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Effects of Maternal Ingestion of Aroclor 1254 (PCB) on the Developmental Pattern of Oxygen Consumption and Body Temperature in Neonatal Rats

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Polychlorinated biphenyl (PCB) is an environmental pollutant that has been implicated in depression of reproductive success in Great Lakes gulls, production of congenital deformities in humans, and increased incidence of carcinogenesis in laboratory mice (Kimbrough 1980). PCB has also been shown to be a thyrotoxin in both adult (Bastomsky 1976) and developing animals (Collins and Capen 1980). Most recently, the hypothyroid effects of PCB exposure have been reported to elicit effects similar to those of hypothyroidism caused by other methods. Meserve and Leathem (1973) demonstrated a delay in maturation of the hypothalamo-pituitary adrenal (HPA) axis in developing animals using a well known thyrotoxin (thiouracil), and similar results have been documented employing PCB as the thyrotoxin (Meserve *et al.* 1992).

Thyroid hormone is important in the development of thermoregulation in mammals and birds (Silvia 1993). Because of the inefficiency of thermoregulatory mechanisms in neonatal mammals, body temperature is regulated by several different systems. The major endocrine regulation is supplied by thyroid hormones and insulin, while the major nervous system response is via the sympathetic nervous system (Silvia 1993). In adult animals, Komvies and Alayoku (1980) showed a depression of body temperature following PCB ingestion. This phenomenon can be attributed to a reduction in thyroid hormones following PCB ingestion.

Because similar studies have not been conducted in developing animals, and given the delicate nature of the development of metabolic parameters, the present study was done to determine the effects of PCB ingestion in pregnant and lactating mothers on the development of thermoregulation in neonatal animals. To this end, the present study evaluated body temperature and rate of oxygen consumption in rat pups on days 4 through 14 after birth. Because the major thermoregulatory hormones are thyroid hormones, thyroid hormone status and thyroid weights were evaluated at the end of the study on postnatal day 15.

MATERIALS AND METHODS

Female Harlan Sprague-Dawley rats (Indianapolis, IN) weighing approximately 225 g were mated to males of the same strain. From the first day of pregnancy, as determined by sperm in vaginal washings, rats were caged singly and fed *ad libitum* either Teklad Rodent Diet (W) 8604 mash (Harlan Teklad, Madison, WI) or mash with the addition of PCB (Aroclor 1254, 125 ppm or 250 ppm, w/w). An anonymous donor provided the PCB, which was originally produced by Monsanto

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Company (St. Louis, MO). The control and 125 ppm groups contained five litters of pups (8 pups per control litter and 6-8 pups per 125 ppm litter) and the 250 ppm group contained four litters (8 pups per litter). PCB was delivered to the pups through the mother across the placental and lactational barriers (Takagi *et al.* 1976). Mother rats were continued on the appropriate diet from conception to completion of the experiment, when pups were 15 days old (day of birth was considered day 0). At 3 days of age, litters were seperated by sex and culled to 8, 4 males and 4 females when necessary, to minimize litter effects (Fiorotto *et al.* 1991). Within gender, culling was done randomly.

Rectal body temperature was determined between 9:00 am and 11:00 am daily from day 4 through 14 using an Omega copper-constantan thermocouple (Springdale, CT) combined with an electric thermometer (Texas Instrument, Dallas, TX). Immediately following the temperature measurements, rate of oxygen consumption (VO_2) was measured employing a flow-through system with constant air flow, as measured by Brooks Instrument type 26-011-3 (Hatfield, PA) flow meter. Initial and final fractional oxygen concentration was measured with a Beckman model C2 oxygen analyzer (Fullerton, CA). The air flow passed over a combination of Collins Baralyme (Braintree, MA) and anhydrous $CaSO_4$ (Drierite, Xena, OH), for removal of carbon dioxide and water vapor, respectively. Each animal was placed for 5 min in a 300 ml air tight Plexiglas chamber kept at room temperature (22 - 23 °C) with a constant air flow. During the final 1.5 min, the expired oxygen concentration was determined by averaging the final oxygen concentrations from readings taken every 30 sec. Calculations for oxygen consumption followed Hill (1972), model 1.

On day 15, the pups were quickly decapitated and trunk blood was collected. The collected blood was centrifuged and the extracted serum was stored at -20 °C until circulating thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) levels were determined. T_4 and T_3 levels were measured by commercially available RIA kits (ICN Diagnostics, Carson, CA). Final body weights and thyroid weights were also determined.

Since no statistical differences were found between the sexes, data were combined within treatment groups regardless of sex. Because of the differences in mean body weights, the thyroid weights were expressed as mg/100 g body weight and VO_2 as ml O_2/kg body weight*min. The data were evaluated by analysis of variance (ANOVA). For the metabolic data, daily VO_2 and body temperature, a two way ANOVA for repeated measures was employed. Development of body temperature and VO_2 was also analyzed by linear regression. The remaining data were subjected to a one way ANOVA, and significant interactions to post hoc analysis using Fisher's protected LSD (Zar 1984). Statistical significance in all cases was ascribed to comparisons where $p < 0.05$.

RESULTS AND DISCUSSION

The present data correspond well with other reports (Meserve *et al.* 1992), with regard to the dose-dependent depression of body weight and the difference in response of the thyroid hormones to PCB(Table 1). T_4 was depressed to less than 1% of control, while T_3 levels were unchanged compared to the control at both dose levels. At the 125 ppm dose, a goitrogenic response was observed as a

43.7% increase in relative thyroid weight, but at the 250 ppm dose, no such effect was observed.

The action of PCB on the thyroid hormones is similar to the response that occurs when an animal is provided an iodine deficient diet in that depression of T_4 occurs without depression of T_3 (Santisteban *et al.* 1982). However when diets are supplemented with thyrotoxins such as propylthiouracil, both T_4 and T_3 are depressed by inhibition of both the production of T_4 and the conversion of T_4 to T_3 through action of type-I 5'-deiodinase (5'D-I) (Tsukahara *et al.* 1990). Dietary thyrotoxins tend to inhibit 5'D-I while iodine deficient diets only limit the production of T_4 in the thyroid. Following PCB exposure, 5'D-I activity may be enhanced rather than depressed. The absence of significant depression of T_3 in either group (Table 1) may be attributed to the enhancement of 5'D-I activity even with depressed T_4 levels.

Table 1. Effects of feeding 125 ppm or 250 ppm Aroclor 1254 to female rats during pregnancy and lactation on body weight, relative thyroid weight, serum total T_4, and serum total T_3 levels in 15 day old rats.

	Body Wt (g)	Thyroid Wt (mg/100 g)	total T_4 (μg/dL)	total T_3 (ng/dL)
Control (40)§	44.1 ±1.5 †	12.6 ±1.2	7.47 ±0.45	100.6 ±5.2
125 ppm (35)	40.1* ±0.8	19.0 * ±1.3	0.64 * ±0.11	113.4 ±7.8
250 ppm (32)	32.1* ±1.0	13.8 ±1.4	0.28 * ±0.05	84.3 ±6.9

§ Number of animals used is in parentheses.
† Data represented as mean ± SEM.
* Statistically different from the control ($p < 0.05$).

The PCB treatment significantly altered devlopment of VO_2, which occurred increasingly across time [$F_{(20,1040)} = 8.39$, $p < 0.001$] (Fig. 1). However, the development of the 125 ppm group was subnormal while the 250 ppm group did not differ from the controls. By day 14, VO_2 for the 125 ppm group stabilized at a level 14.8 ml O_2/kg*min less than that for the controls, but the 250 ppm group was similar to controls (Fig. 1). The VO_2 development of all three groups occurred in a linear fashion, with $r^2 = 0.86$, 0.92, and 0.82 for the controls, 125 ppm, and 250 ppm groups, respectively (Fig. 1).

Because VO_2 depends on many variables, such as body size, hormone status, and nutritional levels (Silvia 1993), body temperature is likely to be a better predictor of overall metabolic activity than VO_2. In the present study, the treatment also significantly affected body temperature development [$F_{(20,1040)} = 18.48$, $p < 0.05$]. The temperature of 125 ppm animals was lower than the controls more days (8 of 11 days tested) than the 250 ppm animals (5 of 11) (Fig. 2). At day 14, the

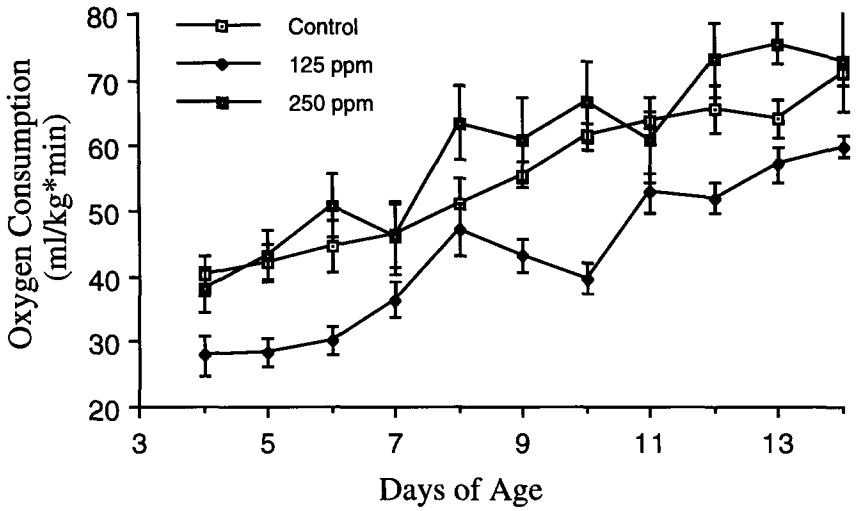

Fig. 1. Effects of feeding 125 ppm or 250 ppm Aroclor 1254 to female rats during pregnancy and 15 days postnatal on oxygen consumption of pups on days 4 to 14. Data are represented as mean ± SEM.

body temperature stabilized at a significantly lower level than controls for both treatment groups [$F_{(2,104)} = 133.75$, $p < 0.0001$], with the 125 ppm group depressed 1.42 °C and the 250 ppm group depressed 0.41 °C from the control. Again, the development of body temperature followed a linear pattern, with $r^2 =$ 0.86, 0.92, and 0.82 for the controls, 125 ppm, and 250 ppm animals, respectively.

Taylor (1960) found that as neonatal rats increased in size, the maximal VO_2 decreased. Because the lactating mother could not survive at a temperature thermoneutral for the pups, the experiments were all carried out at rearing temperature (21 - 22 °C). A higher VO_2 might be expected for the 250 ppm group because the body weights were significantly less than those of the other animals. Since the 250 ppm animals were smaller, they were required to expend more energy to preserve their body temperature, while the larger pups in the 125 ppm group maintained their body temperature at a lower expenditure of energy even if the body temperature was depressed from the controls (Fig. 2). The body weights of the 125 ppm animals were also significantly depressed, but their body temperature was significantly lower than all other animals (Fig. 2). The difference in response of the VO_2 may be attributed to the difference in body weight. The body weights of the 250 ppm animals may have been depressed sufficiently to reveal the compensatory response of the VO_2. The reduced VO_2 of the 125 ppm animals may result from body weight reduction insufficient to cause an increase in VO_2.

Komvies and Alayoku (1980) have shown that the thermogenic system of the adult rat is altered following PCB exposure. Because some PCB congeners are known

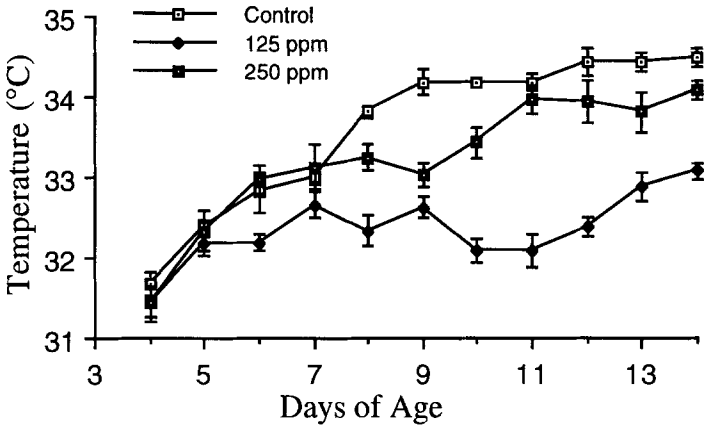

Fig. 2. Effects of feeding 125 ppm or 250 ppm Aroclor 1254 to female rats during pregnancy and 15 days postnatal on body temperature of pups from days 4 to 14. Data are represented as mean ± SEM.

to cross the blood-brain barrier (Seegal and Shain 1992), circulating PCB can act at the hypothalamus to alter the internal setting of body temperature. Therefore, the thyrotoxic result would be counteracted by activation of the sympathetic nervous system (SNS) to increase metabolism and body temperature. The PCB may also act at the pituitary gland, since serum levels of thyrotropin have been suggested to be elevated following PCB exposure (Ness *et al.* 1993). Other major hormone systems may be influenced by PCB exposure. The major hormone besides thyroid hormones that acts on the thermogenesis is insulin (for a review, see Silvia 1993). Because the toxic effects of PCB on the endocrine pancreas have not been studied, further determination of glucose availability in the presence of PCB ingestion will add to the understanding of endocrine controls of the thermogenic response.

In view of the severe reduction of circulating T4 following PCB ingestion (Table 1), it would be anticipated that body temperature would be subnormal. The non-dose dependent response of the body temperature reduction does not follow the results seen in adult rats by Komvies and Alayoku (1980). A possible explanation of the situation where body temperature is close to normal in the face of subnormal T4 levels is a pyrogenic response of the animal to a physiological insult. Because of the damage to the thyroid gland caused by PCB (Collins and Capen 1980), a subsequent immuno-activation may occur. Directly following organ damage, the immune system may undergo a series of responses, including the release of several substances, interleukin 1 (IL-1) being among them. These materials will act at a variety of sites, including the hypothalamus, to increase body temperature (Jones *et al.* 1984). Exposure to PCB results in a depression of specific immune parameters in monkeys, especially the response of T and B-cells, but the IL-1 levels remains unchanged after PCB exposure (Tryphonas *et al.* 1991). The alteration of T-cell function does not impact on the ability of the macrophage to release IL-1 following an insult. If the response of developing animals is similar to that in adults, the actions of IL-1 might be more evident because mechanisms of body temperature regulation are not fully developed (Girard *et al.* 1992).

The present study reaffirmed the thyrotoxic nature of PCB in developing animals. The effects of PCB on the developing thermoregulatory system, first demonstrated in the present study, included a depression of body temperature that was not dose dependent, but the depression of body temperature did correspond to previous studies conducted on adult animals (Komvies and Alayoku 1980). A reduction in VO_2 was seen in the 125 ppm group, but not the 250 ppm group. The differences in body temperature and VO_2 can be attributed to the difference in body weight and the need for an animal with subnormal body size to increase its metabolic rate. Additionally, the activation of IL production by the greater concentration of PCB may allow the development of temperature regulation which is similar to that in normal animals.

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