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Experimental Hepatic Porphyria Induced by Polychlorinated Biphenyls[1]

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Experimental Hepatic Porphyria Induced by Polychlorinated Biphenyls. GOLDSTEIN, J. A., HICKMAN, P. AND JUE, D. L. (1974). *Toxicol. Appl. Pharmacol.* 27, 437–448. Aroclor 1254, which consists of a mixture of polychlorinated biphenyls (PCBs) containing 54% chlorine, produced an experimental hepatic porphyria in rats resembling hexachlorobenzene poisoning and human porphyria cutanea tarda. The PCB-induced porphyria is characterized by delayed development, increased excretion of urinary uroporphyrins, accumulation of 8- and 7-carboxyporphyrins in the liver and increased drug-metabolizing capacity of the liver. Cytochrome P-450 and microsomal heme were increased maximally at 1 week, in the absence of an increase in the rate-limiting enzyme in heme synthesis, δ-aminolevulinic acid (ALA) synthetase. Induction of ALA synthetase and porphyria occurred later, after 2–7 months' exposure to PCBs. No induction of ALA synthetase could be demonstrated prior to the onset of porphyria. Marked induction of ALA synthetase occurred 5 hr after large single doses of Aroclor 1254; however, the doses required were larger than those used to produce porphyria when administered chronically, and induction appeared to be related to the marked increase in cytochrome P-450 seen 24 hr after administration of the drug.

Previously, only hexachlorobenzene, other chlorinated benzenes and chlorinated phenols were known to produce a delayed type of hepatic porphyria resembling human porphyria cutanea tarda. Hexachlorobenzene-induced porphyria is characterized by a delayed onset of action, massive increases in the excretion of 8- and 7-carboxyporphyrins in the urine and accumulation of uroporphyrins in the liver (Ockner and Schmid, 1961; San Martin de Viale *et al.*, 1970). In contrast, porphyrogenic compounds such as allylisopropylacetamide (AIA) produce increased excretion of porphyrins and their precursors within a few hours (De Matteis and Prior, 1962).

Many porphyrogenic chemicals such as AIA apparently cause porphyria by inducing hepatic δ-aminolevulinic acid (ALA) synthetase, the rate-limiting enzyme in heme synthesis (Granick and Urata, 1963; Granick, 1966). The increase is accompanied by a decrease in cytochrome P-450, the terminal cytochrome involved in drug oxidations,

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and the accumulation of breakdown products of heme in the liver (Abritti and De Matteis, 1971). Abritti and De Matteis (1971) and Levin *et al.* (1972) have proposed that destruction of cytochrome P-450 may be related to the porphyrogenic action of these chemicals and have shown that only allyl-containing analogs of AIA and barbiturates induce ALA synthetase and reduce cytochrome P-450. Conversely, a number of xenobiotics including phenobarbital increase liver content of cytochrome P-450, resulting in increased ability to metabolize drugs (Conney, 1967). It has been suggested that ALA synthetase induction may be necessary for the increase in cytochrome P-450. However, most xenobiotics produce large increases in cytochrome P-450 and negligible or moderate increases in ALA synthetase (Wada *et al.*, 1968; Marver, 1967; De Matteis and Gibbs, 1972).

Polychlorinated biphenyls (PCBs) are widespread contaminants of the environment and have been detected in samples of human tissue as well as a variety of wildlife (Risebrough *et al.*, 1968; Yobbs, 1972). Several commercial PCBs have been reported to increase tissue fluorescence in rats, chicks and rabbits and to increase excretion of a porphyrin fraction in the feces of chicks and rabbits (Vos *et al.*, 1970, 1971, Vos and Notenboom-Ram, 1972). However, no detailed study of the porphyria induced by PCBs has been reported.

In the present study, porphyrins and their precursors were identified and quantitated in liver and urine of rats fed a commercial mixture of PCBs. The time course of the porphyria was determined, and the relationship between ALA synthetase, cytochrome P-450 and porphyria was studied to evaluate the role of ALA synthetase in PCB-induced porphyria.

METHODS

Animals. One-month-old female Sherman rats were fed ad libitum ground Purina laboratory chow containing 0 or 100 ppm Aroclor 1254[3] (approximately 7.9 mg kg/day), a commercial mixture of PCBs containing 54% chlorine by weight. Ninety-six rats were divided into 20 groups of 4–6 each, fed 0 or 100 ppm Aroclor 1254, and sacrificed by decapitation after 1, 2, 3, and 7 days and 1, 2, 3, 4, 7 and 13 months. Livers were removed for assay of ALA synthetase, cytochrome P-450, microsomal protein and liver porphyrin content as described below. Urine was collected in metabolic cages for the 24-hr period preceding sacrifice in groups sacrificed at 7 days, 1, 2, 3, 4, 7 and 13 months.

For single dose studies, 36 weanling female rats were starved 24 hr before administration by gastric intubation of a single dose of 0, 5, 10, 100 or 500 mg/kg Aroclor 1254 dissolved in peanut oil, and food was withheld until sacrifice 5 or 24 hr later. Additional rats were dosed with 0 (6 rats) or 1000 mg/kg Aroclor 1254 (6 rats), and urine was collected 48–76 hr and 4 months after dosing.

Liver. Livers were minced, one portion was homogenized with 0.9% NaCl containing 0.5 mM EDTA and 10 mM Tris-HCl buffer for the assay of ALA synthetase. The remainder of the liver was homogenized in 1.15% KCl, a portion utilized for estimation of porphyrins, and the rest was centrifuged at 9000 *g* for 20 min. The 9000 *g* supernatant was then centrifuged for 1 hr at 100,000 *g* in a Beckman model L-2 ultracentrifuge, the microsomal pellet was washed once in 1.15% KCl and resuspended in 1.15% KCl containing 0.1 M K_2HPO_4–KH_2PO_4 buffer, pH 7.4, and 25% glycerol. The resuspended

[3] Aroclor 1254 was a gift from Monsanto, St. Louis, Missouri.

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pellet was stored overnight at 0°C and used for determination of cytochrome P-450 and protoheme.

Assay of ALA synthetase. ALA synthetase was assayed in liver homogenates by the method of Marver *et al.* (1966a). The incubation mixture contained 0.5 ml of homogenate, 200 μmol of glycine, 12.5 μmol of EDTA and 150 μmol of tris-HCl buffer, pH 7.2 in a final volume of 2 ml. After incubation for 30 min at 37° with shaking, the reaction was stopped with 1 ml of 0.3 M trichloroacetic acid. The protein-free supernatants from 7 to 9 flasks were pooled, and ALA was estimated after column chromatography as described below (Marver *et al.*, 1966b).

Microsomal cytochromes and protein. Microsomal cytochrome P-450 and protoheme were measured by the methods of Omura and Sato (1964a,b) using a Cary 14 recording spectrophotometer. Microsomal protein was measured by the method of Lowry *et al.* (1951) using crystalline albumin as the standard.

Assay of ALA, porphobilinogen, and porphyrins.[4] Urinary ALA and porphobilinogen (PBG) were separated by column chromatography and the eluates were assayed after mixing with an equal volume of Ehrlich reagent (Marver *et al.*, 1966b) by the absorbance measured at 556 nm (Mauzerall and Granick, 1956). Urinary porphyrins were routinely estimated in 1- or 2-ml aliquots of urine by a slight modification of the method of Schwartz *et al.* (1951). Briefly, porphyrins were extracted into ethyl acetate at pH 3.1, the uroporphyrin fraction was extracted 4 times with 10% sodium acetate, adsorbed on a 2.5 × 1 cm column of aluminum oxide, washed with 10% sodium acetate and 3% acetic acid, and eluted with 1.5 N HCl containing 1% methanol. The coproporphyrin was then extracted from the ethyl acetate with 1.5 N HCl. Values were estimated with an Aminco fluorometer, with respect to standards of uroporphyrin I and coproporphyrin I. Under these conditions, daily recoveries of coproporphyrin I averaged 100% with less than 1% found in the uroporphyrin fraction, and recoveries of uroporphyrin I averaged 66%, less than 0.2% being found in the coproporphyrin fraction. Values were corrected for recoveries. The only modifications were the substitution of 10% sodium acetate washes for 3% sodium acetate which gave much better separation of uroporphyrin without affecting coproporphyrin recoveries, the addition of 1% methanol to the 1.5 N HCl used to elute uroporphyrin from the aluminum oxide column and preelution of the aluminum oxide column with 1 ml of the eluent.

Liver porphyrins and some samples of urine were methylated, extracted into chloroform, separated by thin-layer chromatography and eluted with chloroform (Doss, 1967). The porphyrin methyl esters in the eluate were estimated by spectrophotometry with a Cary 14 spectrophotometer (Dresel and Falk, 1956) or fluorometry when the amount of porphyrin was too small for spectrophotometric determination.

Results were analyzed by the Student *t* test.

RESULTS

Urinary Porphyrins and Porphyrin Precursors

Figure 1 shows that 4 rats fed 100 ppm Aroclor 1254 became porphyric after a delay of 2–7 months. After this time, urinary porphyrin excretion increased rapidly. The most

[4] δ-Aminolevulinic acid, porphobilinogen, coproporphyrin I and uroporphyrin I were obtained from Sigma Chemical Co., St. Louis, Missouri.

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dramatic increase was in the uroporphyrin fraction. Uroporphyrin excretion was elevated a maximum of 540-fold (588 μg/day vs 1.1 ± 0.2 μg/day for controls), coproporphyrin 27-fold (86 μg/day vs 3.2 ± 1.4 for controls, PBG 50-fold (1266 μg/day vs 24.3 ± 5.5 for controls) and ALA 18-fold (334 μg/day vs 18.3 ± 4.8 for controls)

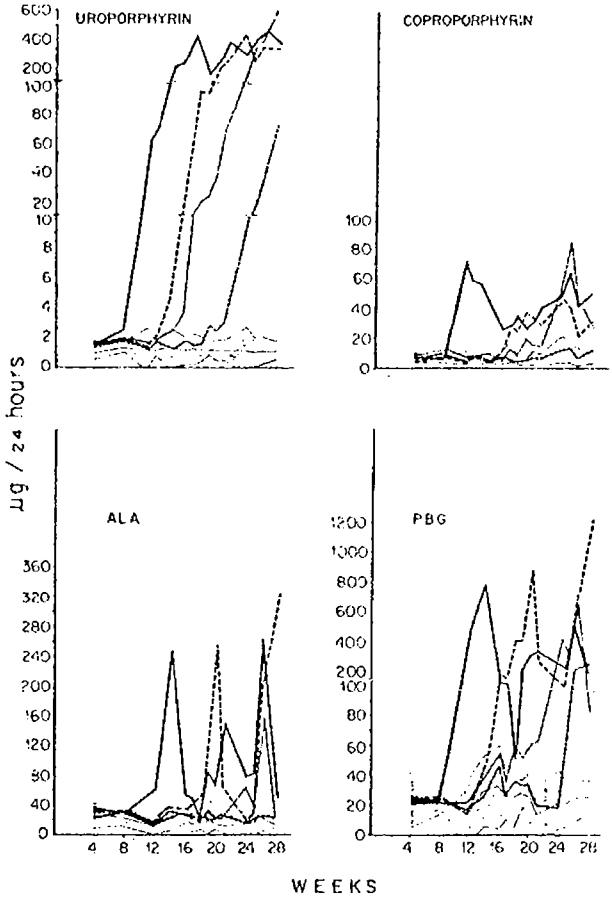

FIG. 1. Effect of 100 ppm Aroclor 1254 on urinary excretion of uroporphyrin, coproporphyrin, ALA and PBG in the female rat. The shaded area represents the 99% confidence limits of the mean for control rats. Each line represents urinary excretion in a single treated rat with time.

The increase in uroporphyrin was the first and most consistent sign of porphyria; variable elevation in coproporphyrin and ALA occurred several weeks after the onset of uroporphyrinuria. An increase in urinary PBG occurred with or slightly later than the increase in uroporphyrin.

More detailed analysis of urinary porphyrins by thin-layer chromatography confirmed the major porphyrin in control rat urine as coproporphyrin (81%), with lesser amounts of 8- and 7-carboxyporphyrins (14%) (Table 1). In contrast, the major porphyrins found in urine from Aroclor-treated rats were 8-carboxyporphyrin (73%

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TABLE 1

COMPOSITION OF URINARY PORPHYRINS IN RATS FED 100 PPM AROCLOR 1254 FOR 1 YEAR[a]

Porphyrin	Control	Aroclor	Control	Aroclor
	μg/24 hr		% of total	
4-COOH	0.87 ± 0.14	10.5 ± 1.4	81%	5%
5-COOH	0.06 ± 0.01	3.0 ± 0.6	6%	1%
6-COOH	ND	9.6 ± 2.6	0%	4%
7-COOH	0.13 ± 0.03	11.7 ± 13.8	14%	16%
8-COOH		151.1 ± 17.7		73%

[a] Urinary porphyrins were methylated and analyzed by thin-layer chromatography. Results are given as the mean ± SE.

and 7-carboxyporphyrin (16%). There was a 10-fold increase in the excretion of coproporphyrin, but the increase in uroporphyrin excretion was 1400-fold. There were also substantial increases in the amounts of 5- and 6-carboxylic porphyrins. Doss *et al.* (1971) found a similar reversal of the coproporphyrin/uroporphyrin ratio in urine of patients with porphyria cutanea tarda.

Liver ALA synthetase, Cytochrome P-450 and Porphyrins

ALA synthetase, cytochrome P-450, microsomal heme and liver porphyrin content were determined in rats sacrificed periodically between 1 day and 13 months of treatment. Urinary porphyrins were determined 24 hr prior to sacrifice. There was a slight (30–60%) increase in the excretion of urinary porphyrins after only 1 week of treatment; however, the increase was slight during the first month (Table 2). Rats became grossly porphyric between 2 and 4 months. There was good correlation between urinary porphyrins and liver porphyrins (Table 2 versus Fig. 2). Only a trace of porphyrins was found in liver samples from control rats (<0.1 μg/liver) after 1 month (0.3 μg/liver) of treatment. However, the increase was appreciable only after 2 months feeding.

ALA synthetase was not increased during the first month of exposure (Fig. 2). After this time, ALA synthetase was elevated 3- to 6-fold in porphyric rats, but was normal in nonporphyric Aroclor-treated rats. Since some porphyrin accumulation occurred in the liver prior to a demonstrable increase in ALA synthetase activity, it is probable that ALA synthetase induction was secondary to the porphyria. There was no correlation between ALA synthetase activity and cytochrome P-450 or microsomal heme. Cytochrome P-450 and microsomal heme were increased throughout the study, but the increase was maximal at 1 week, despite the absence of an increase in the rate-limiting enzyme in heme synthesis, ALA synthetase. Liver weight and microsomal protein were also increased maximally at 1 week (Table 3).

At 4 months, the porphyrin concentration in the livers of rats fed 100 ppm Aroclor 1254 was 1,410 ± 150 μg/g of liver vs <0.1 μg/g for control rats. Only uroporphyrins (75% 8-carboxy and 25% 7-carboxyporphyrins) could be detected in the livers of PCB-treated rats. No fluorescence was observed in the bone marrow. All rats appeared healthy throughout the feeding period; there were no deaths in the rats fed Aroclor 1254 and weight gain was normal.

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TABLE 2

EFFECT OF 100 PPM AROCLOR 1254 ON URINARY PORPHYRIN EXCRETION (μg/24 HR)[a]

Treatment		ALA	PBG	Coproporphyrin	Uroporphyrin
1 Week	Control (4)	22.7 ± 1.3	15.6 ± 0.8	4.5 ± 0.3	1.37 ± 0.07
	Treated (4)	28.0 ± 1.7	15.5 ± 0.9	8.3 ± 1.1[b]	1.86 ± 0.13[b]
1 Month	Control (4)	21.6 ± 1.2	20.6 ± 0.9	3.7 ± 0.3	1.02 ± 0.5
	Treated (4)	32.6 ± 4.6	23.0 ± 0.7	5.8 ± 0.4[b]	1.35 ± 0.04[b]
2 Months	Control (4)	19.7 ± 1.5	18.2 ± 1.5	2.9 ± 0.3	1.08 ± 0.05
	Treated (4)	29.1 ± 4.4 (3)	19.5 ± 2.1 (3)	7.7 ± 2.2 (3)[b]	1.59 ± 0.09 (3)[b]
		43.7 (1)[c]	94.5 (1)[c]	29.5 (1)[c]	11.3(1)[c]
3 Months	Control (4)	20.0 ± 1.4	21.8 ± 1.8	5.9 ± 1.1	2.97 ± 0.61
	Treated (4)	24.1 ± 2.5 (2)	28.0 ± 2.8 (2)	5.7 0 (2)	3.75 ± 1.1 (2)
		268 (1)[c]	650 (1)[c]	167 (1)[c]	337(1)[c]
		30 (1)[c]	41 (1)[c]	47 (1)[c]	5.4 (1)[c]
4 Months	Control (4)	29.6 ± 0.3	25.2 ± 1.7	2.1 ± 0.3	0.85 ±0.04
	Treated (4)[c]	37.5 ± 6.8	38.2 ± 13.7	10.2 ± 1.7[b]	264 ± 121[b]
7 Months	Control (6)	16.8 ± 0.8	21.1 ± 1.1	3.5 ± 0.9	1.13 ± 0.11
	Treated (6)[c]	41.6 ± 7.5[b]	105 ± 33.8[b]	27.6 ± 6.9	262 ± 58[b]
13 Months	Control (6)	16.6 ± 1.7	18.4 ± 1.1	4.5 ± 1.0	0.89 ± 0.16
	Treated (6)[c]	55.1 ± 10.3[b]	220 ± 45[b]	29.6 ± 8.8[b]	306 ± 38[b]

[a] Rats were fed 100 ppm Aroclor 1254 and sacrificed at the times indicated. Urine was collected for a 24-hr period prior to sacrifice for determination of urinary porphyrins. The number of animals per group are shown in parentheses. Values represent the mean ± SE.

[b] $p < 0.05$.

[c] Rats were considered porphyric (total urinary porphyrins > 40 μg/24 hr). At 2 and 3 months, only some of the animals were porphyric and individual values for porphyric rats are given below the mean for the remainder of the group.

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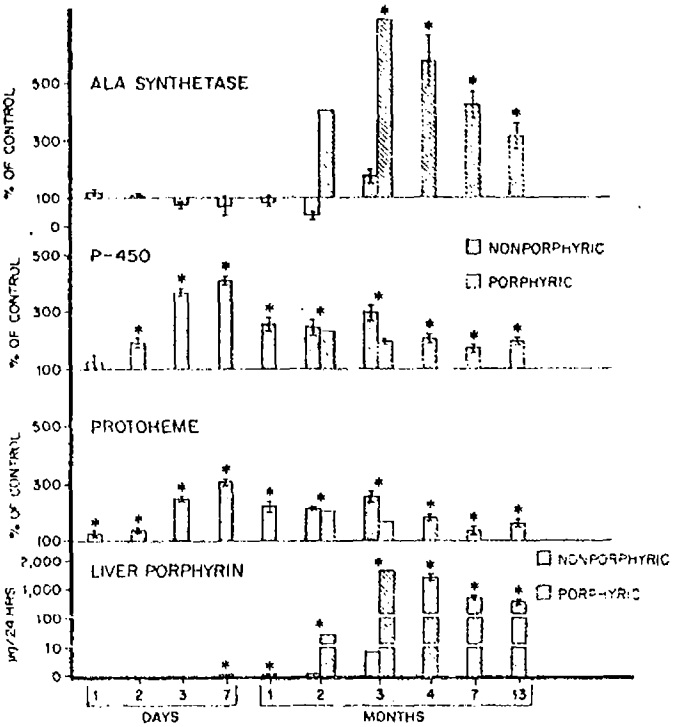

FIG. 2. Effect of 100 ppm Aroclor 1254 on liver porphyrins, ALA synthetase, microsomal P-450 and protoheme in the liver of female rats. Groups of 4–6 rats were killed periodically for 13 months. Rats were designated porphyric when total urinary porphyrin was increased at least 5-fold (all porphyric rats excreted at least 40 μg porphyrin/24 hr). Vertical bars represent means ± SE. Control values for ALA synthetase (23 nmol/g), Cytochrome P-450 (16 nmol/g) and microsomal heme (30 nmol/g) did not vary with age.
* $p < 0.05$.

TABLE 3

EFFECT OF 100 PPM AROCLOR 1254 ON LIVER WEIGHT AND MICROSOMAL PROTEIN*

	Liver/body weight ratio (%)		Microsomal protein (mg/g liver)	
Treatment	Control	Treated	Control	Treated
1 Day	4.36 ± 0.11	4.45 ± 0.14	16.6 ± 0.7	17.0 ± 0.9
2 Days	4.15 ± 0.06	4.60 ± 0.09[b]	16.6 ± 0.7	16.4 ± 0.6
3 Days	4.16 ± 0.08	5.13 ± 0.12[b]	18.6 ± 1.5	24.9 ± 0.8[b]
7 Days	4.50 ± 0.07	5.64 ± 0.21[b]	15.6 ± 0.5	22.5 ± 0.5[b]
1 Month	3.34 ± 0.06	4.34 ± 0.06[b]	17.4 ± 0.2	20.8 ± 1.4[b]
2 Months	2.79 ± 0.10	3.50 ± 0.13[b]	18.0 ± 0.5	23.8 ± 1.6[b]
3 Months	3.01 ± 0.12	3.65 ± 0.06[b]	14.3 ± 0.8	17.4 ± 0.3[b]
4 Months	2.71 ± 0.09	3.46 ± 0.17[b]	20.4 ± 1.2	16.2 ± 1.4[b]
7 Months	2.56 ± 0.05	3.18 ± 0.10[b]	15.1 ± 1.1	16.3 ± 0.6
13 Months	2.39 ± 0.07	3.36 ± 0.12[b]	14.8 ± 1.4	17.1 ± 0.2

* See Fig. 2 for treatment of animals. The results are expressed as mean ± SE.
[b] $p < 0.05$.

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TABLE 4

EFFECT OF A SINGLE DOSE OF AROCLOR 1254 ON RAT LIVER δ-AMINOLEVULINIC ACID SYNTHETASE ACTIVITY AND CYTOCHROME P-450[a]

Dose mg/kg	ALA synthetase activity (nmol/g/hr)		Cytochrome P-450 (nmol/g)		Liver/body weight (% of control)	Microsomal protein (% of control)
	5 Hr	24 Hr	5 Hr	24 Hr	24 Hr	24 Hr
Control (oil)	26.1 ± 2.2	48.9 ± 7.2[b]	11.0 ± 0.6	12.5 ± 1.5	100 ± 3	100 ± 5
5	33.3 ± 5.9	67.2 ± 16.5	9.7 ± 1.3	13.1 ± 2.7	105 ± 5	102 ± 5
10	37.5 ± 7.2	113.7 ± 28.6	9.8 ± 1.3	17.3 ± 0.7[c]	108 ± 4	112 ± 7
50	70.1 ± 17.1[c]	97.1 ± 8.3[c]	10.4 ± 0.7	37.8 ± 4.8[c]	125 ± 5[c]	125 ± 12
100	76.3 ± 6.0[c]	91.0 ± 12.3	11.1 ± 0.4	52.8 ± 8.9[c]	128 ± 5[c]	143 ± 8[c]
500	99.9 ± 19.2[c]	128.9 ± 13.8[c]	11.5 ± 1.4	63.7 ± 5.5[c]	141 ± 4[c]	161 ± 16[c]

[a] One-month-old female rats were starved 24 hr and Aroclor 1254 was administered in peanut oil by gastric intubation (0.005 ml/g body weight). Each value represents the mean ± SE of 6 animals.

[b] Starvation for 48 hr produced a slight increase in δ-aminolevulinic acid synthetase activity.

[c] Significantly different from controls at $p < 0.05$.

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Effect of a Single Dose of PCBs

Table 4 shows the acute effects of single doses of Aroclor 1254 on ALA synthetase and cytochrome P-450 in starved female rats. Fourfold induction of ALA synthetase could be demonstrated 5 hr after large doses of Aroclor 1254 (500 mg/kg). The minimal effective dose was 50 mg/kg, 5-times larger than the dose which produced porphyria when fed chronically in this study. Aroclor 1254 did not affect microsomal P-450 at 5 hr at any dosage level, but at 24 hr, P-450 was increased 5-fold, liver weight 40% and microsomal protein 60%. Administration of a large single oral dose of Aroclor 1254 (1000 mg/kg) increased ALA synthetase 5-fold at 24 hr, but did not result in any increase in urinary porphyrins, ALA or PBG 2 to 3 days after administration or in the surviving rats (2) 4 months after administration.

DISCUSSION

Porphyria occurred in all female rats fed 100 ppm Aroclor 1254 for at least 7 months (16 rats) and was characterized by delayed onset, excretion of large amounts of 7- and 8-carboxyporphyrins in the urine and accumulation of uroporphyrins in the liver. The delayed onset and excretion of large amounts of urinary uroporphyrins is similar to hexachlorobenzene-induced porphyria (Ockner and Schmid, 1961; San Martin de Viale *et al.*, 1970). However, rats fed PCBs exhibited none of the nervous or cutaneous signs associated with hexachlorobenzene poisoning (De Matteis *et al.*, 1961; Pearson and Malkinson, 1965). Induction of hepatic ALA synthetase could be demonstrated only after the onset of PCB-induced porphyria. Some accumulation of uroporphyrins occurred in the liver prior to a demonstrable increase in ALA synthetase. Our results suggest that induction of ALA synthetase is not the primary defect in PCB-induced porphyria in the rat.

The tremendous accumulation of uroporphyrins in the liver and urine of rats fed Aroclor 1254 suggests that PCBs may affect uroporphyrin formation or utilization. Hemosiderin has been reported in the liver of rats treated with Aroclor 1254 and in the liver of patients with porphyria cutanea tarda, indicating an increase in liver iron content (Kimbrough *et al.*, 1972; Uys and Eales, 1963). Kushner *et al.* (1972) recently reported that iron decreases uroporphyrin III cosynthetase activity and suggested iron may also affect uroporphyrin decarboxylase. If a block in heme synthesis is involved in PCB-induced porphyria, heme synthesis is apparently sufficient for synthesis of cytochrome P-450, since microsomal heme and cytochrome P-450 were elevated in porphyric rats.

Many xenobiotics increase cytochrome P-450 and activity of the liver microsomal drug-metabolizing enzymes (Conney, 1967). It has been suggested that increased ALA synthetase activity may be necessary for increased synthesis of P-450 (Marver, 1966a). We found no correlation between changes in ALA synthetase activity and cytochrome P-450 or microsomal heme after feeding 100 ppm PCBs. Cytochrome P-450 and microsomal heme were increased maximally at 1 week, in the absence of an increase in ALA synthetase. Phenylbutazone also increases cytochrome P-450 without affecting ALA synthetase activity (De Matteis and Gibbs, 1972). Administration of large doses of exogenous ALA or heme to rats does not increase cytochrome P-450 levels (De Matteis, 1971), suggesting that synthesis of cytochrome P-450 is regulated by factors other than availability of heme.

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The porphyrogenic compound AIA induces ALA synthetase as early as 5 hr after administration, perhaps as a result of destruction of cytochrome P-450 and accumulation of breakdown products of heme in the liver (Abritti and De Matteis, 1971). Acutely, large doses of Aroclor 1254 produced a 4-fold increase in ALA synthetase. However, Aroclor 1254 did not decrease cytochrome P-450; instead, Aroclor acted as a powerful inducer of drug metabolism, increasing cytochrome P-450 5-fold by 24 hr. The dose of PCBs required to induce ALA synthetase was larger than the dose which produced porphyria when fed chronically, and induction of ALA synthetase by a single large dose of PCBs was probably related to large, rapid increases in cytochrome P-450.

Two European commercial preparations of PCBs have been reported to be contaminated with small amounts of tetrachlorodibenzofuran (Vos *et al.*, 1970). The chlorinated dibenzodioxins and dibenzofurans are very potent chloracneogenic and hepatotoxic agents. (Bauer *et al.*, 1961; Linn Jones and Krizek, 1962). Recently, Poland and Glover (1973) have shown that chlorinated dibenzodioxins induce ALA synthetase in the chick embryo at doses as low as 3 ng/kg. Possibly, contamination with chlorinated dibenzofurans could be responsible for the porphyria produced by PCBs. However, Vos and Notenboom-Ram (1972) have found 2,4,5,2′,4′,5′-hexachlorobiphenyl to be porphyrogenic in the rabbit.

The dietary level of Aroclor 1254 used to produce porphyria in rats (100 ppm) is at least 20 times higher than the 0.1 to 5 ppm temporary tolerances set by FDA for PCBs in food in the United States. The concentration of PCBs in fat of rats fed 100 ppm Aroclor 1254 for 6 months is 1000 ppm in our laboratory (Curley *et al.*, 1971). Although a similar level of PCBs has been detected in the fat of some wildlife (Risebrough *et al.*, 1968), only 5% of human fat samples tested contain more than 2 ppm PCBs (Yobbs, 1972). It is unlikely that present environmental levels of PCBs will result in porphyria in humans. However, an episode of human poisoning due to contamination of rice oil with 2000 ppm Kanechlor 400 occurred recently in Japan, resulting in chloracne and pigmentation of the skin and nails (Kuratsune *et al.*, 1972). Unfortunately, the concentration of porphyrin in the urine was apparently not determined.

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