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THE INTRAUTERINE POSITION PHENOMENON: EFFECTS ON PHYSIOLOGY, AGGRESSIVE BEHAVIOR AND POPULATION DYNAMICS IN HOUSE MICE

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INTRODUCTION

In mice and rats, which are both murine rodents, a significant component of the variation in phenotype among individuals of the same sex is due to whether an animal is contiguous to siblings of the same or opposite sex during intrauterine development. This is referred to as the intrauterine position phenomenon (vom Saal 1981). This phenomenon appears to be due to the fact that male and female fetuses secrete different concentrations of steroid hormones during fetal life, and by some as yet unknown mechanism the hormones produced by one fetus seem to be able to pass into contiguous fetuses and modify their development in a predictable manner.

The first section of this chapter will review differences among females as well as among males that have been found to correlate with prior intrauterine position in experiments with mice and rats. A model concerning the hormonal bases of differences in phenotype among males and among females due to intrauterine position will be described. Also, the implications of these findings to current models of the hormonal regulation of sexual differentiation will be discussed. In the second and third sections of this chapter, the potential implications of differences in phenotype due to intrauterine position (which have been identified in studies of laboratory animals) to individual reproductive success and mouse

population dynamics in natural environments will be reviewed. A new hypothesis will be described, termed the intrauterine hypothesis. This hypothesis proposes that shifts in the proportions of animals from different intrauterine positions within a population can influence population dynamics in mice, and possibly other litter-bearing mammals. The intrauterine hypothesis proposes that variation in phenotype due to the intrauterine position phenomenon evolved due to its adaptiveness. The significance to the study of the genetic basis of behavior of having variation in phenotype mediated hormonally will be discussed.

THE INTRAUTERINE POSITION PHENOMENON

With the exception of the primary sex characteristic, gonadal sex, all other differences between males and females have traditionally been thought to be mediated by androgens secreted by the testes of males during early life (Wilson, George, Griffin 1981). In long gestation species such as humans, sexual differentiation is usually completed by birth, while in short gestation species such as mice and rats, sexual differentiation commences during the last third of gestation and continues into the early neonatal period (Gorski 1979). The testes of male mouse fetuses differentiate around Day 13 of gestation. Differentiation of the accessory sex organs and genitals begins on about Day 15 of gestation in mice (Rugh 1968). Hypothalamic differentiation also begins during the latter part of gestation and continues into the neonatal period (Rugh 1968; Jacobson, Shryne, Shapiro, Gorski 1980). These events occur in rats about two days later than in mice (Weisz, Ward, 1980). Pregnancy in mice usually lasts about 19 days, while in rats, pregnancy lasts about 22 days. Sexual differentiation in rodents is thought to involve two independent processes: masculinization, the development of male characteristics, and defeminization, the loss of female characteristics, although there is also evidence that feminization may be an active process (Baum 1979; Dohler, Hancke, Srivastava, Hofman, Shrine, Gorski 1984; Toran-Allerand 1984).

Prenatal Hormone Titers and Aggression in Female Mice

The decision to conduct experiments concerning the effects of prior intrauterine position on adult reproductive performance in mice stemmed from two independent observations. First, female rats (Clemens, Gladue, Coniglio 1978) and mice (vom Saal, Bronson 1978) that develop in utero between males (2M females) have a larger space between the anus and genital papilla at birth than do females that do not develop contiguous to a male (0M females; see Figure 1). The perineal tissue separating the anus and genital papilla becomes the scrotum in males and elongates during prenatal life in rodents in response to androgen stimulation. The length of this tissue at birth thus serves as a bioassay for prenatal androgen exposure. These data suggested that 2M females had higher blood titers of androgen during fetal life than did 0M females. Subsequently, it was found that on Day 17 of gestation, when male mouse fetuses have over 2.5 - 3 times the amount of circulating testosterone as do female fetuses (see Figure 7), 2M female mouse fetuses have significantly higher amniotic fluid and blood titers of testosterone than do 0M female fetuses (vom Saal, Bronson 1980a). The second observation was that when adult female mice of either the R-S or CF-1 strains were housed together in groups of 6 per cage, one female invariably attacked (and also often mounted) the other females. Since litter size in both R-S and CF-1 mice is usually 12 (6 males and 6 females), it was a simple matter to calculate that if intrauterine position by sex were a random developmental event, which does appear to be the case, then one in 6 females would be a 2M female (vom Saal 1981). This led to the hypotheses that the most aggressive female mice might be those females which were exposed to the highest titers of testosterone during fetal life as a result of developing in utero between male fetuses, and that prior intrauterine position might be correlated with aggressiveness (and other hormone-mediated traits) in female mice.

Before covering differences in aggressiveness and other traits due to intrauterine position, a brief discussion of the literature concerning aggression in female mammals is required (for a review see: vom Saal 1983a). The great majority of articles on aggression in mice have described females as being nonaggressive. The

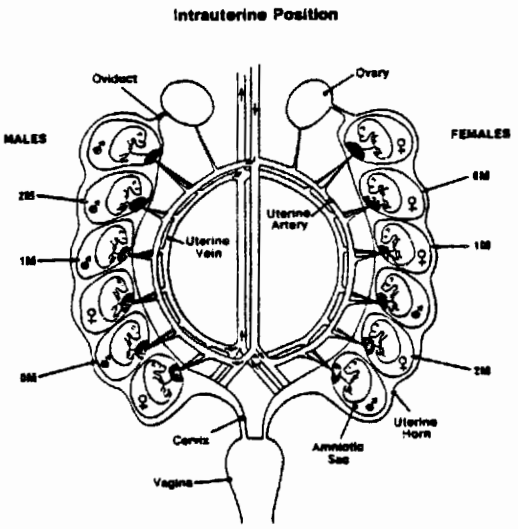

Fig. 1. Schematic diagram of the uterine horns and uterine loop arteries and veins of a pregnant mouse at term. The intrauterine position of fetuses is determined at Cesarean delivery. The labels: 0M, 1M, and 2M refer to the number of male fetuses that an individual is contiguous to (2M = between 2 males, 1M = between a male and a female, and 0M = between 2 females). This scheme is used to identify both male and female fetuses. The arrows indicate the direction of blood flow in the loop artery and vein feeding each uterine horn in both rats and mice based on injections of dye into the maternal heart (arterial flow) and individual placentae (venus flow; F. vom Saal, unpublished observation).

accepted model of the hormonal regulation of aggressiveness among mice (other than lactating females) is that during the perinatal period, androgens act to sensitize the neural substrate mediating aggression, and during adult life, animals sensitized to androgen can be rapidly induced to exhibit aggression when treated with testosterone (the androgen with the greatest effect on behavior). Thus, androgens are thought to sensitize neural areas during early life and activate them later in life

(vom Saal 1983a). Animals not sensitized to androgen during early life eventually exhibit aggression when treated with testosterone in adulthood, but they require a relatively long period of treatment before they respond to the activational effects of testosterone. This is referred to as the sensitization model (vom Saal 1979) and predicts that sex differences in aggressiveness are not "locked-in" (i.e., organized) during perinatal life as had previously been proposed. But, the sensitization model predicts that agggression will only be exhibited by mice that have high circulating titers of testosterone in adulthood. Also, aggression between mice is presumed to be elicited by an androgen dependent pheromone excreted in the urine (Mugford, Nowell 1971). Thus, only gonadally-intact male mice are supposed to behave aggressively, and only gonadally-intact male mice are supposed to elicit intense aggression. This type of aggression is thus referred to as intermale aggression.

Aggression between females has been observed by numerous investigators (Retzlaff 1938; Reimer, Petras 1967; Lloyd, Christian 1969; Christian 1971; Lidicker 1976; vom Saal, Bronson 1978; vom Saal 1983a). But, as mentioned above, most discussions of sex differences in aggression conclude that, in general, female mammals, including female mice, are not aggressive (Moyer 1974). It is important to note that aggression between female mice is sometimes (vom Saal and Bronson 1978), but not always (Yasukawa, Monder, Leff, Christian 1984), less intense than that commonly observed between males. But, interfemale aggression does appear to result in the establishment of dominance hierarchies and also to significantly influence the reproductive success of female mice (Retzlaff 1938; Lloyd, Christian 1969; Lloyd 1975; vom Saal 1981), similar to the effect of aggressivenes on dominance status and reproductive success in male mice (DeFries, McLearn 1972; Christian 1971).

Intrauterine Position Effects on Phenotype in Female Mice

The objective of this section is to review the effects of intrauterine position on the behavior and physiology of inividual animals. Subsequently, this information will provide the basis for speculation concerning the potential significance of variation in

phenotype within a population due to intrauterine position to population dynamics in house mice. In studies involving the effects of intrauterine position in females, the offspring of time-mated CF-1 female mice are delivered by Cesarean section. The intrauterine position of each female is recorded, and individual females are marked using a toe-clipping pattern; all offspring are then raised by foster mothers (for a discussion of methodology see: vom Saal 1981). Most experiments have involved the comparison of 0M and 2M females, but whenever 1M females (that developed between a male and a female fetus) have been tested, they have been intermediate between 0M and 2M females in their characteristics. We do not know the mechanism by which the putative transfer of steroids between fetuses occurs. Thus, we have utilized the most conservative classification scheme, i.e., one that compares only animals situated between fetuses of the same or opposite sex. Fetuses at the ends of the uterine horns have not been examined.

Adult 2M females have been found to be more aggressive than 0M females: 1. when 0M and 2M females (in the diestrous phase of the estrous cycle) were paired, significantly more 2M females attacked (bit and chased) and became dominant over their 0M opponents; 2. after delivering a litter, lactating 2M females exhibited aggression toward a female intruder into the nest area that was significantly more intense than that exhibited by lactating 0M females. Thus, in the absence of exogenous treatment with testosterone, 2M female mice were found to be more aggressive toward other females than were 0M females (vom Saal, Bronson 1978).

The behavior of males toward 0M and 2M females was found to differ considerably: 1. when given a choice of entering a chamber containing a 0M female or a chamber containing a 2M female, males were significantly more likely to choose 0M females over 2M females; 2. males mounted sexually-receptive 0M females at significantly higher rates than 2M females (vom Saal, Bronson 1978); and 3. when placed with a sexually-receptive 0M and 2M female, male mice inseminated 0M females before they inseminated 2M females (Rines, vom Saal 1984). Thus, 0M and 2M female mice differ in their capacity to attract and arouse male mice. In addition, when in estrus, 2M females were found to be less sexually receptive (have a lower lordosis

quotient and lordosis intensity) than 0M females when males attempted to mount them (Rines, vom Saal 1984). In a comparison of 0M and 2M female Sprague Dawley rats, 0M females were also found to exhibit a higher lordosis intensity than 2M females when their flanks were palpated (vom Saal, Coquelin, Schoonmaker, Shryne, Gorski 1984).

Comparisons of the capacity of 0M and 2M female mice to ovulate and then produce and raise young have revealed that adult 2M female mice have longer and more irregular estrous cycles than do 0M females. But, there is no difference between 0M and 2M females in the number of ova shed at ovulation (vom Saal, Bronson 1980b). Similar findings have been reported for 0M and 2M female Sprague Dawley rats (vom Saal 1981). 0M and 2M female mice do not differ in the number or weight of young at birth or at weaning for the first and second litters produced (vom Saal, Bronson 1978), but 2M females cease producing live offspring at a significantly younger age and after fewer litters than do 0M females (vom Saal, Moyer, Rines 1982).

The effect of prior intrauterine position on the timing of puberty has also been examined. 0M female Sprague Dawley rats enter puberty (first vaginal estrus) sooner than do 2M female rats (vom Saal 1981). The regulation of puberty in mice by pheromonal cues emitted in the urine of both males and females makes the study of puberty quite complicated in mice. Male mice emit a pheromone in their urine that accelerates sexual maturation in female mice. Female mice emit a pheromone in their urine that inhibits sexual maturation in other females and blocks the sexual maturation-inducing action of the male pheromone (Bronson 1979). This female-emitted cue thus serves to decelerate population growth when population densities are high (Massey, Vandenbergh 1980). 0M females emit a more potent sexual-maturation inhibiting pheromone and are more sensitive to the presence of the pheromone than are 2M female mice (F. vom Saal, unpublished observation). Thus, when 4 - 5 female mice are placed together in a mouse cage either with or across a wire barrier from a male, 0M females enter puberty later than 2M females, and the first postpubertal estrous cycle is significantly longer in 0M than 2M females. When housed individually with or near a male, however, 0M female mice enter puberty at a younger age than 2M females, and the first postpubertal estrous cycle of 0M females is shorter

than that of 2M females (vom Saal, Bronson 1978; vom Saal, Pryor, Bronson 1981; vom Saal 1981; F. vom Saal, unpublished observation).

Intrauterine Position Effects on Phenotype in Male Mice

Until recently, the above differences between 0M and 2M females were thought to be mediated solely by testosterone, with the development of 2M females being presumed to be altered as a result exposure to high titers of testosterone secreted by contiguous male fetuses. Since testosterone was thought to be the mediator of intrauterine position differences among female mice, it seemed unlikely that males would differ based on their intrauterine proximity to other male or female fetuses. In part, this assumption was based on the observation that treating pregnant female mice with high concentrations of testosterone rendered the female offspring indistinguishable from males in terms of external genital morphology. The prenatally-androgenized females were also similar to males in their aggressiveness toward a male when again exposed to testosterone in adulthood. But, the male offspring of testosterone-treated mothers did not differ from the male offspring of untreated mothers in their external genital morphology or adult aggressiveness (vom Saal 1979). In fact, a review of the literature revealed no evidence that treatment during early life with exogenous testosterone enhances masculinization in males in any mammalian species. On the contrary, treatment with either exogenous androgens or estrogens during early life can disrupt the normal development of sex accessory organs in males (Baranao, Chemes, Tesone 1981). Thus, it did not seem likely that exposure to testosterone secreted by contiguous male fetuses would have any effect on the development of male mice, and differences in phenotype due to intrauterine position were proposed to only occur in females.

In sharp contrast to the above prediction, we found that on Day 17 of gestation, female mouse fetuses have higher amniotic fluid titers of estradiol than do male fetuses, and male mice which are located *in utero* between two female fetuses (0M males) have higher amniotic fluid titers of estradiol than male fetuses that are located *in utero* between two males (2M males; vom Saal, Grant, McMullen, Laves 1983). Estradiol in the amniotic fluid has

been proposed to be in equilibrium with estradiol in the fetal circulation (Belisle, Tulchinsky 1980). The possibility that blood and amniotic fluid concentrations of estradiol are in equilibrium throughout the latter part of pregnancy in mice and rats is currently being examined. Although male mouse fetuses have about three times as much circulating testosterone as do female fetuses (vom Saal, Bronson 1980a), we were surprised to find that 0M and 2M male mouse fetuses did not differ significantly in their blood or amniotic fluid titers of testosterone (vom Saal, Grant, McMullen, Laves 1983). These findings led to the examination of the effects of prior intrauterine position on adult phenotype in male mice and rats.

Comparisons of adult 0M and 2M male mice have revealed that gonadally-intact 0M male mice exhibit more mounts and more intromissions than 2M males when they are paired with a sexually-receptive female for 30 min (vom Saal, Grant, McMullen, Laves 1983). Similarly, gonadally-intact 0M male Sprague Dawley rats exhibit more ejaculations to satiety (30 min without a mount) than do 2M males when they are paired with a sexually-receptive female. These same male rats were then compared for the volume of the sexually-dimorphic nucleus of the preoptic area (SDN-POA) as described by Jacobson, Shryne, Shapiro, and Gorski (1980). The 0M males had a significantly larger SDN-POA volume than did the 2M males (vom Saal, Coquelin, Schoonmaker, Shryne, Gorski 1984). Thus, in male mice exposure to elevated titers of estradiol during fetal life due to developing in utero between female fetuses is correlated with enhanced sexual performance in adulthood. Furthermore, in rats, developing between either male or female fetuses leads to differences in brain structure as well as sexual behavior.

In another set of studies, 0M and 2M male mice were gonadectomized at Cesarean delivery. When treated with testosterone in adulthood, the 2M males required a shorter period of testosterone exposure to be induced to exhibit aggression toward a male opponent than did the 0M males. The 2M males also had larger seminal vesicles after both 2 and 5 weeks of treatment with testosterone than did the 0M males. The length of time required to induce aggression and the rate of seminal vesicle growth in males castrated at birth are both indices of sensitivity to testosterone. Taken together, the results indicate that 0M males are

less sensitive to testosterone than are 2M males in terms of the induction of intermale aggression and seminal vesicle growth, while 0M males are more sexually active than are 2M males (vom Saal, Grant, McMullen, Laves 1983).

It has been hypothesized that testosterone is aromatized to estradiol within some androgen target cells, including areas of the brain thought to be involved in mediating sexual and aggressive behaviors (MacLusky, Naftolin 1981). The aromatization hypothesis proposes that testosterone is a prohormone and that estradiol is actually the hormone that induces masculinization and defeminization during early life. It was previously thought that circulating estrogens could not enter cells in fetuses or neonates due to the presence of high blood concentrations of an estrogen-binding protein (alpha-fetoprotein). It has been presumed, therefore, that for masculinization and defeminization to occur, a prohormone that is not bound and thus inactivated by alpha-fetoprotein (i.e., testosterone) would be required. However, there is now evidence that alpha-fetoprotein which is bound to estrogen may enter brain cells (McEwen, Chaptal, Gerlach, Wallach 1975; Benno, Williams 1978). This finding has raised the possibility that circulating estrogens might directly influence sexual differentiation (McEwen 1980; Dohler, Hancke, Srivastava, Hofman, Shrine, Gorski 1984; Toran-Allerand 1984). The aromatization hypothesis predicts, however, that fetuses which are exposed to elevated titers of estradiol should be more masculinized and more defeminized than are fetuses which are exposed to low titers of estradiol.

In male mice there is a relationship between intrauterine proximity to female fetuses, prenatal titers of estradiol, and adult morphology and behavior. Contrary to the prediction of the aromatization hypothesis, the 0M males (which had the highest amniotic fluid titers of estradiol) were less sensitive to testosterone in terms of the induction of intermale aggression and seminal vesicle growth than were the 2M males. It appears, therefore, that for these traits, elevated titers of estrogen interfere with the normal sensitizing action of testosterone during fetal life. Perhaps this inhibition occurs by estradiol competitively binding to, and thus inhibiting the translocation into the nucleus of, androgen receptors that are present in some hypothalamic areas (as well as in the

seminal vesicles) during prenatal life in both mice and rats (Wilson, George, Griffin 1981; Fox, Olsen, Vito, Wieland 1982). During fetal life (but not necessarily in adulthood) estradiol may thus act as an antiandrogen in some tissues (see Figure 2). The data suggest that the neural areas mediating intermale aggression in mice are sensitized perinatally as a result of testosterone (or possibly its reduced metabolite, dihydrotestosterone; c.f., Lieberburg, McEwen 1980) interacting with cytoplasmic androgen receptors rather than as a result of testosterone being aromatized to estradiol (which would then interact with cytoplasmic estrogen receptors; vom Saal 1983b).

In contrast to the negative relationship of fetal estrogen titers and adult aggressiveness, elevated titers of estradiol during prenatal life appear to facilitate the sensitizing action of testosterone on the neural substrate mediating male copulatory behavior, since sexual performance is enhanced in 0M males relative to 2M males. Thus, in neural tissues mediating male copulatory behavior (mounting, intromitting and ejaculating) and in the neurons of the SDN-POA, testosterone may serve as a prohormone that is aromatized to estradiol prior to interacting with cytoplasmic estrogen receptors and translocation into the cell nucleus. For example, the volume of the SDN-POA is increased by perinatal treatment with estrogens and decreased by perinatal treatment with the antiestrogen, tamoxifen. Similarly, perinatal treatment with either an antiestrogen or aromatase inhibitor interferes with the development of male sexual behavior (Olsen 1983; Dohler, Hancke, Srivastava, Hofman, Shrine, Gorski 1984). Within the nucleus, the estrogen-receptor complex is thought to exert an organizing or sensitizing effect on the genome (vom Saal 1983a). The elevated titers of estradiol observed in the amniotic fluid of 0M male mice may lead to a greater intracellular pool of estradiol and an increase in the number of activated estrogen receptors that are translocated into the nucleus of target cells, thus producing an enhancement of sexual performance during later life (see Figure 2).

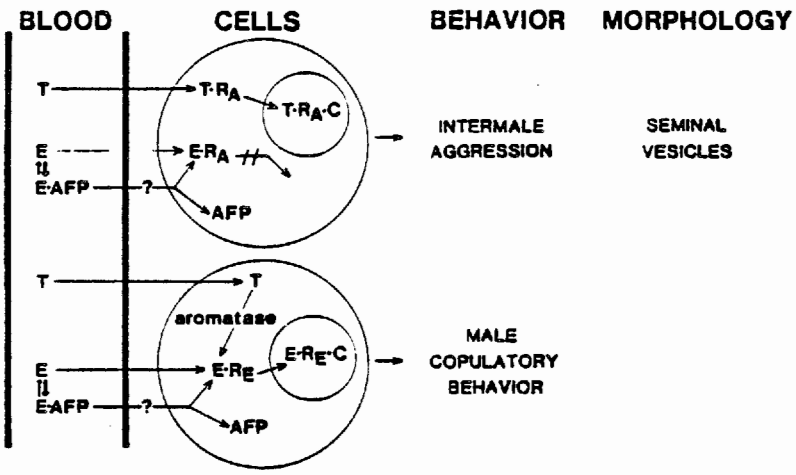

Fig. 2. Schematic diagram of the proposed interaction of estrogens (E) in the fetal blood stream, that are either free or are bound to alpha-fetoprotein (AFP), with androgen receptors (R_A) or estrogen receptors (R_E) in cells containing either androgen or estrogen receptors. The hormone-receptor complex is thought to regulate the functioning of specific genes in chromosomes (C) after translocation from the cytoplasm into the nucleus. Brain areas mediating intermale aggression may contain androgen receptors in fetuses and thus be similar to the seminal vesicles, which are known to contain androgen receptors. The occupation of an androgen receptor by estrogen might inhibit translocation of the occupied androgen receptor into the nucleus, thus interfering with normal development of the tissue. In contrast, during prenatal life the neurons in the SDN-POA as well as other brain cells that mediate male copulatory behavior are hypothesized to contain estrogen receptors and the enzyme aromatase, which converts testosterone to estradiol. High titers of estradiol in the circulation of 0M males are predicted to lead to an increase in the number of estrogen receptors that are translocated into the nuclei of these cells. This model thus explains the decreased aggressiveness, small seminal vesicles, enhanced sexual performance and large SDN-POA of 0M males relative to 2M males in rats and mice.

Prenatal Stress: Elimination of Variance in Phenotype due to Intrauterine Position

Research is currently underway in my laboratory to determine the origin of the sex difference in estradiol concentrations in fetal mice. In humans, female fetuses also have higher amniotic fluid and blood titers of estradiol than do male fetuses (Belisle, Tulchinsky 1980). Considerably more is known about the source of estrogens during pregnancy in humans than in rodents. In humans, most of the estrogens in both the fetal and maternal circulation are of fetal origin. Circulating estrogens are derived from aromatizable androgens (principly dehydroepiandrosterone sulfate) which are secreted from the fetal, and to a lesser degree the maternal, adrenal cortex. These androgens are then aromatized to estrogen in the placenta after they are desulphated (Kime, Vinson, Major, Kilpatrick 1980). In humans, estrogens have been reported to be secreted by the ovaries of female fetuses (Wilson, Griffin, George, Leshin 1981). The available evidence suggests that the ovaries of female rat fetuses do not secrete estrogens in measurable quantities (Schlegel 1967; Weisz, Gunsalus 1973).

Estrogens in the maternal blood stream in rats are of both maternal and fetal/placental origin (c.f., Gibori, Sridaran 1981). In mice, the data suggest that estrogens are also of fetal/placental origin, since female fetuses have over two times the blood concentrations of estradiol as do their mothers (vom Saal, Bronson 1980a). It is possible, however, that this difference could also reflect the presence of high concentrations of circulating alpha-fetoprotein in female fetuses.

In many species androgens secreted from the maternal and fetal adrenals serve as the substrate for aromatase, and adrenal secretions thus determine estrogen concentrations during pregnancy. The adrenal cortex in mice and other rodents is sexually dimorphic after birth. This fact suggests that during prenatal life in rodents, the fetal adrenals may be contributing to the sex difference in estradiol concentrations via the secretion of adrenal androgens, which are then aromatized to estrogens. The evidence is that the secretion of steroids by the fetal adrenal is primarily under the control of adrenocorticotropic hormone (ACTH), which is secreted by

the fetal pituitary, and possibly also chorionic gonadotropin or some other substance secreted by the placenta (Milkovik, Milkovik, Paunovic 1973; Idelman 1978; Krieger 1982; Winter, Fujieda, Faiman, Reyes, Thliveris 1980). In adult mice testosterone inhibits ACTH secretion while estradiol enhances ACTH secretion (Kitay 1961). Also, testosterone inhibits and estradiol enhances adrenal secretions as a result of a direct action on the adrenal cortex (Roy, Mahesh 1964; Stabler, Ungar 1970). It is thus possible that the secretion of high titers of testosterone by male mouse fetuses inhibits ACTH secretion by the fetal pituitary and the secretion of steroids by the adrenal cortex. It would also be expected that the adrenal cortex of fetal males would be hypotrophic relative to that of females, that is characteristic of adult male and female mice (Idelman, 1978). For example, the adrenals of adult virgin female CF-1 mice are almost twice as heavy (mean$\pm$SEM; paired organ weight = 20.6$\pm$2.0 mg/100 g body weight) as are the adrenals of adult males (11.1$\pm$1.2 mg/100 g body weight; t-test, $p < .001$; F. vom Saal, V. Ganjam, unpublished observation).

Corticosterone, but not ACTH, can pass between the maternal and fetal circulation across the placenta (Milkovik, Milkovik 1961). Thus, the corticosterone that is released by the adrenals of pregnant female mice that are subjected to stress should enter the fetal circulation and inhibit ACTH secretion by the fetal pituitary (Milkovik, Milkovik, Paunovic 1973; Kime, Vinson, Major, Kilpatrick 1980). This should result in a decrease in the secretion of adrenal steroids in the fetuses of stressed mothers (see Figure 8). The fact that the pituitary-adrenal axis is functional in fetuses during the period of sexual differentiation suggests that the functioning of this system should be sensitive to changes in maternal physiology, such as the changes in hormone titers which would occur as a result of maternal stress due to high population density (Christian 1971).

The preceding discussion led to the prediction that stressing pregnant mice might modify intrauterine position differences in both male and female offspring as a result of altering fetal steroid concentrations. To examine this possibility, fifty mice were time-mated and stressed by

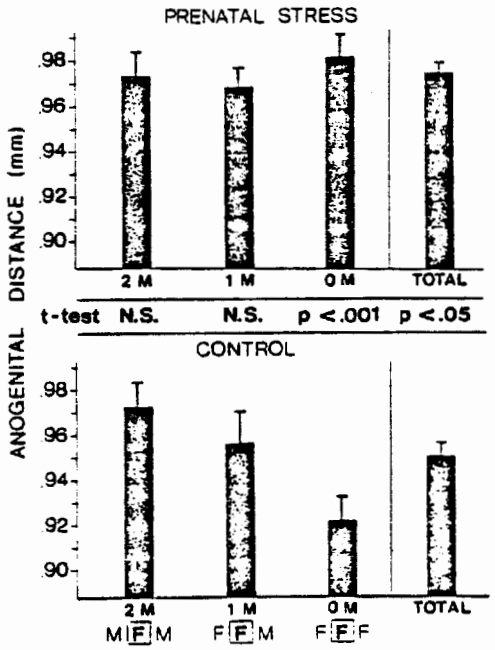

Fig. 3. The mean (± SEM) length of the space between the anus and the posterior aspect of the genital papilla at birth in the 0M, 1M and 2M female offspring of stressed and control mice (n = 40/Group). TOTAL refers to the combined mean for all 0M, 1M and 2M females measured. Significance levels are for t-test comparisons of prenatally-stressed vs. control females from each intrauterine position (N.S. = not significantly different).

being placed under 150 w floodlights for 30 min three times per day during the dark phase of the L:D cycle from Day 13 through 18 of pregnancy (Day 0 = the day of mating). An equal number of control females remained undisturbed.

At Cesarean delivery all offspring of stressed and control mice were weighed. Regardless of intrauterine position or sex, the offspring of stressed mothers weighed significantly (8%) less than did the offspring of control

mothers. No differences in body weight between stressed and control males or females were found when the animals were 24 days old, however, and no differences in prenatal or postnatal survival were observed (vom Saal 1983c). The data presented in Figure 3 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 in control 0M, 1M, and 2M female mice was again found (these measurements are always conducted blind). In adulthood, the control 0M females had shorter (4 - 5 day) estrous cycles than did the control 2M females (6 - 7 day cycles), again confirming previous reports (vom Saal, Bronson 1980b; vom Saal, Pryor, Bronson 1981). But, the prenatally-stressed 0M females had estrous cycles that were characteristic of control 2M females (F. vom Saal, unpublished observation). It is interesting that the 2M female offspring of stressed mothers that did cycle regularly exhibited 6 - 7 day estrous cycles. But, the prenatally-stressed 2M females also exhibited significantly more prolonged 12 - 14 day pseudopregnant-like cycles than did the 0M and 2M control females or the 0M prenatally-stressed females. This finding is consistent with the report of Herrenkohl (1979) that prenatally-stressed female rats exhibited prolonged estrous cycles and a high incidence of pseudopregnancies relative to control females.

A behavioral comparison of 0M and 2M male mice from the stressed and control mothers has been conducted. Prenatally stressed and control 0M and 2M male mice were placed with a female for 30 min, and rates of mounting and intromitting were recorded. The previous finding that control 0M males exhibit higher rates of mounting and intromitting was confirmed in control males, while the behavior of the stressed males was not significantly different from that of control 2M males (i.e., all stressed males exhibited lower rates of mounting and intromitting than control 0M males; Even, vom Saal 1983). Thus, prenatal stress eliminated the effects of developing next to female fetuses in 0M male mice, while there appeared to be no significant effect of prenatal stress on the sexual behavior of 2M males (see Figure 4).

Our findings concerning the effects of maternal stress on the sexual behavior of male offspring are consistent with findings in rats that prenatally-stressed

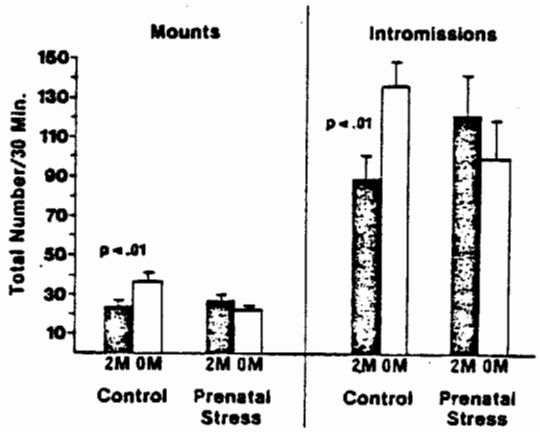

Fig. 4. The mean (±SEM) number of mounts and intromissions exhibited by prenatally-stressed and control 0M and 2M male mice while they were paired with a sexually-receptive 1M female for 30 min. P values are for t-test comparisons of control 0M and control 2M males. All other comparisons are not statistically significant.

males exhibit a decrease in the rate of sexual activity, although prenatally-stressed male mice and rats do not lose the capacity to ejaculate (M. Even, F. vom Saal, unpublished observation; Dunlap, Zadina, Gougis 1978). Our concept of the prenatal-stress phenomenon differs markedly from that of Ward (1972), who has proposed that maternal stress demasculinizes males. The finding that intrauterine position interacts with maternal stress in terms of adult sexual behavior in male mice reveals that maternal stress produces a shift in phenotype, but only in fetuses that develop in utero next to female fetuses. Thus, all prenatally-stressed male and female fetuses have a 2M phenotype, regardless of intrauterine position. To label this shift in phenotype as demasculinization requires that control 2M males be considered to be generally demasculinized relative to control 0M males, since control 2M males exhibit lower rates of sexual activity than do

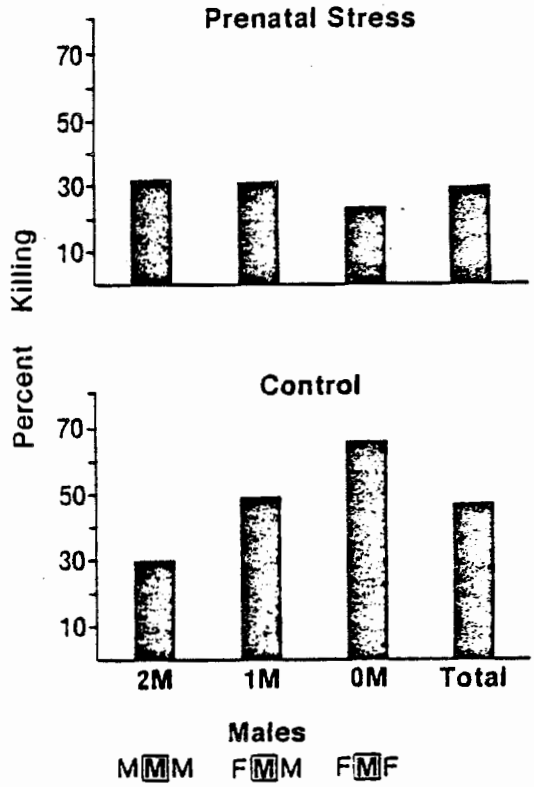

Fig. 5. The percent of adult 0M, 1M and 2M CF-1 male mice (45 - 50 per group) that killed 2 newborn young that were placed into each male's cage for 30 min. The males were housed individually at 35 days of age and tested at 75 days of age. TOTAL represents the combined mean for all 0M, 1M and 2M males tested.

control 0M males. But, 2M males have larger seminal vesicles and are also more aggressive than 0M males, and the conclusion that 2M males are demasculinized relative to 0M males (and therefore that maternal stress demasculinizes male fetuses) is deemed inappropriate.

Other prenatally-stressed and control males were also tested in adulthood for their behavior toward two newborn

young that were placed into a corner of the male's cage for 30 min. The results revealed that differences among males due to intrauterine position in the tendency to commit infanticide were eliminated by prenatal stress, and all male offspring of stressed mothers resembled 2M males in phenotype (few prenatally-stressed males committed infanticide regardless of prior intrauterine position, which is characteristic of 2M but not 0M male mice; see Figure 5; vom Saal 1983c). In both behavioral studies that have been conducted, therefore, maternal stress was found to eliminate differences in behavior among males due to intrauterine position, and all prenatally-stressed males resembled control 2M males in their behavior.

We are currently examining the effects of maternal stress on circulating testosterone, estradiol, corticosterone and cortisol titers during the last 4 days of pregnancy (Days 16 - 19) and the day of birth in pregnant mice and their and male and female fetuses. The accessory reproductive organs and external genitals begin differentiating between Day 15 and 16 of pregnancy, and male and female fetuses are distinguishable based on the appearance of their external genitals on Day 16 (Figure 6). The objective of this experiment was to determine the time during the period of sexual differentiation that maternal stress might alter fetal steroid concentrations in mice. We will subsequently conduct this experiment with fetuses from known intrauterine positions and determine their blood steroid concentrations.

Pregnant mice were stressed as described above and killed just prior to the turning on of lights at 2000 hr (12 hr after the last stress session, which was at 0830 hr) on Days 16, 17, 18 and 19 of pregnancy and the day after birth. The testosterone assays have just been completed, and maternal stress was found to profoundly alter blood testosterone concentrations in both male and female fetuses (see Figure 7). The data are particularly intriguing since the effects of maternal stress on blood testosterone concentrations differ on Days 16, 17 and 18 of pregnancy, suggesting that there are major changes in steroid physiology during this critical period in fetal life (sexual differentiation is occurring during this time). We also collected blood from stressed mice and their male and female fetuses 1 hr after the onset of the stress session, and while there was a significant

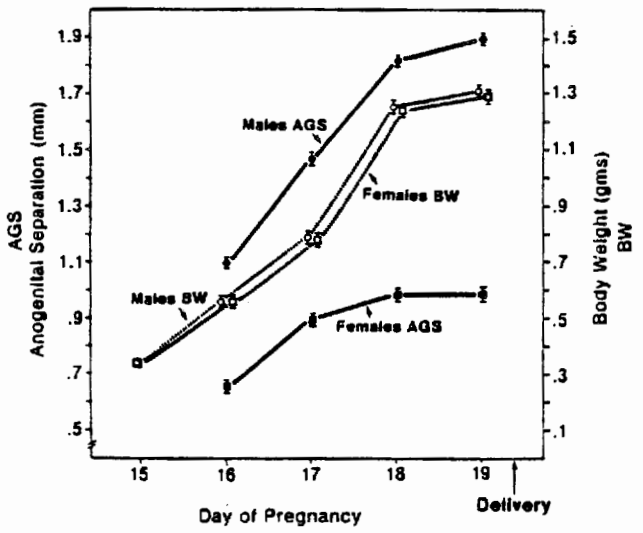

Fig. 6. The mean (±SEM) length of the perineal tissue separating the anus and the posterior aspect of the genital papilla for male and female mouse fetuses during the last 4 days of pregnancy. The perineal tissue elongates in response to stimulation by androgen and becomes the scrotum in males. The mean (±SEM) body weight for male and female mouse fetuses during the last 5 days of pregnancy (Day 0 = insemination). Fetuses were only weighed on Day 15.

elevation in serum testosterone in both the mothers and male fetuses, the stressed female fetuses did not differ significantly from the control female fetuses. Thus, the time course of the endocrine response to maternal stress in male and female fetuses appears to be quite different.

Testosterone is secreted by the testes of male mouse fetuses (F. vom Saal, R. Hammond, unpublished observation; Block, Lew, Klein 1971). While the source(s) of the dramatic increase in testosterone in both male and female rodent fetuses on Day 17 of pregnancy due to maternal

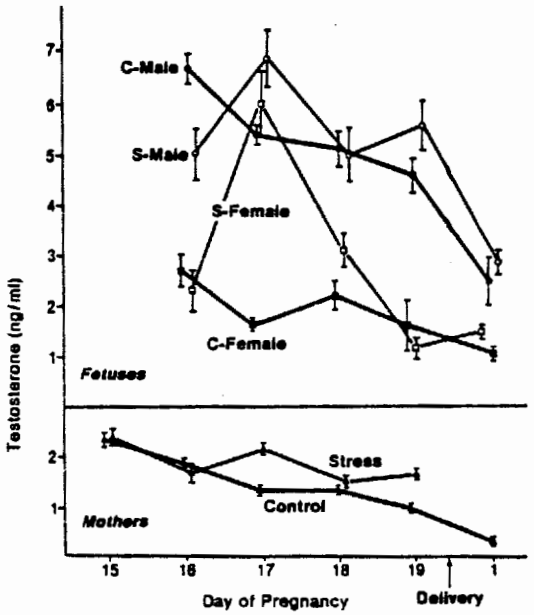

Fig. 7. The mean (±SEM) serum concentrations of testosterone in control (unstressed) and stressed pregnant mice and their male and female fetuses. Stressed females were time-mated (Day 0) and, beginning on Day 13, stressed by being placed under floodlights. Control females remained undisturbed. All animals were killed just before the end of the dark phase of the light:dark cycle. S-MALE and S-FEMALE = offspring of stressed mothers; C-MALE and C-FEMALE = offspring of control mothers.

stress is as yet unknown, the most likely possibility for the site of secretion is the placenta (Soares, Talamantes 1982; 1983). This hypothesis is currently being investigated by determining how stress alters steroid biosynthesis in fetal steroid secreting and metabolizing organs. It is interesting that the fetal pituitary-adrenal axis may become functional in mice around Days 16 to 17 of pregnancy (Rugh 1968). In rat fetuses the pituitary-adrenal axis becomes functional around Days 17 to 18 of pregnancy (Milkovic, Milkovik, Paunovic 1973).

Ward and Weisz (1980) also stressed pregnant rats with the same stress procedure that we used to stress pregnant mice. They collected blood from the male fetuses carried by stressed rats about 2 hr after the end of the last stress session. Ward and Weisz (1980) reported that maternal stress altered the developmental pattern of circulating testosterone secretion in male fetuses, with circulating testosterone being higher in stressed males on Day 17 of pregnancy but lower on Day 18 relative to control males; testosterone concentrations in female fetuses were not reported. Thus, in both rats and mice maternal stress alters the developmental pattern of circulating testosterone concentrations in males, although the exact timing of these changes appears to be different in the two species.

It has been found that exposure to elevated titers of testosterone during early life increases aggressiveness in mice during later life (vom Saal 1979) but decreases the tendency to commit infanticide when adult animals are presented with young (vom Saal 1983c; vom Saal 1984). Thus, during the period in early life when androgens regulate the development of behavior, testosterone has opposite effects on intermale aggression and infanticide in male mice. Consistent with these observations is the finding that 0M male mice are less aggressive toward other adult males than are 2M males, but 0M males are more likely to commit infanticide than are 2M males. Thus, the tendency to be aggressive toward another adult male and the tendency to commit infanticide appear to be negatively correlated. We have not examined intermale aggression in 0M and 2M prenatally-stressed male mice, but this model leads to the prediction that maternal stress should result in an increase in aggressiveness in 0M and 1M males, since they should have the phenotype of a 2M male. There is evidence, however, that the injections of ACTH to pregnant female mice results in a decrease in aggression in male offspring (Simon, Gandelman, 1977). The complexity of the endocrine response to maternal stress thus makes any prediction concerning the effect of maternal stress on a specific behavior of offspring highly speculative.

With regard to the intrauterine position phenomenon in female mice, the finding that maternal stress eliminated the effect of developing in utero next to female fetuses led to the hypothesis that differences

among males and among females due to the intrauterine position phenomenon are mediated by an interaction between exposure to elevated titers of testosterone secreted by contiguous male fetuses and exposure to elevated titers of estradiol secreted by contiguous female fetuses. Control (non-stressed) 2M female mouse fetuses have higher blood and amniotic fluid titers of testosterone than do 0M female fetuses, but 2M female fetuses also tend to have lower blood and amniotic fluid titers of estradiol than do 0M females (vom Saal, Bronson 1980a). Previously, all differences between 0M, 1M and 2M females were thought to be due solely to exposure to testosterone secreted by contiguous male fetuses.

While further experiments are still in progress, the results of these comparisons of stressed and control animals suggest that prenatal stress eliminates the effects of developing next to female fetuses in both male and female mice. 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 fetal adrenal due to negative feedback inhibition of fetal ACTH by maternal corticosterone as well as stimulation of testosterone biosynthesis in the placenta (c.f., Soares and Talamantes, 1982). Thus, if estradiol in mouse fetuses derives primarily from secretions from the fetal adrenals, the suppression of fetal ACTH might reduce estradiol levels in all fetuses, but the greatest reduction in estradiol biosynthesis might possibly be in female fetuses. This could reduce or eliminate differences in estradiol between male and female fetuses. Again, we have already observed that maternal stress results in a marked reduction in the sex differences in circulating testosterone concentrations on Day 17 of pregnancy (12 hrs after the last stress session). The elimination of the effects on littermates of developing next to female fetuses in the offspring of stressed mothers might thus result from a reduction in estrogen secretion in female fetuses, a possibility we are currently examining (see Figure 8).

An interesting problem is why the high concentrations of testosterone in stressed fetuses should not lead to high concentrations of estradiol, since testosterone is aromatized to estradiol by the enzyme aromatase. One possible explanation is that maternal stress results in a

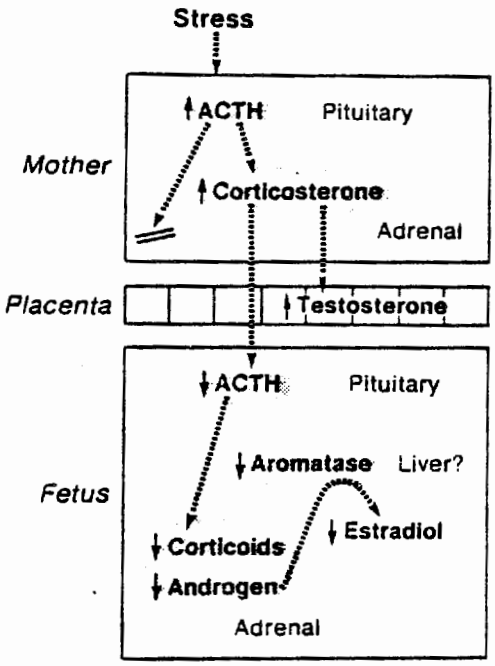

Fig. 8. A schematic diagram depicting the possible mechanism by which maternal stress could result in a decrease in circulating estrogen titers and an increase in circulating testosterone titers in mouse fetuses. In this model maternal ACTH is released in response to stress and stimulates the release of corticosterone from the maternal adrenals; corticosterone (but not ACTH) crosses the placenta and depresses the secretion of ACTH by the fetal pituitary. A decrease in ACTH secretion by the fetal pituitary results in a decrease in the secretion of steroids by the fetal adrenal. Adrenal androgens may be the precursors of circulating estradiol: androgens may be aromatized in the liver (or some other tissue) to estradiol. A decrease in circulating estrogens in the fetuses of stressed mothers might result from both a decrease in adrenal androgen secretion and an inhibition of aromatase in fetuses. Corticosterone might act to increase testosterone secretion from the placenta, which is known to secrete androgens in mice.

decrease in aromatase concentrations and thus a decrease in estradiol even though testosterone concentrations are high. This possibility is also currently being examined in my laboratory.

The intrauterine position phenomenon has thus proven to be a unique, naturally-occurring model system for examining the relationship between fetal hormone titers and adult phenotype. Intrauterine position by sex is a random event (vom Saal 1981), and there should thus be no systematic differences in genotype between 0M, 1M and 2M animals within a litter. Strains of mice, such as the CF-1 strain utilized in my laboratory, are ideal for studying genetic vs. environmental influences on development, since they have very little underlying genetic variation. Thus, the differences in phenotype due to intrauterine position which have been identified are presumed to result from individual differences in hormone concentrations rather than individual differences in genotype.

The use of laboratory mice increases the power of these experiments (by reducing genetic variance) but limits the degree to which findings can be related to the reproductive ecology of wild mice. Experiments involving the effects of intrauterine position on behavior and physiology utilizing the offspring of live-trapped wild house mice are in progress. At this time we have confirmed that intrauterine position influences anogenital distance in the F_2 offspring of wild mice delivered in the laboratory by Cesarean section, similar to the effect of intrauterine position on anogenital distance in control CF-1 female mice (see Figure 4; M. McCarthy, F. vom Saal, unpublished observation). This finding suggests that intrauterine position will be found to also influence the physiology and behavior of wild male and female mice. It is thus of interest to develop hypotheses about: 1. the possible adaptiveness of the intrauterine position phenomenon and 2. what role this phenomenon might play in influencing individual reproductive success and population dynamics in mice. These issues will be addressed in the following sections.

MODELS OF POPULATION DYNAMICS IN SMALL MAMMALS

Some microtine rodents, such as voles and lemmings, have the potential to exhibit irruptive population growth which is often followed by "crashes" in population size. It has been proposed that these changes in population size represent "cycles" (Chitty 1967) in that they are recurrent, although not predictable or periodic. House mice also have the potential to exhibit irruptive increases in population size (Newsome, Corbett 1975). There appear to be factors intrinsic to populations of house mice which can serve to decelerate population growth and which can also lead to declines in population size, even when excess food, water, and shelter are available (Southwick 1958; Christian 1971; Lidicker 1975). For example, female mice emit a pheromone which inhibits sexual maturation in juvenile females when population density becomes high (Bronson 1979).

A number of different hypotheses have been proposed concerning the factors contributing to rapid changes in population densities in small mammals. The two major hypotheses concerning the regulation of population size have emphasized: 1. extrinsic, density-independent factors such as food supply, climate, ground cover, etc. and 2. intrinsic, density-dependent factors, such as aggression between individuals within a population. In this paper, only intrinsic factors will be discussed, but this is not to suggest that extrinsic factors are not relevant to the study of the reproductive ecology of mice (for a review see: Lidicker 1978). Within the density-dependent school, different models concerning the mechanisms by which populations of animals regulate their sizes have been proposed. The two hypotheses that will be discussed will be: 1. the Chitty-Krebs hypothesis, which emphasizes the role of genotype in influencing the physiology and behavior of individuals and, ultimately, population dynamics; and 2. the stress hypothesis of Christian, which emphasizes the role of social interactions, the effects of which are mediated via the brain-pituitary-adrenal axis.

The hypothesis which I will present is termed the "intrauterine hypothesis" and is based on the assumption that intrauterine position influences the reproductive capacity and behavior of individual mice, and shifts in the proportions of animals from different intrauterine

positions within a population can markedly influence population dynamics. One of the predictions of the intrauterine hypothesis is that stress due to overcrowding might influence the individual reproductive success of offspring born in high density environments. This prediction is based on our findings that prenatal stress can significantly alter the physiology and behavior of 0M and 1M mice, at least within a laboratory setting (prenatal stress eliminates differences in phenotype due to intrauterine position in mice, thus resulting in a shift to a 2M phenotype in the 0M and 1M offspring of females which are stressed during pregnancy). The intrauterine hypothesis also provides predictions concerning which individuals should be the most affected in their reproductive performance by increases in population density. The aspects of the intrauterine hypothesis relating to environmental stress thus represent an extension of Christian's stress hypothesis.

Based on studies of microtine rodents, Chitty (1967) and Krebs, Gaines, Keller, Myers, Tamarin (1973) have proposed that as population density increases rapidly during an irruption, aggressive animals drive the less aggressive animals out of the home environment (cf. Krebs 1978). The controversial aspect of this hypothesis is that variation in aggression is related to variation in genotype: there is supposedly an increase in the genotype for aggressiveness within a population during an irruption due to intense selection pressure for this trait; after a "crash", there is then supposedly an increase in genotype for non-aggressiveness within a population. Presumably, animals which are observed leaving the home environment when population density is high have a nonaggressive genotype and are being actively dispersed or driven away by the more aggressive animals (cf. DeLong 1967; Lidicker 1975 for discussions). The Chitty-Krebs model predicts, then, that dispersing animals should differ genetically from animals that remain in high density populations.

A correlate of high aggressiveness is predicted to be a decreased reproductive capacity. In addition, the aggressiveness of the animals that remain in a high density population is proposed to result in a high rate of mortality of adults as well as the death of most or all of the young that are produced. Animals that are highly

aggressive are thus presumed to have a low survival rate relative to animals that are present in a population when population density is low, and this has been proposed to explain why a rapid decrease (crash) in population size occurs when population density becomes high. In summary, the Chitty-Krebs hypothesis predicts that rapid shifts in gene frequencies within a population account for the occurrence of population "cycles" in small mammals.

An alternate hypothesis to the Chitty-Krebs model has been proposed by Christian (1971; 1978). He hypothesized that stress due to overcrowding and increased social contact between animals influences individual survival and reproductive performance, and, ultimately, population dynamics in small mammals. These effects are hypothesized to be mediated by the brain-pituitary-adrenal axis. Briefly, Christian and his co-workers have provided evidence that stress due to an increase in social interaction as population density increases results in an increase in ACTH and glucocorticoid secretion in mice. An increase in ACTH and glucocorticoid secretion is correlated with a decrease in gonadotropin secretion, a delay in sexual maturation in young animals, and an inhibition of reproduction in older animals. Physical growth of the young is also retarded, and the functioning of the immune response system is impaired (Christian 1978).

The Intrauterine Hypothesis.

The intrauterine hypothesis proposes that shifts in the proportions of 0M, 1M and 2M animals within a population as population density increases, due to the aggressive 2M males and 2M females dispersing the less aggressive 1M and 0M animals, will lead to a general increase in aggressive interactions among the 2M animals that remain in the home environment. A high proportion of 2M animals in an environment may thus precipitate a decline in population size, since 2M animals are both aggressive and exhibit a decrement in sexual performance. These are exactly the characteristics proposed by the Chitty-Krebs model as being correlated but mediated genetically rather than hormonally.

The intrauterine hypothesis proposes the existence of a relationship between intrauterine position and: 1. individual survival, 2. the successful production of offspring, and 3. the likelihood that young which are produced will survive and, themselves, reproduce. Speculating about the significance of laboratory experiments to populations of wild animals is a tenuous process at best. But, the pattern of differences that have been found to relate to prior intrauterine position in female mice suggests that when population density is low, 0M female mice might have a reproductive advantage over other females since they would enter puberty at the youngest age and emit the most potent cues that would attract and arouse male mice. 0M females that are dispersed from high density populations and survive may thus have a reproductive advantage over 1M and 2M females that are dispersed (cf. Lidicker 1975). When population density is high, 2M female mice may have a reproductive advantage over other females within the home territory, since 2M females are the most aggressive and also relatively insensitive to the olfactory cues that inhibit sexual maturation and reproduction in crowded female mice.

In this section I will focus on the effects of intrauterine position in females, since female mice exhibit profound changes in physiology and behavior in response to changes in population density (Christian 1978; Yasukawa, Monder, Leff, Christian 1985). In general, however, the effect of intrauterine position in males and females is remarkably similar, with 0M males resembling 0M females while 2M males have the same general phenotype as do 2M females. Thus, the production of both male and female offspring that vary due to the intrauterine position phenomenon is proposed to be adaptive.

Retzlaff (1938) and Lloyd and Christian (1969) have provided evidence that when the population density of a freely-growing population of mice becomes high, only a few females continue to reproduce successfully, and these females are highly aggressive and territorial. Urine marking has been related to the establishment and defense of territories in male mice (Harrington 1976), and dominant males urine mark their environment at a significantly higher rate than subordinate males (Desjardins, Maruniak, Bronson 1973). In this regard, in addition to being highly aggressive, 2M female mice have

also been found to urine mark their environment at a higher rate than 0M females (vom Saal, Bronson 1978). Taken together, these findings suggest that 2M female mice might be more territorial than 0M females.

Christian (1971) reported that with the production of the first wave of offspring at the beginning of the breeding season, there is a rapid increase in population density, and at this point in time, competition for preferred territories is intense. As suggested above, when population density is high (or rapidly increasing), 2M females should have a reproductive advantage over 1M and 0M females within the natal environment. Mice which are born after the initial wave of litters that are produced at the beginning of the breeding season are less likely to survive. Those offspring which do survive are stressed, as assessed by adrenal weight, and are generally smaller, subordinate, and more likely to be dispersed than are animals born early in the breeding season. At any time, subordinate animals are considered to be stressed since they have larger adrenals than dominant animals (Christian 1971). Thus, subordinate (presumably 0M or 1M) females that do manage to reproduce should produce offspring that all have a 2M phenotype, since all prenatally-stressed mice were found to resemble 2M animals. But, even when population density is high, it is possible thatthe dominant (presumably 2M) females, which are presumably not severely stressed, will continue to produce offspring that vary in phenotype as a function of intrauterine position. Whether, in fact, the production of 2M-like young by stressed female mice has evolved due to the adaptiveness of a subordinate female producing 2M-like young when competition within the natal environment is intense cannot be directly tested. Perhaps this phenomenon is just a consequence of the fact that adrenal secretions influence sexual differentiation.

As described above, there is evidence that 0M female mice are more sensitive to the social cues that inhibit sexual maturation than are 2M females (vom Saal, 1981; F. vom Saal, unpublished observation). It is possible that this may actually result in the 0M females in litters that are produced by dominant females near the end of the breeding season having a reproductive advantage over 1M and 2M female siblings. This hypothesis follows from Christian's (1970) proposal that animals which are

inhibited from maturing sexually are not as likely to be attacked (and thus subjected to severe stress) and dispersed as would be the case for sexually mature mice. The immature animals that remain in a population during the winter should therefore serve as the initial breeders at the beginning of the next breeding season.

In summary, it is predicted that for 0M female mice to have a greater probability than 2M females of mating and producing young that survive, population density would need to be low, which would be the case at the end of winter (the beginning of the breeding season). Thus, 0M females that are in litters produced at the end of the breeding season might have the greatest probability of surviving and eventually reproducing the next spring. On the other hand, the 2M females in litters produced at the beginning of the breeding season might have the greatest probability of surviving and producing offspring during that season. Again, a basic assumption of this model is that the overwhelming majority of animals that are dispersed from the natal territory die (Lidicker 1975). Any trait that increases the capacity of an animal to remain and reproduce in the natal territory should thus be adaptive. It is possible, however, that the relative reproductive fitness of any dispersing animal that does successfully colonize a new environment is so high that this premise is invalid. Whether dispersing animals should be considered to have a low reproductive fitness is thus unresolved.

Since multiple uterine residence is obviously genetically based, I propose that the potential for transport of steroids between fetuses (i.e., intrauterine position effects) in mice and rats, and quite likely some other polytocous mammals, evolved due to its adaptiveness. The results of laboratory experiments suggest that 0M, 1M and 2M male and female mice may each have a reproductive advantage in a particular type of environment. Thus, the presence of hormone-mediated variation in phenotype due to intrauterine position may, itself, have been selected for. This hypothesis does not imply the operation of group selection (Wilson 1975). Proponents of group selection believe that intrinsic mechanisms that serve to limit overcrowding in any population evolved for the benefit of the group or population rather than the individual. I propose that the putative relationship between differences

in phenotype mediated by intrauterine position and population dynamics is a consequence of selection for such variance at the individual level rather than for the good of the population. Presumably, in some environmental situations, a mother that produced offspring that were not all similar in phenotype (due to intrauterine position effects) would have a greater probability of some of her offspring surviving than would a mother whose offspring were quite similar in phenotype. Such variation in phenotype should be particularly adaptive in colonizing species which have the potential for irruptive population growth, since offspring born at different times are likely to experience radically different social environments. In this regard, it is interesting that many of the differences in female mice due to prior intrauterine position have been found to relate to the transmission of and response to social cues (vom Saal, Bronson, 1978; vom Saal 1981).

Real (1980) discussed environmental conditions under which the production of offspring that varied would be adaptive and proposed that when resources are uncertain (for instance at the beginning of the breeding season), the strategy for maximizing fitness would involve a mother producing variable offspring. But, when resources are predictable, as would be the case in a high density population where intense competition already existed and resources were already scarce, then production of variable offspring would not be adaptive. Instead, production of offspring that all had a similar phenotype that was optimally suited to the environment (i.e., offspring with a 2M phenotype), would be adaptive. Real (1980) focused on variance in phenotype that presumably reflected variance in genotype. But, the intrauterine hypothesis (that variation in phenotype due to intrauterine position is adaptive in mice) represents an extension of Real's "fitness-uncertainty model", since the intrauterine hypothesis also predicts that in an uncertain environment, diversity is adaptive.

The functioning of the immune system is modulated by gonadal steroids, and animals that have the highest sensitivity to testosterone tend to have the least ability to respond to challenges to the immune system (Cohn 1979). Both 2M males and 2M females appear to be more sensitive to testosterone than 0M males and 0M females (vom Saal,

Bronson 1978; vom Saal, Grant, McMullen, Laves 1983). Since the offspring of stressed mice are all charactistic of 2M animals in phenotype, a correlate of the elimination of the intrauterine position phenomenon in the offspring of stressed females may be a general decrease in resistance to disease in the next generation. This hypothesis is currently being examined. Mice with a 2M phenotype may be found to be less resistant to disease than are mice with a 0M phenotype; this could decrease the reproductive advantage of having a 2M phenotype at times of high population density, when the threat of disease is greatest. But, 2M males and 2M females appear to be the most aggressive and dominant mice. While subordinate animals in high density populations may be severely stressed (and as a result exhibit a decrease in immune function), this does not appear to be the case for dominant animals (Christian 1971). It is possible that future experiments will reveal that there is a general decrease in the potential to withstand a challenge to the immune system in 2M animals relative to 0M animals. But, the prenatal bias toward enhanced aggression and high dominance status (and thus an absense of heightened adrenal function during periods of high population density) may still result in 2M animals having the greatest probability of surviving and reproducing in high density populations.

In an initial study to test one prediction of the intrauterine hypothesis, 8 0M or 8 2M juvenile CF-1 female mice were placed in a 2 ft by 2 ft chamber with 4 nest areas and one adult male (5 replicates per group). The chambers containing the 8 2M females had significantly fewer surviving young than did the chambers containing the 8 0M females when the populations were censused each week, despite the fact that no difference in the number of young produced by the 0M and 2M females was observed on the day the young were born (vom Saal 1981). An important aspect of this study is that the animals were confined together in an environment which did not contain adequate nest areas for all females. Thus, the crowding together of the highly aggressive 2M females in an enclosed environment was predicted to result in a decrease in infant survival.

The finding from the comparison of the reproductive success of CF-1 0M and 2M female mice described above is consistent with the hypotheses of both Christian and

Chitty-Krebs that in an environment in which the animals are highly aggressive, survival of offspring will be reduced (Christian, Davis 1964; Christian 1971; Chitty 1967; Krebs 1978). But, the results suggest that the existance of variation in phenotype due to the intrauterine position phenomenon, rather than variation in genotype as proposed by Chitty (1967), might account for changes in population dynamics as population density increases.

We are now conducting studies with the Cesarean delivered 0M, 1M and 2M offspring of wild house mice (F_{2-4} generation) trapped in a field near Columbia, Missouri. We are utilizing the same environments described above for testing the CF-1 mice. 0M, 1M and 2M wild female mice have been placed together in one environment rather than in separate environments, and the reproductive performance of each individual is being monitored. Other studies utilizing 12 x 20 ft predator-proof enclosures that are located in the field in which the mice were trapped are planned.

THE EVOLUTION OF HORMONE-MEDIATED VARIATION IN PHENOTYPE

An issue that is seldom referred to in the literature on early hormonal effects on adult phenotype is why during early life hormones should regulate the functioning of a substantial segment of the genome. Hormone-mediated traits are presumed to involve a complex interaction of genes, and are therefore hypothesized to be polygenic (cf. vom Saal, Howard 1982). Unfortunately, the complexity of developing models involving selection for polygenic traits has tended to force quantitative geneticists to focus on simple systems. The positive correlation between variation in phenotype within a population and the rate of evolution for the population is well established (see Figure 9). Quantitative geneticists tend to presume that variation in phenotype reflects variation in genotype and thus ignore the potential contribution of nongenetic sources of variation in phenotype (such as hormone-mediated variation) to the capacity of organisms to adapt to environmental change. The component of variance in phenotype that is due to hormonal effects on gene expression therefore tends to end up in a residual term labelled "error", which supposedly is unexplainable. What

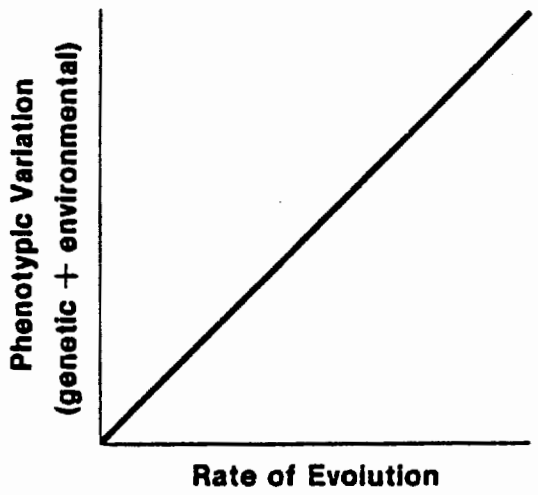

Fig. 9. The relationship between variation in phenotype in a population (mediated by both genetic and environmental factors) and the rate of evolution for the population.

needs to be emphasized is that it is an animal's phenotype which determines whether it is adapted to a particular environment and thus will survive and reproduce. Thus, events which occur as a normal part of development and therefore predictably influence phenotype (such as the possibility of developing next to male or female fetuses in polytocous mammals) also play a role in natural selection.

Territoriality and aggressiveness have a genetic component in mice since there are aggressive strains and nonaggressive strains (Karczmar, Scudder 1969; Simon 1979). But, the expression of genes which regulate aggression is mediated hormonally, and in mouse strains that have the potential to exhibit aggression, the level of aggressiveness is modulated by hormones during early life such that some individuals have a tendency to be more

aggressive than other individuals. For example, the development of aggression varies as a function of fetal hormone titers in both male and female mice based on intrauterine position. Since the tendency of 2M males and 2M females to be aggressive is probably mediated by hormones during early life rather than directly by genotype, this tendency toward high aggressiveness will not be heritable as predicted by Chitty (1967). The intrauterine hypothesis thus predicts that attempts to develop aggressive strains of mice by breeding nondispersing animals and nonaggressive strains by breeding animals that are dispersed from high density populations would be unsuccessful. At this time, there is no supportive evidence that dispersing animals differ from nondispersing animals in terms of genes that influence aggressiveness (cf. Davis 1978; Lidicker 1978).

It might be proposed that all of the characteristics which have been found to vary among siblings due to intrauterine position could somehow have evolved to vary from litter to litter due to shifts in gene frequencies (i.e., one mother might produce an entire litter of 2M-like offspring while another mother might produce an entire litter of 0M-like offspring). In fact, the basic tenet of the Chitty-Krebs hypothesis (Krebs 1978) is that within microtine populations, shifts in the frequencies of alleles for genes mediating aggression and reproductive performance lead to changes in population dynamics when population density becomes high. I suggest that it is unlikely that a shift in gene frequency at one or even many loci could lead to the broad array of differences in phenotype in the next generation that have been found to be due to intrauterine position within a litter. The exception would be alleles that code for the synthesis of an enzyme involved in the production of a gonadal steroid (such as testosterone) or the production of a cytoplasmic steroid receptor in target tissues. This follows from the fact that the expression of genes regulating aggression is mediated by gonadal hormones. In fact, comparisons of inbred C57BL/6J and DBA/2J mice have led to the hypothesis that the observed differences between these strains of mice in aggressiveness and numerous other traits relating to reproduction are mediated by strain differences in the hormonal milieu during fetal life rather than differences in the frequencies of genes independently regulating each trait (Svare, Kinsley, Mann, Broida 1983).

The functioning of steroid hormones requires the presence of hormone receptors in target tissues, and variation in phenotype can also occur due to differences in steroid receptor concentrations. An example of this is the testicular feminization (Tfm) syndrome, which is thought to be an X-linked mutation. This mutation results in a decrease in the production of androgen receptors in genetic males, such that Tfm males secrete testosterone but are unable to respond to it. Tfm males are thus not masculinized during fetal life (for example, Tfm male mice do not exhibit male sexual behavior). This phenomenon occurs in many species including humans, mice and rats (Fox, Olsen, Vito, Wieland 1982). Thus, Tfm male mice differ from normal males in sexual performance, but not because of changes in the frequencies of genes mediating sexual behavior. The assumption that there is a direct relationship between the frequency of an allele and variation in hormone-mediated behaviors in populations of mammals is thus far too simplistic.

A correlate of the hypothesis that hormone-mediated variation in phenotype due to intrauterine position has been selected for is the proposition that other developmental events which would result in a general decrease in reproductive capacity in male and female mice have apparently been selected against. An example is the absence of the phenomenon referred to as the freemartin syndrome in polytocous mammals. The freemartin refers to the situation in cattle (a monotocous species) where a male and a female fetus develop together in utero and the female co-twin virtually always develops ovo-testes and is sterile (Marcum 1974). The male co-twin is also usually sterile or exhibits a decrease in reproductive performance (Dunn, McEntee, Hall, Johnson, Stone 1979). This phenomenon is not mediated by androgens (Jost 1972), but, instead, has been proposed to result from the transfer of tissue between the male and the female via vascular anastomoses between the chorionic membranes of each of the fetuses (Ohno, Christian, Wachtel, Koo 1976). As is sometimes the case with arguments concerning evolution, the reasoning here is circular. I am proposing that since the freemartin syndrome does not occur in polytocous mammals, it may have been selected against. But, it is interesting that even in the rare cases in which the placentae of contiguous mouse fetuses appear to be fused

on gross examination, a connective tissue band is found to separate the individual placentae (McLaren, Michie 1959). This may serve to inhibit the transfer of tissue from one fetus to the other and thus protect against the possibility of sterility of the offspring. Again, the intrauterine position phenomenon in rodents appears to be mediated by the transfer of sex steroids from one fetus to another, which does not result in sterility in any offspring (vom Saal 1981).

In studies involving the genetic bases of behavior in polytocous mammals, controlling for possible litter effects is considered to be essential (for example, by utilizing split-litter fostering techniques, using the mean for all animals in a litter as one data point, or treating litters as an independent variable). What my findings from studies of the intrauterine position phenomenon in CF-1 mice reveal is that, within a litter, 0M, 1M and 2M siblings differ significantly in phenotype from each other, but animals with a 0M phenotype within one litter are very similar in phenotype to animals in other litters that also have a 0M phenotype (the same would be true for 1M and 2M animals).

In summary, the objective of this chapter was to review the differences among male and among female mice which have been found to be due to intrauterine position and to suggest some answers to the questions: 1. which hormones mediate the intrauterine position phenomenon; 2. what might be the selective advantage to a female mouse of having her offspring vary predictably in a broad range of reproductive characteristics as a result of developing by chance next to fetuses of the same or opposite sex; and 3. what effects might intrauterine position have on individual reproductive success and population dynamics in mice. Research is currently being conducted to examine these questions with both laboratory and wild house mice.

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