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Tobacco-Smoke Inhalation Delays Suckling-Induced Prolactin Release in the Rat (38291)

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(Introduced by E. D. Rees)

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A number of clinical reports have correlated tobacco smoking with decreased pregnancy rate, increased abortion rate, infant mortality, still births, neonatal deaths, and lower mean birth weights in those infants which are delivered normally (1–5). Investigations with experimental animals have tended to confirm these observations. Thus, it has been observed that exposure of pregnant rats (6, 7) to tobacco smoke decreases fetal growth and increases the incidence of prenatal mortality, an effect which was mimicked by injection of nicotine (6, 8–10). However, confusion exists in the literature since some investigators have found smoking to have no deleterious effects on reproductive processes (11–13). One source of this confusion may be the fact that often the animal experiments were poorly controlled in terms of smoking parameters and only general parameters of reproduction were measured.

For this and other reasons a number of investigators have begun to examine the effect of nicotine injections on *specific* reproductive parameters, such as the ovulatory surge of luteinizing hormone and the prolactin release known (14) to be induced by suckling. The report of Blake *et al.* (15) was the first to indicate that nicotine injections on the morning of proestrus delays or blocks the ovulatory surge of luteinizing hormone in the rat. Nicotine injections have also been found to inhibit the proestrous surge of prolactin in rats and to block the suckling-induced rise of prolactin in lactating rats (16, 17). The present report goes one step further and presents data showing that, under carefully controlled smoking conditions, smoke inhalation delays the suckling-induced rise of serum prolactin.

Materials and Methods. Adult female Wistar rats (Harlan Industries, Cumberland, Indiana) were maintained under a 14:10 light–dark cycle at 75°F. After acclimatization to laboratory conditions, the rats were mated with males of the same strain and allowed to deliver (21.4-day av gestation). Eight to ten days after parturition, a chronic cannula was inserted into the right atrium according to the procedure of Blake *et al.* (16) and exteriorized at the back of the neck. Using this technique it was possible to collect serial samples by withdrawing blood into heparinized syringes before, during, and after suckling without disturbing the mother or the pups and without anesthetization. The cannulation procedure was performed on the day before the experiment was begun. Eight mothers were designated as "smokers" and six as controls or "nonsmokers". On the day of the experiment, the pups were isolated from their mothers for 8 hr before suckling was permitted. The first blood sample (0.3–0.4 ml) was taken from the mothers 1 hr before the pups were replaced. Fifteen minutes after this sample, the mothers were placed in lucite "smoking" cages (lucite cylinders with adjustable plungers) which in turn were placed on racks of a smoking machine provided by the University of Kentucky Tobacco and Health Research Institute. The machine was programmed to deliver a 2-sec 35-ml puff of 10% smoke (or air in the case of controls) which was held in the smoking chamber and delivered to the animals for 25 sec of the 1-min cycle. Figure 1 shows the configuration of the smoking apparatus. Experimental cigarette No. 1A4 (2.5 mg nicotine/cigarette) was used in all "smoked" groups. All controls (nonsmoked) were subjected to the same conditions (restraint in cages, etc.) except that air was substituted for the smoke so that the same stress induced by con-

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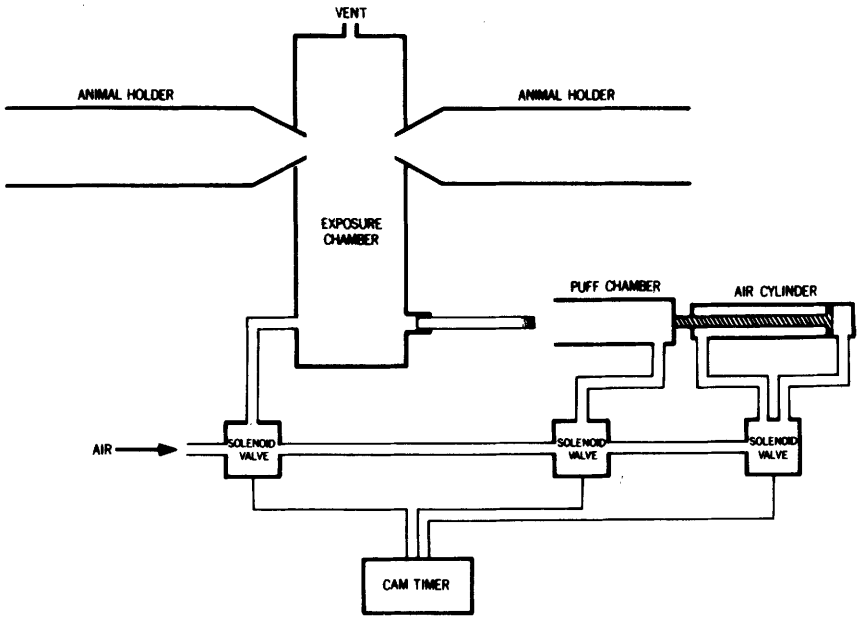

FIG. 1. Schematic of the "smoking" machine. In operation a lighted cigarette is placed in the cigarette holder and the puff chamber (air-cylinder operated) is moved over the cigarette holder forming an air-tight seal against a Teflon disc. Air is then forced into the chamber, mixes with the smoke and flows out through the ports of the exposure chamber through which the animals are breathing. After the puff, retraction of the puff chamber allows the cigarette to free-burn. The solenoid-operated valves which direct the air flow during "smoking" are controlled by switches actuated by a motor driven camshaft. Puff frequency is determined by the motor speed while puff chamber operation (puff dilution and duration) are controlled by cam settings. The flow of air for the puff and dilution are controlled by metering valves and monitored by flowmeters. A manual reset electric counter counts the number of puffs. Puff duration, dilution, and frequency may be varied by adjustment of machine setting. Although only two of the animal holders are shown in place, as many as eight rats may be exposed to smoke at one time.

finement and the noise generated by the smoking machine was experienced by smokers and nonsmokers. Animals were exposed to thirty 1-min cycles during which time a total of 30 puffs of air or smoke were delivered. After treatment they were removed from the smoking cages and placed in regular cages for 15 min before their pups were returned. The pups were replaced (8 per mother) and a blood sample was taken (time 0) from the mother. Unless at least 4 pups were suckling within 3 min no samples (0.3–0.4 ml) were taken at 15, 30, 45, 60, 90, 120, and 180 min after onset of suckling. Heparinized saline (0.5 ml 16.7 U/ml) was reinjected after each collection. Samples were allowed to clot overnight at 4°, centrifuged, and subjected to radioimmunoassay for prolactin using the NIAMDD kit for rat prolactin (18) and the double-antibody procedure of Niswender *et al.* (19). All values are expressed in terms of NIAMDD rat prolactin standard RP-1.

Results and Discussion. Serum prolactin levels of smoked and nonsmoked lactating–suckling female rats are shown in Fig. 2. It is seen that in animals exposed to puffs of air suckling induced a rapid rise in serum prolactin within 15 min after the pups were replaced. This finding is in agreement with previous reports (17). After this time prolactin continued to rise and reached a peak 2 hr after suckling began. In marked contrast, mothers exposed to tobacco smoke showed no rapid prolactin release in response to suckling. Indeed, prolactin levels in these animals were not significantly different ($P < 0.01$) from presuckling values until 90 min postsuckling. After this time prolactin levels continued to rise even in "smoked" mothers and reached a peak at the same time (2 hr after onset of suckling) as in nonsmoked mothers. However, the prolactin levels in the smoke-exposed group were much higher ($P < 0.001$) at this time. The reason for this "overshoot" in prolac-

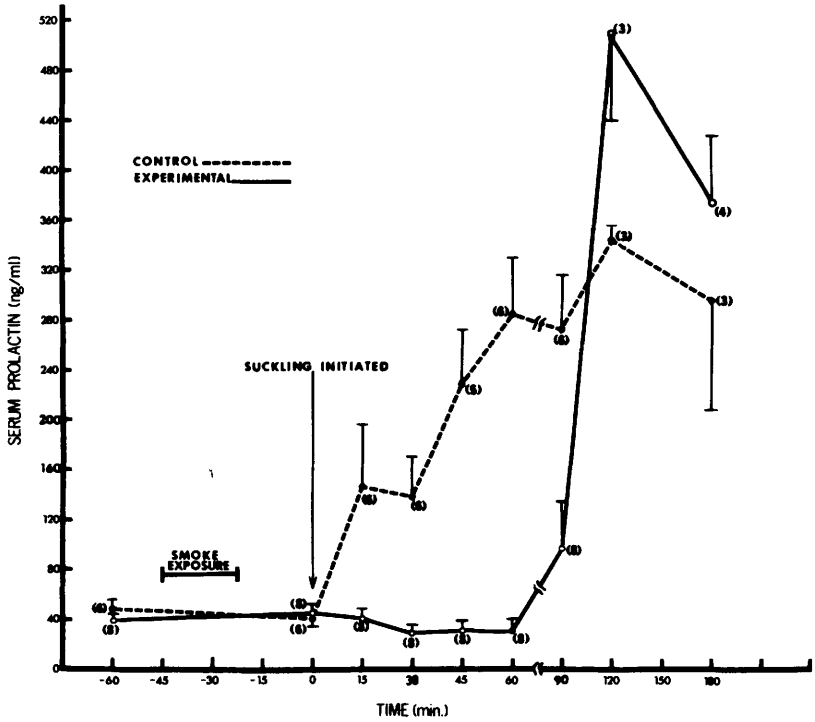

FIG. 2. Serum levels of prolactin (expressed as ng/ml of NIAMDD RP-I) at various intervals before and after smoke (solid line) or "air" (broken line) exposure and comparison of the effects of these treatments on prolactin release induced by suckling. The number of observations at each sampling interval appears in parentheses and the vertical lines at each point indicate the standard error of the mean.

tin secretion following nicotine injections (16) or after smoke exposure (Fig. 1) is not readily apparent. Before speculating, it is necessary to note that only a few observations were made during the interval of the "overshoot". It is possible that the hypersecretion may reflect prolactin accumulated in the pituitary during the nonsuckling interval and further accumulation when the prolactin releasing mechanism was inhibited. Pasteels (20) has noted that prolactin-secreting cells in the rat are engorged with granules after 10 hr of nonsuckling, and Yanai and Nagasawa (21) have observed maximum pituitary prolactin content after nonsuckling intervals of 8 hr. Moreover, the fact that hypersecretion occurred only in animals exposed to smoke suggests interference with neural mechanisms, perhaps a prolactin-releasing factor (22) or a cumulative effect on decreasing the hypothalamic prolactin inhibiting factor (PIF). The last-mentioned possibility seems more likely since it has been shown that the suckling stimulus is most effective in depleting hypothalamic PIF after 30 min of suckling (14). At the 3-hr sampling, no significant difference was seen. It is of interest to note that what was expected to be the "stress" of smoking produced no difference in serum prolactin measured 15 min before and 15 min after smoke exposure. This observation suggests that in contrast to previous studies using nicotine injections (17, 23), smoke inhalation itself does not result in prolonged "stress-induced" discharges of prolactin. However, since this effect was reported (17) to be manifest within 5 min of injection, it is likely that our sampling regimen failed to detect this rise. This possibility is being investigated. It is also of interest that, despite the apparently considerable stress of restraint in smoking cages for 30 min, and in contrast to ether and/or laparotomy stress (17, 23, 24), there was no substantial release of prolactin within the time interval examined.

In the study reported here, comparison of litter weights between groups showed that pups from mothers exposed to smoke weighed significantly

($P < 0.05$) less than control litters when the weights were recorded prior to and just after suckling. Thus it appears that despite the fact that "milkspots", *i.e.*, milk in the stomachs, could be observed in pups from smoked as well as nonsmoked mothers, there is some interference with the milk-release mechanism. This observation is at variance with the report of Blake *et al.* (17) who, noting the presence of milk in the stomachs of pups from mothers injected with nicotine, concluded that the reflex discharge of oxytocin and hence milk release was not disturbed by nicotine.

Thus it appears that, at least in rats, smoke inhalation is capable of temporarily blocking a neuroendocrine reflex. Moreover, since these results are strictly comparable to those obtained with injections of nicotine, it appears that nicotine is the causative agent. The present study is of further value since smoke inhalation should more nearly approximate the situation of habitual use of tobacco smoke than would nicotine injections. The possibility that other constituents of tobacco smoke may be responsible for these effects is currently being investigated utilizing experimental cigarettes of low nicotine content.

Summary. The effects of tobacco–smoke inhalation on a readily quantifiable neuroendocrine reflex, *i.e.*, prolactin release in response to suckling, has been examined in lactating albino rats. After isolation from their pups for 8 hr, mothers exposed to puffs of smoke for 30 min released significantly less prolactin ($P < 0.01$ or less) at 15, 30, 45, 60, and 90 min after suckling began than did air-exposed controls. At 120 min postsuckling, prolactin levels in smoke-exposed animals exceeded those of controls, perhaps reflecting prolactin accumulated in the pituitary during the time its release was inhibited. These results show that tobacco–smoke inhalation delays the suckling induced release of prolactin and provide the first evidence that smoke inhalation (nicotine?) can interfere with neuroendocrine reflexes associated with reproduction.

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