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Effects of Polychlorinated Biphenyls and Nutritional Restriction on Barbituate-induced Sleeping Times and Selected Blood Characteristics in Raccoons (*Procyon lotor*)

W. Edward Montz, William C. Card, and Roy L. Kirkpatrick

Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants and have been implicated in a variety of both lethal and sublethal effects in mammals. Among these sublethal effects are reductions in body weight and diminished appetite (SANDERS et al. 1974; AULERICH and RINGER 1977; BLEVINS et al. 1980), hepatic microsomal enzyme induction (STREET et al. 1969; VILLENEUVE et al. 1971; BITMAN et al. 1972; CONNEY and BURNS 1972; LITTERST et al. 1972; ALLEN et al. 1973; CHEN and DUBOIS 1973), and lowered immune system responses (THOMAS and HINSDILL 1978, 1980).

Since the hepatic microsomal enzyme system metabolizes PCBs (FISHBEIN 1974), significant changes in enzyme activity may alter substrate concentrations in other metabolic pathways. We hypothesized that such changes might be observed by monitoring key blood characteristics. Little information is available on the response of specific blood characteristics to PCB treatment. In addition, many wild animals often undergo seasonal periods of food limitation which might be expected to alter the response of an animal to PCBs.

The purpose of this study was to induce hepatic microsomal enzyme activity in wild-trapped raccoons (Procyon lotor) and to measure selected blood characteristics in an effort to detect responses due to PCB ingestion, nutritional restriction, and their interactions. Barbituate-induced sleeping times were used as an index of hepatic microsomal activity because they have been used reliably by other workers (BITMAN et al. 1972; SANDERS et al. 1974; ZEPP and KIRKPATRICK 1974). Blood characteristics examined in this study were nonesterified fatty acids (NEFA), cholesterol, and three ketone bodies; D-(-)-3-hydroxybutyrate, acetoacetate, and acetone. NEFA and the ketone bodies were selected because of their roles in energy metabolism. Cholesterol plays a major role as precursor in the biosynthesis of reproductive hormones, cortisol, and bile salts.

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METHODS AND MATERIALS

Sixteen wild-trapped raccoons of undetermined ages (13 females, 3 males) were placed in one of 4 blocks (4 animals/block) by weight and randomly assigned within block to one of four treatments in a 2 x 2 factorial design. Experimental factors were nutritional intake (100 or 70% ad libitum based on pre-experiment feeding trials) and PCB treatment (0 or 50 mg Aroclor 1254/kg body weight/da). A 10% solution of Aroclor 1254 in acetone was injected into the center of a sphere (approx. 3-cm dia.) of ground beef at the calculated dosage for each animal. After consuming the PCB treated ground beef, raccoons were given commercial dog food at 100 or 70% ad libitum. Water was supplied to all raccoons ad libitum. Galvanized steel cages (60- x 60- x 30-cm) were used to keep raccoons outdoors during fall environmental conditions.

After 8 days of treatment, all raccoons were weighed and injected intraperitoneally with sodium pentobarbitol (Nembutol; Abbott Laboratories, N. Chicago, IL) at a dose of 30 mg/kg body weight. Sleeping time, the time elapsed from loss to regaining of righting ability, was recorded for each animal. Blood samples were taken by cardiac puncture immediately upon regaining righting ability. Blood samples for serum were obtained using sterile evacuated 15-ml blood collection tubes (Vacutainer, Becton Dickenson and Co., Rutherford, NJ) along with 20-gauge 38-mm needles and were immediately placed on ice. Sterile heparin-coated 10-ml Vacutainers were used to collect uncoagulated whole blood for ketone body determinations. Serum and deproteinized whole blood fractions obtained were stored in capped polyethylene culture tubes and frozen at -10°C until analyzed.

Colorimetric procedures were employed to determine concentrations of NEFA (NOMA et al. 1973), cholesterol (ZAK 1957), D-(-)-3-hydroxybutyrate (WILLIAMSON and MELLANBY 1974), acetoacetate (MELLANBY and WILLIAMSON 1974) and acetone (PEDEN 1964). Cuvette concentrations of ketone bodies were adjusted by appropriated dilution factors to quantify concentrations in whole blood (BERGMAYER et al. 1974:315).

Analysis of variance was used to determine significance of treatment effects. Duncan's multiple range test was used to distinguish differences among groups.

RESULTS AND DISCUSSION

All raccoons survived treatment, and the mean body weight of all 4 groups increased regardless of treatment (Table 1). Increases in weight were probably due to the large number of growing juvenile and subadult animals. The 70% restriction did not entirely prevent weight gain in younger animals. Decreases in activity due to confinement may have reduced the severity of restriction. However, animals not given PCBs gained significantly more weight than those receiving PCBs ($P<0.02$). PCB treatment did cause a noticeable voluntary food restriction; however, this could not be readily quantified.

Mean sleeping times were 30% lower for PCB treated groups than for untreated groups but were not significantly different ($P=0.13$). Groups fed PCBs did have 30% lower mean sleeping times than controls. Small sample sizes and large variability reduced ability to detect statistical differences among treatments. Our pentobarbitol sleeping times are comparable to those of STREET et al. (1969) who found that rats fed 50 to 100 ppm of Aroclor 1248 and 1268 for 15 days had hexobarbital sleeping times which were reduced 35% and 48%, respectively, when compared with controls.

NEFA concentrations were elevated in raccoons receiving either PCB treatment or nutritional restriction but these differences were not significant ($P=0.10$). The observation that nutritional restriction increased NEFA concentrations is consistent with many previous studies which have demonstrated this response (ANNISON 1960:; REID and HINKS 1962; RUSSELL et al. 1967; KARIHALOO et al. 1970). Our results also show a significant interaction between PCB treatment and nutritional restriction ($P<0.03$).

Cholesterol concentrations were significantly lower ($P<0.03$) in the two PCB treated groups than those not given PCBs. This finding suggests increased metabolism of cholesterol possibly caused by enhanced microsomal enzyme activity. Our results differ with those of TANAKA et al. (1969) who reported that PCBs fed to rats at 100 mg/kg body weight/da for 28 days caused elevated serum cholesterol levels. By contrast, VILLENEUVE et al. (1971) found that female rabbits fed 10 mg/kg body weight/da demonstrated no difference in serum cholesterol levels. These discrepancies may be explained by differences among mammalian species in toxic responses to PCBs, differing dose rates among the studies, degree of voluntary food restriction, and/or reduction in physical activity.

TABLE 1

Characteristics measured to assess the effects of PCBs and nutritional restriction in 4 groups of raccoons (means ± S.E.).

Characteristic	100% ad libitum diet		70% ad libitum diet	
	no PCB	PCB	no PCB	PCB
(n)	(4)	(4)	(4)	(4)
Weight gain (g)	715 ± 178[a,1]	144 ± 364[b]	591 ± 176[a]	177 ± 114[b]
Sleeping time (min)	163.8 ± 53.8[a]	103.3 ± 24.0[a]	129.0 ± 58.0[a]	102.5 ± 41.3[a]
NEFA (uM)[2]	228.0 ± 98.1[a]	368.9 ± 102.6[a]	619.3 ± 124.4[a]	255.2 ± 85.6[a]
Cholesterol (mg/dl)[2]	159.1 ± 28.0[a]	109.7 ± 26.0[b]	169.9 ± 20.8[a]	87.2 ± 22.2[b]
D-(-)-3-hydroxybutyrate (uM)[3]	86.1 ± 35.6[a]	264.1 ± 75.3[b]	293.4 ± 84.6[b]	362.7 ± 70.8[b]
Acetoacetate (uM)[3]	16.3 ± 5.8[a]	43.1 ± 8.8[a]	34.8 ± 5.9[a]	24.1 ± 6.0[a,4]
Acetone (uM)[3]	88.4 ± 18.9[a]	78.9 ± 15.0[a]	100.7 ± 20.4[a]	95.3 ± 11.8[a]

[1]Means with different letter superscripts are significantly different ($P<0.05$, Duncan's Multiple Range Test).
[2]Serum concentrations.
[3]Whole blood concentrations.
[4]n=3.

D-(-)-3-hydroxybutyrate concentrations were significantly higher ($P<0.05$) in restricted and PCB-fed animals than the group fed uncontaminated food ad libitum. A highly significant interaction ($P<0.002$) between PCB treatment and nutritional restriction was observed in acetoacetate concentrations. Acetoacetate can be converted to D-(-)-3-hydroxybutyrate in the liver (GANONG 1979:232). Highest concentrations of D-(-)-3-hydroxybutyrate were found in restricted PCB-fed animals which also exhibited the lowest acetoacetate concentrations of PCB treated groups.

Acetoacetone and acetone concentrations did not differ significantly among the 4 groups. Further experiments are needed to elucidate the role of PCB ingestion in altering biochemical pathways.

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