and with respect to cellular protein (0.7 to 12 mg) within the ranges used in these experiments.

The requirements for AHH from phytohemagglutinin-stimulated lymphocytes (Table 1) are similar to the requirements of AHH from rat liver microsomes as well as from hamster embryo cells (*1*, *3*). The enzyme has characteristics typical of the microsomal mixed-function oxidases: a requirement for NADPH, inhibition by carbon monoxide, suggesting an involvement of the P-450 hemoprotein, and inhibition by 7,8-benzoflavone, a potent inhibitor of the AHH system (*8*). The protein nature of the catalysis is further indicated by its sensitivity to trypsin digestion.

Activity of AHH in resting, in mitogen-stimulated, and in BA-treated lymphocytes is shown in Table 2. The AHH activity of lymphocytes compared to rat liver microsomes or hamster embryo cells is relatively low. Mitogen treatment alone increases AHH activity about twofold. The difference in AHH between resting and mitogen-treated cells, however, is amplified by treatment with BA. Thus, mitogen-treated cells exposed to BA exhibit a three- to eightfold greater AHH activity than resting cells treated with BA.

The finding of highest AHH activity in PWM-stimulated, BA-treated cells is biochemical evidence consistent with the morphologic observation that PWM stimulates proliferation of endoplasmic reticulum more intensely than does phytohemagglutinin (*9*). Therefore, AHH may be useful for biochemical detection of quantitative changes in the endoplasmic reticulum of lymphocytes at various stages of their differentiation. Purification of lymphocytes by column absorption techniques, as in this study, may preferentially remove "B"-type lymphocytes (*10*). The latter are thought to be the progenitors of antibody-producing plasma cells (*11*) and may be the precursors of the plasmacytoid cells seen in PWM-stimulated cultures (*9*). Our finding of a significantly greater increase in AHH activity after PWM than after phytohemagglutinin stimulation suggests either that B cells are not completely removed by the lymphocyte purification technique, or that blast cells with increased endoplasmic reticulum may arise from PWM stimulation of the remaining T cells.

Investigation of AHH in humans is relevant to several problems. AHH may play a role in chemical carcinogenesis; furthermore, specific inhibitors, such as 7,8-benzoflavone (*8*), or inducers, such as polycyclic hydrocarbons or phenobarbital (*12*), may be useful in reducing the toxic or carcinogenic effects of exposure to certain environmental compounds.

Individual variations in response to drug therapy, susceptibility to drug toxicity, and drug synergism or antagonism may be related to differences in drug-metabolizing enzymes (*13*).

This is the first report of the presence of a drug-metabolizing enzyme system in an easily available human tissue. The detection of other microsomal enzymes in human lymphocytes may require assays more sensitive than those presently available. The study of AHH and other drug-metabolizing enzyme systems in lymphocytes may help to reveal the genetic and environmental factors and the molecular mechanisms involved in the regulation of this enzyme system, which is important in the organism's response to exogenous chemicals.

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Nicotine Blocks the Suckling-Induced Rise in Circulating Prolactin in Lactating Rats

Abstract. *In lactating rats the rapid suckling-induced release of pituitary prolactin into circulating blood is inhibited by a subcutaneous injection of nicotine. This treatment does not block the lesser "stress-induced" rise in prolactin in response to either. Although nicotine may impair milk production by its effect on prolactin release, it does not appear to block milk ejection from the lactating mammary gland.*

Nicotine has been implicated as the constituent of tobacco smoke that is responsible for a high incidence of reproductive disorders during pregnancy and an increased mortality rate in infants (*1*). Until now, no one has specifically investigated the effects of nicotine on the neural events that initiate the suckling-induced rise in circulating prolactin and oxytocin, two hormones which are necessary to maintain milk production and discharge, respectively. It is well established that suckling causes a rapid release of prolactin and oxytocin from the pituitary gland in the rat (*2*). More recently, stress has been shown to cause prolactin output (*3*), and the mechanisms involved in prolactin release after suckling and after exposure to ether have been shown to be different (*4*). We now report that nicotine inhibits the suckling-

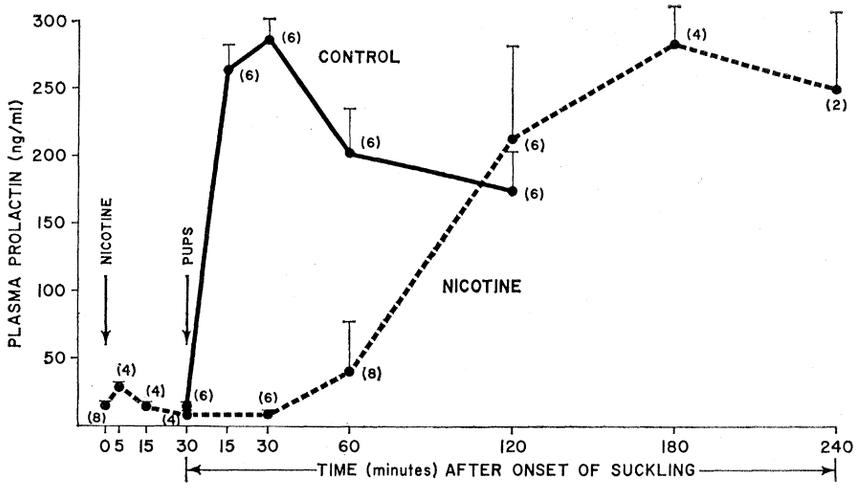

Fig. 1. Effects of nicotine on circulating prolactin in suckling rats. Prolactin concentrations in nicotine-treated rats are represented by the dashed line and in control rats by the solid line. The vertical bars represent the standard errors of the mean, and the number of samples taken at each time period is shown in parentheses. No more than five samples were collected from each mother. Highly significant ($P < .01$) differences in plasma prolactin levels are observed between the two groups during the first hour of suckling.

induced rise but does not block the mechanism involved in the ether-induced rise in prolactin or the suckling-induced ejection of milk.

Virgin female Sprague-Dawley rats (220 ± 10 g) were maintained under standard conditions (*5*). Rats were mated and a chronic cannula was inserted into the right atrium of the heart 7 to 10 days after parturition. Two days after cannulation six-pup litters were separated from mothers for 8 hours before suckling was permitted or before the mother was exposed to ether fumes for 6 minutes. After 6 minutes of exposure the mother was removed from the ether jar for 45 minutes and then returned for an additional 6 minutes. Blood (0.5 ml) was withdrawn via the cannula before and at various times during experimentation from rats apparently unaware they were being bled. Unless four to six pups were actively suckling within 2 minutes of pup return, blood was not withdrawn. Blood samples were centrifuged to collect plasma for radioimmunoassay of prolactin (*6*). In similar experiments in noncannulated mothers, the pups were killed at 10, 30, or 60 minutes after suckling, and their stomachs were examined for milk content. In all experiments a total of 2 mg of nicotine tartrate (0.65 mg of nicotine) was administered to the mothers in two subcutaneous injections 5 minutes apart. The second injection preceded the return of the pups by 30 minutes or placement of the mother in ether by 10 minutes.

Suckling caused a rapid increase in circulating prolactin (Fig. 1), with near maximal levels of discharge at 15 minutes after suckling, a finding in agreement with earlier reports (*4*). Nicotine itself caused a transient "stress-induced" rise in prolactin which returned to preinjection levels before the pups were returned. Except for one nicotine-treated mother which had elevated prolactin levels within 1 hour after suckling, a substantial increase was not seen until 2 hours after suckling or 2.5 hours after nicotine. Prolactin concentrations were high thereafter. Ether caused a rapid 2-fold increase in circulating prolactin (Fig. 2). The latency to onset of release was rapid, but maximal levels of discharge were small in comparison with the 17-fold increase found after suckling. Again, nicotine treatment itself caused a "stress-induced" rise in prolactin, but levels approached baseline values within 15 minutes. It can be clearly seen in Fig. 2 that nicotine was without effect on the ether-induced rise in prolactin. Prolactin levels in both groups returned to baseline within 45 minutes after ether treatment, and both groups were equally responsive to a second exposure to ether at that time.

It was previously reported in a more detailed study (*4*) that suckling and ether appear to activate different mech-

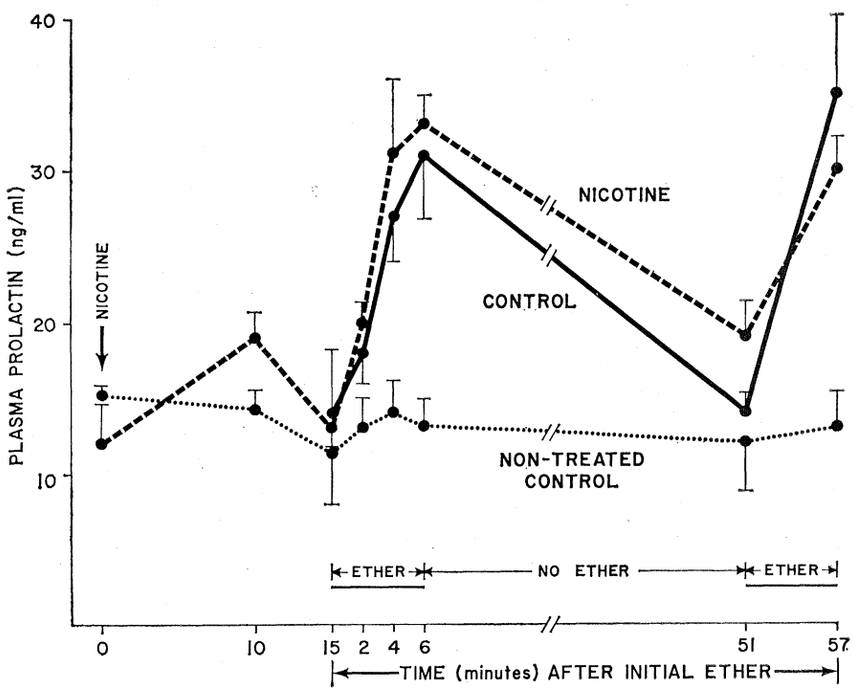

Fig. 2. Effects of ether and nicotine on circulating prolactin in suckling rats. Each point represents the mean of eight samples except for the points on the dotted line which represent the means of four mothers that were not given nicotine or exposed to ether.

anisms in releasing prolactin. It has been shown that suckling acts at the hypothalamic level by inhibiting prolactin inhibiting factor (PIF) activity (*7*). Our study supports the concept of two different mechanisms of prolactin control since nicotine blocks the suckling-induced release of prolactin, probably by stimulating PIF, but does not block the other mode of release.

Although nicotine was completely effective in inhibiting the reflex release of prolactin, oxytocin secretion was apparently not blocked since milk release was indicated by the presence of milk in the pups' stomachs after 10, 30, and 60 minutes of suckling from nicotine-treated mothers.

These data suggest that nicotine has the ability to cause severe malfunction in milk production by blocking prolactin output in lactating mothers even though the milk release mechanism is apparently unaltered by nicotine. With the knowledge that tobacco smoking can cause severe reproductive disorders in women (*1*) the present results cannot be ignored by lactating mothers who are heavy smokers. Although the smoker's consumption of nicotine would certainly be much less than our rat's intake on a per kilogram basis, species differences can make weight-basis comparisons meaningless.

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Subtle Consequences of Methylmercury Exposure: Behavioral Deviations in Offspring of Treated Mothers

Abstract. *Overt neurological impairment is the endpoint currently used to document a case of methylmercury poisoning. No consideration is given to possible subtle consequences. Offspring from mice exposed to methylmercury on day 7 or 9 of pregnancy were apparently unaffected during postnatal development. However, subtle behavioral differences between treated and control offspring were found when the overtly normal animals were tested in an open field and evaluated in a swimming apparatus at 1 month of age. Brain weight, protein, choline acetyltransferase, and cholinesterase were not significantly altered.*

Obvious neurological dysfunction is the only criterion used in officially recognizing a case a methylmercury poisoning and in setting standards for the Allowable Daily Intake (ADI) of methylmercury. No consideration is given to possible behavioral, biochemical, carcinogenic, or other subtle effects such as lowered intellectual capacity and premature senility (*1*, *2*). We think subtle alterations are important and should be considered.

Furthermore, attention should be directed to the fetus since the unborn organism is much more susceptible to the toxic effects of methylmercury than the adult. Methylmercury easily crosses the placental barrier and is concentrated in fetal tissues; it has a greater affinity for the embryonic central nervous system than for that of the adult (*1*, *3*, *4*).

Our studies with mice from mothers exposed to methylmercury show that, although offspring are overtly normal, they are significantly different from controls when tested in an open field and evaluated in a swimming apparatus. However, data from subsequent analysis of brain weight, protein, and acetylcholine enzyme activities revealed no significant difference between treated and control offspring. As a result of our research, we believe that screening for subtle behavioral deviations (from normal) in offspring from treated mothers may be one of the most sensitive indicators of the toxic effects of methylmercury.

On day 7 or 9 of pregnancy, primiparous 129/SvSl mice (approximately 4 months old) were weighed and each was given a single intraperitoneal injection of saline or 0.16 mg of methylmercury dicyandiamide (MMD) per 20 g of body weight (*5*). This dose [approximately ⅓ to ¼ the LD_{50} (*6*)] and these days of treatment were selected because, in a previous study to determine effects of MMD on prenatal development (*4*), all offspring born of similarly treated 129/SvSl females were apparently normal. Objectives of the present study were to determine if subtle behavioral, biochemical, or neurological deviations are detectable during postnatal development of these so-called normal offspring.

One day before term (day 18), pregnant mice were put in separate cages and allowed to deliver. Young remained with their biological mother until weaned at 21 days of age. Therefore, offspring were potentially exposed to methylmercury in utero and during nursing. There was no difference in average litter size (6.70) or in appearance and weight (1.2 g) of individual young. When 30 days old, each offspring was tested in the open field on 2 consecutive days (at approximately the same time each day) and then evaluated in a swimming apparatus. At 60 days the brains were removed and weighed, and protein, choline acetyltransferase, and cholinesterase determinations were made. All evaluations were done "blind."

Treated and control mothers were observed for possible differences in rearing (nursing, retrieving, grooming, defense) of young, open-field behavior, and swimming performance. No differences were found.

A daily session in the open field (*7*) consisted of first placing the experimentally naive animal in the center of the field under a light-tight chamber for 15 seconds. The chamber was then removed and eight behavioral parameters were recorded during the ensuing 2-minute interval.

Table 1 summarizes the results of open-field testing. A two-way least-squares analysis of variance (*8*) was used to examine the behavioral responses as a function of treatment and sex. There was a significant difference