

Allen, J.E.

MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE LIVER OF RATS FED POLYCHLORINATED BIPHENYLS

J. R. ALLEN and L. J. ABRAHAMSON

Department of Pathology, University of Wisconsin Medical School, and
Regional Primate Research Center, University of Wisconsin,
Madison, Wisconsin 53706

Rats fed diets containing 0.1 percent of three polychlorinated biphenyls (PCBs) (Arochlor 1248, Arochlor 1254, Arochlor 1262) for six weeks show a progressive enlargement of the liver. This liver hypertrophy is attributed to proliferation of the smooth endoplasmic reticulum, development of large membranous concentric arrays, and increase in lipid droplets within the cytoplasm of the affected liver cells. Liver homogenates show increased levels of protein and RNA and reduced concentrations of DNA. Microsomal fractions have increased levels of protein and phospholipids, and reduced levels of cholesterol. Also, there are modifications in the activity of certain hepatic microsomal enzymes. By the sixth week, the animals have progressed from a stimulatory effect on the liver by the PCBs to a stage where regressive hepatic changes are occurring, such as a decreased activity of microsomal enzymes, dissolution of concentric membrane arrays, vesiculation of the endoplasmic reticulum, and accumulation of lipid droplets within the cytoplasm of the affected cells.

Interest was manifested in the polychlorinated biphenyls (PCBs) as early as 1881 (Schmidt and Schultz 1881) and they found widespread commercial use by 1930 (Penning 1930). Workers responsible for the manufacture of these and closely related compounds periodically developed acneform lesions primarily of the face (Jones and Alden 1936, Good and Pensky 1943). Also, there have been isolated reports of hepatic dysfunction following contact with PCBs (Drinker *et al.* 1937, Flinn and Jarvik 1939). In 1968, over 1,000 persons consumed rice oil contaminated with PCBs (Katsuki 1969). In addition to acne, the exposed persons developed hyperpigmentation of the skin and subcutaneous edema. There was a decided increase in serum triglycerides and proliferation of the smooth endoplasmic reticulum (ER) of the liver hepatocytes. Experimental animals given PCBs also experienced liver hypertrophy primarily as the result of proliferation of the hepatic ER (Vos 1972). Also, there have been isolated reports of modifications in various hepatic microsomal enzyme activities following exposure to PCBs (Norback and Allen 1970, Fujita *et al.* 1971, Allen and Abrahamson 1972).

The study reported here shows that the symptoms and lesions in rats produced by three of the more common PCB mixtures are quite similar. The decided increase in liver size of these animals is attributed to a proliferation of the smooth ER, formation

of large cytoplasmic concentric membrane arrays (CMAs), and the accumulation of lipids within the hepatic cells. In addition to an increase in number and a change in the composition of the membrane, there are modifications in the activity of certain microsomal enzymes that persist throughout the period of examination.

Materials and methods

Male, Sprague-Dawley rats, having an average weight of 100 grams, were fed *ad libitum* on a ground commercial diet (Rockland Mouse/Rat Diet, Tekland, Inc., Monmouth, Illinois) containing 0.1 percent of one of three PCBs (Arochlor 1248, Arochlor 1254, and Arochlor 1262) (Monsanto Company, St. Louis, Missouri). After having been deprived of food for 24 hours, four rats from the three experimental groups and the one control group were weighed and killed on the 1st, 3rd, 7th, 14th, 21st, and 28th day and at the end of the sixth week. The rats were sacrificed by exsanguination. Blood collected at this time was analyzed for total white blood cell count, hemoglobin, hematocrit, differential white count, total serum protein, and blood urea nitrogen. For histologic evaluations, small sections of the tissues were placed in 10 percent neutral formalin for 24 hours. They were subsequently dehydrated, embedded in paraffin, sectioned at five microns, and stained with hematoxylin and eosin. For electron microscopic studies the hepatic tissue was cut into small cubes, fixed in osmium tetroxide and buffered with veronal acetate (Caulfield 1957) for $1\frac{1}{2}$ hours. Subsequently, they were dehydrated through a graded series of ethanol and embedded in an epoxy resin mixture (Mollenhauer 1964). Sections of the tissues were cut on an ultramicrotome, placed on uncoated copper grids, stained with uranyl acetate, and examined with an electron microscope. The remaining portion of the liver was chopped into small pieces and homogenized in two volumes of KCl solution (1.15% W/V) at 0°C using a Potter homogenizing tube and a teflon pestle. Levels of protein (Lowry *et al.* 1951), and RNA and DNA (Munro and Fleck 1966, Ceriotti 1952) were determined on the homogenates. A postmitochondrial supernatant was obtained by centrifugation at $10,000 \times g$ for 20 minutes. The pellet was recentrifuged under the same conditions after one wash with KCl solution. The two supernatants were combined and spun at $105,000 \times g_{max}$ for 85 minutes to pellet the microsomes. The pellets were washed in KCl solution and repelleted for 80 minutes. The resulting pellets were resuspended in a volume of KCl solution equal to three volumes of liver and stored at -70°C. Activities of aromatic hydroxylase, nitroreductase, *N*-demethylase, nitrophenylacetate hydroxylase, and cholesterol were determined from the microsomes as previously described by Norback and Allen (1972).

Results

Throughout the course of the experiment, all of the rats ate well. However, those that are given PCBs in the diet do not gain weight as rapidly as do the controls; the Arochlor 1248-fed rats are most severely retarded followed by the Arochlor 1254-and, lastly, the Arochlor 1262-fed rats. Other than being reduced in size, there are no gross abnormalities as a result of ingesting PCBs. Hemograms of the animals display

Table I. Hematological Changes in Rats Fed Various Polychlorinated Biphenyls

Blood analysis (unit) ^b	Hematological value ^a after feeding listed PCB for stated period								
	Arochlor 1248			Arochlor 1254			Arochlor 1262		
	1 day	4 wk	6 wk	1 day	4 wk	6 wk	1 day	4 wk	6 wk
Hb (w/v%)	13.9 ± .6	18.1 ± 1.5	20.9 ± 1.5	14.6 ± .8	17.5 ± 1.1	17.9 ± .6	13.8 ± .5	16.3 ± .4	17.9 ± 1.2
HCT (%)	41.0 ± 1.4	54.3 ± 5.5	64.8 ± 5.9	43.4 ± 2.0	49.8 ± 3.9	53.5 ± 2.4	40.6 ± .9	45.9 ± 1.3	49.1 ± 2.5
WBC (x10 ⁹)	6.8 ± .6	12.2 ± 3.4	9.3 ± 1.7	6.3 ± 2.0	10.8 ± 1.9	10.6 ± 2.6	6.6 ± 1.1	6.6 ± 2.3	8.2 ± 2.6
Npl (%)	8.3 ± 2.5	18.5 ± 6.1	52.8 ± 3.2	8.0 ± 1.2	15.3 ± 5.0	42.5 ± 10.1	6.3 ± .5	9.0 ± 8.7	20.4 ± 14.5
Lct (%)	91.8 ± 2.5	81.5 ± 6.1	47.0 ± 3.4	92.0 ± 1.2	84.3 ± 5.7	59.0 ± 9.2	93.8 ± .5	90.5 ± 8.4	79.6 ± 14.5

^aMean ± standard deviation.

^bHb = hemoglobin; HCT = hematocrit; WBC = total white blood cells; Npl = neutrophiles; Lct = lymphocytes.

Table II. Six-Week Organ Weights of Rats Fed Polychlorinated Biphenyls

Organ	Weight of organ, % of total body weight, after feeding listed PCB			
	Controls	Arochlor 1248	Arochlor 1254	Arochlor 1262
Lungs	0.58 ± .04	.92 ± .11	1.03 ± .30	.72 ± .10
Heart	0.38 ± .02	.57 ± .05	0.46 ± .03	.43 ± .06
Liver	2.77 ± 1.0	8.88 ± .08	9.30 ± .94	9.85 ± 1.18
Kidney	0.87 ± .03	1.34 ± .08	1.13 ± .09	.98 ± .11
Spleen	0.21 ± .01	.26 ± .03	0.22 ± .04	.20 ± .02
Thymus	0.22 ± .02	.09 ± .08	0.09 ± .03	.15 ± .04
Testes	1.21 ± .12	1.51 ± .39	1.80 ± .16	1.79 ± .17
Brain	0.58 ± .05	1.14 ± .03	1.02 ± .12	.85 ± .15

* Data from four rats, mean ± standard deviation.

a gradual increase in levels of hemoglobin and hematocrits (Table I). No major changes are found in the total white cell count. However, a decided relative neutrophilia occurs in the Arochlor 1248-fed animals and, to a lesser extent, in the Arochlor 1254- and Arochlor 1262-fed groups. The levels of blood urea nitrogen and total serum protein do not change appreciably during the PCB-ingestion period.

The reduction in body weight of the Arochlor fed-rats is further exemplified when compared to organ weights that were obtained at necropsy (Table II). The organs from the experimental animals comprise a greater percentage of the total body weight, when compared to those of the control animals, primarily as a result of the slower growth rate and reduced amount of body fat. The increase in liver size is obvious within one day and becomes larger as the experiment progresses. At the same time, there is hypertrophy of the liver, thymic regression occurs in the Arochlor 1248- and Arochlor 1254-fed animals.

Within two to three weeks, small cystic spaces develop throughout the hepatic tissue of animals fed Arochlor 1248 and Arochlor 1254, and, to a lesser extent, in the Arochlor 1262-fed animals (Fig. 1). In these focal areas, the hepatocytes first accumulate large numbers of small fat droplets. The coalescence of the fat droplets is followed by a breakdown of the cell membranes and disruption of the cell. As groups of closely associated cells disintegrate, the cysts become apparent. Remnants of the cell membrane, proteinaceous material, and a few inflammatory cells are obvious in many of the cystic spaces.

In addition to the cystic areas, relatively large, well-circumscribed areas of necrosis

are present along the capsular surface, particularly in the livers of the Arochlor 1262-fed rats (Fig. 2). Instead of the cells becoming enlarged and filled with lucent droplets, as is the case in the genesis of the cysts, the hepatic parenchymal cells become shrunken, markedly eosinophilic, and their nuclei picnotic. Numerous acute inflammatory cells are dispersed among the dead cells. As the lesions became older, lymphocytes and monocytes predominate.

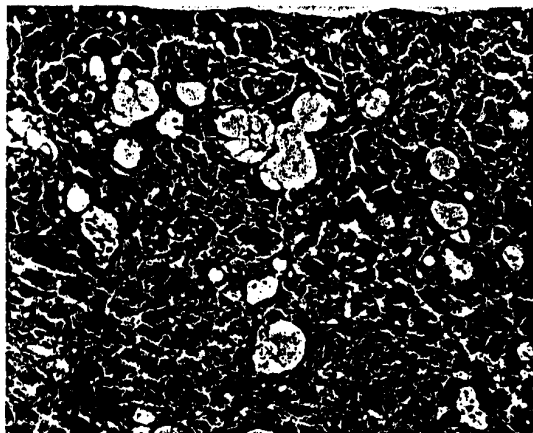


Fig. 1. Variable sized cystic spaces occur in the liver section of a rat that had received PCB in the diet for six weeks. The fenestrated structures (→) within some of these cysts represent the remnants of cell membranes. Hematoxylin and eosin stain; X 165.

The hepatic cells not involved in the focal necrosis contain abundant cytoplasm that is filled with heavily stained acidophilic material and numerous small clear droplets. Sections of the epon-embedded tissue clearly demonstrate the presence of numerous small fat vacuoles, proliferated ER, and large concentric membrane arrays (CMAs) throughout the hepatic tissue. This increase in smooth ER becomes apparent within one day and progressively greater throughout the course of the experiment. Small CMAs are observed in Arochlor 1248- and Arochlor 1254-fed rats within one day and within one week in the Arochlor 1262-fed animals. The fine structure of the hepatocytes exhibits an increase in smooth ER which is readily apparent in the livers of all animals



Fig. 2. Liver section of a rat fed PCB showing well-circumscribed areas of necrosis predominantly adjacent to the capsule, particularly in those ingesting Aroclor 1262. The lesion depicted is from a rat that had been fed the PCB for six weeks. Note the more chronic nature of the lesion as characterized by the development of connective tissue components around the periphery (→) and a predominance of chronic inflammatory cells within the enclosure. Adjacent hepatic cells appear relatively normal. Hematoxylin and eosin stain; X 165.

ingesting PCBs (Fig. 3) but not so apparent in the livers of control animals (Fig. 4). Particularly in the tissues taken during the second and third weeks, the large CMAs occupy a good portion of the cytoplasm of the hepatic cells (Fig. 5). These elaborate membranes are similar in structure to, and continuous with, the rough and smooth ER. Encircling of lipid droplets and other cytoplasmic organelles by the CMAs is a constant observation. There also appears to be a moderate increase in lysosomes which are filled with a variety of cell debris. Other organelles, including the nucleus, are normal in appearance.

By the sixth week the hepatocytes of the experimental animals contained fewer CMAs, and lipid droplets, lysosomes and autophagic vacuoles were more prevalent. In numerous cells the smooth ER appeared as fine tubular aggregates and the rough ER became vesiculated. The latter changes were more numerous in the livers of animals fed Aroclor 1248.



Fig. 3. Liver section of a rat showing decided proliferation of the smooth endoplasmic reticulum which occurs within 24 hours following the ingestion of PCBs. Uranyl acetate stain; X 5900.

Biochemical data obtained on the liver homogenates clearly indicate their hypertrophic state (Table III). As a result of membrane proliferation and more numerous ribosomes, there is an increase in levels of protein and RNA of the affected livers. Indications are that the primary effect on the livers involves the cytoplasm of the affected hepatic cells. The apparent decrease in DNA, when expressed in relation to liver weight, further signifies the proliferated state of the cytoplasmic components. Generally speaking, modifications in the protein, RNA, and DNA of the liver homogenates taken from Arochlor 1248- and Arochlor 1254-fed animals attain their maximum level by the second week of the experiment and tend to level off or decrease from the fourth to the sixth week. However, in the homogenates of livers from animals fed Arochlor 1262, maximum differences are attained by the fourth week and level off or decrease thereafter.

There are compositional changes in the microsomes of all test animals (Table IV). An increase in the protein content of the microsomes is apparent soon after the compound is ingested and persists throughout the period of PCB feeding. Also, levels of phospholipids are moderately increased. However, there is a decided decrease in cholesterol and RNA in all of the liver microsomes from PCB-fed animals. As is the case

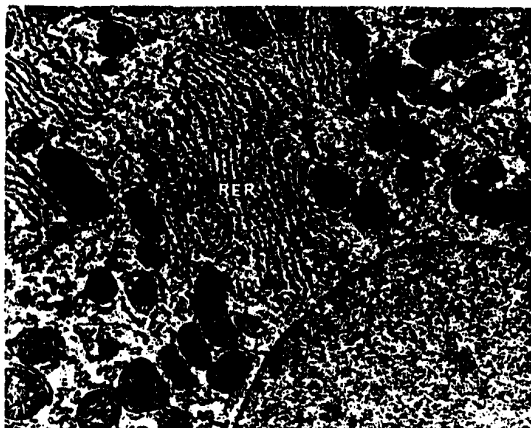


Fig. 4. Hepatocyte from the liver of a rat on a control diet. The abundant rough endoplasmic reticulum (RER) assumes a lamellar array. Isolated membranes of the smooth endoplasmic reticulum ($\rightarrow$) are dispersed among the other organelles. Uranyl acetate stain; X 10,000.

in the liver homogenates, the modifications in composition occur earlier in the Arochlor 1248- and Arochlor 1254-fed groups than in the Arochlor 1262-fed animals. However, these modifications persist at a higher level for a longer period in the hepatic microsomes from Arochlor 1262-fed animals.

An increase in activity of the microsomal enzyme nitroreductase develops during the first week in liver microsomes from animals fed Arochlor 1248 and Arochlor 1254 and the activity returns to near normal values by the second week (Tables V, VI, VII). Subsequently, the activity of nitroreductase continues to decrease through the sixth week of the PCB feeding. A much greater increase in enzyme activity of nitroreductase is experienced in the microsomes of the Arochlor 1262-fed animals. This increase occurs during the first week and persists throughout the period of examination. Increased activity of *N*-demethylase develops in all of the experimental microsomes soon after rats are placed on the diet and persists throughout the six-week feeding period, although toward the end of the period there is a gradual decline in activity. Aromatic hydroxylase, glucose 6-phosphatase, and esterase activity begin to decrease almost immediately

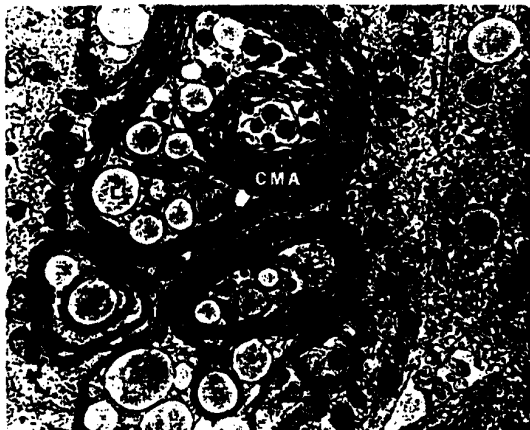


Fig. 5. Liver section of a rat showing the large concentric membrane arrays (CMA) which become prominent in the livers of all the PCB-fed rats within 2 weeks. There is continuation of these structures with the rough and smooth endoplasmic reticulum (-). Lipid droplets (L) are particularly prominent within the confines of the CMAs. Uranyl acetate stain; X 6600.

following the administration of the compounds and continues to decline throughout the period of feeding in the Arochlor 1248- and Arochlor 1254-fed animals. Similar changes are also observed in the glucose-6-phosphatase and aromatic hydroxylase activities of the Arochlor 1262-fed animals. However, a slight increase in esterase occurs by the first week, drops to near normal values by the second week, and levels off thereafter.

The aforementioned data on enzymatic activity is expressed in relation to microsomal protein. However, when these data are expressed in relation to the total liver, a decided increase occurs in the activity of all the enzymes assayed.

Discussion

There is considerable variation in the response of animals to the PCBs. In man (Jones and Alden 1936, Good and Pensky 1943) and in the subhuman primate (Allen

Table III. Changes in the Liver of Rats Fed Various Polychlorinated Biphenyls

PCB	Ratio* of indicated liver components, % of respective control value, after feeding listed PCB for given periods								
	Protein/DNA			RNA/DNA			DNA/liver wt.		
	2 wk	4 wk	6 wk	2 wk	4 wk	6 wk	2 wk	4 wk	6 wk
Arochlor 1248	132 ± 6	118 ± 7	118 ± 9	137 ± 3	113 ± 3	121 ± 4	62.3 ± 2.3	68.5 ± 4.2	60.4 ± 6.0
Arochlor 1254	164 ± 10	104 ± 7	112 ± 13	164 ± 4	130 ± 2	117 ± 6	51.7 ± —	63.6 ± 4.1	72.4 ± 5.0
Arochlor 1262	170 ± 13	174 ± 31	197 ± 25	172 ± 10	212 ± 10	141 ± 8	53.3 ± 3.0	42.3 ± 1.6	56.5 ± 4.4

*Data from four rats, mean ± standard deviation.

Table IV. Compositional Changes in Hepatic Microsomes of Rats Fed Various Polychlorinated Biphenyls

PCB	Indicated ratio*, % of respective control value, after feeding listed PCB for given period								
	Protein/Unit of liver wt			Phospholipid/Protein			Cholesterol/Protein		
	2 wk	4 wk	6 wk	2 wk	4 wk	6 wk	2 wk	4 wk	6 wk
Arochlor 1248	156 ± 13	110 ± 15	81 ± 8	117 ± 8	124 ± 8	154 ± 13	67.0 ± 3.5	71.1 ± 7.2	110 ± 14
Arochlor 1254	138 ± 10	160 ± 13	125 ± 3	104 ± 12	139 ± 6	141 ± 20	49.1 ± 3.4	69.9 ± 10.0	88.8 ± 12.9
Arochlor 1262	218 ± 12	244 ± 20	231 ± 9	132 ± 28	127 ± 7	127 ± 4	71.6 ± 11.7	57.0 ± 13.6	69.2 ± 7.4

*Data from four rats, mean ± standard deviation.

MONS 085644

Table V. Enzymatic Changes in Hepatic Microsomes of Rats Fed Arochlor 1248

Enzyme (unit)	Enzyme assays*, % of control level, at indicated feeding period		
	2 wk	4 wk	6 wk
Aromatic Hydroxylase (nmol <i>p</i> -aminophenol/30 min)	24.9 ± 1.1	29.0 ± 5.0	14.4 ± 2.7
<i>N</i> -demethylase (nmol formaldehyde/30 min)	136 ± 11	153 ± 8	150 ± 12
Nitroreductase (nmol <i>p</i> -aminobenzoate/hour)	104 ± 35	52.0 ± 14.0	
Glucose-6-phosphatase (μmol PO ₄ 15 min)	54.9 ± 14.0	26.6 ± 6.0	40.3 ± 19.1
Esterase (μmol <i>p</i> -nitrophenol/min)	68.4 ± 11.6	28.4 ± .5	36.9 ± 2.2

*Represents data from four rats, mean ± standard deviation.

Table VI. Enzymatic Changes in Hepatic Microsomes of Rats Fed Arochlor 1254

Enzyme (unit)	Enzyme assay*, % of control level, at indicated feeding period		
	2 wk	4 wk	6 wk
Aromatic Hydroxylase (nmol <i>p</i> -aminophenol/30 min)	20.9 ± 4.2	29.9 ± 4.5	32.0 ± 1.4
<i>N</i> -demethylase (nmol formaldehyde/30 min)	169 ± 10	116 ± 13	158 ± 2
Nitroreductase (nmol <i>p</i> -aminobenzoate/hour)	101 ± 18	84.2 ± 23.5	93.8 ± 19.3
Glucose-6-phosphatase (μmol PO ₄ 15 min)	29.9 ± 1.5	36.3 ± 6.0	32.0 ± 6.0
Esterase (μmol <i>p</i> -nitrophenol/min)	58.4 ± 3.5	45.8 ± 2.4	38.0 ± 6.8

*Represents data from four rats, mean ± standard deviation.

MONS 085645

Table VII. Enzymatic Changes in Hepatic Microsomes of Rats Fed Arochlor 1262

Enzyme (unit)	Enzyme assay*, % of control, at indicated feeding period		
	2 wk	4 wk	6 wk
Aromatic Hydroxylase (nmol <i>p</i> -aminophenol/30 min)	35.9 $\pm$ 6.5	41.6 $\pm$ 4.0	37.8 $\pm$ 8.0
<i>N</i> -demethylase (nmol formaldehyde/30 min)	151 $\pm$ 38	161 $\pm$ 16	131 $\pm$ 6
Nitroreductase (nmol <i>p</i> -aminobenzoate/hour)	394 $\pm$ 153	302 $\pm$ 27	359 $\pm$ 47
Glucose-6-phosphatase (μ mol PO ₄ 15 min)	33.0 $\pm$ 5.4	32.3 $\pm$ 2.7	27.3 $\pm$.5
Esterase (μ mol <i>p</i> -nrophenol/min)	89.7 $\pm$ 49.1	104 $\pm$ 7	86.5 $\pm$ 17.5

*Represents data from four rats, mean $\pm$ standard deviation.

et al. 1972), acneform lesions of the skin are characteristic of PCB intoxication while in the rat these skin changes are absent. Lymphopenia, atrophy of the cortex of the thymus, and regression of lymphoid germinal centers of the spleen and lymph nodes are found in rabbits (Vos 1971). Splenic hypoplasia is present in chickens (Flick *et al.* 1965) and a decrease in antibody-forming cells is observed in the lymph nodes of guinea pigs fed PCBs (Vos 1972). In the rat and infant monkey (Allen and Abrahamson 1972, Abrahamson and Allen 1972), there is regression of the cortex of the thymus; however, the spleen and lymph nodes appear normal. The effect of the PCBs on the lymphopoietic system of man appears to be of a minor nature (Kuratsume 1972). The development of neutrophilia takes place in all animals that experience lengthy exposure to PCBs (Kuratsume 1972, Allen *et al.* 1972) as is the case in the rats of the presently reported experiment. The reason for the increase in circulating neutrophils is not obvious. It seems unlikely that the isolated focal areas of necrosis that are present in the livers of animals fed PCBs is sufficient to elicit an increase in neutrophils. Also, it is of interest that hemograms of PCB-intoxicated animals return rapidly to normal values when the PCBs are removed from the diet.

Liver hypertrophy is a constant finding in all animals exposed to the PCBs (Vos 1972, Allen *et al.* 1972, Kuratsume 1972). This increase in liver size of the rats is a result of hypertrophy of the hepatic parenchymal cells. As viewed electron microscopically and substantiated biochemically, the cytoplasmic components of the hepatic cells experience the greatest modification. Almost immediately following exposure to the PCBs, there is proliferation of the smooth ER and, within three to seven days, large

concentric membrane arrays (CMAs) develop as extensions of the rough and smooth ER. As a result of the proliferative state of the ER, there is an increase in protein and RNA of the liver homogenates and, more specifically, of the microsomal fractions obtained from the affected livers.

The increase in ER is associated with modifications in microsomal enzyme activity. Although there is increased activity of all of the enzymes assayed, when expressed in relation to the total liver, some enzymes, such as *N*-demethylase and nitroreductase, are more active than glucose-6-phosphatase and aromatic hydroxylase. Also, there are moderate variations in the stimulatory effects produced by the PCBs tested. As an example, nitroreductase activity of the microsomes from the Arochlor 1262-fed rats increases more rapidly to a greater degree of activity for a longer period than do those of the Arochlor 1248- and Arochlor 1254-fed rats.

Litterer *et al.* (1972) have also evaluated the activity of hepatic microsomal enzymes obtained from rats given PCBs at levels ranging from 0.5 to 500 parts per million in the diet for a period of four weeks. The highest level used in their study is approximately half that employed in the presently reported experiments. They, too, find an increase in activity of *N*-demethylase and nitroreductase, and a decrease in glucose-6-phosphatase (per gram of microsomal protein). In addition, they also report an increase in phenobarbital hydroxylation with all of the four PCBs that were tested. In the present experiments, increased activity of aniline hydroxylase is found only for the Arochlor 1262-fed animals. This difference can be related to the particular substrates employed in the two laboratories.

Liver hypertrophy, proliferation of the ER, and increase in microsomal enzyme activity is greater in rats than in subhuman primates exposed to the PCBs (Allen *et al.* 1972). Instead of a fourfold increase in liver size, as found with rats, the monkey livers increase twofold in size. The proliferation of the ER is limited primarily to an increase in smooth ER. Only isolated CMAs are present in the monkey hepatocytes as compared to the elaborate membrane systems that occupy approximately 15 percent of the cytoplasmic profile of rat hepatocytes. Although increased activity of the microsomal enzymes *N*-demethylase, nitroreductase, esterase, aniline hydroxylase, and glucose-6-phosphatase occurs in the liver microsomes of the monkeys (when expressed as activity in total liver), it is not as great as that found for rats.

Functional significance can be placed on the morphological and biochemical modifications in the ER, particularly in the CMAs. These elaborate membranes are invariably associated with lipid droplets. Since the chlorinated hydrocarbons are lipid soluble, it seems reasonable that the CMAs possibly serve as means by which the PCBs present in the lipid droplets are metabolized. In addition, the increase in membrane phospholipids could enhance the capacities of the membranes to sequester the PCBs. The reduced levels of membrane cholesterol possibly are related to a less stable structure, thereby

making regions available for insertion of exogenous compounds among the fatty acids and phospholipids (Norback and Allen 1972). It seems likely that the enzymatic and compositional alterations present in these newly formed membranes would make them well suited for sequestration of the PCBs, or their metabolites, for subsequent excretion.

The stimulatory effect on the liver by the PCBs, as previously described, is followed by what can possibly be termed a regressive period. The latter period is characterized by a decrease in activity of the hepatic microsomal enzymes, dissolution of CMAs, vesiculation of the ER, formation of tubular aggregates of the smooth ER, and accumulation of numerous lipid droplets throughout the cytoplasm. Such changes are followed by a gradual disintegration of the severely affected hepatic cells. The time required to progress from the proliferative to the regressive stage depends, to a large extent, upon the PCBs that are employed in the study. The compounds with the lower percentage of chlorine are conducive to more rapid progression of the toxicity, even though greater liver hypertrophy is experienced with the higher chlorine PCBs. In addition, the species of animals that are exposed to the PCBs also influences the rate with which the lesions develop. Rats and dogs appear to tolerate larger doses for longer periods than do subhuman primates (Keplinger *et al.* 1971, Allen *et al.* 1972). This possibly is also related to the means by which the compounds are metabolized and excreted by different species. Age of the animals exposed to the PCBs can also influence their response. Indications are that infant monkeys are able to tolerate the PCBs better than adults (Abrahamson and Allen 1972). This, too, possibly is related to the metabolism and excretion of the compounds.

Acknowledgments

This study was supported in part by USPHS grants ES-AM-00472 and RR 00167 and the Wisconsin Sea Grant Program. Technical assistance was rendered by T. Durkin, J. Scheffler, and S. Edgerton.

References

- Abrahamson, L. J., and J. R. Allen: The biological response of infant nonhuman primates to a polychlorinated biphenyl. *Environmental Health Perspectives* (1973).
- Allen, J. R., L. J. Abrahamson, and D. H. Norback: Biological effects of chlorinated biphenyls and triphenyls on the subhuman primate. *Environmental Research* (1973).
- Allen, J. R., and L. J. Abrahamson: Enzymatic changes in the liver of rats fed polychlorinated biphenyls, triphenyls and DDT. In preprints of papers presented at the 164th ACS National Meeting, Division of Water, Air, Waste Chemistry, August 28-September 1, 1972. 12 (2), 97 (1972).
- Caufield, J. B.: Effects of varying the vehicle for O_2 in tissue fixation. *J. Biophys. Biochem. Cytol.* 3, 827 (1957).

- Cerlotti, G.: A microchemical determination of deoxyribonucleic acid. *J. Biol. Chem.* 196, 297 (1952).
- Drinker, C. K., M. F. Warren, and G. A. Bennett: The problem of possible systemic effects from certain chlorinated hydrocarbons. *J. Ind. Hyg. Toxicol.* 19, 283 (1937).
- Flinn, F. B., and D. E. Jarvik: Action of certain chlorinated naphthalenes on the liver. *Proc. Soc. Exp. Biol. Med.* 35, 118 (1936).
- Flick, D. F., R. G., O'Dell, and V. A. Childs: Studies on the chick edema disease. 3. Similarity of symptoms produced by feeding chlorinated biphenyls. *Poultry Sci.* 44, 1460 (1965).
- Fujita, S., H. Tsuji, K. Kato, S. Saeki, and H. Tsukamoto: Effects of biphenyl chloride on rat liver microsomes. *Fukuoka Acta Medica* 62, 30 (1971).
- Good, C. K., and N. Pensky: Halowax acne (cable rash): Cutaneous eruption in marine electricians due to certain chlorinated naphthalenes and diphenyls. *Arch. Dermatol. Syph.* 48, 251 (1943).
- Jones, J. W., and H. S. Alden: An acneform dermatogiosis. *Arch. Dermatol. Syph.* 33, 1022 (1936).
- Katsuki, S.: Foreword, Report on the study group for "Yusho" (chlorobiphenyls poisoning). *Fukuoka Acta Medica* 60, 403 (1969).
- Keplinger, M. L., O. E. Fancher, J. C. Calandra, and E. P. Wheeler: Toxicological studies with chlorinated biphenyls. Proceedings of NIEHS Polychlorinated Biphenyl Meeting, Dec. 20-21, 1971.
- Kuratsume, M.: An epidemiologic study in "Yusho" or chlorobiphenyls poisoning. *Fukuoka Acta Medica* 60, 513 (1969).
- Litterst, C. L., T. M. Farber, A. M. Baker, and E. J. Van Loon: Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat. *Toxicol. Appl. Pharmacol.* 23, 112 (1972).
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall: Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265 (1951).
- Mollenhauer, H. H.: Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* 39, 111 (1964).
- Munro, H. M., and A. Fleck: The determination of nucleic acids. In D. Glick (ed.): *Methods of biochemical analysis*, vol. 14, p. 113. New York-London: Interscience (1966).
- Norback, D. H., and J. R. Allen: Enzymatic and morphologic alterations of hepatic endoplasmic reticulum induced by chlorinated aromatic hydrocarbons. *Fed. Proc.* 29, 816 (1970).
- : Chlorinated triphenyl induced extension of the hepatic endoplasmic reticulum. *Proc. Soc. Exp. Biol. Med.* 139, 1127 (1972).
- Penning, C. H.: Physical characteristics and commercial possibilities of chlorinated biphenyls. *Ind. Eng. Chem.* 22, 1180 (1930).

Schmidt, H., and G. Schultz: Patent for manufacture of pentachlorobiphenyl. Ann. 207, 338 (1881).

Vos, J. G., and R. B. Beems: Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. Toxicol. Appl. Pharmacol. 19, 617 (1971).

_____. Toxicology of the PCBs for mammals and for birds. Environmental Health Perspectives 1, 105 (1972).

Manuscript received November 1, 1972; accepted December 26, 1972