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Postnatal Growth in Rats Prenatally Exposed to Cigarette Smoke or Carbon Monoxide

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Previously, we demonstrated that cigarette smoke exposure during pregnancy lowered the intrauterine growth of fetuses in rats (Tachi and Aoyama 1983; 1986; 1989) as well as earlier results in human and animals (Essenberg et al. 1940; Lowe 1959; Frazier et al. 1961; Younoszai et al. 1969; Comstock et al. 1971; Butler et al. 1972; Meyer et al. 1974; Reznik and Marquard 1980), and that the effects of cigarette smoke on fetal growth seemed to be evoked wherever the exposure was applied in pregnancy, although the strength of influences was largest when the inhalation was accomplished during late gestation.

In contrast to the well-known events during pregnancy, effects of prenatal exposure to the smoke on the growth of newborns have not been extensively investigated yet. In human, Butler and Goldstein (1973) reported that height of 11-year-old children whose mothers had smoked during pregnancy was 1.0 cm shorter than those born to nonsmoking women with a significance, and that these children from smoking mothers were 3-5 months retarded on reading, mathematics, and general ability. Other authors also revealed that physical and intellectual development was harmfully affected in those born to smoking mothers, although there have been conflicting publications in which no long term effect was observed (Hardy and Mellits 1972; Naeye and Peters 1984; Fogelman and Manor 1988). However, it is difficult in humans to distinguish the effects of cigarette smoke during pregnancy on the growth or development after birth from those of passive smoking after birth or other factors influencing growth.

Thus, the present study was attempted, using rats, to clarify whether cigarette smoke exposure during fetal period had any influences on growth or development even after birth. In addition, the effects of prenatal carbon monoxide inhalation on growth after birth were also investigated, because the gas is a constituent of the smoke and has been suggested to disturb the fetal growth (Tachi and Aoyama 1983).

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MATERIALS AND METHODS

Female Wistar rats were individually housed under the condition of lights (12L12D, lights on 0600h) and temperature (22.5-26.5℃). Animals were mated overnight on the day of proestrus with proven males. Pregnant females were placed in a chamber (0.532 m^3), and exposed to cigarette smoke or carbon monoxide (CO) at the concentration (around 1200 ppm) similar to the smoke inhalation, twice a day for 81 min., from days 0 (spermatopositive smears taken) to 20 of pregnancy, according to the manner previously described (Tachi and Aoyama 1983). Controls inhaled room air. Animals were checked their delivery at noon, and the day of discovery of newborn pups was assigned as day 0 of age. At the same time, pups weighed, and litter size was adjusted to 6 juveniles randomly selected (each 3 males and females, if possible).

The following observations of pups were performed from days 1 to 30 of age: Body weight and physical signs (pinna unfolding, fore and hind digit detachment, upper and lower incisor eruption, and ear and eye

Table 1. Description of reflex responses

Reflex	Eliciting stimuli and criteria of achievement
Righting reflex	When pups were placed on back on a flat surface, they could turn over on to ventral surface within 5 sec.
Cliff avoidance	When pups were put on edge of bench with nose and fore feet just over edge, they could withdraw their head and both fore feet, and move away from cliff.
Negative geotaxis	When pups were placed on a 30-degree slope with their head downward, they could turn at least 135 degrees to face up the slope.
Auditory startle	When a sudden sound stimulus was made on pups, they responded brief extension of hind limbs.
Free-fall righting	When pups were dropped back downward from 30 cm on to cotton wool pad, they could turn in mid-air to land on all four legs.

When responses fulfilled the criteria for consecutive 3 days, the first day of appearance in responses was recorded, except for auditory startle in which only first appearance of the response was checked.

opening) of offsprings were observed around 0900h every day. Also, reflex responses of progenies were tested, and the day on which the responses were achieved at a certain level, as shown on Table 1, was recorded.

On day 21 of age, pups were removed from their mothers, and each litter was housed in a cage.

The open field study was accomplished on day 30 of age in all progenies. The field was a 60 cm long square with the walls 30 cm high, and divided equally to 25 small squares (12 cm long) with lines. After animals were placed on a center square, latency time within a center, and numbers of squares across (enterance of at least 3 legs), grooming, rearing, defecation, and urination were measured for 2 min.

Data was used from 6 litters which size was kept 5 or 6 throughout the experiment in each group. Duncan's new multiple range test was employed for a statistical significance between groups.

RESULTS AND DISCUSSION

The present study failed to show a difference in duration of pregnancy between groups (22.2 ±0.2, 22.2±0.2, and 22.0±0.3 days, mean± S.E. for the groups of cigarette smoke and CO exposure, and control, respectively), while it has been published that nicotine administration, one of major components of the smoke, delayed the parturition in pregnant animals (Becker et al. 1968). It could be considered that

Table 2. Litter size and body weight of newborns on day 0 of age.

Treatment	Litter size	Body weight (g)
Cigarette smoke	12.5±1.1 (6)	5.02±0.05* (75)
Male	6.3±0.7 (6)	5.14±0.07* (38)
Female	6.2±1.0 (6)	4.90±0.07* (37)
Carbon monoxide	10.2±1.1 (6)	5.17±0.07* (61)
Male	7.0±0.5 (6)	5.30±0.07* (42)
Female	3.2±0.9 (6)	4.88±0.11* (19)
Control	12.2±1.3 (6)	5.89±0.09 (73)
Male	6.2±1.0 (6)	5.95±0.14 (37)
Female	6.0±1.0 (6)	5.82±0.12 (36)

Mean±S.E. An asterisk reveals a significant difference ($P<0.05$) from the corresponding control. Numeral in parenthesis is number of samples.

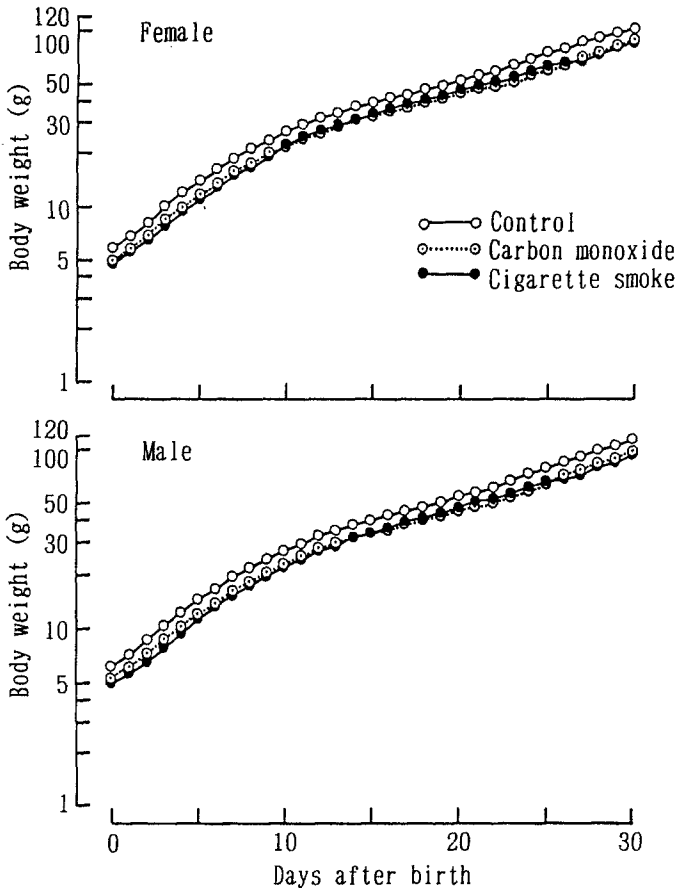

Figure 1. Body weight of female (upper panel) and male (lower) pups from days 0 to 30 of age. Each point represents the mean weight of more than 13 pups. Significant difference ($P<0.05$) is obtained in both sexes throughout the experiment between groups of control and cigarette smoke or CO exposure.

absorption of nicotine by the rat was smaller in the smoke exposed group of this study than the previous experiments mentioned above. There was no significant difference in litter size on day 0 between groups, although number of newborn females was some 3 pups less in the group of CO exposure than other groups (Table 2).

The weight of infants was significantly smaller ($P<0.05$) on day 0 of age in the groups of cigarette smoke and CO inhalation than controls in either sex (Table 2). Differed from the earlier observations in which fetuses weighed heavier in CO exposure than the group exposed to the

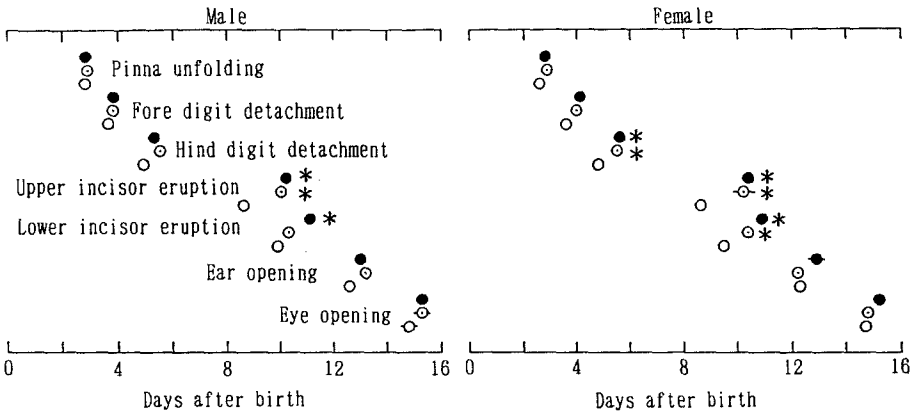

Figure 2. The day of appearance in various physical signs in pups prenatally exposed to cigarette smoke, carbon monoxide, or room air. Each point represents mean ±S.E. of more than 13 offsprings. Solid, dotted and open circles indicate the groups of cigarette smoke and CO exposure and control, respectively. *; Significant difference from the control ($P<0.05$).

smoke (Tachi and Aoyama 1983; 1986), the weight of newborns was analogous in both groups.

The body weight of pups selected was always 15-20% larger in the control than other groups with a significane ($P<0.05$) throughout the observation period (Figure 1), although it progressed at a similar rate in all groups. The value was almost equal in the weight of progenies exposed to cigarette smoke and CO.

The appearance of upper and lower incisors was delayed in either sex of progenies prenatally exposed to the smoke or CO, compared with the control (Figure 2). Other indices were not different among groups, except for the hind digit detachment in females which was slightly but significantly postponed in smoke and CO exposed groups. The delay in incisor eruption might be responsible for the finding that the weight of infants exposed to the smoke or CO could not catch up with the control, due to lower ability to take chow.

In reflex responses observed, full response in righting reflex and free-fall righting was delayed around 1 day in infants of prenatal exposure to the smoke or CO (Figure 3). The appearance in negative geotaxis was also significantly later in females exposed to the smoke or CO. These observations implied modified development in neuromotor functions in smoke and CO exposed groups.

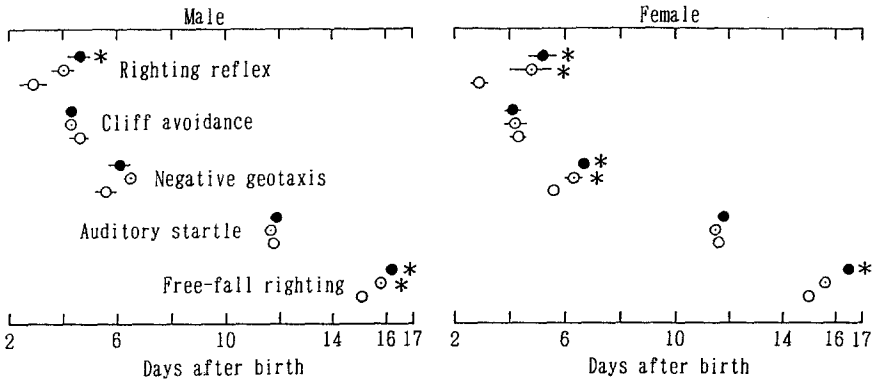

Figure 3. The day of appearance in various reflex responses.
See the legend for Figure 2.

Table 3. Variables of the open field study for 2 min. on day 30 of age.

	Latency time (sec)	No. of area across	No. of grooming
Female			
Cigarette smoke (17)	5.26±0.75	54.1±3.2	2.4±0.5
Carbon monoxide (13)	6.67±1.05	65.2±3.9	0.8±0.3
Control (18)	5.08±0.40	56.3±3.7	1.8±0.5
Male			
Cigarette smoke (18)	6.48±1.68	51.1±2.8	1.6±0.3
Carbon monoxide (22)	5.72±1.33	60.5±4.2	1.3±0.3
Control (17)	5.70±1.43	48.7±4.5	2.0±0.5

	No. of rearing	No. of defecation	No. of urination
Female			
Cigarette smoke	25.3±1.4	0.4±0.2 *	1.2±0.4
Carbon monoxide	25.8±1.1	0.9±0.4	1.2±0.5
Control	25.9±1.7	1.6±0.4	1.6±0.5
Male			
Cigarette smoke	22.4±1.6	0.6±0.2	1.0±0.3
Carbon monoxide	24.4±1.6	1.7±0.4	1.8±0.3
Control	23.8±2.4	1.5±0.4	1.8±0.6

An asterisk represents a significant difference from control ($P<0.05$).
Numeral in parenthesis is number of offsprings.

No meaningful difference was observed in the open field study, although number of defecation was lower in females prenatally exposed to the smoke, compared to the control (Table 3). This result seemed likely to imply little effect of smoke or CO exposure on emotional development in offsprings. However, since previous authors reported that open field activity was lowered in preweaning pups exposed prenatally to 150 ppm of CO, and that learning and memory deficits appeared in these offsprings afterwards (Fechter and Annau 1977; Mactutus & Fechter 1984), it should be undertaken to examine more precisely.

The findings obtained in this study seemed to indicate that prenatal inhalation of cigarette smoke could affect the physical growth or development of pups even after birth. This result agreed with the earlier studies in which several authors observed harmful influences of smoking during pregnancy on postnatal physical and mental development in human, (Butler and Goldstein 1973; Naeye and Peters 1984; Fogelman and Manor 1988), whereas Hardy and Mellits (1972) reported that no long-term effect was indentified in children who survived the perinatal period.

Alternatively, effects of the smoke on mothers lasted not only during pregnancy but during the lactating period, so that they may have been unable to nurse their offsprings enough to grow, compared to the control. However, since the body weight or its growth of mothers was comparable during nursing period, and the weight of mammary glands was not different on the following day of the weaning in all groups (data not shown), the mothers seemed likely to take care of their progenies similarly in all groups.

Finally, the present study demonstrated that exposure to cigarette smoke during fetal period affected the postnatal growth and development, as well as the intrauterine growth in rats. Furthermore, it was suggested that the effects of the smoke was at least in part due to CO in the smoke, because of the similar results shown in the groups of cigarette smoke and CO exposure.

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