibrosis and contained collagen, and often the ducts which were formed by the epithelial pale-staining cells were

markedly dilated. The dilated ducts contained mucus or necrotic debris, and at times formed small cysts measuring up to 0.5 cm in diameter. Small dilated ducts were lined by columnar epithelium, and larger ones by flattened epithelium (Fig 1).

In areas of extensive fibrosis, and also adjacent to blood vessels in areas of normal hepatic parenchyma (Fig 2),

clusters of small glandular cells were seen in 15 of the livers which resembled the epithelium observed normally in the pancreas. These small clusters of pancreatic-like tissue have not previously been described in the livers of rats or of other species. Special stains for esterase, esterase with sodium taurocholate, and tryptophan showed a positive reaction indicative of salivary gland tissue. The significance of these cells is presently not understood. Normally tissue of a pancreatic type is not observed in the livers of our rats. Clusters of small columnar epithelial cells resembling pancreatic tissue also occurred in portal triads without being surrounded by typical adenofibrosis. In the present study, one to two rats at each killing showed these small columnar cells with highly acidophilic cytoplasm and a small, round nucleus.

In the older animals, the fibrosis was quite extensive in some instances and the glandular proliferation was present mainly at the periphery of the lesion. Signs of regression of the lesion were not noted, except for the disappearance of epithelial cells from the center of the lesion. Furthermore the lipid accumulation and the hypertrophy of the hepatic cells remained constant throughout the study. Extensive areas of necrosis were observed in three of the 40 livers.

In addition to the change described previously,¹ electron microscopic examinations of sections of liver tissue showed numerous small lipid vacuoles in the cytoplasm of many hepatocytes, particularly in the latter part of the recovery phase (Fig 3). The smooth and rough endoplasmic reticulum was arranged in a disorderly fashion (Fig 4). Many of the mitochondria were atypical and surrounded by double membranes.

The electron microscopic appearance of the brown pigment present in the macrophages and Kupffer cells is illustrated in Fig 5. Large clusters of dark granular material, intermixed with lipid vacuoles, were observed, as well as round clusters of granular material about the size of mitochondria. In our previous studies it was found that at least some of this pig-

ment contained iron¹ but in addition high concentrations of uroporphyrin (75% 8-carboxyprophyrins and 25% 7-carboxyprophyrins) were found in the livers,⁵ which could account for some of the pigment. In addition to these findings, oil red O stain demonstrated granules of red-staining lipid material in the area of the brown pigment.

Since the morphological changes in the liver did not disappear, the remaining seven rats were killed after a ten-month recovery phase and their adipose tissue and livers analyzed for Aroclor. In adipose tissue, the concentrations of PCBs ranged from 924 ppm to 1,688 ppm, with an arithmetic mean of 1,192 ppm. The concentrations in the liver ranged from 17.30 ppm to 26.24 ppm, with an arithmetic mean of 22.65 ppm. The PCB levels in control adipose and liver tissues were <1.0 ppm.

The gas chromatogram obtained from the EC analysis of adipose tissue did not differ from that of liver, but was significantly different from standard Aroclor 1254. An average of seven peaks was observed as a residue in the tissues. Under these gas chromatographic conditions, at least 18 peaks have been observed in the standard material (Fig 6). The difference is gas chromatographic patterns between stored and ingested Aroclor has been observed before in this laboratory and by other researchers in the field.^{6,7}

The total ion current (TIC) chromatogram for the liver tissue composite is shown in Fig 7. Mass spectral analysis revealed the presence of only three major Aroclor components with masses of 324, 358, and 392. These components contained isotopic clusters indicative of the presence of Cl_5 , Cl_6 , and Cl_7 , respectively. Adipose tissue analysis by mass spectrometry revealed the same patterns. Although only three different molecular components were present, examination of the EC and TIC chromatograms coupled with measured intensities from multiple scanning of eluting peaks indicates that stereoisomers of these three molecules are obviously present. Peaks 1 to 3 (Fig 7) did not contain any

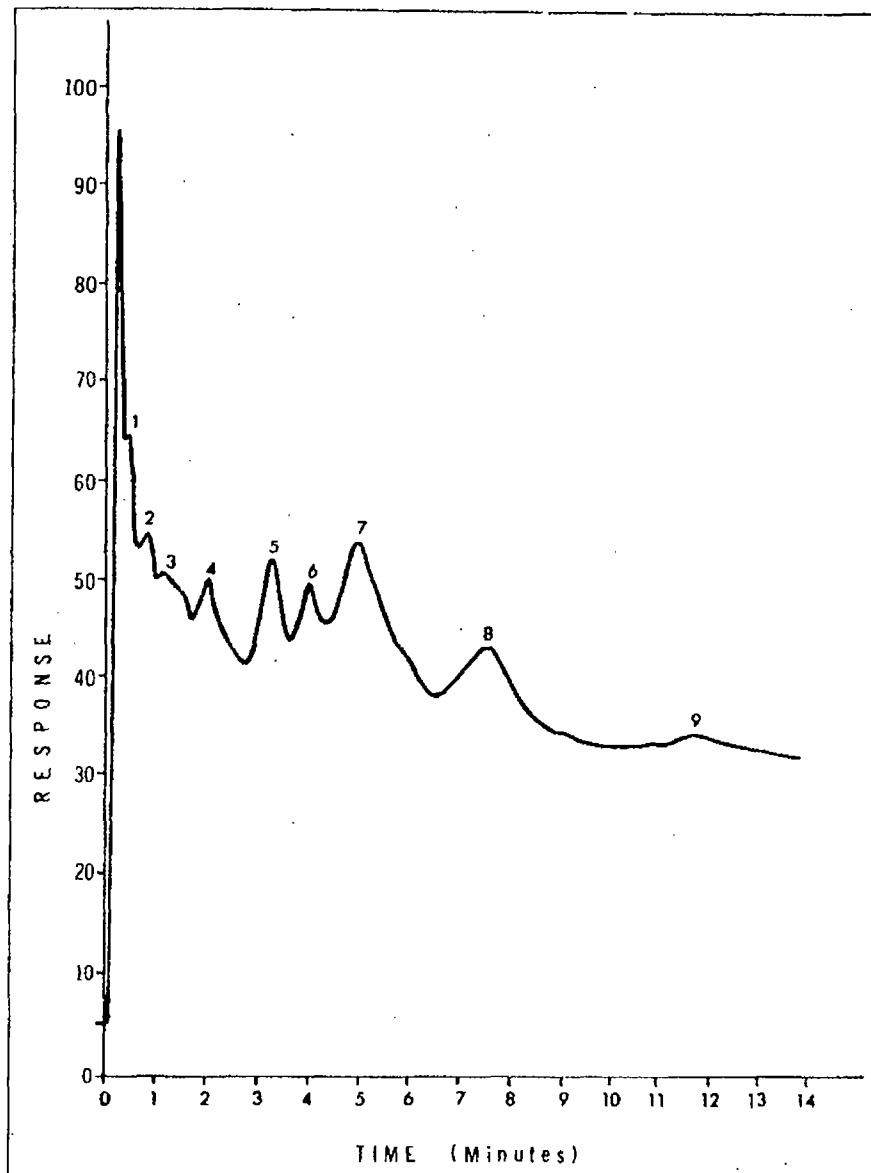


Fig 7.—Total ion current chromatogram of sample of composite liver. Response expressed as percentages, with 100% = 10.0 mv.

chlorinated moieties, while other peaks observed in the liver TIC contained the following molecular ions: peak 4 (324); peaks 5 to 7 (392 and 358); and peaks 8 to 9 (392).

Comment

The adenofibrosis of the liver produced in this study and the one previously reported¹ is the result of Aroclor consumption. Whether the Aroclor itself, or a contaminant such as a chlorinated dibenzofuran, is responsible still needs to be resolved.

A study of the lesion shows that

epithelial proliferation occurs in the periphery, while the central portion of the lesions contains primarily fibrous tissue. The present study does not completely resolve the question of reversibility of this lesion since the storage levels of PCBs in adipose tissue and liver may have been sufficiently high to stimulate continued growth of the altered hepatic tissue. The levels in the liver suggest that a constant low-level turnover of PCBs takes place in the rats. In a previous study, Curley et al³ found levels of 10,000 ppm Aroclor 1254 in adipose tissue of rats that had been fed 500

ppm of the mixture in their diet for eight months. This finding indicates that a great deal of the Aroclors had been mobilized from the adipose tissue and excreted in the present study. The storage levels found ten months after onset of recovery are in the same range of those of rats fed 100 ppm PCBs in their diet for eight months.³ The dietary level of 100 ppm produces a much lower incidence of adenofibrosis,¹ and it is possible that adenofibrosis represents a permanent lesion, particularly since the percent of body weight of the livers had decreased.

The Aroclors found in the tissues differed from those fed in the diet. Metabolism of lower chlorinated biphenyls is considered as a possible explanation for the differences observed analytically between consumed and stored PCB. Earlier work of Block and Cornish⁸ has shown that biphenyls and chlorobiphenyls can be hydroxylated and excreted in the urine of rabbits. A more recent study by Hutzinger et al⁹ indicates that the rat is capable of hydroxylating mono-, di-, and tetrachlorobiphenyls, but not hexachlorobiphenyls. Since Aroclor 1254 averages five chlorines per molecule, it is conceivable that the heavier chlorinated moieties could remain as a residue.

P-dimethylaminoazobenzene also produces adenofibrosis. Edwards and White¹⁰ and Bennett et al¹¹ described the lesion in rats when a mixture of chlorinated naphthalenes and chlorinated diphenyls were fed. Unfortunately, the finding was not well described in Bennett et al's report, but Edwards and White mentioned Bennett et al's finding as another example of the induction of adenofibrosis by chemicals in an excellent description of the lesion. A critical review of the histopathogenesis of this lesion was made by Stewart and Snell¹².

The presence of the brown pigment in Kupffer cells and macrophages is of interest and was to a lesser extent

also observed in the earlier study.¹ Electron microscopic examination of the pigment shows that particularly the large clusters of it resemble very closely illustrations of ceroid pigment.¹³ Experimentally ceroid pigment can be produced by hemorrhage into fatty tissues rich in unsaturated fatty acids, particularly in the absence of an antioxidant (vitamin E deficiency). Ceroid pigment is commonly referred to as lipofuscin. These lipid pigments are at times associated with hemosiderin and may contain iron-positive granules as in the present case.¹ Ceroid granules were also described by Edwards and White in the livers of rats fed butter yellow. Bennett et al also observed a brown pigment in rats fed a mixture of chlorinated naphthalenes and chlorinated biphenyls. These authors claimed that it failed to stain in a manner characteristic of either hemosiderin or hemofuscin. The porphyrins demonstrated in the livers of our rats fed Aroclor may also contribute to the overall pigment deposits. Ceroid pigment increases in people with age and has therefore been called "Abnutzungspigment" by the Germans. Attempts have been made to relate it to senility. It has been associated with neuronal ceroid-lipofuscinosis (Batten disease) in man. In minks, a disease called yellow fat disease¹⁴ is associated with the accumulation of excessive amounts of ceroid in adipose tissue. The accumulation of ceroid in mink can be prevented by addition of vitamin E to the diet. It occurs when the food rations contain high percentages of fish scrap. However, Gorham et al suggest that other factors may also be involved, since high dietary percentages of fish scrap did not promote growth as well as the stock ration, even when vitamin E was added to the diet. These observations raise the question whether high doses of vitamin E, and possibly the addition of choline to the diet, could prevent or

minimize the observed effects of PCBs on the liver of our rats.

Richard L. Moore assisted with the animal tests.

Linda W. Anderson assisted with preparation and interpretation of the electromicrographs.

Annie R. Alford performed the tissue preparations.

H. L. Stewart, MD, and the tissue laboratory of the National Cancer Institute, Bethesda, Md., performed evaluations of liver slides.

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