Sexual Characteristics of Adult Female Mice are Correlated with their Blood Testosterone Levels during Prenatal Development

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leitic (oceanic) basalt at the base of the crust (11). A schematic northwest-south- 
east cross section taken along profile AA’ is shown in Fig. 1. Also shown are veloci-
ties variations in the upper mantle
east cross section taken along profile
3. Lonselle, thesis, Massachusetts Institute of
12. G. W. Putman and J. W. Sullivan, Geology 7,
14. We benefited from discussions with B. C.
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Sexual Characteristics of Adult Female Mice Are Correlated with
Their Blood Testosterone Levels During Prenatal Development

Abstract. Mice produce litters containing many pups, and the female fetuses that
develop between male fetuses have significantly higher concentrations of the male
sex steroid testosterone in both their blood and amniotic fluid than do females that
develop between other female fetuses. These two types of females differ during later
life in many sexually related characteristics. Thus, individual variation in sexual
characteristics of adult female mice may be traceable to differential exposure to
testosterone during prenatal development because of intrauterine proximity to male
fetuses.

Differentiation of mammalian fetuses
into the masculine phenotype depends
primarily on the secretion of androgens
from the testes. The female phenotype is
thought to occur if the fetus remains rela-
tively free from the effects of androgens
during the time of sexual differentiation (1).
Inherent in this traditional concept of the
“normal” development of a mamma-
lian female is the assumption that fe-
male exposed to androgens during fetal
development may be abnormal. Indeed, exper-
iments in which female fetuses are ex-
posed to increased concentrations of
androgens, either by way of administra-
tion of hormones to the mother or be-
cause of a metabolic error that results in
an increased production of adrenal
androgens by the fetus, are often cited as
evidence supporting this assumption (2).
Recent studies with rodents, which
produce litters containing many pups,
have shown that in both mice (3) and
rats (4) there is considerable variability
among adult females in terms of repro-
ductive characteristics, and that part of
this variability can be traced to the
former intrauterine proximity of females
to male fetuses during prenatal develop-
ment. For example, female mice that de-
veloped in utero between two male fetus-
es (referred to as 2M females) were
found to differ morphologically, physio-
logically, and behaviorally from females
that did not develop next to a male fetus
(0M females) (3). When these two types of
females were compared, 2M females
had larger anogenital spaces at birth and
in adulthood, were more aggressive in a
variety of test situations, marked their
environment with urine at a higher rate,
and had longer and more irregular adult
estrous cycles than 0M females. The 0M
females appeared to be more attractive
and sexually arousing to males. Prior in-
trauterine position was also found to in-
teract with housing density in terms of
the time of onset of puberty in female
mice (3). These findings suggest that in
species that produce litters containing
many pups, the reproductive character-
istics of females may vary depending on
their intrauterine proximity to male fe-
tuses, and that such variation is normal
in polytocous animals.

Fig. 1. Concentrations of testosterone, pro-
gesterone, and 17β-estradiol in the serum and
amniotic fluid of 17-day-old 0M and 2M fe-
tale fetuses. Hormones in blood serum are
expressed as nanograms per milliliter of
serum; amniotic fluid values are expressed as
picograms of steroid extracted from the
amniotic fluid of each fetus.
It has been proposed (5) that the course of development of female fetuses that are contiguous to males in utero is altered by exposure of these females to increased concentrations of androgens, particularly testosterone. Presumably, androgens produced by male fetuses diffuse across the fetal membranes separating individual fetuses and into the amniotic fluid and blood of contiguous female fetuses. We designed the experiments described herein to investigate whether the differences in the reproductive characteristics of OM and 2M females were related to differences in steroid hormone concentrations during fetal or adult life. Both blood and amniotic fluid were collected from OM and 2M female fetuses and assayed for the presence of the sex steroids testosterone, 17β-estradiol, and progesterone. Other OM and 2M females were raised to adulthood at which time the concentrations of testosterone in their blood and their attractiveness to males were compared.

We found that the 2M female fetuses had significantly higher concentrations of testosterone both in their blood and in their amniotic fluid than did the OM female fetuses; adult OM and 2M females did not differ in their blood testosterone concentrations, but adult OM females were significantly more attractive to male mice than were 2M females.

One group of timed-mated CF-1 female mice was killed by decapitation on day 17 of pregnancy (6), and their blood was collected for later hormone analyses. The pups were then removed from the uterine horns without rupturing the fetal membranes surrounding each individual fetus so that the amniotic fluid could be collected (7). The sex of each fetus, determined initially by examining the length of the anogenital space, was subsequently confirmed by autopsy. We collected blood and amniotic fluid from 125 OM and 125 2M female fetuses, and blood from 125 male fetuses. In each experiment we pooled 25 samples to obtain five replicates that we then subdivided into separate chambers of a test apparatus so that a male could jump from a platform into either the chamber containing a 2M female or the chamber containing a 0M female. Of the 24 adult males tested, 19 chose a 0M female (χ2; P < .01), thus replicating our previous finding (5).

The results presented here indicate that male fetuses have three times more circulating testosterone than female fetuses, and that 2M female fetuses have significantly higher concentrations of testosterone in both their blood and amniotic fluid than OM females. Concentrations of testosterone in the mothers’ circulation did not appear to account for these differences. In adulthood, 0M and 2M females differed markedly in their attractiveness to males but not in their blood levels of testosterone. Taken together, these results support the hypothesis that the normal variation observed in some sexual characteristics of female mice is in part traceable to differential exposure to testosterone during prenatal

![Figure 2](image-url)
life, which in turn is due to intrauterine proximity to male fetuses. In both mice and rats there is evidence that exposure to high concentrations of testosterone shortly after birth can result in the complete loss of both estrous cyclicity and the capacity to ovulate (10). This period of maximum neural sensitivity to the defeminizing action of testosterone occurs after female pups have been removed from the influence of proximal male fetuses. Exposure of 2M females to higher concentrations of testosterone than OM females in utero does not influence their capacity to ovulate, exhibit female sex behavior, or produce and raise normal offspring in an optimum laboratory environment (3). But, variation in numerous characteristics that could influence reproductive success is related to prior intrauterine position. We propose that under certain ecological conditions females with a particular set of characteristics might be more likely to reproduce than other females. For example, 2M females might have a reproductive advantage over OM females when population density is high, because they are highly aggressive toward other females but not toward males, they fiercely defend their young while lactating, and they enter puberty sooner than OM females when housed in groups. In contrast, OM females may be more likely to reproduce than 2M females when population density is low, because OM females are highly preferred by males and enter puberty sooner and have shorter estrous cycles than 2M females when housed individually. Thus, it appears that OM females are neither more nor less “normal” than 2M females, since intrauterine proximity to male fetuses does not influence a female’s basic capacity to reproduce.

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References and Notes


8. During day 17 of gestation numerous tissues, whose course of development is influenced by androgens, are defeminizing. For instance, the rate of elongation of the perineal tissue in males as well as 2M females is at a maximum at this time (F. vom Saal, unpublished observation; R. Rugh, The Mouse (Burgess, Minneapolis, 1966)). Within a single uterine horn, the percentages of female fetuses that develop to 2.1 or 0 male fetuses are 25, 50, and 25 percent, respectively (F. vom Saal, unpublished observation), indicating that positioning of fetuses by sex occurs at random.

9. The amniotic fluid from individual fetuses, collected on filter paper (No. 2) strips, was placed in 10 ml of acetone and incubated at 40°C for 1 hour with periodic agitation. The filter paper was removed, and the extract was dried under nitrogen. Two milliliters of sterile water was added to each sample, which was then frozen. Subsequently, 25 samples were combined prior to extraction of steroids.

10. Blood plasma was collected in 50-ml heparinized pipettes from individual decapitated fetuses. Blood collected from 25 fetuses from the same group was added to a single test tube and, after centrifugation, the serum was frozen. Blood serum and amniotic fluid were extracted with 10 volumes of a mixture of benzene and hexane (2:1) after the addition of appropriate H-labeled steroids to monitor losses incurred during extraction and isolation. The extracts were evaporated and quantitatively transferred to a chromatographic column packed with Sephadex LH-20 (J by 60 mm). Steroids (progesterone, testosterone, and 17β-estradiol) were eluted with cyclohexane, toluene, and methanol at a flow rate of 0.5 ml/min. Fractions were measured by procedures previously validated for rat blood. Elution density was 20 (17β-estradiol and progesterone: F. H. Bronson and C. Desjardins, Endocrinology 94, 1658 (1974); and testosterone: F. H. Bronson and C. Desjardins, ibid., 101, 993 (1977)).

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Monte Carlo Simulation of Water Behavior Around the Dipeptide N-Acetylalanyl-N'-Methylamide

Abstract. Applications of Monte Carlo and molecular dynamics computer simulations indicate that they are potentially powerful tools for understanding biological systems at the molecular level. The Monte Carlo technique can be used to study the solvent structure around a small peptide and the effect of the aqueous environment on the conformational equilibria of the peptide.

That solvent plays a crucial role in determining the structure and function of biological molecules is now dogmatic. The study and elucidation of solvent in biological systems is, however, far from trivial and lags far behind structural studies of the corresponding biomolecules themselves (1, 2). We now show how the Monte Carlo technique, a powerful computer simulation method long used in the field of statistical physics of liquids, may be used to provide insight into the effect of solvent on molecular structure and conformational equilibria. For this purpose, we have applied the Monte Carlo method to the study of the water structure around the dipeptide analog N-acetylalanyl-N'-methylamide.

The dipeptide unit is the fundamental architectural unit of peptides and proteins. Because of this position, the conformation in vacuo of N-acetylalanyl-N'-methylamide has been extensively studied by theoretical methods (3). In most cases, the effect of the solvent has either been neglected or included simply by introducing a dielectric constant. A few calculations have approximated the effect of solvent with the use of such models as the hydration shell (4), the “supermolecule model” (5, 6) or the continuum reaction field (7), but none of these takes into account both the configurational fluctuations and individual molecular interactions characteristic of solvent (8–13).

The structure of water was studied around N-acetylalanyl-N'-methylamide fixed in both the aH (φ = 60, ψ = 50), and C3 (φ = -90, ψ = +90) conformations. Here we report mainly the results of the former simulation. The dipeptide was placed in the center of a 21.73-Å cubic unit cell and 338 water molecules were packed around it to simulate infinitely dilute solution conditions. The calculated density of this system is 1.001 g ml⁻¹ (for comparison, the experimental density was 1.004 g ml⁻¹) (14). The usual periodic boundary conditions (15) were used to avoid solvent-vacuum edge effects. Interactions between water molecules more than 6.2 Å apart and interactions between water molecules and the dipeptide more than 14.3 Å apart were neglected. The simulation is effectively at infinite dilution, since 14.3 Å was chosen such that no dipeptide-dipeptide interactions are included. Two simulations were carried out for each conformation in order to study the potential dependence of the results. In the first, the water-water interactions were calculated with the Rowlinson (16) po-