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Effect of Early Postnatal Exposure to Polychlorinated Biphenyls (PCBs) on Fertility in Male Rats

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Polychlorinated biphenyls (PCBs) are persistent industrial pollutants that have been found widely distributed in the global ecosystem (Wasserman et al. 1979) including in the milk of women (Slorach and Vaz 1985). Numerous investigations have demonstrated that PCBs have various toxic effects including reproductive dysfunction in female mammals (Kimbrough 1985). Although information concerning the effects of PCBs on reproduction in male mammals is limited (McConnell 1980), PCB treatment of adult male rats appears to have little effect on fertility, testes weight, incidence of chromosomal abnormalities or on number of spermatogonial cells in mitosis (Dikshith et al. 1975; Green et al. 1975a, 1975b; Garthoff et al. 1977). In contrast, in our laboratory we have observed in rats that exposure to a PCB mixture (Aroclor 1254) early postnatally via the dam's milk (Takagi et al. 1976) does have an effect on subsequent reproductive function in males as well as females (Sager 1983; Sager and Girard 1983).

Males exposed to PCBs during lactation exhibited reduced fertility, i.e., reduced incidence of implantation in normal females mated to experimental males. However, a reduced weight gain during the time of treatment in the pups exposed to the higher doses of PCBs was also observed. After treatment, weight gain was comparable or greater in the experimental pups and by the time of mating and autopsy, body weights in all groups were comparable. The present experiments were designed 1) to determine if the early reduced weight gain (previously observed) has any influence on fertility, and 2) to investigate the effect of early postnatal exposure to PCBs on sperm counts and the ability of sperm to support normal development.

MATERIALS AND METHODS

Sperm positive pregnant rats were obtained from the Holtzman Rat Co., Madison, Wisconsin, on Day 1 of gestation and housed separately. On Day 1 of age (Day 0 of age = day of birth), litters were reconstituted at eight by removing excess females and

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redistributing males; weight of the reconstituted litter was recorded and treatment of the dams initiated. PCBs (Aroclor 1254) were dissolved in peanut oil and administered orally to lactating dams on days 1, 3, 5, 7, and 9 (in approximately 0.2cc of peanut oil) in the following doses: 8mg/kg (PCBI), 32mg/kg (PCBII), and 64mg/kg (PCBIII). Normal controls (CI) and underfed nutritional controls (CII) received 0.2cc peanut oil/treatment. During lactation dams in CII were restricted to approximately 70-75% of the food intake of CI so that the weight gain of CII pups matched the reduced weight gain of PCBIII pups. After weaning at 23 days, the male offspring were housed in groups of 3 until mating or autopsy. Dams, litters, and individual rats were weighed periodically throughout the experiment. All animals were fed Purina Lab Chow and were maintained on a 14L:10D schedule at 22±2°C.

Beginning at 130 days of age, males were mated with normal females (300-350gr) obtained from the Holtzman Rat Co. Each male was housed with 2 or 3 females until a positive sperm smear was obtained from one of the females or until the male had been exposed to four proestrus/estrus stages of the female. The stage of the estrous cycle of the female and the presence of sperm were determined by daily saline lavage. Sperm-positive females were housed separately until autopsy. In the first experiment, autopsy was done on Day 11 or 12 of gestation (Day of insemination = Day 0), and body weight, number of implantation sites, number of embryos, number of corpora lutea, and ovarian weights were recorded. In the second experiment, females mated to the same male were autopsied on Day 2 after mating (expected 2-4 cell stage of development) or on Day 4 after mating (expected morula/blastocyst stage). The fertilized eggs were obtained by flushing the right oviduct and uterine horn with saline. If no eggs were found, the same procedure was repeated using the left oviduct/uterine horn. The flushed eggs were collected and examined microscopically.

Male offspring were autopsied after mating of all groups was completed (not less than 8 days after mating). Body weights were recorded and the cauda of one of the epididymides was weighed and frozen for subsequent determination of sperm number (Robb et al. 1978). Data from the first experiment were analyzed using nested analysis of variance techniques. Ratio data were first transformed using the arcsine square root transformation. Treatment group means were then compared using Fisher's protected least significant difference test (LSD) using the litter mean square as error term. Data from the second experiment were analyzed using one-way ANOVA or chi-square techniques.

RESULTS AND DISCUSSION

In the first experiment, normal females mated to adult males exposed to PCBs during suckling had significantly fewer implants, fewer embryos, and a reduced proportion of ovulated eggs that implanted as compared to both normal controls and underfed

nutritional controls (Table 1). The fact that reduced fertility was not observed in the nutritional controls (CII) suggests that the reduced weight gain observed in PCBII and PCBIII pups during treatment, but not subsequently, is not involved in the reduced fertility of the experimental males. At the time of mating and autopsy, body weights in all groups were comparable.

In the second experiment, we did not observe a significant difference in sperm counts among the groups at the time of autopsy (215.3±32.6 - 281.0±23.6 million, one cauda). However, significantly fewer of the normal females mated to PCBII and PCBIII males had eggs in the expected stage of development: 2-4 cell on Day 2 (Table 2) or morula/blastocyst on Day 4 (Table 3). The average number of blastocysts found in one uterine horn on Day 4 was significantly reduced in the same groups. Comparison of embryonic development in the two females mated to the same male indicates that early PCB exposure influenced fertility of the PCBII and PCBIII males in such a way that they could either support normal development to the 2-4 cell stage, but not through to the blastocyst stage, or neither to the 2-4 cell stage nor to the blastocyst stage (Table 4).

It is interesting to note that if on Day 2, females mated to experimental males that had eggs not only in the expected stage (2-4 cells) but also at the 1-cell stage are included in the data, a significant difference among the groups is not observed (Table 3). This suggests that fertilization/initial development may be occurring, but may be delayed.

Previous studies examining effects of early exposure to PCBs on fertility in rodents are limited. Kihlstrom et al. (1975) observed no effect on fertility when male offspring exposed to PCBs during suckling were mated to normal females, although fewer implanted ova resulted when both males and females had been exposed during suckling. Orberg (1978) also failed to observe any significant effect on reproductive capacity in male mice after exposure to two pure chlorobiphenyls pre- and early postnatally via treatment of the dams.

Precisely how PCBs, when given early postnatally, work to affect fertility in males remains to be determined. Sperm counts are normal, but motility of sperm may be a factor. When fertilization is delayed, subsequent development has been found to be abnormal (Blandau 1969). Of interest is the fact that initial stages of spermatogenesis, i.e., formation of type A, intermediate, and type B spermatogonia, are occurring during the time of exposure to PCBs (Clermont and Perey, 1957). Sensitivity of the stem cells to toxic substances at this time may be such that exposure to PCBs results in a long-term effect on the quality of sperm produced (Meistrich, 1986). As a consequence, the ability of these sperm to fertilize eggs and/or support normal development is impaired.

Table 1. Fertility of Male Offspring[1] after PCB Exposure During Lactation

Group	# of Litters	# of Males	# of Corpora lutea	# of Implants	# of Embryos[2]	Implants/ Corpora lutea
			Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
CI	7	24	17.1 (.76)	13.8 (.58)	13.3 (.58)	0.827 (.04)
CII	6	21	18.1 (.68)	14.6 (.86)	13.2 (.89)	0.819 (.05)
PCBI[3]	7	25	17.0 (.68)	10.9 (1.1)*	9.5 (1.1)**	0.664 (.07)
PCBII[3]	6	24	16.9 (.56)	5.7 (1.2)***	5.0 (1.2)***	0.337 (.07)***
PCBIII[3]	7	24	16.3 (.56)	4.7 (1.3)***	4.5 (1.3)***	0.276 (.08)***

1 - mated to normal females
2 - day 11/12 of gestation
3 - PCBI = 8 mg/kg; PCBII = 32 mg/kg; PCBIII = 64 mg/kg
* - significantly different from CII (P< .02)
** - significantly different from CI, CII (P< .02)
*** - significantly different from CI, CII (P< .001)

Table 2. Reproductive Success of Females Mated to PCB Exposed Males: Day 2 After Mating[1]

Group	# of Litters	# of Males	No. of females with[2] no eggs	eggs at 1-cell stage	eggs at 2-4 cell stage	eggs at 1-4 cell stage
CI	4	12	0	3	12	12
CII	4	12	1	2	11	11
PCBI	4	12	0	0	11	11
PCBII	4	12	0	3	7*	10
PCBIII	4	12	3	5	7*	9

[1]Day 0 = Day of insemination [2]one or more eggs present
*significantly different from CI+CII (pooled)($P<0.001$)

Table 3. Reproductive Success of Females Mated to PCB Exposed Males: Day 4 after Mating[1]

Group	# of Litters	# of Males	No. of females with[2] presence of morula/ blastocyst	absence or only abnormal eggs	Found in one uterine horn no. of morula Mean(SE)	no. of blastocysts Mean(SE)
CI	4	12	12	0	.25(.18)	4.75(.63)
CII	4	12	12	0	.75(.68)	3.25(.68)
PCBI	4	12	10	2	.75(.43)	3.33(.96)
PCBII	4	12	7*	5*	1.67(.63)	.92(.36)**
PCBIII	4	12	3*	9*	.33(.26)	.08(.08)**

[1]Day 0 = Day of insemination [2]One or more eggs present
*Significantly different from CI+CII(pooled)($P<0.001$) **Significantly different from CI, CII ($P<0.01$)

Table 4. Embryonic Development[1] on Day 2 and Day 4[2] in the Females Mated to the Same PCB-Exposed Male

group	no. of litters	no. of males	No. of males that supported development of: normal embryos on both D2 & D4	normal embryos on D2, abnormal embryos on D4	abnormal embryos on both D2 & D4
CI	4	12	12	0	0
CII	4	12	11	0	0
PCBI	4	12	9	2	0
PCBII	4	12	6*	4*	1
PCBIII	4	12	3**	6**	3

[1]Normal: Embryos at the expected developmental stages (2-4 cell on D2; morula/blastocyst on D4)
Abnormal: Embryos not at the expected developmental stages, absence of eggs, or presence of abnormal eggs
[2]Day 0 - Day of insemination
*Significantly different from CI+CII(pooled)($P<.005$)
**Significantly different from CI+CII(pooled)($P<.001$)

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