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In Vivo Metabolism of Exogenous Progesterone by PCB Treated Female Rats

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The physiological manifestations elicited by exposure to polychlorinated biphenyls in mammals has received significant attention (PEAKALL, 1967; NOWICKI and NORMAN, 1972; GOOD and WARE, 1969). Included in the diversity of these manifestations are effects related to the alteration of steroid hormone metabolism (NOWICKI and NORMAN, 1972; VILLENEUVE, et al., 1971). Experimentation to date in the area of enhanced steroid metabolism by the induction of mixed function oxidases has largely been concerned with in vitro studies resulting in an increase in polar metabolites from testosterone, estradiol, B^{-17} and progesterone. The hepatic microsomal drug metabolizing enzymes aniline hydroxylase and aminopyrine N-demethylase are both indicated to be induced by the administration of 10 mg/kg body weight of Arochlor 1254 (VILLENEUVE, et al., 1971). These authors suggested that Arochlor 1254 may increase the hydroxylation of naturally occurring steroids in vivo. However, no study has been undertaken to elucidate or quantitate the degree of induced steroid metabolism via an in vivo approach. The in vitro demonstration of enhanced hepatic metabolism of estrogens and androgens may not be reflective of in vivo metabolism in that compensatory homeostatic mechanisms may be operating to maintain adequate hormone levels.

Methods and Materials

Nine female Spague-Dawley rats weighing 343 g ($\pm$ 5.2 g) were equally divided into three groups. Two of the groups received intraperitoneal 0.25 ml injections of Arochlor 1254 (Monsanto Chemical Co.) in sesame oil of 10 and 40 mg/kg respectively. The third group designated the controls received 0.25 ml volumes of sesame oil. Injections were given every third day for a two week period.

On the sixteenth day after PCB exposure was initiated all animals received an intravenous introduction of progesterone -4^{14}C (specific activity 47.2 mCi/mM) diluted with non-radioactive progesterone (Sigma Chemical Co.) resulting in a working specific activity of 12.6 uCi/uM. The parent radiolabeled progesterone demonstrated a single peak of radioactivity with the solvent system utilized. Daily

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urine samples were collected from all animals for six consecutive days. The analytical scheme utilized for metabolite determination was to extract a 5 ml volume urine with 20 ml of dichloromethane evaporated to dryness and then dissolved in 1 ml of methanol. A 200 ul aliquot was spotted on a TLC plate (silicic acid) and developed in a solvent system consisting of decalin, nitromethane and methanol (2:1:1). Following development 1 cm sections were scraped into scintillation vials' a fluor added (Bray's solution) and counted on a Packard Tri-Carb Counter (Model No. 2420). An extraction efficiency of 92.6 ± 3% was obtained for progesterone utilizing this analytical scheme.

The c.p.m. (counts per minute) obtained for each 1 cm scraping was plotted against distance from origin for each treatment for every day of urine collection. The amounts of metabolites formed were calculated from areas under the utilizing a planimeter. The amount of progesterone metabolized refers to the summation of all metabolites formed and assumes that there was no isotope effects in the conversion of progesterone, 4-^{14}C to its corresponding metabolites.

Results and Discussion

Typically three major peaks were obtained from urine collected from control and PCB treated female rats when cpm were plotted against distance from the origin, indicating two major metabolic products were formed. The values presented under metabolites in Table 1 are the summation of the relative areas under these two peaks while parent progesterone values represent relative areas under that single peak of radioactivity. R_f values for the two metabolites were 0.11 and 0.29 respectively while the parent progesterone had an R_f value of 0.52.

Control animals excreted parent progesterone and a small amount of metabolized progesterone throughout the experiment period with a peak of activity at day 3 followed by a decrease in excretory rate (Table 1). PCB treated animals also demonstrated similar excretory patterns with peak excretion at day 3 but the amount of metabolites found was increased at both 10 and 40 mg/kg exposures. The ratio of parent progesterone (P) to its corresponding metabolites (M) demonstrates than in increased rate of metabolism was accomplished by the PCB pretreated animals. Pretreatment with PCB resulted in an approximately 2.6 fold increase in metabolism of progesterone when compared to the controls.

TABLE 1. Time dependent production of metabolites obtained from ip introductions of progesterone, 4-^{14}C to female Sprague-Dawley rats pre-exposed for 14 days to 10 and 40 mg/kg Arochlor 1254. Values presented are relative units calculated from areas under the curve generated from plotting cpm of each 1 cm section of a developed TLC plate against its distance from the origin.

	TREATMENT								
	Control			10 mg/kg			40 mg/kg		
DAY	Parent (P) progesterone	Metabolites (M)	Ratio P/M	Parent progesterone	Metabolites (M)	Ratio P/M	Parent gesterone	Metabolites (M)	Ratio P/M
1	150(12.6)[a]	16(6.2)	9.3	196(13.2)	49(9.6)	4.0	226(19.2)	58(4.3)	3.8
2	525(9.7)	46(4.7)	11.4	679(21.1)	98(6.9)	6.3	757(25.2)	111(9.6)	6.8
3	463(20.6)	37(5.6)	12.5	409(19.3)	105(12.2)	4.8	365(19.2)	94(9.3)	3.9
4	327(12.2)	26(4.9)	12.6	201(16.2)	68(6.5)	3.0	190(1.27)	63(9.3)	3.0
5	265(15.7)	20(3.2)	13.2	160(19.3)	49(6.8)	3.2	151(16.2)	53(6.6)	2.8
6	120(6.9)	29(9.6)	4.1	112(6.9)	86(9.6)	2.0	136(11.6)	70(6.9)	1.9

[a]Values represent mean and standard deviations of 3 animals per treatment.

The data presented in Table 1 clearly demonstrate that pretreatment of Spague-Dawley female rats with 10 and 40 mg/kg Arochlor 1254 elevated the metabolism of progesterone. The metabolic increase of 2.6 fold over the controls obtained in this study for progesterone agrees well with that reported by PEAKALL (1967) who obtained an *in vitro* value of 2.3 utilizing DDT as an hepatic microsomal inducer in pigeons. The *in vitro* metabolism of progesterone by pigeon microsomes also demonstrated the same type of chromatographic separation of parent progesterone and metabolites obtained in this study. Similarly NOWICKI and NORMAN (1971) reported a 2.7 fold increase in the *in vitro* metabolism of estradiol B-17 in chickens when pretreated with Arochlor 1254.

The degree of *in vivo* metabolism of progesterone compared to the *in vitro* values reported by other workers demonstrates that self-compensating homeostatic mechanisms may not be operable in maintaining sufficient progesterone levels. However, further experimentation is needed before concluding that physiologically deleterious manifestations are caused by exposure to compounds capable of inducing mixed function oxidases which they can hydroxylate naturally occurring steroids.

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