Time-Course of Induction of Microsomal Enzymes Following Treatment with Polychlorinated Biphenyl

by

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Concern over environmental contamination by industrial chemicals has recently disclosed the widespread presence of polychlorinated biphenyls (PCBs) in various aquatic and terrestrial ecosystems. Several authors have recently documented the ability of PCBs to induce hepatic microsomal drug-metabolizing enzymes. LITTERST et al. (1972) have shown that dietary administration of Aroclor® 1254 to rats for 30 days produced increases in the activity of some oxidative enzymes and a marked increase in reductive reactions. In addition, LITTERST and VAN LOON (1972) showed that the inducing activity of PCB on a molar basis was equal to or greater than that of either DDT or phenobarbital. VILLENEUVE et al. (1972) reported that the pentobarbital sleeping time of rats was decreased following dietary administration of several PCB isomers. After parenteral administration of PCB to pregnant rabbits, increases in the activities of certain oxidative liver microsomal enzymes were observed (VILLENEUVE et al. 1971). Recently BICKERS et al. (1972) attempted to correlate changes in mixed-function oxidase activity with chlorine content of administered PCBs. In all studies reported to date, apparently arbitrary durations of exposure to PCB have been utilized to induce the enzymes studied, even though no work has been conducted to determine the optimal inducing dose, the rate at which PCB produces enzyme induction, or how stable the induced enzymes are after PCB administration is discontinued. The purpose of the present communication is to provide information on the latter two questions.

Methods

Male Osborne-Mendel rats weighing 125-150 g were used for all experiments. Rats were housed two per cage, fed Purina Laboratory Chow, and allowed free access to water. For dietary administration of the compounds, Aroclor® 1254 (obtained through the courtesy of Monsanto Chemical Co., St. Louis, Mo.) or phenobarbital sodium (PB) were incorporated into the diet at a level of 50 mg of compound per kg of feed (50 ppm) as previously described (LITTERST and VAN LOON 1972). Prior to use, control feed was analyzed for content of PCB and chlorinated hydrocarbon insecticides and found to contain 0.01 ppm PCB and 0.02 ppm DDT (DDT + DDE). For single-dose studies, the agents were administered via stomach tube at a dose of 50 mg/kg of body weight; PCB

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was prepared in corn oil and PB in 0.85% saline. Control animals were given equivalent volumes of vehicle. Experiments designed to demonstrate the stability of PCB-induced enzymes were conducted by feeding rats a diet containing 50 ppm of PCB for a period of 7 days. A group of rats was then killed (0 time) and microsomal enzymes and components were determined. The experimental diets were then replaced with control diet for the remaining animals and other groups were killed 3, 5, 10, and 14 days later for analysis.

PCB or PB were each administered to six rats; control groups also consisted of six rats. After treatment, the rats were killed by cervical dislocation, and their livers were excised, weighed, and homogenized in mannitol-sucrose buffer as previously described (LITTERST et al. 1972). All subsequent manipulations were conducted in a cold room at 4°C. The six livers from each treatment group were pooled in groups of two each for enzyme assays, which were conducted in duplicate. The supernatant fraction from a 9000 x g centrifugation of the liver homogenate was used for assays of N-demethylation of ethylmorphine, nitroreduction of p-nitrobenzoic acid, and hydroxylation of ^{14}C-pentobarbital. The 9000 x g supernatant was centrifuged for 1 hour at 105,000 x g and the resulting pellet was used for determination of microsomal protein and cytochrome P-450 content. All enzyme and component assays have been described in detail in a previous communication (LITTERST et al. 1972). Differences between treated groups and control groups were judged significant by a two-tailed Student's t test at $P=0.05$.

Results

Table 1 summarizes the results of experiments in which PCB or PB was fed to rats at a dose level of 50 ppm in the diet. Food consumption and weight gain have been shown to be constant for control and experimental animals at this dose level (LITTERST and VAN LOON 1972). The 50 ppm dietary level is approximately equivalent to 5 mg PCB or PB consumed per kg of body weight per day. Both PCB and PB produced significant increases in levels of hepatic microsomal drug-metabolizing enzymes as early as 7 days after exposure. At 28 days, all parameters continued to be elevated but the activities of hydroxylase and demethylase appeared to be increasing at a much less rapid rate than earlier, while nitroreductase and P-450 continued the rapid increase in activity.

The results of oral administration of a single dose of 50 mg/kg of PCB or PB are shown in Table 2. Most parameters reached peak values by 24 hours after treatment, although in animals treated with PCB, hydroxylation apparently did not reach a peak in activity until at least 48 hours after treatment. The relative quantities of cytochrome P-450 detectable in microsomal pellets appeared to be more closely related to activity of ethylmorphine demethylase than to activity of the other enzymes studied. PCB

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TABLE 1

Development of Induction in Microsomes of Rat Livers Following Dietary Administration of PCB or Phenobarbital (PB)[a]

Days on Diet	Treatment[b]	Liver: Body Weight Ratio	Protein (mg/g)	P-450 (nmoles/mg)	Demethylation (nmoles/g/30 min)	Nitroreduction (µg/g/30 min)	Hydroxylation (µg/g/30 min)
7	0	0.033±0.003	28.1±0.6	0.62±0.02	4125±438	49.2±7.4	0.22±0.02
	PCB	0.037±0.001	34.6±4.4	0.78±0.06[c]	8750±575[c]	148.1±18.2[c]	0.44±0.04[c]
	PB	0.035±0.004	33.9±2.2[c]	0.78±0.02[c]	8263±1285[c]	105.0±10.2[c]	0.40±0.08
14	0	0.033±0.002	21.7±0.7	0.66±0.05	3406±781	57.0±4.5	0.16±0.02
	PCB	0.036±0.002	25.4±0.9[c]	1.03±0.11[c]	8354±2020[c]	149.3±8.6[c]	0.40±0.04[c]
	PB	0.034±0.001	23.4±0.9	0.75±0.04	6896±1236[c]	88.0±6.2[c]	0.28±0.01[c]
28	0	0.030±0.002	29.5±1.5	0.66±0.01	3660±387	60.5±0.5	0.28±0.02
	PCB	0.037±0.004	41.4±1.6[c]	1.28±0.16[c]	7729±1385[c]	212.8±46.0[c]	0.66±0.16[c]
	PB	0.034±0.003	33.9±1.2	0.86±0.05[c]	6605±337[c]	105.0±7.2[c]	0.40±0.02[c]

[a]Values are means ± S.D. of 3 replicates except nitroreductase activity at 7 days where n=2.

[b]Rats were given diets containing 50 ppm of Aroclor® 1254 or phenobarbital for the times indicated.

[c]Statistically different from control at $P<0.05$.

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TABLE 2

Effect of a Single Oral Dose of PCB or Phenobarbital (PB) on the Activity of Microsomal Drug-Metabolizing Enzymes in Rats[a]

Hours after Treatment	Treatment[b]	Liver: Body Weight Ratio	Protein (mg/g)	P-450 (nmoles/mg)	Demethylation (nmoles/g/30 min)	Nitro reduction (µg/g/30 min)	Hydroxylation (µg/g/30 min)
6	0	0.035±0.002	32.2±0.3	0.61±0.02	6262±388	81.2±6.2	0.24±0.02
	PCB	0.037±0.001	31.6±1.0	0.64±0.06	4938±840	74.6±3.2	0.20±0.01
	PB	0.038±0.002	32.8±1.3	0.62±0.02	7104±1315	88.7±12.1	0.22±0.04
12	0	0.030±0.002	32.5±1.9	0.65±0.01	5969±219	65.9±3.4	0.26±0.02
	PCB	0.036±0.001[c]	36.0±2.2	0.93±0.09[c]	7417±449[c]	95.5±5.7[c]	0.28±0.02
	PB	0.033±0.002	40.0±1.3[c]	0.73±0.01[c]	7281±221[c]	103.0±2.0[c]	0.28±0.04
24	0	0.035±0.002	26.9±0.3	0.68±0.08	7592±192	78.2±5.8	0.26±0.03
	PCB	0.040±0.001[c]	38.8±1.8[c]	1.25±0.01[c]	13479±1515[c]	180.4±41.8[c]	0.38±0.02[c]
	PB	0.039±0.003	36.0±2.4[c]	1.25±0.03[c]	16354±2153[c]	207.7±35.1[c]	0.44±0.08[c]
48	0	0.035±0.001	32.0±1.0	0.57±0.01	5094±654	73.8±18.8	0.20±0.02
	PCB	0.042±0.002[c]	40.8±4.1	1.06±0.09[c]	10875±2378[c]	200.8±28.9[c]	0.44±0.14[c]
	PB	0.040±0.002[c]	39.4±1.8[c]	0.93±0.13[c]	12500±2558[c]	169.3±32.5[c]	0.42±0.09[c]

[a] Values are means ± S.D. of 3 replicates.

[b] Rats were given 50 mg/kg PCB or phenobarbital by stomach tube.

[c] Statistically different from control at $P<0.05$.

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and PB both appeared to be equally effective in their abilities to increase microsomal activity and component content, and both generally demonstrated the same approximate rates and extents of induction.

Table 3 shows decay of microsomal enzymes and components following induction by dietary treatment with PCB and PB. The levels of enzymes and components at 0 day demonstrate that all enzymes were induced before the experimental diets were removed and that the amount of induction was comparable to that in other experiments 7 days after dietary treatment (see Table 1). After rats had been fed the control diet for 10 days, values for all enzymes and components except hydroxylation had returned to normal or near-normal. PB-induced enzymes appeared to return to control levels more rapidly than did PCB-induced enzymes.

Discussion

Dietary administration of PCB has previously been shown to increase the activity of microsomal drug-metabolizing enzymes (LITTERST and VAN LOON 1972; LITTERST et al. 1972; VILLENEUVE et al. 1972); Table 1 demonstrates the approximate rate at which this induction occurs. P-450, nitroreductase, and demethylase levels from PB-treated rats appeared to reach the maximum level of induction within 7 days of treatment. In PCB-treated rats, the maximum induction of demethylation had also occurred in 7 days, but P-450 and nitroreductase levels in this group continued to increase during the entire 4 weeks of treatment. Both PCB and PB produced the same qualitative response in hydroxylation and protein content; each compound demonstrated two peaks of activity, one after 7 days and the other after 28 days of treatment, with a decline in activity between the two peaks.

PCB and PB both had the same effect on microsomal enzymes after a single oral dose; activities increased until 24 hours after treatment and then either remained elevated or declined. With both compounds, enzyme activities were still significantly increased over control values 48 hours after the single administration. Six hours after the single dose of PCB, all enzyme activities were slightly decreased. Although this decrease was not statistically significant, it is consistent with unpublished data from this laboratory in which low doses of PCBs (0.5 and 5.0 mg/kg) produced similar trends in these same parameters in three separate experiments.

The results of this study demonstrate that treatment with low dietary doses of PCB or PB produce significant levels of enzyme induction within 7 days and that with PCB, but not PB, this level of induction continues to increase. Single oral doses of PCB and PB produced similar responses in enzyme activity; the activity

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TABLE 3

Stability of Induced Enzymes or Components Following Removal of the Inducing Substance[a]

Days on Control Diet	Treatment	Liver: Body Weight Ratio	Protein (mg/g)	P-450 (nmoles/mg)	Demethylation (nmoles/g/30 min)	Nitro reduction (µg/g/30 min)	Hydroxylation (µg/g/30 min)
0	0	0.032±0.002	27.5±0.8	0.40±0.01	4818±254	42.7±2.2	0.22±0.02
	PCB	0.048±0.003[b]	32.5±1.6[b]	0.56±0.02[b]	8727±375[b]	104.6±16.1[b]	0.47±0.05[b]
	PB	0.044±0.001[b]	31.4±0.6[b]	0.48±0.03[b]	7567±574[b]	94.4±4.6[b]	0.41±0.04[b]
3	0	0.037±0.001	27.0±0.8	0.48±0.03	4875±775	55.0±4.0	0.26±0.04
	PCB	0.041±0.001[b]	32.5±0.4[b]	0.49±0.03	8362±512[b]	76.5±5.5[b]	0.48±0.04[b]
	PB	0.042±0.004[b]	30.1±0.9[b]	0.49±0.04	6687±262[b]	62.5±3.5[b]	0.38±0.02[b]
5	0	0.041±0.002	37.2±2.5	0.42±0.03	5640±196	46.0±0.6	0.28±0.02
	PCB	0.049±0.003[b]	44.2±2.4[b]	0.58±0.03[b]	10980±770[b]	72.4±10.3[b]	0.44±0.08[b]
	PB	0.045±0.001	40.5±2.2	0.42±0.02	6640±315[b]	53.6±6.3	0.28±0.02
10	0	0.042±0.002	45.0±1.6	0.38±0.01	4680±368	47.2±2.0	0.28±0.04
	PCB	0.041±0.002	47.7±1.1	0.44±0.01[b]	5730±531	57.7±1.9[b]	0.42±0.02[b]
	PB	0.041±0.002	45.4±2.0	0.41±0.05	4800±300	50.6±1.6	0.28±0.02

[a]Rats were maintained on diets containing 50 ppm PCB or phenobarbital for 7 days; at day 0 the treated diet was replaced with control diet. Values are means ± S.D. of 3 replicates obtained after the indicated times on control diet.

[b]Statistically different from control at $P<0.05$.

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reached a peak 24 hours after dosing and then slowly declined to normal. Discontinuation of PCB or PB resulted in a slow decay of the induced enzyme activity to approximately control steady-state levels after 10 days. The enzyme activity in PB-treated rats returned to normal somewhat more rapidly than did that in PCB-treated rats.

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