The Interaction of Circulating Oestrogens and Androgens in Regulating Mammalian Sexual Differentiation

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1. Introduction

In mammals males are the heterogametic sex, and the Y chromosome codes for differentiation of the gonads into testes. With the exception of the primary sexual characteristic, gonadal sex, all other differences between males and females are thought to be mediated hormonally via secretions from the testes. In the absence of gonads, mammalian foetuses develop into phenotypic females (Foote 1972). The ovaries of female foetuses differentiate significantly later in development than do the testes, and it appears that the ovaries of female foetuses are not teratogenic (cf., Gibori and Shlazar 1981). In mammalian species in which the period of gestation is relatively long, sexual differentiation is usually completed by birth. In short-gestation species, such as rats and mice, however, sexual differentiation commences during the last third of pregnancy and continues during the first week to 10 days of postnatal life. Previously, it was erroneously assumed that the differentiation of sexual behaviour in mice and rats began shortly after birth (Barradough and Leuchten 1954, Young et al. 1964). There is a critical coupling of the timing of the secretion of testosterone, the primary androgens secreted by the testes of foetuses and adults, and the development of both neural and peripheral androgen-target tissues. Extrinsic factors that interfere with this normal coupling (such as maternal stress or exposure to exogenous hormones) can radically alter the course of sexual differentiation.

Two processes have been identified as occurring during the normal development of behaviours that are influenced by sex steroids: masculinization, the development of male-typical behaviours, and determination, the loss of the potential to behave in a female-typical fashion (in this review complete physiological de masculinization will refer to both the loss of the capacity of specific brain areas to respond to rising oestrogen titers by triggering periodic surges in luteinizing hormone that result in ovulation, and the loss of the production of olfactory cues that stimulate mating behaviour in males). There is now considerable evidence that behavioural masculinization and behavioural feminization are independent processes that occur during different periods of sexual differentiation in some species (Whalen 1974, Baum 1979).

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2. The Intrauterine Position Phenomenon: Hormone-Mediated Variation in Phenotype

During the course of evolution, selection has resulted in a broad range of morphologi-
cal, physiological and behavioural traits being regulated by sex steroids during both
the period of sexual differentiation in early life and in adulthood. One of the
consequences of having hormones regulate sexual differentiation is that variation in
phenotype is guaranteed, regardless of the amount of genetic variation within a pop-
ulation. Both the environment of the mother and intrauterine environment of a foetus
are sources of variation in terms of the titre of hormones that a foetus is exposed
to in utero. The titres of hormones that foetuses are exposed are modulated by the
environment of the mother via the pituitary-adrenal axis of the mother. The
intrauterine positioning of female and male foetuses has also proven to be an impor-
tant variable which influences prenatal hormone titres and thus phenotype in both
rats and mice. The intrauterine position phenomenon refers to the fact that during
foetal life, foetuses are positioned randomly next to other foetuses of the same or
opposite sex. Male and female foetuses score different titres of steroid hormones
during the prenatal period of sexual differentiation in mice as well as every other
mammal that has been studied (cf., von Saal and Breden 1989a). Steroids secreted
by one foetus are somehow transmitted to contiguous foetuses, with the result that
variation among males and among females in a breed range of characteristics is due
to an individual's intrauterine proximity to foetuses of the same or opposite sex.

The mechanisms by which hormonal communication between foetuses occurs is
unknown. The report by Meisel and Ward (1981) that the uterine vasculature might
be involved in transmitting hormones between foetuses was based on an erroneous
interpretation of an anatomical study (cf., De Campo and Guttler 1972), which did
not assess the size of the direction of uterine blood flow in the rat as Meisel and
Ward stated. Uterine blood flow in the rat and mouse is actually quite complex:
both arterial and venous flow are bidirectional and in opposite directions (blood
enters the uterine loop artery leading each separate uterine horn cranially from the
descending aorta and caudally from the internal iliac; blood leaves the placenta of
foetuses located near the cervix and flows caudally in the uterine vein, which drains
into the iliac vein, while blood from the placenta of foetuses located near the ovary
flows cranially in the uterine vein, which leads into the ascending vena). The anat-
omical proximity of the uterine loop arteries and veins in rats and mice, the fact
that at virtually all points blood flow in a uterine loop artery and vein is in an oppo-
site direction (McLaren and Machle 1960, von Saal, unpub.), is interesting in that it
is suggestive of a counter-current exchange system.

The mechanism by which steroids are transmitted between foetuses could involve
passage of steroids through the amniotic fluid, lymphatic system in the uterus, or
maternal vasculature. In the studies that will be described in this paper, the scheme
that is utilized to designate the intrauterine position of animals of both sexes involves
utilizing male foetuses as the reference for labelling foetuses: 2M = between two
males, 1M = between a male and a female, and OM = not next to a male (between two
females; see Fig. 1). The use of male foetuses as the reference occurred at a time
when the intrauterine position phenomenon was assumed to be mediated solely by
secreted from male foetuses rather than also by secretions from female foetuses. This assumption is now thought to be incorrect. The continued use of the 2M, 1M, 0M scheme thus does not imply that proximity to females is unimportant.

To obtain male and female foetuses from known intrauterine positions, female mice are time-mated. The young are removed from the uterus by Caesarean delivery shortly before parturition on Day 19 of pregnancy. The young are then raised in foster-litter groups by mothers that had delivered a litter within the preceding 24 h (Fig. 1).

2.1 The Intrauterine Position Phenomenon in Female Mice and Rats

The testes of male mice differentiate and begin secreting testosterone during early foetal life (about day 13 of gestation; Block et al. 1971), thus marking the onset of sexual differentiation. Male CF-1 mouse foetuses (Mus musculus) have about three times more testosterone circulating in their blood than do female foetuses on Day 17 of gestation, which is during the time that sexual differentiation is occurring (von Seil and Bronson 1980b). In addition, the intrauterine positioning of female foetuses...
relative to males influence testosterone titers in female foetuses: females that develop between 2 male foetuses (2M females) have higher amniotic fluid and blood titers of testosterone than do female fetuses that do not develop next to a male (OM females; see Fig. 2). Females located in utero between a male and a female fetus (1M females), the third possible intruterine position, have not been examined for foetal hormone titres, but after birth 1M females are intermediate in phenotype between OM and 2M females. OM female foetuses also tend to have higher amniotic fluid titers of oestradiol than 2M female foetuses, although the difference was not statistically significant (von Saal and Bronson 1980a). Intruterine position thus results in a complex pattern of hormonal differences in female foetuses during the prenatal period of sexual differentiation of both the brain and peripheral organs in mice. It is presumed that differences in hormone titers between OM and 2M females occur during prenatal but not early postnatal life due to the transmission of steroids from one foetus to another. By adulthood, OM and 2M female mice do not differ in their blood titers of testosterone during the diestrus phase of the oestrous cycle (von Saal and Bronson 1980a). It is likely, however, that OM and 2M females will be found to differ in the pattern of gonadal and pituitary hormones secreted during different stages of the oestrous cycle, since OM and 2M females differ in the length of their oestrous cycles (von Saal and Bronson 1980a).

Numerous comparisons of female mice and rats from different intruterine positions have been conducted. By way of a brief summary, OM, 1M and 2M female mice have been found to differ: (1) in their morphology—the space separating the anus and genital papilla at birth is longest in 2M females and shortest in OM females (von Saal and Bronson 1978), which has been interpreted as indicating that 2M females are masculinized due to their proximity to male foetuses (this measure is a sensitive index for prenatal testosterone exposure in rodents); (2) in their physiology—OM, 1M and 2M females differ in the timing of puberty (von Saal and Bronson 1978, von Saal 1981, von Saal et al. 1981) and in the length of subsequent oestrous cycles (2M females have longer and more irregular oestrous cycles than OM females; von Saal and Bronson 1980b); OM females are also more sexually attractive and sexually arousing to males than are 2M females (males choose OM females over 2M females, males exhibit more mounts and intromissions toward OM than toward 2M females, and males ejaculate into OM females prior to ejaculating into 2M females when given a choice; von Saal and Bronson 1978, 1980a, von Saal et al. 1982); 2M females also cease producing live offspring at a younger age than do OM females when they are allowed to repeatedly mate and produce litters (von Saal et al. 1982).

Intruterine position effects have also been found in studies involving Sprague Dawley rats: 2M females have larger genital organs at birth than do OM females (Clemens et al. 1976). This difference is eliminated by treating pregnant females with antiandrogens in both rats (Clemens et al. 1979) and mice (von Saal 1978). 2M female rats also enter puberty (first vaginal oestrus) significantly later and have longer adult oestrous cycles (5.7 ± 0.3 days) than do OM female rats (4.7 ± 0.1 days; p < 0.01; unpubl.). Most of these studies involving comparisons of OM and 2M females have been previously reviewed (von Saal 1981).

The emphasis of this section will be on behavioural comparisons of OM and 2M female mice. Studies that will be described will thus involve comparisons of animals
from these two extreme intraterine positions. OM and 2M females were compared for their aggressiveness toward a female opponent. Intact OM and 2M female mice (in the dioestrus phase of the oestrous cycle) were paired for 30 min after being matched for age and weight. Aggression was observed in 20 of 26 pairs, and in 17 cases, the 2M female was the aggressor. 2M females are thus significantly more aggressive toward another female than are OM females. In contrast, neither OM or 2M females are aggressive toward male mice. Aggression between females appears to result in the establishment of dominance hierarchies among females, and dominance status has been observed to correlate with reproductive success in female mice (Lloyd and Christian 1969) as well as male mice (DePries and McLeam 1970).

Fig. 2. A Mean (± SEM) blood titres of testosterone (expressed as nanograms/millilitre of serum) for male (10 pups; 21 fetuses/pups) and female (OM and 2M female 5 pups; 25 fetuses/pups) mouse fetuses on Day 17 of gestation. B mean (± SEM) lutenizing hormone (number of luteins x 100) for OM and 2M female mice (10/group). All females were ovariectomized when 99 days old and tested for their sexual receptivity when 150 days old. Forty-eight h before testing, the females were injected with 5 μg estradiol benzoate, and 4 h before testing with 200 μg progesterone. A OM and 2M female were then matched for age and weight and placed into the cage of a 1M stud male for 30 min. C The percent of OM and 2M female mice that exhibited aggression (tail-rattling, chasing, and biting) during a 30-min test. Twenty-eight matched pairs of OM and 2M females were observed for aggression when both females were in the dioestrous phase of the oestrous cycle. In 8 pairs no aggression was observed. Results are based on the 20 pairs in which aggression was observed. Significance levels are for A and B t-test and C Chi-square comparisons.
Aggression toward a female is not a masculine trait, and it cannot be concluded that 2M females are behaviorally masculinized relative to OM females based on this finding. Rather than label 2M females as masculinized, it is more appropriate to state that a significant component of the variance in interfemale aggression is feminine rather than masculine trait) is due to intrauterine position. 2M females have also been found to urine mark their environment at a higher rate than 40 OM females (von Saal and Brillmoen 1978). Rates of urine marking in male mice are correlated with dominance status (Denenberg et al. 1975), and urine marking also serves to delineate territorial boundaries (Harrington 1976).

The fact that OM and 2M female mice differ in the length of their estrous cycles makes studying this behavior in intact OM and 2M females very difficult. Thus, OM and 2M females (C57 group) were ovariectomized, and 2 months later they were treated with estradiol benzoate and progesterone to induce sexual receptivity (the frequency of lordosis responses when mounted by a male). When placed with a 1M male for 30 min, OM females had significantly higher lordosis quotients (number of lordoses/number of mounts \( \times 100 \)) than did the 2M females (see Fig. 2). It can thus be concluded that 2M females are feminized in terms of their sexual behavior, capacity to exhibit regular estrous cycles, and the production of sex-attracting pheromones (most likely pheromones) relative to OM females (von Saal et al. 1982). What is readily apparent from the data presented in Fig. 2 is that 2M females are more aggressive toward other females than are OM females, but 2M females exhibit a decrement in sexual performance relative to OM females. Intersex aggression and sexual performance are thus negatively correlated in female mice.

2.2 The Intrauterine Position Phenomenon in Male Mice and Rats

Based on the finding that 2M female mouse fetuses had higher amniotic fluid and blood titers of testosterone than did OM female fetuses, it was predicted that 2M male mouse fetuses (that develop in utero between two male fetuses) would also have higher amniotic fluid and blood titers of testosterone than OM male mouse fetuses (that develop in utero between two female fetuses). However, OM and 2M male mouse fetuses were not found to have significantly different amniotic fluid or blood titers of testosterone, at least on Day 17 of gestation (when male fetuses are secreting higher titers of testosterone). Instead, female mouse fetuses were found to have about twice the amniotic fluid titers of testosterone as did male fetuses on Day 17 of gestation, and OM males were found to have about 50% higher titers of estradiol in their amniotic fluid than did 2M male fetuses (von Saal et al. 1983; see Fig. 3). In humans, female fetuses also have high amniotic fluid and blood titers of estradiol relative to male fetuses (total blood and amniotic fluid concentrations of androgens are presumed to be in equilibrium; Forte and Vulchanov 1980).

The data presented in Fig. 3 reveal the same relationship of intrauterine position to aggressiveness and sexual performance in OM and 2M mice that was observed in comparison of OM and 2M female mice. 2M males appear to be more aggressive toward other males than are OM males. This behavior study consisted of placing a sexually receptive OM female into the cage of an initially intact
Fig. 3. A Mean (± SEM) anesthetic fluid titres of oestradiol (expressed as picograms/fetus) for female (5 litters; 25 fetuses/litter) and 0M and 2M male (5 litters; 25 fetuses/litter) mouse fetuses on Day 17 of gestation. B Mean (± SEM) number of mounts and intromissions (stimulating movements) during a 30-min test with a sexually receptive 1M female for gonadally intact, 90-day-old 0M and 2M male mice (20/group). C Percent of neonatally castrated, 90-day-old 0M and 2M male mice (20/group) that exhibited a 3:5 sustained biting attack toward a 1M male stimulator within 15 days after the 0M and 2M males were implanted with a silastic capsule containing testosterone. Ten-min tests for aggression were conducted every other day after replacement of testosterone. Significance levels are for A and B and C. Chi Square comparisons.

0M or 2M male (10 males/group) for 30 min and recording the number of mounts and intromissions by the males. 0M males exhibited significantly more mounts and intromissions than did 2M males (von Saal et al. 1983). In a similar experiment, gonadally intact, adult male Sprague-Dawley rats were tested to sexual satiety (30 min without a mount) when paired with a sexually receptive 1M female. The 0M males exhibited significantly more ejaculations to satiety (6.5 ± 0.37) than did the 2M males (3.1 ± 0.37) (unpublished observation). Thus, in both rats and mice, sexual performance is enhanced in 0M males relative to 2M males.

Intermale aggression was examined in 0M and 2M male mice that had been gonadectomized within 1 h of Caesarean delivery. The main objectives of this experiment were to: (1) eliminate possible differences between 0M and 2M males in the titres of gonadal steroids that they were exposed to during postnatal life by castrating the males at birth, and (2) to assess the sensitivity of the neural substrate mediating intermale aggression to the aggression-inducing action of a known amount of testosterone administered in adulthood via silastic capsules. When implanted with a silastic capsule
containing testosterone and tested every other day for 10 min for aggression against a 1M male mouse that had had its olfactory bulbs removed, significantly more 2M than 0M males exhibited a 5-s sustained attack within 16 days after implantation of testosterone (see Fig. 2). This finding was replicated with another group of 0M and 2M male mice, and in addition, after both 2 and 5 weeks of testosterone treatment, 2M males were found to have significantly heavier seminal vesicles (von Saal et al. 1983). Thus, 2M males are more sensitive to testosterone than 0M males, both in terms of the trophic action of testosterone on seminal vesicle growth and the induction of intermale aggression.

0M and 2M male mice that had been castrated at birth were also examined for the capacity to elicit mounting from a 1M stud male and to elicit the female sexually receptive posture (vulva-spread) when mounted during a 30-min test. The 0M and 2M males were treated with only estradiol benzoate or both estradiol benzoate and progesterone prior to being tested with a 1M male. Little mounting was observed toward either the 0M or 2M males that were only treated with estradiol benzoate, but significantly more 0M than 2M males that were treated with estradiol benzoate and progesterone elicited mounting by the 1M male (see Fig. 4). Too few 2M males elicited mounting to allow a comparison of the lordotic quotients of the 0M and 2M males (von Saal et al. 1983). These results reveal that 2M males are more determined than 0M males during foetal life, at least in terms of the loss of the cues involved in eliciting mounting by a stud male. This difference between 0M and 2M males appears to reflect a loss in the capacity of 0M males to respond to progesterone. As reviewed above, 2M female mice also elicit fewer mounts by stud males than do 0M females, indicating that 2M females are more determined than are 0M females.

Fig. 4. The percent of sexually castrated, 30-day-old 0M and 2M male mice that elicited mounting by a 1M stud male during a 30-min test. All 0M and 2M males received injections of estradiol benzoate 48 h before testing. The 0M and 2M males were then divided into two groups (15 males/treatment condition): 0 h before testing one-half of the males received an injection of progesterone, while the remaining males received an injection only of oil. The animals were administered hormones and tested at weekly intervals for 4 weeks. Data are for the percent of 0M and 2M males in each group that were mounted during both the third and fourth weeks of testing. Significance levels are for Chi Square comparisons (N.S. not statistically significant).
3. The Evolution of Variance Due to Intrauterine Position in Polytocous Mammals

I have proposed that prior intrauterine position influences individual reproductive success in both male and female mice (van Sool 1981). For example, 2M males and 2M females appear to be more aggressive than OM males and OM females. This enhanced aggressiveness should confer a reproductive advantage upon 2M males and 2M females when population density is high. OM females are more sexually attractive and arriving at males and at low population densities, OM females enter puberty earlier and have shorter and more regular oestrous cycles than 2M females. OM females thus have a phenotype that may confer a reproductive advantage upon them when population density is low. Whether OM male mice will be found to be more sexually attractive to females remains to be examined. Based on these observations, it is proposed that there is no absolute advantage or penalty to developing between male or female foetuses in utero. It is interesting in this regard that the traditional concept that in small mammals all fertile females are either pregnant or lactating (Croninow 1971) does not appear to be true. In fact, as described above, dominance status influences reproductive success in female mice, and when population densities are high, only a small proportion (10%–15%) of female mice may actually be able to reproduce (Lloyd and Christian 1969). Christian (1971) has related this finding to the disruptive effects of stress due to crowding on reproductive capacity in subordinate mice, since mice that live in high density populations exhibit signs of heightened adrenal activity.

House mice are opportunistic colonizers that are almost global in their distribution, mice having spread throughout the world as commensals of man (Bronson 1979). In addition, in mice as well as many other small mammals, population densities increase and decrease dramatically as a function of food availability and climatic conditions, and also other as yet unexplained factors that periodically lead to dramatic "crashes" in population size (Christian 1971). The reason that this point is raised is that it is proposed that the capacity of foetuses to communicate with each other hormonally evolved in response to positive selection pressure due to the adaptiveness of this phenomenon, not to the species (which would imply group selection), but to an individual pregnant female. The presence of variance in phenotype due to the intrauterine position phenomenon may increase the likelihood that at least some of the offspring produced by an individual female may have a phenotype that renders them highly competitive to reproduce in whatever environment that they
happen to be born into. Any factor, whether genetic or environmental, that increases variance in phenotype increases the capacity of some animals in a population to survive and eventually reproduce. In terms of the issue of genetic versus environmental influences on phenotype, it is important to emphasize that intraspecies positioning by sex is a random developmental event, and random events are highly predictable with large sample sizes. Males and females from particular intraspecies positions thus do not differ systematically in genotype (vom Saal 1981). In conclusion, the intraspecies position phenomenon may have evolved since it provides a predictable degree of variance in phenotype, regardless of the amount of underlying variance in genotype (the CF-1 mice utilized in the experiments described above are maintained as an outbred strain in a closed colony, but are presumed to have very little underlying genetic variation; the CF-1 strain is derived from a single brother-sister mating after 20 generations of inbreeding).

In contrast to the intraspecies position phenomenon, which appears to be adaptive, other clearly maladaptive developmental events are also found to occur. For example, the freemartin syndrome in cattle (a single-fetal species), which is most likely due to the formation of vascular anastomoses between contiguous fetuses and the sharing of tissue, results in both male and female offspring being chimeric and sterile in virtually all situations in which dizygotic male and female twins develop in utero together (Marcum 1974, vom Saal 1981). There appears to have been intense selection against this possibility in polytocous species. In house mice, for example, even in situations in which the placenta of contiguous fetuses are in contact (a rare event), a connective tissue band separates the placenta of individual fetuses, thus providing a barrier to the potential formation of vascular anastomoses between the chorionic membranes of fetuses of the opposite sex (McLaren and Michie 1959).

The argument that the intraspecies position phenomenon has evolved not simply as a correlate of the overwhelming need of a prey species to produce many young at once and thus to have multiple uterine residence, but due to the adaptiveness of this phenomenon, also suggests that intraspecies position differences may only be highly adaptive in colonizing species, such as house mice, in which the density of population is found to vary widely. In contrast, in litter-bearing species in which population size tends to be stable (K-selected species; Wilson 1975), variance in phenotype due to intraspecies position may not be found to occur.

4. The Interaction of Oestogens and Androgens in Mediating Differences in Phenotype Due to Intraspecies Position

While it has been known for some time that there are sex differences in fetal oestrogen titres in some species, foetuses have been assumed to be protected from circulating oestrogens by blood proteins that bind and thus inhibit oestrogens from entering cells (bound or conjugated steroids are presumed to be inactive while free steroids are presumed to be active). In mice and rats, foetuses have high circulating concentrations of alpha-fetoprotein, which binds oestrogens but not androgens with high capacity. Thus, the high concentrations of oestradiol that are present during the
latter stages of pregnancy were previously not believed to influence sexual differentiation (at least in mice and rats, since it is unclear whether circulating oestrogen-binding proteins occur in high concentrations during foetal life in other species; MacLusky and Naftolin 1981).

It has been proposed that in neurons in the brain that respond to testosterone, testosterone acts on the genome to influence neural development after being converted to oestradiol (i.e., aromatized) by aromatase enzymes. Oestradiol produced by intracellular aromatization of testosterone is presumed to be tranlocated into the cell nucleus by oestrogen receptors; oestrogen receptors have been found to be present in the brains of foetal mice and rats (Vito and Fox 1982). In other tissues such as the prostate, testosterone acts during foetal life to influence development by being reduced to dihydrotestosterone (Keech et al. 1977), which is then translocated into the nucleus by androgen receptors.

The proposition that oestradiol is actually the hormone that induces masculinization and feminization, thus making testosterone a prohormone, is referred to as the aromatization hypothesis (MacLusky and Naftolin 1981). Testosterone was thought to be needed as a prohormone due to the fact that circulating oestrogens would be inhibited from entering cells by alpha-foetoprotein. There is evidence from studies with rats that the development of male sex behavior involves the aromatization of testosteron to oestradiol. For example, aromatase inhibitors interfere with the development of male sex behavior (Gladic and Clennets 1980), while administration of oestrogens to neonatally castrated male rats can facilitate the development of male sex behavior (Booth 1977). The finding that OM males are exposed to higher titers of oestradiol than 2M males during foetal life, and in adulthood OM males are more sexually active than 2M males, is consistent with the aromatization hypothesis. Thus, in neural tissues mediating male copulatory behavior (mounting, intromitting and ejaculating), testosterone may serve as a prohormone that is aromatized to oestradiol prior to interacting with cytoplasmic oestrogen receptors and translocation into the cell nucleus. Within the nucleus, the oestrogen-receptor complex is thought to confer an organizing or sensitizing effect on the genome. The elevated titers of oestradiol observed in the amniotic fluid of OM male mice may lead to a greater intracellular pool of oestradiol and an increase in the number of activated oestrogen receptors that are translocated into the nucleus of target cells, thus producing an enhancement of sexual performance during adult life (see Fig. 5).

The finding that OM males are less sensitive to the effects of testosterone on intermale aggression and seminal vesicle growth suggests that exposure to high titers of oestradiol during foetal life interferes with the organizational effects of testosterone in the neural substrates mediating aggression and in the seminal vesicles. Oestradiol may bind to (and inhibit the translocation into the nucleus of) androgen receptors that are present in some hypothalamic neurons and in the seminal vesicles during prenatal life in both mice and rats. During foetal life (but not necessarily in adulthood) oestradiol may thus act as an antiandrogen in these tissues. Comparisons of OM and 2M males suggest that the neural areas mediating intermale aggression in mice are organized perinatally as a result of testosterone (or possibly its reduced metabolites, dihydrotestosterone e.g., Lieberburg and McIwenn 1980) interacting with cytoplasmic androgen receptors rather than as a result of testosterone being aromatized to oestradiol.
Fig. 5. Schematic diagram of the proposed interaction of oestrogen (E) circulating in the foetal blood stream, that is either free or bound to alpha-fetoprotein (AFP), with putative oestrogen receptors (ER) in the cytoplasm of cells in brain areas mediating intimate aggression or putative oestrogen receptors (AR) in brain areas mediating male copulatory behaviour. Brain areas mediating intimate aggression may contain only oestrogen receptors in foetuses, and high blood concentrations of oestrogen may thus act to block masculinization in these tissues by competitively binding to cytoplasmic oestrogen receptors. The testosterone (T)/androgen receptor complex is thought to regulate the functioning of specific genes in chromosomes (C) after translocation from the cytoplasm into the nucleus, thus leading to an increase in aggressiveness in adulthood. The occupation of an androgen receptor by oestrogen might inhibit translocation of the occupied androgen receptor into the nucleus. There is thus a correlation between high concentrations of oestradiol in 0M male foetuses and low aggressiveness in adulthood. In contrast, during prenatal life brain cells that mediate male copulatory behaviour are hypothesized to contain only oestrogen receptors and aromatase enzymes, which convert testosterone to oestradiol. The occupation of an oestrogen receptor by oestradiol might inhibit aromatase activity and thus a decrease in the number of oestrogen receptors that are translocated into the nuclei of these cells. This would then explain the enhanced sexual performance of 0M males relative to 2M males in both mice and rats.

(which would then interact with cytoplasmic oestrogen receptors). Thus, as circulating oestradiol titres increase in foetuses, there is a decrease in the response to testosterone in the neural areas mediating intimate aggression, which results in a decrease in the severity of these sequelae to the aggression-inducing action of testosterone during later life (see Fig. 5).

The obvious question that arises is how could oestradiol titres influence sexual differentiation if alpha-fetoprotein really does inactivate all circulating oestradiol during fetal life? One possible answer to this question may be that alpha-fetoprotein may actually pass into brain cells (possibly into selected cells due to active endocytosis mechanisms). The evidence for this is that alpha-fetoprotein has been found in brain cells in rats (McKean et al., 1975; Benno and Williams, 1978). Whether alpha-fetoprotein actually transports oestrogen from the circulation into brain cells is
unknown. But, alpha-fetoprotein does not appear to be synthesized in the brain (Schuhler and Toran-Allerand 1982).

It is important that the effects of steroids during early life (referred to as organizational effects, which are permanent) be distinguished from the effects of steroids during adult life (referred to as activational effects, which are usually transient). During postnatal life tissues may acquire different enzymes and receptor systems, and it is possible that oestradiol may serve to inhibit development of a tissue during foetal life, while the same steroid might have a stimulating effect on the same tissue at a later time in life.

5. Sex Differences in Foetal Adrenal Physiology and the Intrauterine Position Phenomenon

The previous findings demonstrate that there are sex differences in oestradiol titres during foetal development in mice. In other mammals, including humans, female foetuses also have higher titres of oestradiol than do male foetuses (Reyes et al. 1974, Ileside and Tuchinsky 1980). One hypothesis that has been developed from comparisons of 0M and 2M male mice is that differences in foetal oestradiol titres due to intrauterine proximity to females lead to differences in phenotype in 0M, 1M and 2M male mice (and also contribute to differences in phenotype between 0M, 1M and 2M female mice). At present, however, this hypothesis is only based on correlational studies. This hypothesis does not discount the importance of individual differences in either the rate of secretion of or tissue sensitivity to testosterone in mediating variation in phenotype in males. But if future research confirms that variation in phenotype among male mice is mediated by at least in part by differences in foetal oestradiol titres due to intrauterine proximity to female foetuses, then differences between males and females should also be mediated in part by the known sex differences in oestradiol titres during foetal life.

The above hypotheses lead to an important question, namely, where do oestrogens in the foetal and maternal circulation come from? Considerably more is known about the source of oestrogens during pregnancy in humans than in rodents. In humans, most of the oestrogens in both the foetal and maternal circulation are of foetal origin. Circulating oestrogens are derived from aromatizable androgens (principally dehydroepiandrosterone sulfate, DHEAS) which are secreted from the foetal, and to a lesser degree the maternal, adrenals. These androgens are then aromatized to oestrogens in the placenta after they are desulphated (Kime et al. 1980). The fact that adrenal androgens are sulphated prior to secretion has been considered to be quite important, since sulphated steroids are hydrophilic and have been thought to have little ability to enter cells and thus influence sexual differentiation. This is most likely untrue, however (cf., Vignon et al. 1980).

The available evidence suggests that the ovaries of female foetuses do not secrete oestrogens in measurable quantities, and in rats as in humans, oestrogens in the maternal blood stream are of foetal-placental origin (cf., Gibori and Trudinger 1981). In mice, the data suggest that oestrogens are also of foetal-placental origin, since female foetuses have over two times the blood concentrations of oestradiol as to their
mothers (vom Saal and Bronson 1980a). Unfortunately, the source of this osestradiol within the foetal-placental unit in rodents is unknown. In rats, for example, the placenta appears to have little or no capacity to aromatize testosterone to osestradiol, and the adrenal cortex of rats appears to secrete low concentrations of androgens relative to humans (Kim et al. 1980).

These considerable evidence that the adrenal cortex of males and females in mice and other mammals is sexually dimorphic after birth (Christian 1971, Kim et al. 1980). It is proposed that the adrenals of mice are also sexually dimorphic during foetal life. Secretions from the foetal adrenals may thus be contributing to the sex difference in amniotic fluid osestradiol concentrations in mice, either directly in terms of the secretion of androgens which are then aromatized, perhaps within the liver which contains aromatase (Gibbs and Szilassy 1981), or indirectly through effects of glucocorticoids on both aromatase enzymes or rates of clearance of substances in the liver or placenta. The evidence is that the secretion of steroids by the foetal adrenal is primarily under the control of ACTH, which is secreted by the foetal pituitary, and possibly also choric gonadotropin or some other substance secreted by the placenta. In adult mice testosterone inhibits ACTH secretion while osestradiol enhances ACTH secretion (Kim et al. 1980), thus accounting for the known sex differences in adrenal morphology and physiology. It is possible that testosterone and osestradiol also influence the secretion of ACTH during foetal life in mice. The secretion of high titres of testosterone by the testes of male foetuses may inhibit ACTH secretion by the male's pituitary, which would result in a decrease in the secretion of steroids by the adrenals. It would also be expected that the adrenal cortex of foetal males would be hypotrophic relative to that of females, which is characteristic of adult male and female mice (Kim et al. 1980). In addition, since 2M female mouse foetuses have higher amniotic fluid and blood titres of testosterone than 0M female foetuses, it would also be expected that 2M female foetuses should have lower titres of osestradiol than 0M females due to inhibition of ACTH, which is suggested by the available evidence (vom Saal and Bronson 1980a).

6. The Effects of Prenatal Stress on Differences in Phenotype Due to Intrauterine Position

Corticosterone, but not ACTH, can pass between the maternal and foetal circulation across the placenta. Thus, corticosterone released by the adrenals of pregnant female mice that are subjected to stress should enter the foetal circulation and inhibit ACTH secretion by the foetal pituitary (Kim et al. 1980). This should result in a decrease in the secretion of adrenal steroids in the foetuses of stressed mothers. The fact that the pituitary-adrenal axis is functional in foetuses during the period of sexual differentiation, when steroids are regulating the development of sex phenotype, suggests that the functioning of this system should be sensitive to maternal stress, such as stress due to high population density in mice.

While the previous discussion is highly speculative, it is proposed that adrenal secretions interact with testicular secretions in regulating normal sexual differentia-
tion. Thus, it is possible that stressing pregnant mice might inhibit secretions from the foetal adrenal, and possibly attenuate or eliminate sex differences in foetal adrenal secretions. The result would be an attenuation or elimination of intrasuterine-position related differences in phenotype. To test this hypothesis, forty-five mice were time-mated and stressed by being placed under 150 W flood lights (350 fc; 34°C) three times per day from Day 13 through 16 of pregnancy. An equal number of control females remained undisturbed. Ward and Weisz (1980) have reported that this procedure advances the timing of the maximum release of testosterone in male rat foetuses. In adulthood, prenatally stressed male rats are less masculinized (exhibit lower levels of male copulatory behaviour) and less feminized (exhibit higher levels of female copulatory behaviour) than are normal males (Ward 1972).

At Caesarean delivery all offspring of stressed and control mice were weighed. Regardless of intrasuterine position or sex, the offspring of stressed mothers weighed significantly (8%) less (1.25 g) than did the offspring of control mothers (1.36 g). No difference in body weight between stressed and control males or females was found when the animals were 24 days old. The data presented in Fig. 6 reveal that all of the female offspring of stressed mothers resembled 2M females in the length of the anogenital space at birth. The previously reported difference in anogenital distance of control OM, 1M, and 2M female mice was again found. In adulthood, the control OM females had shorter oestrous cycles than did the control 2M females, again confirming previous reports (vom Saal and Bronson 1980b, vom Saal et al. 1981). But the prenatally stressed OM females had oestrous cycles that were characteristic of control 2M females (unpublished observation.). It is interesting that the 2M female offspring of stressed mothers exhibited significantly more pseudopregnant cycles than did any of the other females. This finding is consistent with the report by Herrenkohl (1979) that prenatally stressed female rats exhibited prolonged oestrous cycles and a high incidence of pseudopregnancies relative to control females. If maternal stress had only disrupted the secretion of testosterone by the testes of male foetuses as the findings of Ward and Weisz (1980) suggest, then the loss of females with a 2M phenotype would have been expected. Unfortunately, Ward and Weisz (1980) did not measure foetal oestradiol titres.

A comparison of the behaviour toward young of OM, 1M and 2M male mice from the stressed and control mothers has been conducted. In control OM, 1M and 2M males, there are differences in the tendency to commit infanticide or behave parentally toward young: most OM males commit infanticide while most 2M males behave parentally. Prenatal stress eliminated this difference, and all male offspring from stressed mothers resembled 2M males (most behaved parentally toward young) regardless of prior intrasuterine position (vom Saal 1983). Thus, prenatal stress eliminated the effects of developing between female foetuses in male mice, while there appeared to be little or no effect of prenatal stress on the behaviour of 2M males. In summary, all offspring from stressed mothers have resembled 2M animals in phenotype in the studies that have been completed at this time.

2M male mice and rats have previously been found to be the least sexually active of all offspring. Thus, we proposed that the previous report (Ward 1972) that prenatal stress resulted in a decrement in male sexual behaviour in rats was explained by the fact that prenatal stress results in the elimination of variance due to intrasuterine
position in the offspring of stressed mothers and a shift in the population mean for sex behaviour toward that characteristic of 2M males. OM and 2M male mice from stressed and control mothers were compared in terms of their sexual activity and this prediction was confirmed (Even and von Saal 1983). Animals with a OM and 1M phenotype thus do not appear to be produced by female mice or rats that are subjected to severe stress during the last third of pregnancy. It is proposed that the elimination of intrauterine position differences in the offspring of stressed mice is mediated by the suppression of steroid secretion by the foetal adrenal due to negative feedback inhibition of foetal ACTH by maternal corticosterone. If the enhanced titre of oestradiol in the amniotic fluid of female mouse foetuses derive from secretions from the foetal adrena, then suppression of foetal ACTH might reduce oestra dioil levels in female foetuses and eliminate the effects on siblings of developing between female foetuses. The findings from comparisons of the OM and 2M offspring of stressed and control mice are thus consistent with the hypothesis that the high rate of secretion of oestradiol is female mice (and thus intrauterine proximity to female foetuses) plays an important role in mediating the intrauterine position phenomenon in both male and female mice.
7. Summary and Conclusions

During fetal life circulating oestrogens may interact with androgens secreted by the fetal testes in regulating both differences in phenotype between males and females and in producing variation in phenotype among males and among females (i.e., differences in phenotype due to intrauterine position). The evidence from studies involving human foetuses is that circulating oestrogens are derived from androgens (primarily DHEA-S) secreted by the fetal adrenals, and the adrenals of females secrete higher titres of steroids than the adrenals of males. It is thus also proposed that in mice, the observed sex difference in amniotic fluid titres of oestriadiol reflects a sex difference in fetal adrenal physiology. The fetal adrenal may thus play a critical role in modulating normal sexual differentiation in mammals. The ability of secretions from the maternal adrenals to cross the placental barrier and depress the secretion of steroids from the fetal adrenals provides a mechanism via which the environment of the mother can influence foetal development. In addition, the intrauterine environment of foetuses, i.e., their intrauterine proximity to other foetuses of the same or opposite sex, provides another source of variation in phenotype in mice and rats, and possibly other mammals in which multiple uterine residencies occurs.

The way in which oestriadiol might interact with testosterone to regulate sexual differentiation is obviously complex, since elevated foetal titres of oestriadiol are correlated with enhanced sexual performance and decreased aggression and decreased seminal vesicle size in male mice. The negative relationship between aggressiveness and sexual performance observed in comparisons of OM and 2M male mice presents an interesting problem. Psychobiologists typically refer to males that exhibit a decrement in male sex behaviour (usually due to some hormonal manipulation or to prenatal stress) as being less masculinized than are males which exhibit more mounts, intromissions or ejaculations when paired with a female. Thus, if only sexual behaviour had been examined in 0M and 2M male mice, 0M males would have been labelled as more masculinized than 2M males. But the induction of aggression in response to testosterone treatment is also utilized by psychobiologists as an index of prenatal masculinization, and if only aggression had been examined in 0M and 2M male mice, 2M males would have been labelled as more masculinized than 0M males. Obviously, if two behaviours are commonly used as indices of masculinization and they are found to be negatively correlated, labelling one male as more or less masculinized than another male in an experiment in which only one of the behaviours is analyzed is meaningless. Thus, 0M, 1M and 2M males are all masculinized in that they are all capable of reproducing, but variance in both aggressiveness and sexual performance in male mice is due in part to prior intrauterine position.

In conclusion, a significant component of the variance in a broad range of morphological, physiological and behavioural characteristics in populations of mice and rats has been found to be due to prior intrauterine position. The intrauterine position phenomenon is thus a valuable model system for examining the relationship between foetal hormone titres and adult phenotype.

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