Postnatal Behavioral Effects of Prenatal Treatment with PCBs (Polychlorinated Biphenyls) in Rats

By

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Introduction

Polychlorinated biphenyls (PCBs) have been recognized to be of environmental and toxicologic concern for the last several years. A number of studies have been conducted on the biological effects of PCBs in experimental animals and man (for recent reviews see Ad Hoc Committee on Environmental Health Research, 1972, and Fishbein, 1974). The present author (1976) recently demonstrated that two commercial mixtures of PCBs (Kanechlor 300 and 500) administered orally during pregnancy adversely affect the development of rat offspring, and this effect persists for a certain period after birth. Some attempts have been made to investigate the long-lasting deleterious effects of the prenatally exposed PCBs on postnatal development in man (Taki et al., 1969, Kuratsune et al., 1972), but the postnatal development of the Yusho babies has not thoroughly been followed up.

On the other hand, diverse behavioral changes have been observed in animals exposed to some chemicals or ionizing radiation in utero. There is an increasing amount of evidence which suggests that sub-teratogenic doses of some pharmacological agents can cause enduring behavioral abnormalities in the offspring of treated females (Ordy et al., 1966, Joffe, 1969, Butcher et al., 1972, Golub and Kornetzky, 1974, Hutchings et al., 1973, Hutchings and Gaston, 1974). It is important to investigate some subtle effects of prenatally administered environmental substances on postnatal growth and behavior of the offspring.

The purpose of the present study was to investigate certain aspects of postnatal growth and behavior in rats prenatally exposed to PCBs.

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This kind of study could contribute to the elucidation of possible behavioral changes caused by prenatally administered environmental chemicals.

**Materials and Methods**

Four-week-old male and female rats of Sprague-Dawley-JCL strain were purchased from CLEA, Japan, Inc. (Takatsuki), and reared in an air-conditioned laboratory with the diet, OA-2 (CLEA, Japan, Inc.), and tap water *ad libitum*. The room temperature was maintained at 22±2°C and the relative humidity at 55±5%. The lighting was on a 12 hr light-dark cycle.

Each virgin female rat of 12-16 week old was placed overnight (from 6:00 a.m. to 9:00 a.m.) with a potent male rat of the same strain. The day on which a vaginal plug was found was taken as day zero of pregnancy. The females with vaginal plugs were assigned to one of the following six treatment.

- **Group I.** 100 mg/kg dose of Kanechlor 500 (1 g/100 ml dissolved in olive oil) daily on days 8–14 of gestation.
- **Group II.** 20 mg/kg dose of Kanechlor 500 (0.2 g/100 ml dissolved in olive oil) daily on days 8–14 of gestation.
- **Group III.** 10 ml/kg dose of olive oil daily on days 8–14 of gestation.
- **Group IV.** 100 mg/kg dose of Kanechlor 500 (1 g/100 ml dissolved in olive oil) daily on days 15–21 of gestation.
- **Group V.** 20 mg/kg dose of Kanechlor 500 (0.2 g/100 ml dissolved in olive oil) daily on days 15–21 of gestation.
- **Group VI.** 10 ml/kg dose of olive oil daily on days 15–21 of gestation.

The dosages were administered using stainless gastric tube.

About a half of the females of each group, randomly selected, were sacrificed on day 21 in order to observe term fetuses. The numbers of implantations, resorptions, and live and dead fetuses were recorded. The live fetuses were sexed, weighed, and inspected for gross malformations.

The remaining half of the pregnant females were allowed to litter naturally. The newborns were examined, weighed, and sexed shortly after birth, and the state of the young, live, dead or malformed was recorded. The live pups were branded for identification and allowed to suckle the mother. On the fourth day, the litter was reduced to 8 offspring comprising 4 males and 4 females if possible. If the litter was smaller than 8, pups were fostered from a female of the same treatment group, which had delivered pups on the same day. They were weighed weekly thereafter, and weaned at 3 weeks of age. The young of the same group and same sex were housed 3–5 to a wire mesh cage until the completion of testing.
Behavioral Testing Procedures

At 12 weeks of age, the young were tested individually in an open field. The open field was a 115 cm square surface that was painted flat black, divided by white lines into 25 squares, and covered by a sheet of transparent acrylic plate. The field was enclosed by wooden walls, 30 cm in height, which were also painted flat black. During testing, the field was illuminated by an overhead fluorescent lamp, and low-noise masking was provided by the continuous operation of an air-conditioner. Each animal was tested in the field 5 times on the day. In each trial, subjects were placed individually in a corner square of the field and allowed to explore freely for 5 minutes. The measures taken were: (1) The total number of squares entered with all four legs, (2) the total number of activities such as defecation and edging. The acrylic plate was wiped clean after each trial to avoid position cues to successive animals.

The maze testing was commenced when the animals (used in open field testing) were 13 weeks old. The maze was a multiple T pattern similar to that described by Biel (1940). The sides of the maze were 150 cm × 150 cm and each alley was 15 cm wide and 45 cm high. The maze was situated in a tank 170 cm square and 45 cm deep. The water in the tank and maze was maintained at a depth of approximately 20 cm and its temperature at 23 ± 2°C. The water in the maze was changed daily during testing. Trials were given the animals approximately at the same time each day. On the first day of the testing period, swimming ability of each animal was tested in the straight channel. On each trial, the subject was placed in the water at one end of the channel and allowed to swim to an goal ramp placed at the opposite end. The elapsed time required by the subjects to traverse the channel was recorded as a measure of swimming ability. This testing was given each animal 5 times on the day. On the three succeeding days, the subjects were given 3 trials of maze testing each day. Two measures of maze performance were recorded: (1) elapsed time between entry into the water at the starting point and contact with the goal ramp, (2) numbers of errors defined as a head and shoulder entry into an incorrect alley. To prevent exhaustion of the animals, no subject was allowed to remain in the maze for more than 10 minutes in any trial. Such trials were terminated by guiding the subject to the goal ramp and removing it from the apparatus. Trials were separated by at least 30 minutes and animals were dried with a towel and returned to their home cages between trials.
Results

Effects upon dams and fetuses

One of the ten females of group V died on day 18 of gestation (4th day of administration). As a result of necropsy, no marked pathological changes were found except slight petechiae in lungs. Administration of 100 mg/kg/day of Kanechlor 500 from day 15 (group IV) caused abortion on the 4th day of administration in 2 out of the 8 females. All of the other females maintained their pregnancy normally until the day of sacrifice or parturition.

The results of observation of term fetuses are summarized in Table 1. No significant difference was shown in the means of implants and viable fetuses, and the resorption rates between the Kanechlor and placebo groups. The mean weight of viable fetuses from Kanechlor treated dams was less than that of the controls, and the difference from the control was significant (p < 0.05) for female fetuses of group I. The only gross malformation observed was club foot, and its occurrence was very sporadic. These data confirmed the previously observed fetotoxic effects of Kanechlor 500 in rats (Shiota, 1976).

One of the three females of group IV which were allowed to litter died soon after completion of parturition. The remaining two females

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>No. of mothers</th>
<th>Total Implants</th>
<th>No. of resorptions (%)</th>
<th>Average litter size</th>
<th>Mean weight of fetuses; mean±SE (g)</th>
<th>No. of externally malformed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>I</td>
<td>100 mg/kg/day</td>
<td>4</td>
<td>50</td>
<td>12 (24.8)</td>
<td>9.5</td>
<td>4.83 ± 0.085</td>
<td>4.63* ± 0.075</td>
</tr>
<tr>
<td>II</td>
<td>20 mg/kg/day</td>
<td>4</td>
<td>52</td>
<td>6 (11.4)</td>
<td>11.5</td>
<td>5.38 ± 0.125</td>
<td>5.05 ± 0.112</td>
</tr>
<tr>
<td>III</td>
<td>Placebo</td>
<td>4</td>
<td>50</td>
<td>7 (13.6)</td>
<td>10.8</td>
<td>5.53 ± 0.256</td>
<td>5.28 ± 0.144</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>100 mg/kg/day</td>
<td>3</td>
<td>36</td>
<td>5 (14.7)</td>
<td>10.3</td>
<td>5.37 ± 0.250</td>
<td>4.99 ± 0.055</td>
</tr>
<tr>
<td>V</td>
<td>20 mg/kg/day</td>
<td>4</td>
<td>49</td>
<td>11 (21.8)</td>
<td>9.5</td>
<td>5.20 ± 0.329</td>
<td>4.92 ± 0.277</td>
</tr>
<tr>
<td>VI</td>
<td>Placebo</td>
<td>4</td>
<td>46</td>
<td>4 (7.4)</td>
<td>10.5</td>
<td>5.64 ± 0.151</td>
<td>5.37 ± 0.190</td>
</tr>
</tbody>
</table>

* Significantly different from group III; p < 0.05 (t test).
of this group gave birth to pups, but all of the pups were immature and died within a week. The young of group IV therefore could not be subjected to behavioral testings. In all of the other groups, all litters were delivered without accident. One newborn pup of group II was found to have omphalocele, but this anomaly disappeared within a week and the pup survived normally thereafter. One female pup from a dam of group V suffered from imperforate anus and absence of tail, and this defective pup could not survive until 1 week of age. Control pups were all essentially normal.

No significant differences were evident between groups in weaning rate and survival rate at the time of behavioral testings.

The numbers of the animals subjected to the behavioral testings were: 13 in group I, 22 in group II, 10 in group III, 0 in group IV, 20 in group V, and 19 in group VI.

Open field test

The open field performance of the animals in all groups was characterized by large within-group variability.

No statistically significant differences between treatments were observed in the mean numbers of squares traversed and other emotional variables such as defecation and edging during the 5 minute testing period (Table 2). During the open field testing, one pup in group I showed a sign of locomotor impairment sustaining purposeless locomotion over a circular course.

Table 2. Results of open field test for the offspring from dams treated with Kanechlor 500

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>No. of squares traversed; mean±SE</th>
<th>No. of boluses; mean±SE</th>
<th>Edging; mean±SE</th>
<th>Gait abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>9</td>
<td>77.0±12.0</td>
<td>4.1±1.7</td>
<td>10.8±3.3</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>47.0±10.5</td>
<td>2.0±1.0</td>
<td>10.1±3.2</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>73.5±5.5</td>
<td>0.8±0.7</td>
<td>8.4±2.8</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>9</td>
<td>52.2±3.8</td>
<td>1.8±0.9</td>
<td>12.4±0.7</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>7</td>
<td>25.4±9.6</td>
<td>1.3±0.2</td>
<td>7.7±3.1</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>98.5±29.5</td>
<td>1.0±0.0</td>
<td>10.3±0.3</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>12</td>
<td>64.0±9.1</td>
<td>0.1±0.1</td>
<td>16.9±1.8</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>69.8±1.8</td>
<td>0</td>
<td>11.3±1.8</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>11</td>
<td>50.9±11.6</td>
<td>0.1±0.1</td>
<td>12.7±3.5</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>12</td>
<td>68.7±6.3</td>
<td>1.5±1.2</td>
<td>19.6±2.5</td>
<td>0</td>
</tr>
</tbody>
</table>
Water-filled multiple T-maze

Because difference between male and female subjects in the parameters of water-filled multiple T-maze testing was not statistically significant in any group, sex of the subject was disregarded in analysis of the differences between groups.

The result of pretest swimming trials in the straight channel (day 1) revealed that subjects could swim adequately and the mean elapsed time was not significantly different between the Kanechlor and placebo groups.

The average required time to achieve the maze testings for each of the 5 groups is presented in Table 3. Statistical difference was proved between groups V and VI on day 3, and between day 3 and days 2 and 4 of group VI (Student's t test).

Table 3. Elapsed time per trial in water maze test

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>80.8±5.88</td>
<td>55.7±6.17</td>
<td>75.0±10.70</td>
</tr>
<tr>
<td>II</td>
<td>77.7±9.28</td>
<td>76.0±12.58</td>
<td>65.5±12.58</td>
</tr>
<tr>
<td>III</td>
<td>73.2±12.23</td>
<td>65.1±14.41</td>
<td>82.6±17.83</td>
</tr>
<tr>
<td>V</td>
<td>98.0±11.26</td>
<td>94.7±5.20*</td>
<td>92.2±10.37</td>
</tr>
<tr>
<td>VI</td>
<td>98.3±7.99</td>
<td>36.4±7.19**</td>
<td>76.4±11.27</td>
</tr>
</tbody>
</table>

* Significantly different from group VI; p < 0.05.
** Significantly different from days 2 and 4; p < 0.05.

Fig. 1. Means of errors made in water maze testing by the offspring of females given Kanechlor 500 on days 8-14 of gestation.
The mean number of errors per trial exhibited by the subjects apparently reduced day by day in any of the groups (Figs. 1 and 2).

Analyses of the error data indicated that groups II and V on day 3 and group III on day 4 were significantly different from the corresponding controls. The means of errors exhibited in total 9 trials, however, did not significantly differ among groups. Figs. 1 and 2 indicate that the "learning" effect as measured by the improvement of maze solving was less apparent in the Kanechlor-treated groups than the controls.

**Discussion**

The observation of term fetuses confirmed previous findings (Shiota, 1976) indicating that prenatal administration of a high dosage of Kanechlos suppress the fetal body weight but do not induce congenital malformations. In addition, this study revealed that a high dosage of Kanechlor 500 administered in later stage of gestation may cause abortion and increase neonatal mortality.

The findings on behavior demonstrated that prenatally exposed Kanechlos can alter the learning ability by performance on the particular test used. It is of interest that some subtle learning impairment was observed in the maze testing although there was no remarkable change in their brain structure. Considering that the open field
activities and swimming ability of the Kanechlor and control offspring were similar, it is unlikely that the differences in achieving the maze performance were due to simple motor dysfunction. The observed results concerning the maze learning ability appear to show the dose-effect relationship. To confirm that this learning impairment is exactly due to the prenatal administration of the Kanechlor, appropriate reference agents for positive controls may be required. In addition, further comparative data of the behavioral teratology should be accumulated.

As mentioned above, prenatal treatment with subteratogenic doses of some chemicals can cause behavioral abnormalities in the offspring. In interpreting these findings, determinants of such kinds of behavior should be argued. Various factors can affect the subtle behavioral changes in experimental animals. It is noted that the food consumption by dams given a high dose of the Kanechlor was suppressed. Coyle and Singer (1975) stated that maternal malnutrition and medication during pregnancy can prevent the offspring from interacting with the postnatal environmental factors, and that this unresponsiveness may be the direct cause of some of the symptoms in the offspring. Similarly, maternal medication during pregnancy has been shown to produce relative unresponsiveness in human infants for some considerable time (Brazelton, 1970, Conway and Brackbill, 1970). Coyle and Singer (1975) also pointed out the importance of housing conditions in which the subjects are reared. That is, prenatally determined behavioral impairment can be obscured by rearing the subjects in a deprived environment. On the other hand, behavioral changes ranging from increased open field activity to enhanced performance on learning tasks have been reported to result from enriched rearing conditions (Forgays and Forgays, 1952, Denenberg et al., 1968, Rosenzweig et al., 1972). In the present study, the subjects were reared under similar condition, being housed 3-5 in a cage.

A note of caution is necessary regarding the limitations of such kinds of research. If the behavioral effects of prenatal drug administration is extremely subtle, it seems difficult at present to extrapolate the animal data to humans. In other words, the relevance of such effects to human drug evaluation programs is still unclear.

Behavioral teratology is a relatively new field of investigation and it could contribute to the elucidation of some unfavorable outcomes in offspring which cannot be evaluated by other measures. Psychopharmacological assessment in behavioral teratology is further expected.

Abstract

Sprague-Dawley rats received 20 or 100 mg/kg/day of Kanechlor 500 on days either 8-14 or 15-21 of gestation. Controls were given the
solvent, olive oil, only. Offspring were evaluated for activity and emotionality on the open field and for maze-learning ability on the water-filled multiple T-maze. The results showed that the prenatal treatment with PCBs altered learning ability as measured by the water maze, although activity and emotionality were similar to those of controls. On the maze-learning test, the offspring exposed to the Kanechlor in utero learned the maze slower than the control rats, and dose-response relationships appeared evident in this parameter. These results were discussed with reference to previous findings in this area, and the potential values of behavioral teratology were stressed.

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References


