leiitic (oceanic) basalt at the base of the crust (*11*). A schematic northwest-southeast cross section taken along profile *AA'* is shown in Fig. 1. Also shown are velocity variations in the upper mantle derived from teleseismic P-wave residuals (*4*), which indicate that the upper mantle is characterized by slightly lower velocities beneath the Appalachians. The time-term analysis suggests that the Appalachians have a slightly thicker crust than the Grenville and can be divided into two well-defined layers. The upper 15-km layer probably corresponds to rocks that have been subjected to a high degree of compression and crustal shortening during the Taconic and Acadian orogenies. The rocks of the Appalachian belt probably were associated with a cycle of oceanic opening and closure. It is therefore possible that the chemistry of the lower crust was strongly affected by tectonic interaction of these sediments with the underlying basement during orogeny. Thus, interaction of ocean floor within the Appalachians would account for the higher velocities found in the lower crust relative to the predominantly ensialic crust of the Grenville Province.

The homogeneous character of the crust in this portion of the Grenville Province is consistent with the hypothesis that the crust underwent substantial reactivation, thickened, and became vertically uniform during the Grenville orogeny (*12*). After the thickening, the crust was eroded to relatively deep levels, as evidenced by the surface exposure of granulite terrains (*12*).

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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 relatively free from the effects of androgens during the time of sexual differentiation (*1*). Inherent in this traditional concept of the "normal" development of a mammalian female is the assumption that females exposed to androgens during fetal life may be abnormal. Indeed, experiments in which female fetuses are exposed to increased concentrations of androgens, either by way of administration of hormones to the mother or because 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*).

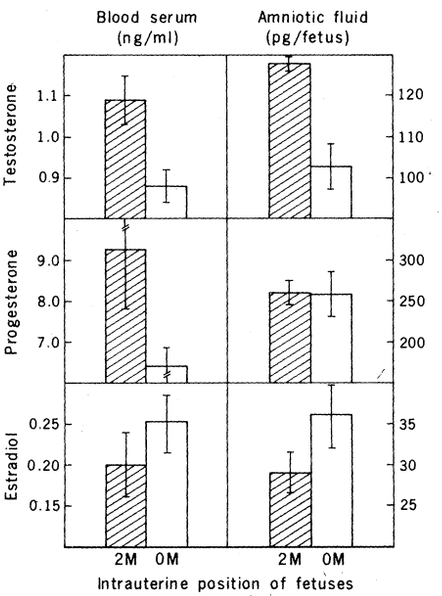

Fig. 1. Concentrations of testosterone, progesterone, and 17β-estradiol in the serum and amniotic fluid of 17-day-old 0M and 2M female fetuses. Hormones in blood serum are expressed as nanograms per milliliter of serum; amniotic fluid values are expressed as picograms of steroid extracted from the amniotic fluid of each fetus.

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 reproductive characteristics, and that part of this variability can be traced to the former intrauterine proximity of females to male fetuses during prenatal development. For example, female mice that developed in utero between two male fetuses (referred to as 2M females) were found to differ morphologically, physiologically, 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 intrauterine position was also found to interact 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 characteristics of females may vary depending on their intrauterine proximity to male fetuses, and that such variation is normal in polytocous animals.

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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 0M and 2M females were related to differences in steroid hormone concentrations during fetal or adult life. Both blood and amniotic fluid were collected from 0M and 2M female fetuses and assayed for the presence of the sex steroids testosterone, 17β-estradiol, and progesterone. Other 0M 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 0M female fetuses; adult 0M and 2M females did not differ in their blood testosterone concentrations, but adult 0M 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 0M 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 for radioimmunoassay of testosterone, 17β-estradiol, and progesterone (*8*).

Blood testosterone concentrations differed significantly between male and female fetuses (mean ± standard error: males, 3.00 ± 0.14 ng/ml; females, 0.98 ± 0.05 ng/ml; $P < .001$, t-test). We found no sex differences in either blood 17β-estradiol (males, 0.18 ± 0.03 ng/ml; females, 0.23 ± 0.05 ng/ml; $P > .05$) or progesterone (males, 6.2 ± 0.3 ng/ml; females, 7.8 ± 0.9 ng/ml; $P > .05$).

The results of comparing hormone concentrations in the blood and amniotic fluid of 0M and 2M females are presented in Fig. 1 and reveal that only testosterone levels varied as a function of intrauterine position. The 2M females had significantly elevated levels of testosterone in both their blood ($P < .05$) and amniotic fluid ($P < .01$) relative to 0M females. Neither 17β-estradiol nor progesterone levels differed significantly ($P > .05$). Such differences could have been due to the fact that litters containing 2M females generally contain more male pups than do litters containing 0M females. Litters containing many males conceivably could increase the concentrations of testosterone in the mother's circulation and, in turn, increase blood concentrations of this hormone in the entire litter. To test for this possibility, we measured the sex steroids in blood collected from mothers that were killed on day 17 of pregnancy and were carrying either nine male and three female fetuses or three male and nine female fetuses (ten mothers per group). Since we found no differences in the serum testosterone, 17β-estradiol, or progesterone concentrations between the two groups of mothers (Fig. 2), the difference in testosterone levels between 0M and 2M female fetuses appears not to be mediated by the maternal circulation.

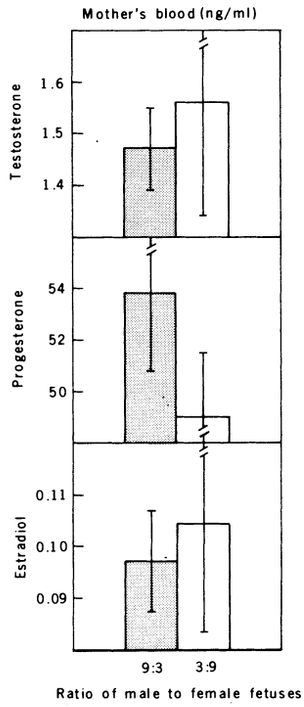

Fig. 2. Concentrations (nanograms per milliliter) of testosterone, progesterone, and 17β-estradiol in the serum of mothers killed on day 17 of pregnancy and carrying nine male and three female versus three male and nine female fetuses.

From a second group of randomly chosen, timed-mated females we obtained by cesarean section the 0M and 2M offspring on day 19 of pregnancy just prior to parturition. The pups were fostered to mothers that had just delivered naturally. These 0M and 2M females were raised to adulthood at which time we compared their serum testosterone levels and their relative attractiveness to male mice.

Ten 0M and ten 2M females in diestrus (as indicated by a vaginal smear and uterine weight subsequent to death) were killed when 120 days old, and blood levels of testosterone were determined. We found no significant differences in the serum concentrations of testosterone between these 0M and 2M females (mean ± standard error: 0M females, 299 ± 87 pg/ml; 2M females, 228 ±73 pg/ml). Thus, behavioral differences between 0M and 2M females cannot be attributed to differential exposure to endogenously produced testosterone in adulthood. However, this experiment cannot rule out the possibility that adult 0M and 2M females differ in their sensitivity to testosterone (*9*). However, in previous experiments in which both physiological and behavioral measures were used, there was no difference in sensitivity to estradiol between adult 0M and 2M females (*3*).

The remaining adult 0M and 2M females were tested for their relative attractiveness to male mice. This experiment was conducted as a control measure, since it was deemed important to replicate one of the tests on which 0M and 2M females previously had been found to differ significantly. Using a procedure described elsewhere (*3*), we enclosed diestrous 0M and 2M females in wire-mesh cages that were placed in 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 (χ^2; $P < .01$), thus replicating our previous finding (*3*).

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 0M 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

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 0M 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 0M females when population density is high, because they are highly aggressive toward other females but not toward males, they fiercely defend their young when lactating, and they enter puberty sooner than 0M females when housed in groups. In contrast, 0M females may be more likely to reproduce than 2M females when population density is low, because 0M 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 0M females are neither more nor less "normal" than are 2M females, since intrauterine proximity to male fetuses does not influence a female's basic capacity to reproduce.

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6. During day 17 of gestation numerous tissues, whose course of development is influenced by androgen, are differentiating. 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, 1968)]. Within a single uterine horn, the percentages of female fetuses that develop next 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.
7. 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.
8. Blood plasma was collected in 50-μl 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 ^{3}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 (9 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 mouse blood [17β-estradiol and progesterone: F. H. Bronson and C. Desjardins, *Endocrinology* **94**, 1658 (1974); and testosterone: F. H. Bronson and C. Desjardins, *ibid.* **101**, 939 (1977)].
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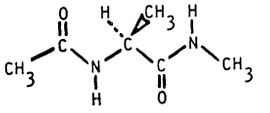
Monte Carlo Simulation of Water Behavior Around the Dipeptide *N*-Acetylalanyl-*N*-Methylamide

Abstract. *Applications of Monte Carlo and molecular dynamics computer simulation techniques 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 by 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 the solvent (*8*–*13*).

The structure of water was studied around *N*-acetylalanyl-*N'*-methylamide fixed in both the α_R ($\phi = -60$, $\psi = -50$), and C_7 ($\phi = -90$, $\psi = +90$) 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-

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