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Effect of Restricted Food Supply to Pregnant Rats Inhaling Carbon Monoxide on Fetal Weight, Compared with Cigarette Smoke Exposure

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Although many studies have shown that cigarette smoking during gestation retarded the intrauterine fetal growth, resulting in the decreased birth weight in babies born to smoking mothers (Lowe 1959; Frazier et al. 1961; Butler et al. 1972), neither causal substance nor mechanism of action to disturb fetal growth has been firmly established yet.

Based on the human and animal studies, Astrup et al. (1972) implied fetal hypoxia induced by carbon monoxide (CO) in the cigarette smoke to be responsible for the event mentioned above. Tachi and Aoyama (1983) demonstrated that pregnant rats which had inhaled either cigarette smoke or CO at the similar CO concentration, had smaller conceptuses than those in the control group, and that cigarette smoke could more deleteriously exert the effect on conceptuses, compared with CO exposure. This finding suggested that CO in the cigarette smoke was important but not sole factor to contribute to the fetal growth retardation.

A shortage in energy intake in smoking mothers also has been suspected to cause the retardation in fetal development (Rush 1974). In the previous results (Tachi & Aoyama 1983), the weight increment in CO exposed animals was greater than that in the smoke exposed group. The phenomenon seemed to indicate that the reduction in the food intake occurs in animals which inhale the cigarette smoke, and induces the disturbance of fetal development in association with CO.

In the present study, so as to evaluate the role of energy intake upon the fetal development in utero, the experiment of paired feeding with pregnant rats exposed to cigarette smoke is designed in animals which inhale the cigarette smoke, CO, or room air, following after the observation of the quantity of food taken by mothers exposed to cigarette smoke, CO, or room air.

MATERIALS AND METHODS

Female Wistar rats were individually housed in a wooden cage (17.5×28.0 ×20.0 cm) under the lighting of 12L12D (lights on 0600h) and temperature at 22.5-26.5℃. Animals were mated overnight, and the following day on which sperms were found in the vaginal smear was designated day 0 of pregnancy. Commercial diet (CLEA CE-2, CLEA Japan Inc., Tokyo, Japan) and tap water were given ad libitum except for the inhalation period

unless otherwise mentioned.

Pregnant animals were exposed twice daily around 1000h and around 1500h from days 0 to 20 of pregnancy either to cigarette main smoke manually generated (CIG), to carbon monoxide (CO), or to room air as a control (CONT), according to the procedure described in the previous study (Tachi and Aoyama 1983). Duration of each inhalation was 81 minutes; for twenty one minutes, smoke or CO was introduced into the chamber in which animals had been placed, and they were exposed for another 60 minutes in the chamber. CO concentration in a chamber was at 1210±112 (mean±S.D.), and 1290±63 ppm for CIG, and CO groups, respectively. Preliminary results displayed that carboxyhemoglobin saturation in venous blood reached 57% or 54% at the termination of exposure in rats which had inhaled CIG or CO respectively.

In the first experiment, daily food consumption in pregnant rats exposed to CIG, CO, or room air (CONT) was observed between 0730 and 0900h throughout the experiment. Daily intake was defined as the difference between the weights of container filled with chow on a given day and that remained on the following day.

Five groups, each of which consisted of four pregnant rats, were assigned to the second experiment; CIG, CO, CONT, pair-fed and room air exposed (PF), and pair-fed and CO exposed (PF+CO) groups.

Animals in PF and PF+CO showed similar body weight and daily food intake before conception to each corresponding pair in CIG.

From days 0 to 20 of pregnancy, similar bulk of food to that ingested by the rat exposed to CIG in a given day of pregnancy was supplied to the corresponding pair in PF or PF+CO on the same getational day; approximately one third of the amount of chow was given around 0800h and the rest around 1700h. Other three groups of rats had free access to food. Tap water could be available anytime except for during the exposure in all groups. The daily food intake was measured in CIG, PF, and PF+CO, and neither in CO nor CONT groups.

Mothers in the groups of CIG, CO, PF, PF+CO, and CONT were cesarean sectioned around 1100h on day 21 of pregnancy to observe the individual weight of fetuses.

Duncan's new multiple range test was used for the statistical analysis of data between groups. A p value less than 0.05 was considered as a significance.

RESULTS AND DISCUSSION

Food intake was expressed as a weekly consumption, sum of daily food intakes for 7 days (Fig. 1). Weekly food intake was significantly decreased throughout pregnancy in the group of CIG, compared with others. Especially, in the first week, the mean intake in rats exposed to CIG falled to 65% of the amount of food ingested by mothers in CONT, while it was more than 80% in the following two weeks. The food consumption in the first and second weeks in animals which had inhaled CO was slightly but significantly reduced about 10% lower than CONT, although there was no

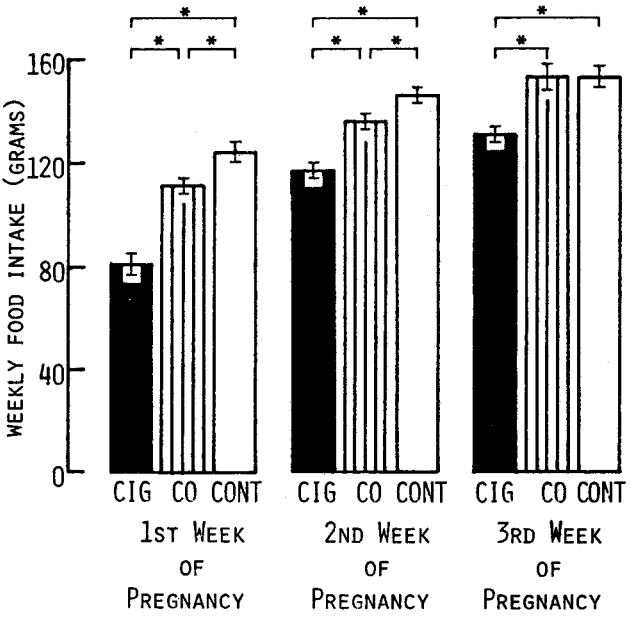

Figure 1. Food intake during each week of pregnancy in rats exposed to cigarette smoke (CIG, solid column), carbon monoxide (CO, striped column) and room air (CONT, open column). Each column represents mean ± S.E. An asterisk indicates a significant difference ($P<0.05$) between groups connected by a line. Number of animals is eight in all groups.

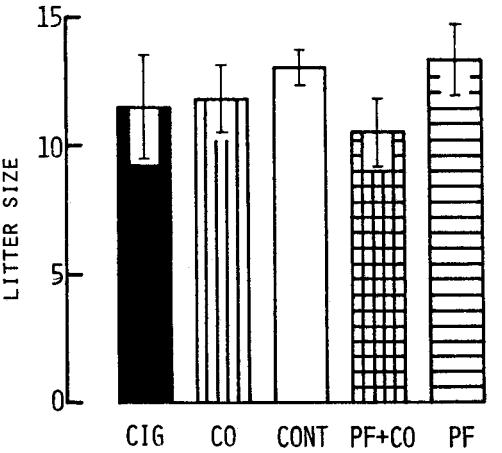

Figure 2. Litter size in each group of rats exposed to cigarette smoke (CIG), carbon monoxide (CO), or room air (CONT), or pair-fed and exposed to carbon monoxide (PF+CO), or to room air (PF). Number of rats is four in all groups.

difference in the last week of gestation.

Earlier authors (Rush 1974) assumed that in human, decreased appitite in smoking women might contribute to fetal retardation, and others (Chow and Lee 1964; Berg 1965) have reported that restricted supply of chow reduced the fetal weight in rats. In the present study, food intake was observed to fall in rats which had inhaled CIG, compared with those in the groups of CO and CONT, and this decline might be able to disturb the fetal growth. The second experiment of paired feeding was then designed to clarify the role of lowered food intake upon the fetal development.

In the second study, the litter size was not statistically different between groups, although that in CIG, CO, and PF+CO was slightly lower than other two groups (Fig. 2).

Fetal weight in CONT was the largest in all groups in the second experiment (Fig. 3). Fetuses from either CIG or CO exposed mothers were retarded in weight to 72% or 80%, respectively, of CONT, whereas those in the CIG group were smaller than CO with a significant difference. This result agrees with the previous study (Tachi & Aoyama 1983) in which authors suggested that CO in the cigarette smoke played an important role to reduce the conceptus weight but was not a single factor.

Rats in the groups of CIG, PF+CO, and PF ingested similar bulk of chow; 331.4 ± 15.1 (mean$\pm$S.E.), 330.4 ± 16.0 and 324.0 ± 10.8 grams, respectively, as a total intake during pregnancy.

Both groups of pair-fed animals had larger progenies than rats exposed to CIG (Fig. 3). The fetal weight in the group of PF was slightly but significantly less than CONT. The result was in accordance with the earlier investigations (Haworth & Ford 1972; Bassi et al. 1984). The reduction in caloric intake thus makes fetuses smaller under the condition of room air inhalation. On the other hand, progenies in the group of PF+CO displayed similar weight to those in CO exposed mothers which had had free access to food. Therefore, shortage in energy intake failed to enhance the adverse effect of CO inhalation by mothers on the fetal growth. These results seemed to indicate that since the progenies of which growth have been already retarded by CO have no need to take nutrients so much, the fetal weight can not be influenced even if their food supply is partly removed.

Meanwhile, body weight increase of mothers from days 0 to 21 of pregnancy was similarly lowered to less than 80 grams in the groups of CIG and PF + CO, compared with other groups where animals gained around 100 grams (Fig.4). Thus, animals in CIG could utilized nutrients as much as those in PF+CO. In spite, mothers in CIG had smaller progenies than in PF+CO. The finding implies that the transference of nutrients from mothers to fetuses may be influenced adversely in CIG, differed from in PF+CO. In human, histological examination revealed disadvantage in the placental vascular system in smoking women (Asmussen 1977), and amino acid pool has been reported to be reduced in smoker's placenta in vitro (Rowell 1981). These investigations seemed to suggest that placental morphological and functional changes can be responsible for the shortage in nutritional transference between mothers and progenies.

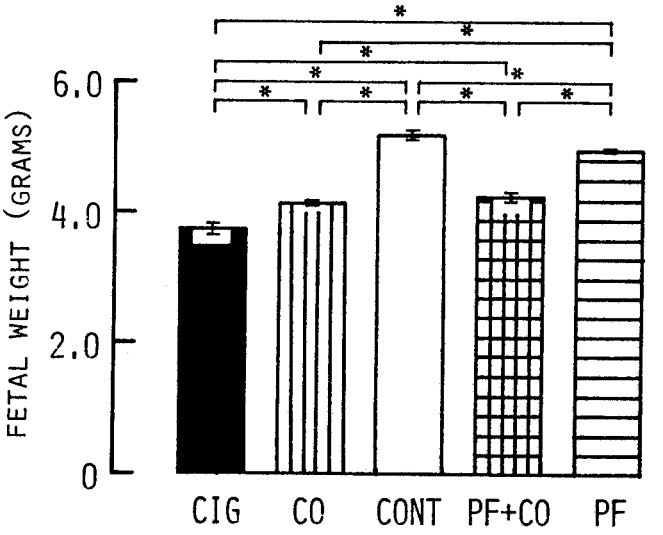

Figure 3. Weight of fetuses from mothers in the groups of CIG, CO, CONT, PF+CO, and PF. Number of fetuses is 46, 47, 52, 42, and 53 for CIG, CO, CONT, PF+CO, and PF.

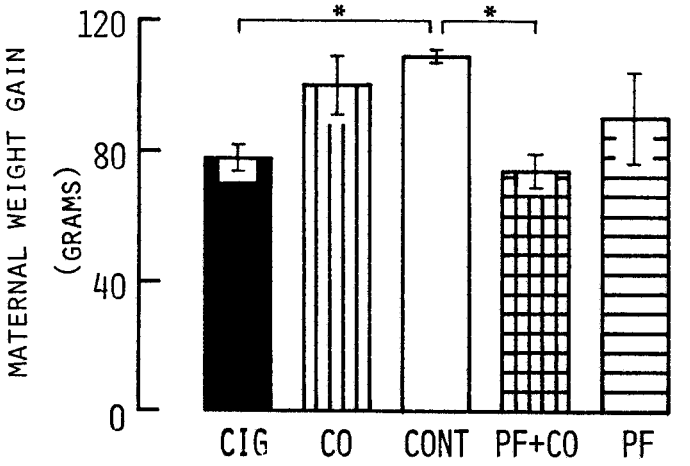

Figure 4. Increase in body weight of gravid rats from days 0 to 21 of pregnancy in each group. Number of animals is four in all groups except for in PF where it is three because of missing data in body weight on day 21 of pregnancy in one rat.

However, it remains unclear at present why reduction in food ingestion failed to affect the fetal weight under CO exposure, while it could decreased the weight in normal condition.

Finally, the present results indicated that the decrease in food consumptions in pregnant rats exposed to cigarette smoke could not always disturb the fetal growth in utero when mothers had inhaled carbon monoxide. Recent epidemiological studies have complained of the reduction of dietary intake in smoking gravida, and showed no difference between smokers and nonsmokers (Haworth et al. 1980). Therefore, the reduced weight of offsprings in smoking mothers is supposed to be induced by carbon monoxide and other factors except for the caloric intake of mothers.

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