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Polychlorinated Biphenyl-Induced Alteration of Biologic Parameters in the Rat[1,2]

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Polychlorinated Biphenyl-Induced Alteration of Biologic Parameters in the Rat. BRUCKNER, J. V., KHANNA, K. L. AND CORNISH, H. H. (1974). *Toxicol. Appl. Pharmacol.* **28**, 189–199, Aroclor 1242, a commercial polychlorinated biphenyl (PCB) mixture, was administered ip to rats for 10 weeks. Principal findings included: loss of body weight; hepatic and renal damage, with some animals exhibiting renal papillary epithelial hyperplasia; slight reduction in erythrocyte count, diameter, and hemoglobin content, with an elevation in serum iron; diminished plasma corticosteroid and glucose concentrations; increased urinary excretion of protein, sugars, and coproporphyrin.

The effect of PCBs on several hepatic microsomal enzymatic parameters was also evaluated. Maximal hydroxylation and *N*-demethylation activities were observed 3–10 days following a single ip injection (100 mg/kg). Each remained significantly higher than control values after 20 days, with hydroxylation activity still 150% of controls after 40 days. The minimal effective single dose for induction of hydroxylation activity was approximately 5 mg/kg. Induction of hydroxylation activity was found to be dose-dependent; however, a smaller degree of correlation was noted between N-demethylation activity and dose. Values for cytochromes P_{450} and b_5 and NADPH–cytochrome *c* reductase activity were observed to roughly parallel hydroxylation and N-demethylation activities, the highest degree of correlation was manifest between enzymatic activity and cytochrome P_{450} values.

Commercial polychlorinated biphenyl (PCB) products consist of mixtures of PCB isomers, differing from one another in extent of chlorination. Although their use has recently been voluntarily restricted in the United States to closed-system heat transfer applications, global environmental contamination and accumulation in food chains has been demonstrated (Risebrough and deLappe, 1972). Humans have been exposed to PCBs, either via accidental contamination of foodstuffs by quantities large enough to elicit toxic symptoms (Kuratsune *et al.*, 1972), or via PCB residues in everyday foods. The latter mode of exposure has apparently resulted in deposition of detectable concentrations ($\geqslant$1 ppm wet weight) of PCBs in human adipose tissue within the general population of the United States (Price and Welch, 1972).

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Despite a number of recent investigations, toxic manifestations of PCB exposure in mammals are not well understood. Some of the major findings to date include: renal and/or hepatic injury in mice and monkeys (Nishizumi, 1970), rabbits (Vos and Beems, 1971), and rats (Kimbrough *et al.*, 1972; Bruckner *et al.*, 1973); porphyria in rabbits (Vos and Notenboom-Ram, 1972); hepatic microsomal enzyme induction in rats (Fujita *et al.*, 1971; Litterst *et al.*, 1972; Bruckner *et al.*, 1973); alteration of steroid metabolism in boars (Platonow *et al.*, 1972) and lipid metabolic alteration in rats (Nagai *et al.*, 1971).

In order to gain better insight into the toxic nature of PCBs, a representative commercial product (Aroclor 1242) was administered to rats. Emphasis was placed upon effects on hepatic and renal function, hematologic parameters, hepatic microsomal enzyme stimulation, porphyria induction, and steroid metabolism.

METHODS

Male, Sprague-Dawley rats[3] were used in both subacute and hepatic microsomal enzyme induction studies. The animals were housed in stainless steel cages in air-conditioned quarters, with Rockland rat and mouse chow and tap water available ad libitum.

Subacute study. The subacute dosage regimen consisted of ip injections (100 mg Aroclor 1242[4]/kg) given twice weekly for 6 weeks, then weekly thereafter for 4 weeks to a group of 9 rats. Each rat therefore received a total dose of 1.6 g/kg. Dilutions of Aroclor 1242 were made with peanut oil to an average injection volume of 0.4 ml. A control group of 4 rats was given an equivalent volume of peanut oil by ip injection.

Histopathology. Tissue samples of liver, kidney, spleen and adrenal from rats in the subacute investigation were processed and stained with hematoxylin and eosin. Liver and kidney specimens were also stained for lipid with Sudan IV.

Hematology. Hematologic examinations were performed by standard techniques. Total blood hemoglobin concentrations were measured by a standard technique involving cyanmethemoglobin formation (Davidsohn and Henry, 1969). Mean corpuscular hemoglobin concentration (MCHC) was computed by dividing hemoglobin concentration in g/100 ml by hematocrit in percent. Blood urea nitrogen (BUN) concentrations were determined with a Nesslerization technique (Frankel and Reitman, 1963).

Serum iron and bilirubin concentrations were measured by standard procedures (Frankel and Reitman, 1963), as were blood glucose values (Mark and Zimmer, 1967). The technique of Solem and Brinck-Johnsen (1965) was utilized for analysis of plasma corticosteroids.

Urinalysis. Urinary sugars and proteins were estimated by established procedures (Davidsohn and Henry, 1969). Twenty-four-hour excretion of coproporphyrin (Schwartz *et al.*, 1951) and of 17-ketosteroids (Mark and Zimmer, 1967) was also determined.

Microsomal enzyme induction studies. Rats weighing 250–300 g were housed in groups of 2 or 3 animals per cage for dose-response and time-effect studies. Dilutions of Aroclor 1242 were made with peanut oil to an average injection volume of 0.15 ml. Controls received 0.15 ml peanut oil by ip injection. Rats were divided into 6 groups of 4 rats each

[3] Spartan Research Farm, Lansing, Michigan.

[4] Lot No. KA-419. Kindly supplied by Monsanto Chemical Company, St. Louis, Missouri.

for the dose-response study, with each group receiving 100, 50, 25, 5, 1, or 0 mg Aroclor 1242/kg as a single ip injection. Animals were sacrificed 72 hr after dosing. For the time-effect study, a single ip injection of 100 mg Aroclor 1242/kg was given to each of 5 groups of rats (6 PCB-treated and 3 controls per group). A group of rats was sacrificed at intervals of 1, 5, 10, 20 and 40 days post injection.

Microsomal assays. Hepatic microsomal hydroxylation activity was quantitated by measurement of *N*-acetyl-*p*-aminophenol formation from acetanilide, *N*-demethylation activity by measurement of 4-aminoantipyrine formation from aminopyrine. Activity of each was calculated in terms of μg product formed/mg microsomal protein/20 min. Procedures used were described in a previous study (Bruckner *et al.*, 1973), as were methods for the determination of cytochromes P_{450} and b_5. Each cytochrome concentration was expressed as nmol/mg microsomal protein. Determination of NADPH–cytochrome *c* reductase activity was performed according to the technique of Baron and Tephly (1969), and expressed as nmol cytochrome *c* reduced/mg microsomal protein/min. Microsomal protein concentrations were determined by the biuret method (Robinson and Hogden, 1940).

Statistical analysis. All results were analyzed by Student's *t* test. Correlation coefficients (*r*) were computed to examine relationships between certain microsomal parameters.

RESULTS

Subacute Study

The effect of the subacute dosage regimen on body weight is illustrated in Fig. 1. A marked reduction in mean body weight gain of PCB-treated rats became evident after 5 weeks of twice weekly injections of 100 mg Aroclor 1242/kg.

Histopathologic alterations were evident only in the liver and kidneys of rats which received Aroclor 1242 over the 10-week period. Sudanophilic vacuolation was present in

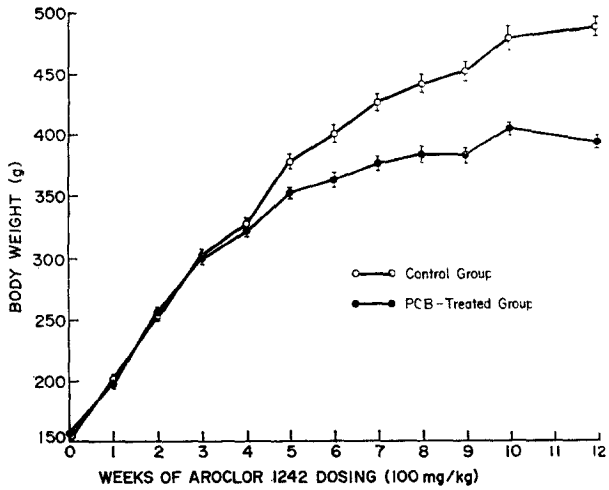

FIG. 1. Body weight gain of rats dosed with Aroclor 1242 (100 mg/kg ip) twice weekly for 6 weeks, then once weekly for 4 weeks thereafter. Plots represent mean values ± SE of the control group (4 rats) and of the PCB-treated group (9 rats).

midzonal areas of each hepatic lobule, leaving relatively unaffected cells in centrolobular and in portal zones. Multiple, tiny vacuoles were distributed uniformly through the cytoplasm of affected hepatocytes. Widely scattered, necrotic foci were also present in the liver of each PCB-dosed animal.

Kidneys of Aroclor 1242-dosed rats exhibited a moderate degree of dilation of proximal convoluted and collecting tubules, often with proteinaceous casts. Diffuse areas of sudanophilic vacuolation were evident within renal epithelium of proximal convoluted tubules. A unique finding in the present study was a marked dilation and degeneration of collecting tubules at the tip of the renal papilla of some PCB-dosed rats. The papillary capsular epithelium in such instances showed extensive vacuolation and hyperplasia (Figs. 2 and 3).

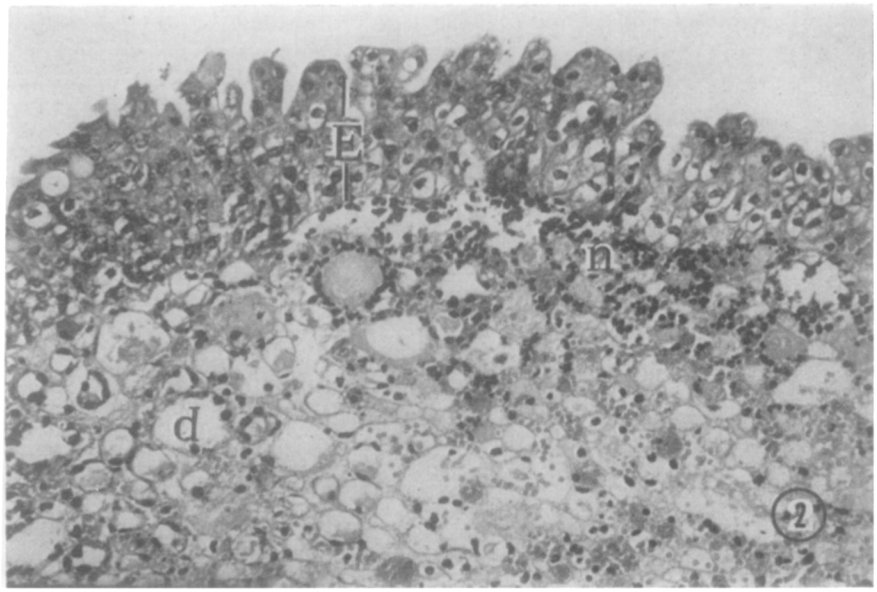

FIG. 2. Renal papilla of a rat maintained on the subacute dosage regimen. The hyperplastic epithelium (E) overlies a damaged area containing both necrotic (n) and dilated (d) collecting tubules. Hematoxylin and eosin. ×170.

Hematologic findings are shown in Table 1. Hematocrit, erythrocyte count, erythrocyte diameter, MCHC, and hemoglobin concentration were found to be somewhat lower in PCB-dosed animals than in controls. An elevation in the leucocyte count was noted in the blood of PCB-dosed rats, with a large increase in the proportion of circulating neutrophils. Erythrocytic anisocytosis was evident in PCB-treated rats when compared to controls, though no reticulocytosis or other morphologic abnormalities were evident. Osmotic fragility tests revealed that these erythrocytes were no more resistant to lysis in hypotonic saline than those from control rats.

Additional hematologic findings are presented in Table 2. Serum iron of PCB-dosed rats was found to be elevated when compared to control values. BUN and serum bilirubin values, though, were almost identical in both groups. Plasma corticosteroid and glucose concentrations were both diminished by Aroclor 1242 administration. Food

TABLE 1

EFFECT OF REPEATED ADMINISTRATION OF AROCLOR 1242 ON HEMATOCRIT, ERYTHROCYTE COUNT, ERYTHROCYTE DIAMETER, HEMOGLOBIN, MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) AND LEUCOCYTE COUNT[a,b]

	Hematocrit[c]	Erythrocyte count ($\times 10^6$/mm^3)	Erythrocyte diameter (μm)	Hemoglobin (g/100 ml)	MCHC (%)	Leucocyte count ($\times 10^3$/mm^3)
Controls	52.6 ± 0.7	13 ± 0.5	8.5 ± 0.04	16.4 ± 0.15	31.1 ± 0.6	9 ± 1
PCB-treated	49.1 ± 0.6	11 ± 0.2	8.1 ± 0.04	14.7 ± 0.18	29.8 ± 0.6	13 ± 1
p[d]	<0.01	<0.001	<0.001	<0.01	<0.3	<0.02

[a] Results are expressed as mean ± SE.
[b] Dosage regimen consisted of ip injections (100 mg/kg) given twice weekly for 6 weeks, then weekly thereafter for 4 weeks.
[c] Mean values represent average of 2 daily determinations.
[d] p values denote difference between control group (4 rats) and PCB-treated group (9 rats).

TABLE 2

EFFECT OF REPEATED ADMINISTRATION OF AROCLOR 1242 ON SERUM IRON, BUN, BILIRUBIN, PLASMA CORTICOSTEROIDS AND BLOOD GLUCOSE[a,b]

Group	Serum iron (μg/100 ml)	BUN (mg/100 ml)	Serum bilirubin (mg/100 ml)	Plasma corticosteroids (μg/100 ml)	Blood glucose (mg/100 ml)
Controls	212 ± 5	15.7 ± 0.7	0.16 ± 0.03	50.2 ± 2.7	143 ± 9
PCB-treated	269 ± 12	15.5 ± 0.3	0.16 ± 0.03	38.4 ± 2.8	117 ± 6
p[c]	<0.01	NS[d]	NS	<0.02	<0.05

[a] Results are expressed as mean ± SE.
[b] Dosage regimen consisted of ip injections (100 mg/kg) given twice weekly for 6 weeks, then weekly thereafter for 4 weeks.
[c] p values denote difference between control groups (4 rats) and PCB-treated group (9 rats).
[d] NS = not significant.

TABLE 3

EFFECT OF REPEATED ADMINISTRATION OF AROCLOR 1242 ON URINARY VOLUME AND URINARY CONCENTRATIONS OF PROTEIN, SUGARS, COPROPORPHYRIN, AND 17-KETOSTEROIDS[a,b]

	Volume[c] (ml/24 hr)	Protein[d] (mg/24 hr)	Sugars[d] (mg/24 hr)	Coproporphyrin (μg/24 hr)	17-Ketosteroids (μg/24 hr)
Controls	24 ± 3.0	359 ± 39	147 ± 16	0.69 ± 0.11	30.2 ± 2.9
PCB-treated	19 ± 1.2	681 ± 19	263 ± 18	6.67 ± 0.98	29.0 ± 1.3
p[e]	<0.1	<0.001	<0.01	<0.001	<0.4

[a] Results are expressed as mean $\pm$ SE.
[b] Dosage regimen consisted of ip injections (100 mg/kg) given twice weekly for 6 weeks, then weekly thereafter for 4 weeks.
[c] Mean values represent average of 5 daily determinations.
[d] Mean values represent average of 2 daily determinations.
[e] p values denote difference between control group (4 rats) and PCB-treated group (9 rats).

was not withheld prior to blood collection, in an effort to avoid stress-induced alteration of blood glucose and plasma steroid concentrations.

Results of urinalyses are given in Table 3. A reduction in the mean daily urine output of Aroclor 1242-dosed rats was measured, but variability was such that the p value was slightly more than 0.05. Mean urinary protein and sugar values of both control and PCB-treated groups were relatively high. This phenomenon was probably the result of the collection procedure, in which voided urine may have been contaminated by fecal and food materials. Still, the Aroclor 1242-dosed rats exhibited elevated urinary protein and sugar concentrations. Coproporphyrin excretion was markedly higher in PCB-treated animals; however, 17-ketosteroid excretion was equivalent in each group.

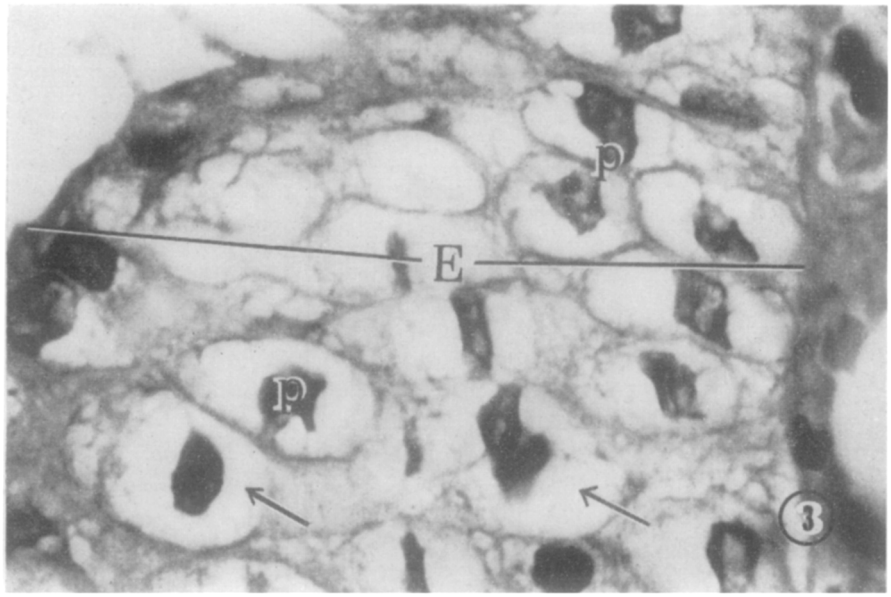

FIG. 3. Higher magnification of papillary epithelium (E) pictured in Fig. 2. Observe the severe cytoplasmic vacuolation (arrows) and the pyknosis (p). Hematoxylin and eosin. ×1100.

Microsomal Studies

The effects of a single ip injection of 100 mg Aroclor 1242/kg on microsomal enzymatic parameters are illustrated in Fig. 4. Hydroxylation and N-demethylation activities were significantly ($p < 0.025$) elevated 24 hr after dosing. Although each appeared maximal after 5 days, neither 5-day value was significantly ($p < 0.05$) different from its respective 3- or 10-day values. Hydroxylation activity showed the most marked rise, reaching a value almost 500% of controls. Both remained significantly ($p < 0.05$) higher than controls after 20 days, though N-demethylation activity was equivalent to control values after 40 days. Hydroxylation activity remained approximately 150% of control values 40 days following the single injection. Cytochromes P_{450} and b_5 and NADPH-cytochrome c reductase activity all showed significant ($p < 0.05$) increases 3 days after dosing. Each remained elevated over controls at 20 days post injection, but returned to control values after 40 days.

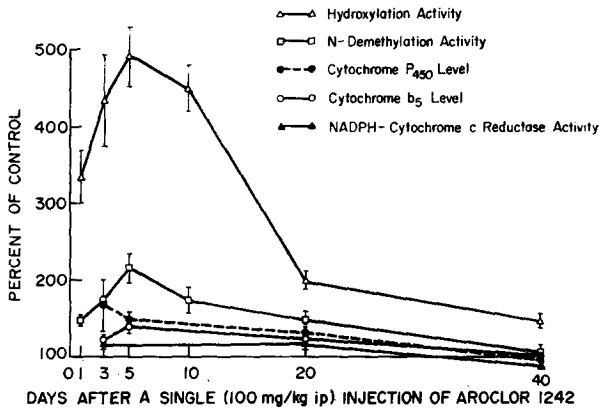

FIG. 4. Effect of a single dose of Aroclor 1242 (100 mg/kg ip) on hepatic microsomal enzymatic parameters. Each point represents the mean value of 6 PCB-treated rats vs that of 3 control rats; vertical bars represent the SE.

Dose-response relationships of the selected enzymatic parameters are shown in Fig. 5. No elevation of any parameter was noted in response to a single 1 mg/kg dose. Hydroxylation activity showed a small increase in response to 5 mg/kg, though individual variability created a rather large SE. Significant ($p < 0.01$) increases in both hydroxylation and in N-demethylation activity were measured in response to 25, 50, and 100 mg/kg doses of Aroclor 1242. Although hydroxylation activity was dose-dependent ($r = 0.98, p < 0.01$), a smaller degree of correlation between dose and N-demethylation activity ($r = 0.72$, $p < 0.2$) was manifest. Cytochromes P_{450} and b_5 and NADPH–cytochrome *c* reductase activity exhibited initial increases over controls in rats receiving 50 mg/kg. Cytochrome P_{450} values were even greater in rats dosed with 100 mg/kg; however, cytochrome b_5 and NADPH–cytochrome *c* reductase activity remained equivalent to their respective 50 mg/kg values.

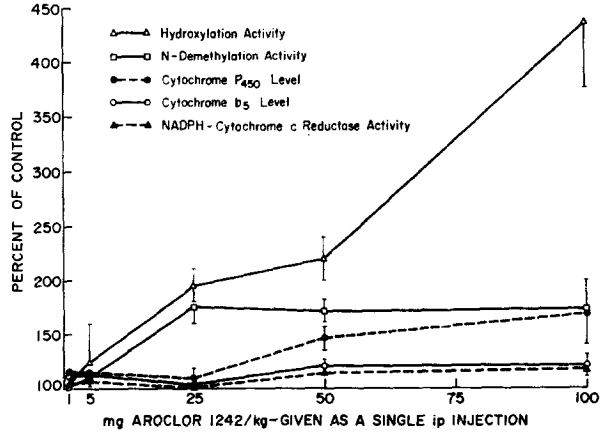

FIG. 5. Dose-response relationships of hepatic microsomal enzymatic parameters, measured 72 hr after a single injection (100 mg/kg ip) of Aroclor 1242. Each point represents the mean value of 4 PCB-treated rats vs that of 4 control rats; vertical bars represent the SE.

DISCUSSION

Our finding of reduction in body weight gain after 4–5 weeks of administration of Aroclor 1242 concurs with reports of other investigators (Nagai *et al.*, 1971; Vos and Notenboom-Ram, 1972), Although modes and regimens of administration of PCB compounds and isomers varied in these reports, adverse effects on body weight gain were noted within 4–6 weeks of initiation of dosing.

Pathologic alterations in the liver and kidneys of subacutely dosed rats were quite similar to those observed in an earlier subacute study (Bruckner *et al.*, 1973). Hepatic vacuolation was also noted by Koller and Zinkl (1973) to precede midzonal necrosis. Renal papillary degeneration and epithelial alterations, however, were unique to the present study. Visual observation of urine of Aroclor 1242-dosed rats revealed an abnormally dark coloration, as well as a reduction in urine volume. Renal injury was further evidenced by the demonstration of glycosuria and proteinuria. As blood glucose values of these animals were lower than those of controls, it may be assumed that glycosuria did not result from exceeding the resorptive threshold of the renal epithelium. Rather, the loss of glucose via the urine may have contributed to the depressed blood glucose concentration. The presence of proteinuria suggested glomerular damage; however, light microscopic examination revealed no apparent structural anomalies.

Aroclor 1242-induced increases in hydroxylation and in N-demethylation activities appeared maximal from 3–10 days after the single injection of Aroclor 1242. N-demethylation activity returned to normal 40 days after a single injection (100 mg Aroclor 1242/kg ip), although hydroxylation activity remained 150% of the control value. Similar peak activity times and prolonged induction periods for hepatic microsomal *O*-demethylase (Benthe *et al.*, 1972) and aniline hydroxylase (Fujita *et al.*, 1971) activities have been elicited in rats by a single dose of PCBs. Such prolonged elevation of microsomal enzyme activity indicates that PCBs may alter the rate of metabolism of a variety of substances for extended periods of time.

The minimal effective dose of Aroclor 1242 for induction of hydroxylation activity was approximately 5 mg/kg in this 10-week study, however, individual variation produced a value of p greater than 0.05. Although hydroxylase activity appeared dose-dependent, 50 and 100 mg/kg doses produced no more an inductive effect of N-demethylase activity than did 25 mg/kg. Dose-related increases in hydroxylase, demethylase, and nitroreductase activities in rat liver microsomes have been reported (Litterst *et al.*, 1972) in response to a 4-week feeding regimen.

Microsomal values for cytochrome P_{450}, cytochrome b_5, and NADPH–cytochrome c reductase activity tended to parallel microsomal enzymatic activity. However, marked differences were evident between the magnitude of these 3 cytochrome parameters and that of corresponding enzymatic activity. Greim and Remmer (1966) reported a similar phenomenon in rats in response to DDT. In the present study the P_{450} concentration was the most reliable index of hydroxylase activity $r = 0.67$, $p < 0.001$ with NADPH-cytochrome c reductase activity ($r = 0.46$, $p < 0.01$) and the b_5 value ($r = 0.20$, $p < 0.2$) less reliable indices. Some correlation of N-demethylase activity with P_{450} ($r = 0.65$, $p < 0.001$), b_5 ($r = 0.60$, $p < 0.001$), and NADPH–cytochrome c reductase activity ($r = 0.36$, $p < 0.02$) was demonstrable. It appears unlikely that induction of microsomal enzyme activity resulted from a selective stimulation of any one of these cytochrome

parameters. Rather, the level of each was likely elevated in response to enhanced activity of the entire microsomal oxidative sequence.

The administration of many drugs and insecticides has been shown to be associated, in hepatic microsomes, with stimulation of drug and steroid hydroxylation, proliferation of smooth endoplasmic reticulum, increased cytochrome P_{450}, and diminished bioeffects of drugs and steroids (Gillette *et al.*, 1969). Plasma adrenocorticosteroid concentrations of Aroclor 1242-dosed rats in our own study were lower than controls, suggesting a PCB-mediated increase in corticosteroid metabolism and excretion. Concomitant blood glucose values of PCB-treated rats were also lower than controls. This latter finding may have resulted from diminished plasma adrenocorticosteroids and/or loss of glucose in the urine via damaged renal epithelium. In contrast, Aroclor 1242 appeared to have no effect on urinary excretion of 17-ketosteroids.

Studies have demonstrated a number of diverse compounds to elicit concurrent induction of microsomal enzyme activity and porphyria (Wada *et al.*, 1968; Baron and Tephly, 1970). Our own results confirmed that Aroclor 1242 produced microsomal enzyme induction and porphyria, the latter evidenced by marked rises in urinary coproporphyrin excretion.

Hematologic effects of Aroclor 1242 included slight decreases in hematocrit, erythrocyte count and diameter, and hemoglobin concentration. A decreased incorporation of iron into protoporphyrin was suggested by the elevation of serum iron and decrease in hemoglobin concentration.

Contribution by Aroclor 1242 to the mild reduction in erythrocyte count via a hemolytic action was discounted by the finding of identical bilirubin values in serum of PCB-dosed and that of control rats. No alteration in osmotic fragility was observed, since erythrocytes from PCB-dosed rats were no more resistant to lysis in hypotonic saline than those from control rats.

The findings of renal and hepatic damage, hematologic effects, porphyria, altered plasma glucose and corticosteroid concentrations, and hepatic microsomal enzyme induction for extended periods of time indicate that PCBs affect a wide variety of biologic parameters. The long-term elevation of microsomal enzyme activity after a single dose of PCBs also suggests that these compounds have a great potential for interaction with the biologic responses of mammals to other chemical and environmental stresses.

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