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# Effect of Exposure to Cigarette Sidestream Smoke on Growth in Young Rats

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Sidestream smoke from cigarettes contains many toxic constituents at concentrations higher than those found in mainstream smoke (Schmeltz et al. 1975). Nonsmokers occupying the same indoor area are forced to inhale these materials. An increase in the incidence rate of lung cancer has been reported by Hirayama (1981) and Trichopoulos et al. (1981) in women whose husbands are smokers. Children living with smokers frequently develop lower respiratory functions, and chronic obstructive respiratory diseases (Cameron et al. 1969; Harlop and Davies 1974; Targer et al. 1979).

Few studies have been conducted to demonstrate the effects of sidestream smoke on infant growth. While Ferris et al. (1985) found children shorter whose parents smoked than those who did not, the study could not clarify the difference in the growth rate between children of smoking and nonsmoking parents, and failed to distinguish the effect on the growth in height from exposure during the fetal period from that during childhood.

The present study was conducted to investigate the effects of sidestream smoke on the growth of rats. At the same time, the effect of the sole exposure to carbon monoxide at the concentration produced by sidestream smoke was also evaluated, since the gas has been suggested as one of the important factors reducing intrauterine fetal growth in pregnant rats exposed to cigarette smoke (Tachi and Aoyama 1983; 1986).

## MATERIALS AND METHODS

Five male or female Wistar rats, maintained in a cage (25 X 40 X 20 cm) under the condition of lighting (12L

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12D, lights on 6 AM) and temperature at 22.5-26.5°C, were exposed twice daily around 10 AM and 3 PM for 29 days, starting from 23-24 days of age, to a sidestream of cigarette smoke (SS), carbon monoxide (CO) at a concentration near to that in SS exposure, or room air as a control (CONT). Water and food were given ad libitum anytime except for the inhalation period during which time they were removed.

Ten rats, five males and females each, transferred to the inhalation cages (five per cage, 20 X 30 X 15 cm), were placed in a chamber (60 X 54 X 50 cm), in which SS was manually generated by the complete combustion of commercial-brand cigarettes described earlier (Tachi and Aoyama 1983); five cigarettes, each of which was put in the chamber immediately after the lighting outside, were completely combusted for 7.5 min. for the first 7 days of exposure by using a one-way flow pump located outside of the chamber and connected to a cigarette in the chamber by a rubber tube. The mainstream smoke was conducted outside of the chamber. The number of cigarettes combusted was raised from 5 to 8 for 12 min. during the remaining period of exposure. Animals were exposed for another 60 min. after combustion. In CO inhalation, 140 ml of CO gas was introduced for 7.5 min. into the chamber in which animals had been placed, and the volume was changed to 220 ml for 12 min. after the 8th day of exposure. Others were manipulated as for SS exposure. Rats in the CONT group were placed in the chamber on the same time schedule as in the other groups, and further treatment such as pumping the room air was not performed.

Body weight of animals was recorded every day throughout the experiment. On the 29th day of exposure, animals were anesthesized by ether immediately after one exposure, and the blood was collected from the jugular vein to determine blood hemoglobin (Hb) concentration, and percent concentrations of oxyhemoglobin ($O_2Hb$), carboxyhemoglobin (COHb), and methemoglobin (metHb) by IL 282 CO-Oximeter. The weights of brain, heart, lung, liver, spleen, and both kidneys were also obtained in these animals after heart puncture.

Statistical analysis was performed by Duncan's new multiple range test, and $P<0.05$ was considered as significance.

## RESULTS AND DISCUSSION

Although CO concentration in the chamber was about 10% lower in CO inhalation (1390±82 ppm, means±S.D.) during the latter 22-day period of exposure than in SS inhalation (1550±69 ppm), the blood Hb concentration, and the

percent concentrations of $O_2Hb$, COHb, and metHb showed substantially corresponding values between these two groups in either sex (Table 1). Therefore, it would be reasonable to suppose that animals exposed to SS or CO inhaled an equal amount of CO during the exposure. The finding that blood Hb concentration was higher in the group with SS and CO exposure than in the CONT group implied some physiological modification to compensate for hypoxia induced by CO, and agreed with the earlier results of CO inhalation by rats (Wilks et al. 1959).

Table 1. Blood Hb concentration and percent concentrations of $O_2Hb$, COHb, and metHb in animals exposed to SS, CO, or CONT.

| MALES | Hb conc. (g/dl) | % $O_2Hb$ | % COHb | % metHb |
|---|---|---|---|---|
| SS | 16.9 ± 0.2* | 40.8 ± 5.7* | 55.2 ± 2.1* | 2.0 ± 0.6 |
| CO | 17.1 ± 0.2* | 35.3 ± 2.1* | 53.0 ± 1.6* | 2.3 ± 0.8 |
| CONT | 13.9 ± 0.4 | 67.2 ± 5.7 | 1.3 ± 0.4 | 1.7 ± 0.8 |
| FEMALES | | | | |
| SS | 16.6 ± 0.4* | 39.2 ± 3.2* | 51.7 ± 2.7* | 2.6 ± 0.8 |
| CO | 16.5 ± 0.2* | 37.2 ± 2.4* | 53.7 ± 2.8* | 2.1 ± 1.0 |
| CONT | 13.6 ± 0.2 | 75.9 ± 2.3 | 3.1 ± 0.3 | 1.2 ± 0.3 |

Mean ± S.E.
Asterisk: indicates a significant difference from CONT.
Number of animals: 5 in all groups of either sex.

Growth in body weight was much inhibited by SS exposure in both sexes. Body weight on the day of autopsy was 25% and 27% less in males exposed to SS than those in CO and CONT groups, respectively, and 14% and 12% smaller in females (Figure 1).

While Ferris et al. (1985) reported that children whose parents were smokers were shorter in height than those of nonsmoking parents, the authors failed to determine whether the effect was induced by the involuntary smoking during childhood life, or by that during the fetal period. The present study clearly demonstrated that the postnatal inhalation of SS reduced the growth in body weight in rats.

CO, which has been recognized as one of the factors to decrease the intrauterine fetal growth (Tachi and Aoyama 1983; 1986), had no influence upon the body weight growth, which similarly progressed in CO exposed animals to those in CONT group. Thus, CO seemed unlikely to be a cause of retarded growth in SS-exposed rats.

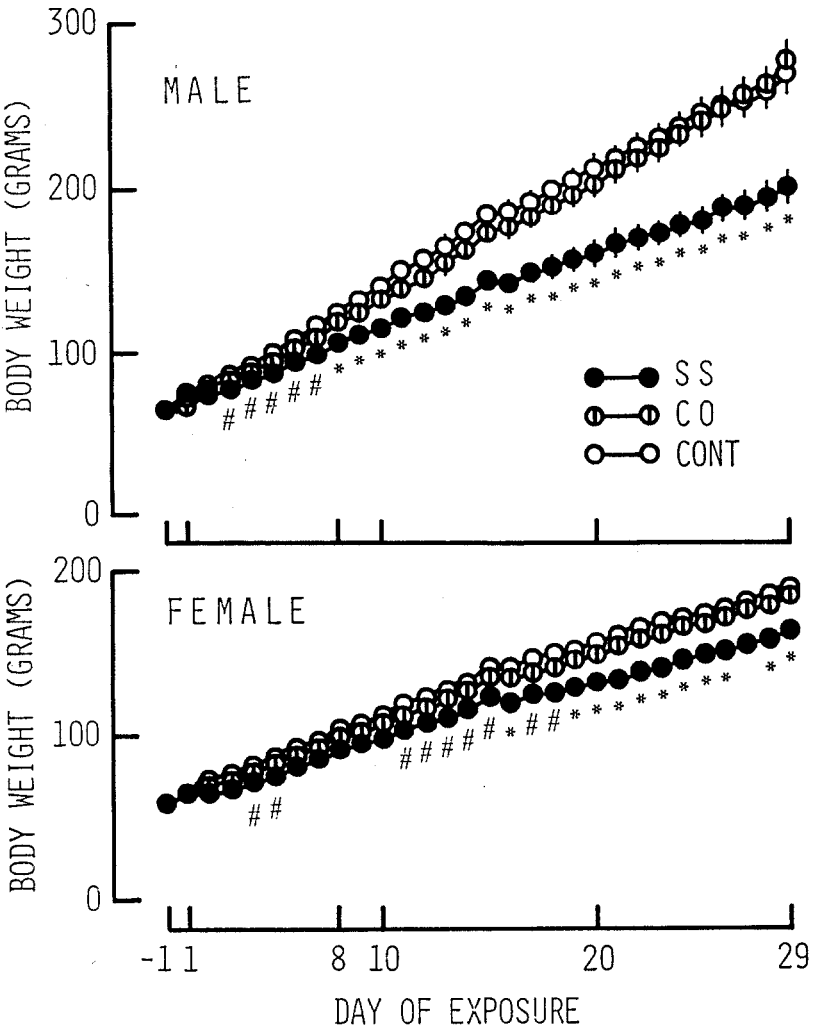


Figure 1. Body weight in both sexes of animals exposed to SS, CO, or CONT.
Each point represents the mean ± S.E. grams of 5 rats in any group. # and * indicate a significant difference from CONT, and from CONT and CO, respectively.

At present, the cause(s) of the growth retardation in body weight by SS exposure remain unclear. However, SS has been reported to contain many toxic substances at higher concentration than in mainstream smoke (Schmeltz et al. 1975). Nicotine, for example, has been known to decrease body weight in rats (Schechter and Cook 1976; McNair and Bryson 1983; Grunberg et al. 1984). One or a certain combination of them may have been responsible for the events observed in this study.

Although the weight of the heart in both sexes, and that of the lung in females were not significantly different between groups, other organs weighed less in

Table 2. Organ weights in both sexes of animals exposed to SS, CO, or CONT.

| | | BRAIN | HEART | LUNG | LIVER | KIDNEY | SPLEEN |
|---|---|---|---|---|---|---|---|
| MALE | SS | 1.74±0.02*# | 1.12±0.08 | 1.10±0.08* | 8.36±0.59*# | 1.52±0.10*# | 0.43±0.05*# |
| | CO | 1.86±0.02 | 1.20±0.08 | 1.28±0.04 | 11.28±0.72 | 2.06±0.08 | 0.78±0.04 |
| | CONT | 1.86±0.02 | 1.04±0.05 | 1.36±0.07 | 11.20±1.00 | 2.04±0.07 | 0.78±0.06 |
| FEMALE | SS | 1.56±0.04* | 0.80±0.05 | 1.12±0.15 | 6.40±0.27*# | 1.22±0.04*# | 0.44±0.02*# |
| | CO | 1.64±0.02 | 0.82±0.04 | 1.02±0.04 | 7.26±0.13 | 1.42±0.07 | 0.52±0.02 |
| | CONT | 1.66±0.02 | 0.76±0.02 | 1.00±0.03 | 7.80±0.30 | 1.52±0.07 | 0.52±0.04 |

Mean±S.E. grams. Number of animals is five in any group.
*, and #: a significant difference from CONT and CO exposed groups, respectively.



Table 3. Percentages of organ weights to body weight in both sexes of animals exposed to SS, CO, or CONT.

| | | BRAIN | HEART | LUNG | LIVER | KIDNEY | SPLEEN |
|---|---|---|---|---|---|---|---|
| MALE | SS | 0.88±0.05*# | 0.56±0.01*# | 0.55±0.03# | 4.16±0.07 | 0.76±0.01 | 0.24±0.02*# |
| | CO | 0.68±0.03 | 0.43±0.02 | 0.46±0.01 | 4.07±0.12 | 0.75±0.03 | 0.28±0.00 |
| | CONT | 0.70±0.03 | 0.39±0.01 | 0.51±0.01 | 4.14±0.22 | 0.76±0.03 | 0.29±0.01 |
| FEMALE | SS | 0.96±0.03*# | 0.49±0.03* | 0.68±0.09 | 3.92±0.06 | 0.75±0.02 | 0.27±0.02 |
| | CO | 0.88±0.01 | 0.44±0.02 | 0.55±0.02 | 3.93±0.07 | 0.77±0.03 | 0.28±0.01 |
| | CONT | 0.88±0.02 | 0.40±0.02 | 0.53±0.01 | 4.11±0.08 | 0.80±0.03 | 0.28±0.02 |

Mean±S.E. percent. Number of animals is five in any group.
*, and #: a significant difference from CONT and CO exposed groups, respectively.

SS-exposed animals than in CO or CONT exposed group (Table 2).

Since the body weight was smaller in the group of SS exposure, the percentage of the organ weight to the body weight was calculated and compared among groups. The indices indicated similar values in liver and kidney in both sexes, and spleen and lung in females between groups (Table 3). The percentages of brain and heart were larger in the SS group than in the other groups. The rate of lung or spleen was the smallest in CO- or SS-exposed males, respectively. The reasons for this in organ weights or their ratio to the body weight remain unknown, whereas it was obvious that the phenomena which occurred in SS-exposed animals were not due to CO inhalation.

Finally, the present study revealed that SS exposure from juvenile to adult life served to reduce the body weight growth in rats, and to modify the development in some organs. CO in the smoke proved not to be responsible for the events. Thus, the involuntary smoking in children was supposed to have a harmful effect on their growth and health as shown previously (Cameron et al. 1969; Harlop and Davies 1974; Targer et al. 1979; Ferris et al. 1985). However, the concentration used in SS and CO inhalation in this study was considered high, and the inhalation should be done at a lower concentration.

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