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8. The colony was bioassayed periodically to monitor for changes in susceptibility. The bioassay procedure was similar to that used on the original colonies except that three replicate bioassays were done on each generation tested, and eventually the upper dose was raised to 2000 mg/kg. Data from the three replicates were pooled for calculating dose-mortality regressions.
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Androgens and Prenatal Alcohol Exposure

McGivern *et al.* (*1*) reported that altered nonreproductive, sexually dimorphic behavior is associated with exposure of rats to ethanol during development. They concluded that the results were evidence of a direct effect of ethanol on testicular or adrenal function. There have been many studies of monoamine control, direct and indirect, of neuroendocrine function (for example, inhibition of prolactin release by dopamine) which, in turn, may influence sexually dimorphic behaviors. In 1974 I reported that nonreproductive, sexually dimorphic behaviors could be altered by treating pregnant rats early during gestation with drugs that affect the synthesis or storage of such monoamine transmitters (*2*), so I am not questioning the observations of McGivern *et al.* (*1*). However, there are alternative interpretations of their results.

Caution should be used when data from other laboratories, based on studies with different experimental designs, are used to support one's own experimental design decisions or interpretation of results. For example, if other laboratories had studied the same behavioral variables and reported that cross-fostering has no effect on that measure of behavioral teratogenicity in fetal alcohol-exposed (FAE) animals, then McGivern *et al.* could use such studies to defend their decision not to remove the pups from their biological mothers at term. The early experience literature supports the possibility that residual ethanol effects (more likely withdrawal) in the dams may have affected the outcome of the experiment. Furthermore, neonatal withdrawal might also have acted alone or in concert with altered maternal behavior to contribute to or be responsible for the outcome. It would have been preferable to cross-foster half of each litter to pair-fed dams and the other half to ethanol-fed dams to clarify this issue. While perinatal birth weights are not given for experiment 1, McGivern *et al.* report a significant reduction in body weight of day-old ethanol-exposed males and females, weights being not significantly different from controls at 35 or 90 days of age in experiment 2. Even if greater malnutrition did not occur on succeeding days, when maternal or neonatal withdrawal might have been more severe, the fact that a significant weight differential existed at this early stage is problematic.

The suggestion that adrenal steroids may be responsible for at least the masculinization of adult FAE females, because others have reported increased brain and plasma levels of corticosterone in 1-day-old pups after prenatal alcohol exposure, begs the question related to the contribution of perinatal withdrawal and malnutrition and its associated stresses. We have recently reported such a potential source of epiphenomena in studies of the effects of opiates during development (*3*). We used opiate-naive subjects, rendered neonatally undernourished to an extent essentially identical to some and less severe than others exposed to opiates during development (*4*). These subjects showed significant differences in basal body temperature and in hyperthermic and behavioral responses to morphine administration later in life compared to fully nourished littermates. Similar results have been offered by others as evidence of a direct perinatal opiate effect in mature subjects whose body weights were even more severely reduced at the time of testing. While it may be necessary to include an isocaloric pair-fed group as a control, it is not a sufficient control if significant body weight differences of offspring emerged during the perinatal period, unless the data are interpreted within a framework that includes the concept that exposure to ethanol prenatally invariably leads to undernutrition or runting in offspring and that this effect is part of the fetal alcohol syndrome. Thus it may be premature to attribute to testosterone the effects observed by McGivern *et al.* until there is evidence that identical behavioral outcomes can be obtained in subjects with testicular hormone production suppressed in some other manner.

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Our work demonstrating long-term alterations in saccharin preference and maze performance of animals exposed to alcohol in utero was based on the hypothesis that an alcohol-induced inhibition of fetal testicular steroidogenesis would feminize adult male behaviors that are organizationally dependent on perinatal androgen concentrations (*1*). Both saccharin preference and maze behavior are such behaviors. Moreover, alcohol is well known to inhibit testicular steroidogenesis in adult rats and humans (*2*). Our results revealed a clear feminization of both behaviors in adult fetal alcohol exposed (FAE) males. Our pair-fed control dams received the same number of calories during pregnancy as the alcohol-fed dams, but no alcohol. Offspring from these control dams showed the normal sex differences reported by numerous investigators over the past 20 years [see

(*3*) for a review], indicating that nutrition was not a primary factor in the reversal of normal sex differences of adult FAE animals that we reported. Unexpectedly, we also observed a masculinization of FAE females. We suggested that our findings in FAE males were consistent with an interpretation of an alcohol-induced inhibition of fetal testicular androgen production during the critical perinatal period for neurobehavioral sexual differentiation. Since alcohol is also a potent activator of the adrenal gland, which produces several steroids with weak androgenic properties, we suggested that such an action during pregnancy might be the source of the long-term masculinization of FAE females. This interpretation, which is consistent with many data on the organizational effects of androgens during the perinatal period in animals and humans (*4*), was not, however, our conclusion, nor was it stated as such.

We found no data in referreed journals on the influence of neurotransmitter storage or synthesis during perinatal development on nonreproductive sexually dimorphic adult behaviors. We therefore chose not to speculate in our report on the possible contribution of such influences to our results. However, we agree with Sparber that such a contribution may be present, especially in view of recent work demonstrating long-term depletions in norepinephrine concentrations in the hypothalamus of FAE animals (*5*).

Prenatal alcohol exposure consistently produces lower birth weights in animals and humans. This decrease is positively correlated with the amount of alcohol consumed by the mother during pregnancy (*6*). The fact that we observed a decrease in the birth weights of FAE animals compared to pair-fed controls indicates that nutritional status alone cannot account for our results. Using an experimental design that equates the nutritional intake of alcohol-exposed and control animals, Randall *et al.* (*7*) showed that restricted caloric intake cannot account for the teratogenic effects observed. Although nutritional factors can interact with alcohol exposure to produce higher blood alcohol levels (*8*), it is clear that the alcohol in the body, rather than nutritional factors, is the teratogen during pregnancy. In the rat, catch-up growth is often observed (*9*) following exposure to the dose of alcohol we used. We have observed that this catch-up growth is evident by postnatal day 8 in FAE animals raised with their biological dams. Since we used a nutritional control, the relevance of the effects of malnutrition found by Sparber and Lichtblau (*10*) seems tangential to our results until these authors demonstrate a long-term influence on behaviors that are organizationally dependent on circulating androgen levels during the perinatal critical period.

The role of withdrawal in the long-term effects of prenatal alcohol exposure is not clear. Dams reduce their consumption of the ethanol diet by 70 to 100 percent between 24 and 48 hours before parturition, and blood alcohol levels in the fetus and the dam are undetectable at parturition (*9*). Therefore, the occurrence of maternal or fetal withdrawal would be expected to be precipitated prior to parturition, and thus may be difficult to bring under experimental control. We have not observed any influence of prenatal ethanol exposure on maternal lactation, as evidenced by the amount of milk present in the stomachs of the offspring 24 to 48 hours postnatally compared to pair-fed controls.

To what extent cross-fostering procedures should be used is open to question. Previous work has shown no influence of fostering or cross-fostering procedures on maze learning or avoidance conditioning of adult FAE animals (*11*). Most investigators of the long-term behavioral influences of prenatal alcohol exposure are attempting to find an animal model that mimics the human condition. Perhaps for similar reasons, in his preliminary study of postpuberal effects on female open-field behavior from prenatal α-methyl-*p*-tyrosine treatment, Sparber also did not include a cross-fostering procedure (*12*).

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