

Effects of Sidestream Smoke on Pregnant Mice and their Offspring

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Introduction

Numerous studies suggest a causal relationship between cigarette smoking during pregnancy and certain detrimental effects on the fetus and neonate. The adverse effects of maternal smoking on fetal growth, marked by a lower mean birth weight, was first observed almost 30 years ago (30). The risk of delivering a low birth weight infant (i.e., less than 2500 g) is approximately doubled in smoking mothers (21). An increase in perinatal mortality has also been repeatedly associated with cigarette smoking during pregnancy (9, 19, 21, 22, 31).

Animal studies using rats (4, 12, 16, 28, 36), mice (34), and rabbits (29) have shown that maternal exposure to cigarette smoke reduces birth weight of offspring. However, a number of authors have not observed this association (7, 27, 35). In addition, some investigators have reported that smoke exposure results in smaller litter size and increased perinatal mortality (12, 29), while others have not (7, 16, 27, 35). These conflicting studies together with the limited research regarding the effects of sidestream smoke (passive smoking) on reproduction and development prompted the present investigation.

Materials and Methods

Adult virgin female Cox-Swiss outbred mice were mated during the night and examined the next morning for evidence of impregnation (sperm positive vaginal smear). Pregnant mice were randomly assigned to experimental and control groups. They were maintained in separate cages under environmental conditions controlled with respect to room temperature (20-25°C), humidity (55-60%), and photoperiod (12 hr light, 12 hr dark). Both groups were fed Lab-Blox pellets (Wayne Feed Division, Continental Grain Co.) and water *ad libitum* between trials.

Experimental mice were exposed daily to the sidestream smoke from one filter-tip cigarette for 40 min in a plexiglass smoking chamber (25.5 cm × 30.5 cm × 21.0 cm) containing 6 airholes of 6.5 mm diameter on two opposite sides. The cigarettes, which contained 17.0 mg tar, 1.0 mg nicotine, and 15.6 mg carbon monoxide (14), were obtained from commercial sources. Control mice were divided into two groups: sham controls and shelf controls.

At the end of each treatment period, dams were returned to their respective cages. Treatment was terminated at parturition, and the dams were allowed to nurture their litters until they were weaned on postnatal day 21. On postnatal day 1 (within 16 hr of birth) all litters were sexed, weighed, and dead fetuses removed. The pups were also weighed on postnatal days 3, 5, 10, 15, and 21. Cages were observed daily to record offspring deaths.

In a parallel study, carboxyhemoglobin concentrations of peripheral blood (vena cava) of female mice subjected to the conditions described above were determined using a Model 282 CO-Oximeter (Instrumentation Laboratories, Lexington, Mass.) and taken as an index of the amount of smoke inhaled.

Results are reported as means $\pm$ S.E. Chi-square analysis was used to evaluate percent mortality data. Differences between means were evaluated using Student's *t* test and a P value of less than 0.05 was considered to be significant.

Unless specifically noted, sham treated mice are the controls of reference.

Results and Discussion

Dams exposed to the sidestream smoke of one cigarette for 40 min had a carboxy-hemoglobin (COHb) value of 23.74% (± 0.78 ; $N = 48$). Control dams had a COHb concentration of 2.64% (± 0.12 ; $N = 32$). The COHb concentration was reduced to 4.36% (± 0.40 ; $N = 9$) one hour following smoke exposure, and to 3.64% (± 0.24 ; $N = 10$) 2 hr postexposure. After 24 hr, COHb levels were within the range of control dams (2.27% ± 0.16 ; $N = 6$).

Exposure of pregnant female mice to sidestream cigarette smoke significantly reduced mean litter size, lowered birth weight, and increased perinatal mortality. Gestation time was unaffected, and there were no obvious teratogenic effects. Results concerning the effect of sidestream smoke on sex ratio were inconclusive (Table 1).

TABLE 1. Effects of sidestream smoke on mouse litter size¹, birth weight (g), sex ratio (M:F), and gestation period (days). Number of litters given in parentheses.

Treatment	Litter Size	Birth Weight	Sex Ratio	Gestation Time
Con-Shelf (25)	11.4 $\pm$ 0.43	1.49 $\pm$ 0.02	1.38 $\pm$ 0.12	20.4 $\pm$ 0.25
Con-Sham (64)	11.2 $\pm$ 0.40	1.50 $\pm$ 0.01	0.89 $\pm$ 0.09 ¹	20.5 $\pm$ 0.30
Smk-Exp (70)	9.2 $\pm$ 0.40 ²	1.40 $\pm$ 0.01 ¹	1.34 $\pm$ 0.17	20.8 $\pm$ 0.20

1. Within 16 hr of birth; equivalent to postnatal day 1.

2. Significantly different from both control groups, $P < 0.01$.

3. Significantly different from shelf control and experimental groups, $P < 0.05$.

The average control litter in this study had two more pups (11.2) than did the average experimental litter (9.2). In addition to this 18% difference in litter size, the mean birth weight of control pups were 6.7% greater (1.5 g) than smoke-exposed pups (1.4 g). In an attempt to determine whether these differences could be predicted late in pregnancy, an in utero study was conducted. Counts and weights of pups of sidestream smoke-exposed mice were compared to those of controls on day 18 of gestation (Table 2). Although there was no significant difference (4%) in the number of offspring,

TABLE 2. Comparison of litter size and weights (g) of pups at birth¹ to litter size and weights in utero on day 18 of gestation. Number of litters given in parentheses.

Treatment	Mean Litter Size			Mean Weight		
	Birth	In Utero	% Diff	Birth	In Utero	% Diff
Self Con	11.4 $\pm$ 0.43 (25)	12.1 $\pm$ 0.49 (7)	5.8	1.49 $\pm$ 0.02 (25)	1.05 $\pm$ 0.03 (7)	29.5
Sham Con	11.2 $\pm$ 0.40 (64)	12.4 $\pm$ 0.29 (19)	8.9	1.50 $\pm$ 0.01 (64)	1.06 $\pm$ 0.02 (8)	29.5
Smk-exp	9.2 $\pm$ 0.40 (70)	11.9 $\pm$ 0.16 (21)	22.7	1.40 ² $\pm$ 0.01 (70)	0.90 ² $\pm$ 0.03 (9)	36.1
% Diff (Shelf vs Smk)	19.3	1.7		6.0	14.3	
% Diff (Sham vs Smk)	17.8	4.0		6.7	15.4	

1. Within 16 hr of parturition; equivalent to postnatal day 1 and day 21 of development.

2. Significantly different from both control groups, $P < 0.01$.

experimental pups weighed significantly less (15.4%) than controls. This difference between in utero weights and the fact that there was no significant difference in length of gestation is consistent with human data. It has been suggested that the increased incidence of low birth weights among smokers is primarily due to intra-uterine growth retardation rather than prematurity (1). Perhaps the lower weight of experimental pups in utero leads to still-births and neonatal deaths. This would account for a decrease in litter size at birth, as well as the smaller (but significant) difference in experimental and control birth weights compared to day 18 in utero weights of the two groups.

When all control and experimental litters are compared, survivorship over the 21 day preweanling (lactation) period was approximately 20% greater for control offspring (78.3%) than for those of smoke-exposed mothers (59.7%) (Table 3A). The highest rate of offspring mortality occurred between day 1 and day 3. During this period 26.2% of the smoke-exposed pups died compared to 7.6% of the controls. From day 5 to day 21 no significant difference in mortality was observed between

TABLE 3. Percent mortality of preweanling mice at day 1-3, 3-5, 5-10, 10-15, 15-21, and 1-21 intervals. Number of litters given in parentheses.

Group	Treatment	Percent Mortality					
		Day Intervals					
		D1-D3	D3-D5	D5-D10	D10-D15	D15-D21	D1-D21
A'	Shelf Con (25)	—	—	—	—	—	21.2 ± 2.6
	Sham Con (35)	7.6 ± 0.03	5.8 ± 0.03	6.6 ± 0.03	1.3 ± 0.01	2.2 ± 0.01	21.7 ± 2.5
	Smk-exp (41)	26.2 ± 0.06 ¹	5.7 ± 0.04	6.5 ± 0.02	3.9 ± 0.03	4.5 ± 0.01	40.3 ± 2.9 ¹
B'	Shelf Con (23)	—	—	—	—	—	15.2 ± 3.2
	Sham Con (32)	5.3 ± 0.37	3.5 ± 0.02	4.9 ± 0.02	1.3 ± 0.02	2.2 ± 0.01	16.2 ± 2.9
	Smk-exp (31)	9.3 ± 0.03	5.1 ± 0.03	6.2 ± 0.02	1.3 ± 0.01	4.5 ± 0.02	24.0 ± 4.0 ⁴

1. All litters, including those with 100% mortality between day 1 and day 21 preweanling.

2. 23/25 (92.0%) self control, 32/35 (91.4%) sham control, and 31/41 (75.6%) smoke-exposed litters that did not experience 100% mortality between days 1 and 21.

3. Significantly different from control, $P < 0.01$.

4. Significantly different from control, $P < 0.05$.

experimental and control pups. In 24.4% (10/41) of the experimental litters, all of the pups died prior to weaning. Only 8.6% (3/35) control litters experienced 100% preweanling mortality. Exclusion of litters of control and smoke-exposed dams in which all the pups died during the lactation period produced less but still significant differences in mortality rates of the two groups (Table 3B). Cannibalism, which has been shown to accompany perinatal death in rats (12), was observed on numerous occasions among experimental litters, but was rarely observed among controls. Since day 1 observations were made as late as 16 hr after birth, it is possible that cannibalism was partly responsible for the difference in day 1 litter size between experimental and control groups. If this is the case, perinatal mortality would be correspondingly higher.

Cigarette smoke is a complex mixture of about 2000 chemicals (1). Therefore, the fetus is exposed to many possible causes of damage. Most research has focused on two of these components: nicotine and carbon monoxide (CO). Action of nicotine on uterine vessels, direct action of nicotine on the fetus, chronic fetal hypoxia associated

with carboxyhemoglobin, and enzyme poisoning by CO are a few of the mechanisms proposed that could result in fetal damage (28, 36).

Injection of nicotine in concentrations of 3 mg/kg or greater has been shown to cause deleterious effects on fetal and neonatal development. Several studies have reported a reduction in live offspring (5, 23, 32). Nicotine can also affect mortality of neonates. Giving pregnant rats twice-daily injections of 5 mg/kg nicotine resulted in 34% mortality of their pups prior to weaning (5). Similar doses have produced even higher rates of perinatal mortality (32, 26). However, Hammer and Mitchell (13) found no significant increase in perinatal mortality with nicotine concentrations in this range. Although dose levels were approximately the same in each of these studies, the time of administration during pregnancy and the duration of exposure differed. This indicates the importance of each of these factors relative to the effects produced.

In the blood, carbon monoxide, which has a higher affinity for hemoglobin than oxygen, combines with hemoglobin to form carboxyhemoglobin. This produces as much as a 12% reduction of the oxygen-carrying capacity of the blood. In addition, carbon monoxide increases the affinity of oxygen for hemoglobin and impairs oxygen unloading (1).

Exposure of pregnant animals to concentrations of CO that produce COHb values of 11 to 28% lowers birth weight (2, 24, 25), decreases litter size (11), and increases perinatal mortality (20). A mean COHb concentration of 23.7% (range 15% to 42%) immediately following smoke exposure under the conditions of this investigation are well within the effective range.

Perinatal mortality of 26% in this study by day 3 and 40% by day 21 of smoke-exposed mice compared to 7% and 22%, respectively, for the control groups, corresponds to the findings of Hoffman and Campbell (17). They subjected rats to continuous exposure of 230 ppm CO (26% COHb) for 3 weeks prenatally and 3 weeks postnatally. This exposure resulted in offspring mortality of 28% by 3 days and 64% by 21 days of age. Astrup et al. (2) found that exposure of rabbits to 180 ppm CO (16-18% COHb) throughout pregnancy caused a neonatal mortality of 35% within the first 24 hours compared to less than 1% mortality in the control group. Essenberg et al. (12) reported that 13.5% of rats exposed to cigarette smoke lost an entire litter prior to weaning. In this study, 24% of the smoke-exposed mice lost a complete litter, but only 8.6% of the control litters experienced 100% mortality by day 21.

The data regarding the effect of sidestream smoke on sex ratio is inconclusive. Experimental pups showed a significantly elevated sex ratio (1.34) compared to sham controls (0.89), but no difference in sex ratio compared to shelf controls (1.38) (Table 1). Although some studies associated with the effects of tobacco smoke or smoke constituents have tended toward a greater preponderance of male offspring (6, 15), the sex ratios were not significantly higher than those of the control groups. The reason for shifts in sex ratio is not clear, but it has been suggested that female fetuses may be more sensitive to hypoxic states than are male fetuses (33).

There was no significant difference between the average weight of experimental and control pups by preweaning day 10. This can be attributed to the high perinatal mortality among experimental pups. With fewer pups, the dam can more efficiently nurse those remaining. Consequently, their weights catch up with those of controls.

The findings of this study are in general agreement with some investigations (12, 29), but contradict others (3, 10, 27, 35). Most studies have involved smoking machines, which subject the test animal to smoke drawn through the cigarette and released in regulated, periodic puffs. Smoke drawn through the cigarette in this manner is referred to as mainstream smoke. This study used sidestream smoke (smoke from the lit end of the cigarette) instead. It has been reported that the CO concentration is about

2.5 times higher in sidestream than in mainstream smoke. The concentrations of tar and nicotine are also higher in sidestream smoke, being 1.7 times higher for tar and 2.7 times higher for nicotine (18). Therefore, the difference between the effects of cigarette smoke reported in this study compared to other animal studies may be due in part to the type of smoke exposure used. Another factor might be that mice served as the research animal in this investigation, whereas rats have been used in most smoking research. It has been shown that the effects of cigarette smoke tend to be species-specific (8).

In summary, these data suggest that sidestream smoke (passive smoking) which produces carboxyhemoglobin concentrations of 23-24% can significantly affect pre- and postnatal development in mice and that the final days of gestation may be especially critical.

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