GONADAL HORMONE REGULATION OF BEHAVIOUR IN THE MONGOLIAN GERBIL

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ABSTRACT

Gonadal steroids have been found to influence a variety of behaviours in mammals. However, many of the models and hypotheses concerning the organizational and activational effects of gonadal steroids are based on data obtained primarily from the rat. Examination of the effects of gonadal steroids on behaviour in rodent species which differ from the rat in their ecological niche would aid in determining the generality of such hypotheses and models. The Mongolian gerbil, a relatively new laboratory animal, inhabits an environment quite different from that of the rat and could provide useful comparative data. The general purpose of the present study was to examine the effects of gonadal steroids on feeding and sexual behaviour in the female gerbil. In addition, the hypothesis that the extent of perinatal androgen-induced defeminization can be used to predict whether androgens or estrogens will be the primary regulator of food intake and body weight in adulthood (and vice versa) was tested.

In the first series of experiments, it was found that, in the female gerbil, as in other female rodents, ovarian steroids are primary regulators of sexual receptivity. When compared to other female rodents, the system regulating receptivity in the gerbil appeared to be less sensitive to estrogen as well as more dependent on the synergistic action of progesterone with estrogen for the induction of receptivity.

The second series of experiments indicated that adrenalectomy significantly decreased sexual receptivity in the
female gerbil, an effect not observed in other rodents. Further examination of this effect suggested that adrenal steroids, in addition to ovarian steroids, were essential for the elicitation of high levels of receptivity. However, although particular combinations of adrenal steroids did restore normal responding in animals tested with estrogen alone, they failed to eliminate the decreased receptivity observed when estrogen and progesterone were administered. Thus, in the gerbil, unlike other rodents, adrenalectomy appears to decrease the sensitivity of the system regulating receptivity to progesterone.

The effects of ovarian steroids on food intake and body weight in male and female gerbils were examined in the third set of experiments. It was found that, in the gerbil, as in most other rodents, ovarian steroids appeared to be the primary hormonal regulators of food intake and body weight. However, unlike effects observed in other rodents, ovarian steroids were not found to be responsible for the sex difference in food intake and body weight, indicating the involvement of a non-hormonal factor. It was also suggested that interspecific differences in hormone-neurotransmitter interactions might be responsible for the differences in the hormonal effects on food intake and body weight.

The findings that ovarian steroids were the prime hormonal regulators of food intake and body weight in the gerbil led to the prediction that perinatal androgens would defeminize the gerbil. At present, only rodents in which ovarian steroids exert the primary hormonal influence on food intake and body weight are defeminized by perinatal androgens. The final set of
experiments indicated that, like other such rodents, the gerbil was also defeminized by perinatal androgens. In contrast to effects in the rat, none of the experimental treatments resulted in behavioural masculinization although significant physiological masculinization was observed. The ability of present models of the differentiation process to account for these results was examined and a more general concept proposed. The concept proposed also provides a possible link between interspecific differences in the differentiation of sexual behaviour and the differentiation of other hormonally mediated behaviours.

The results of the present study indicate that the organization and activational effects of sex steroids in the Mongolian gerbil are unique in several respects and that further investigation should aid in developing and refining present models of the hormonal regulation of development and behaviour.
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INTRODUCTION

Gonadal steroids have been found to influence a large number of behaviours in vertebrates. Not only do they determine the morphology of the external genitalia, they also influence cognitive abilities, food consumption, aggression, and a variety of other behaviours not directly associated with sexual behaviour.

It is now generally accepted that gonadal steroids, the so-called sex hormones, exert two types of effect, depending on the developmental stage of the animal. During prenatal development and, in some species, early postnatal life, sex hormones exert an organizing effect on hormone sensitive tissues. This action is responsible for the morphology of the external genitalia, changes in neuronal organization, and the sensitivity of various tissues to specific gonadal steroids. The activational effects of sex steroids are most apparent at the time of sexual maturity, when hormone levels rise and lead to further changes in body morphology and behaviour. For example, increased sexual activity, menarche, the development of pubic, axillary, and facial hair, and the deepening of the male voice are all attributable to the activational effects of gonadal steroids.

The effects of sex hormones are also observed in a variety of behaviours although, in the human, these effects are not nearly as obvious as they are in other species. One of the major confounds in determining the effects of sex steroids on human behaviour arises from the difficulty in separating cultural from biological influences. Money & Ehrhardt (1972)
found that cultural and social influences during development were more important than genetic constitution or particular hormones in determining psychosexual development and gender identity. That is, the sex of assignment and rearing can override biological determinants. Nonetheless, Ehrhardt and coworkers (1967, 1968) have found that females exposed to androgens prenatally exhibit toy preferences, fantasy contents, and certain cognitive abilities that resemble those seen in males. However, the evidence is far from convincing and many argue that the differences are due to biased rearing rather than, or in addition to, any innate sex difference (Carlson, 1980, pp. 334). It is doubtful that the study of humans alone will ever provide sufficient information to allow the separation of cultural and biological contributions to human behaviour.

In order to investigate the influence of hormones on sexual differentiation and behaviour in the absence of strong cultural influences, researchers have turned to non-human animals. Indeed, the use of non-human primates has provided evidence that at least some of the sex differences in play behaviour, such as amount of rough and tumble play, are influenced by early hormonal environment (Goy, 1968, 1970; Phoenix, 1974). However, the primate, although most similar to the human in many respects, is not often used for research on the more basic organizational and activational effects of sex steroids. The highly developed and intelligent nature of the non-human primate raises ethical questions concerning its use as a research animal in studies that do not require these characteristics. Furthermore, primate research costs are becoming increasingly
more prohibitive as the availability of primates for research continues to decline. On the other hand, the laboratory rodent possesses all of the essential features required for research into the fundamental aspects of the hormonal regulation of differentiation and behaviour. Thus, many researchers have turned to the laboratory rodent with the aim of developing models of the influence of hormones on behaviour and development. It is from research such as this that most of our present knowledge of hormonal influences is derived.

Investigations in the laboratory rodent have indicated that the organizing actions of androgens during sexual differentiation are responsible for a great many sex differences in behaviour observed in adulthood. One of the most obvious sexually dimorphic behaviours is sexual behaviour itself. In the female rodent, sexual behaviour is determined primarily by the circulating levels of the ovarian steroids estrogen and progesterone. In most laboratory rodents, the estrous cycle is four to five days in length with estrus, the period of receptivity, lasting slightly under one day. Low levels of estrogen and progesterone characterize non-receptive periods, or diestrus, while increasing estrogen levels plus a progesterone surge bring about estrus (Komisaruk, 1978). The cyclic nature of estrogen and progesterone secretion reflects the cyclic nature of hypothalamic, and thus pituitary, hormone release. Under experimental conditions, artificial estrus can be induced by the administration of estradiol benzoate (EB) and progesterone (P) in the proper dose and temporal pattern. Sexual behaviour in the male rodent, on the other hand, is
dependent upon circulating androgens. In male rodents, the release of hypothalamic and pituitary hormones is tonic rather than cyclic and the secretion of androgens is relatively constant. Castration, which removes the major source of endogenous androgens, results in a loss of sexual activity while injections of testosterone propionate (TP) restore sexual activity. Females given androgens around the time of birth fail to exhibit cyclic release of pituitary hormones and males castrated at birth will, under appropriate conditions, exhibit the cyclic pattern of hormone release. This indicates that perinatal androgens, rather than any strictly genetic factor, are responsible for the tonic hormone release observed in the male while the absence of perinatal androgens permits the cyclic pattern of hormone release observed in the female.

The same generalizations can be made about the organizational effects of androgens on a variety of behaviours and can be summarized as follows: In those behaviours which are influenced by the organizational action of androgens, the presence of androgens during the period of sexual differentiation will produce responses to sex steroids in adulthood that are typical of the male while the absence of androgens during this period will produce responses in adulthood that are typical of the female. This much is consistent in all rodents studied to date and there seems little controversy about the matter.

Most of the generalizations about the effects of perinatal androgens on behaviour are based on studies done in the rat. This has led to a tendency to overgeneralize. For example, many
researchers refer to the defeminizing action of perinatal androgens, implying that this is a necessary consequence of perinatal androgen exposure in female rodents. The hamster, which is not defeminized by perinatal androgens, provides a clear demonstration that the end result of the organizational action of androgens is not necessarily identical in all species. It is somewhat surprising then that the hamster frequently receives little more than a paragraph in papers dealing with the organizational effects of androgens. For example, Plapinger & McEwen (1978) devote one paragraph to the hamster out of eight pages on androgen's effects on sexual differentiation of behaviour patterns in rodents and merely inform the reader that the hamster is different. The hamster is not even mentioned in Ward's (1974) otherwise comprehensive chapter on the differentiation of sexual behaviour. Surely the hamster, differing from other rodents, could provide some important comparative information that would increase our understanding of the hormonal mediation of behaviour. Yet there seems to be a pervasive tendency in the literature on hormonal influences in rodents to ignore or dismiss without comment the results obtained in the hamster. Although never overtly stated, the general impression one gets is that because the rat, mouse, and guinea pig are so similar in many respects, they comprise the norm while the hamster, being overtly different, is abnormal. Clearly, the rat has become the comparison species, probably because it is used far more often than any other rodent for hormonal research. There is nothing wrong with using the rat as a standard for comparison. However, researchers tend to forget
that the laboratory rat (classified as *Rattus albinus* by Lockard, 1968) is as domesticated as the dog and is about as comparable to a wild rat (*R. norvegicus*) as a dog is to a wolf. After about a century of selective breeding, the common laboratory rat exhibits many differences when compared with the wild rat. For example, in the laboratory rat, body length and weight are greater, the pituitary, thymus, thyroid, and parathyroid are larger while the brain, heart, spleen, adrenals, liver, and kidneys are smaller than in the wild rat (Lockard, 1968). Furthermore, the laboratory rat exhibits less aggression and different social, maternal, and feeding behaviours when compared to the wild rat (Lockard, 1968). Given these differences, the experimental findings in the laboratory rat should not be taken as necessarily indicative of behaviour in the wild rat, much less the behaviour of other rodents.

The unfortunate tendency of some researchers to view the hamster as an exception among rodents can result in concepts, theories, models, and hypotheses that are oversimplified and restricted in their applicability. It should be clear that no model or hypothesis concerning general principles of hormone action can be based upon the results obtained for a few similar species. Yet, we find that many of the points made by Beach (1971) in his timeless paper on the hormonal factors controlling differentiation and copulatory behaviour in the ramstergig and related species still hold true today. There is still a tendency to act as if all laboratory rodents are like each other and are also like the ramstergig. In the ramstergig, "all relationships between its anatomical, physiological, and
behavioral characteristics are completely known and never subject to controversy." (Beach, 1971, p.249). Furthermore, "the neurontogeny of the ramster gig follows precisely current theories describing the "organizational" action of gonadal hormones." (Beach, 1971, p. 249). Finally, male and female sexual behaviour are "mutually exclusive in ramster gigs, and therefore the ramster gig is what is technically known as a "straight" species. Males always engage exclusively in masculine sexual behaviour and females display only the feminine mating pattern." (Beach, 1971, p.251).

The tendency to look for similarities between rodents may have been advantageous in the early stages of hormone research, and could be now if researchers would also look for differences and develop models which could account for both interspecific differences and similarities. However, there is little room for the investigation of between species differences in the hormonal regulation of differentiation and behaviour when the total number of commonly used laboratory rodents does not exceed five (rat, mouse, hamster, guinea pig, gerbil). Of these five, the guinea pig is no longer used frequently, the rat and mouse are fairly similar in known hormone-behaviour relationships, the gerbil is a newcomer, and the hamster differs from the rat and mouse in many respects. Furthermore, the rat is used primarily for studies on hormones and differentiation, sexual behaviour, and feeding behaviour, the mouse is used extensively and almost exclusively for studies on inter-male aggression, the guinea pig was used for studies on differentiation, and the gerbil is used primarily for studies on scent marking and aggression. Thus,
even for the laboratory rodents available, each is used primarily for the study of the hormonally mediated behaviour it displays best. For example, both hamsters and gerbils scent mark. However, the marking response and the effects of hormones on scent gland tissue are most obvious in the gerbil, which has become the animal of choice for this type of research. This type of selective examination of behaviours within a species results in a relative paucity of information in some areas where comparisons would be useful. It also provides a plethora of information, often redundant, in a few specific areas for a few specific species. For example, there are exceedingly few studies that have examined the effects of perinatal TP administration on sexual behaviour in the mouse although there are a large number which have examined the effects of perinatal TP on aggression in this species. It would seem that two lines of action need to be taken to remedy this situation. First, areas of sparse information in existing laboratory rodents should be identified and steps taken to fill these gaps in our knowledge. Second, every attempt should be made to increase the number of rodent species presently available in the laboratory. Furthermore, rodents which live in very different natural habitats would be the most desirable new addition. Studying the differences between rodent species of different environments will allow researchers to determine which hormone-behaviour relationships reflect underlying principles of hormonal action and which hormone-behaviour relationships reflect specific adaptations to particular environments. Granted, it is not simple to introduce a rodent into a laboratory setting and begin
a breeding colony. However, bringing new rodents into the laboratory is essential to the study of general principles and mechanisms which underlie physiological and behavioural development in rodents and indeed, in mammals.

Until recently, the hamster appeared to be the only unusual laboratory rodent. However, the use of the Mongolian gerbil as a laboratory rodent has increased greatly in the last decade and there are clear indications that the gerbil differs in many respects from other laboratory rodents. The hormonal mediation of food intake and body weight is one example of this difference. To date, the gerbil is the only rodent examined in which sex steroids may not be primarily responsible for sex differences in food intake and body weight. The gerbil also differs from other rodents in the ability to utilize saline and conserve water (Winkelmann & Getz, 1962; Wong, 1977), the type of territorial behaviours displayed (Rieder & Reynierse, 1971; Yahr, 1981), social and socio-sexual behaviour (Ågren, 1976, 1980; Ågren & Meyerson, 1973, 1975, 1977a,b, 1978; Roper & Polidiioukis, 1977), and perhaps a variety of other behaviours as well. In addition, like the hamster and unlike the rat, the major adrenal steroid of the gerbil is cortisol rather than corticosterone (Oliver & Péron, 1964). Furthermore, the gerbil is a desert animal, as is the hamster, and one would expect this harsh environment to produce a variety of physiological and behavioural adaptations not seen in the rat. Thus, the information concerning the hormonal regulation of differentiation and behaviour in the gerbil would aid in developing and refining present models of the actions of sex
steroids on development and behaviour in rodents.

Although the gerbil has been used extensively for studies on the hormonal regulation of scent marking, aggression, and, in the male, sexual behaviour, relatively little work has been done on other hormonally mediated behaviours. This is especially the case in the female gerbil, which has not received a great deal of attention. Apparently there are only two studies (Maass & Wade, 1977; Roy, Maass & Wade, 1977) that have examined the effects of sex steroids on food intake and body weight in the gerbil. Furthermore, there appears to be only one study in which neonatal TP treatment was administered for any reason and in this study, scent marking, rather than sexual behaviour, was examined (Turner, 1975). Finally, although reproductive behaviour in the intact female gerbil has been studied (e.g., Barfield & Beeman, 1968; Norris & Adams, 1974, 1981a,b,c; Vick & Banks, 1969) and the behaviour repertoire described (Burley, 1979), few studies have manipulated sex steroids to determine their influence on sexual behaviour. Kuehn & Zucker (1968) found that the administration of 6 μg EB 45 hr before testing plus the administration of 400 μg P 9 hr prior to testing would elicit high levels of receptivity in the female gerbil. McDermott & Carter (1980) and McDermott, Fischer, & Carter (1980) have done some preliminary work which indicates that acute EB administration is not sufficient to elicit receptivity and that P initially facilitates receptivity in these animals while later exerting an inhibitory effect. To the best of my knowledge, there are no other studies that have examined the effects of sex steroids on these behaviours in the gerbil. It
would appear that very little is known of the influence of sex hormones on sexual differentiation, sexual behaviour, and food intake and body weight regulation in the gerbil.

The gerbil is from an environment quite unlike that of the rat. It is inevitable that a variety of adaptations to this environment have occurred, some of which will be reflected in hormonally mediated behaviours. The present series of studies in the female gerbil will: (1) determine the dose and temporal parameters of ovarian steroid mediation of sexual receptivity, (2) examine the effects of ovarian steroids on food intake and body weight regulation, (3) investigate the effects of perinatal androgens on sexual differentiation and the capacity to display sexual behaviour in adulthood, and (4) examine the possibility that the extent of naturally occurring sexual differentiation can predict interspecific differences in the hormonal regulation of food intake and body weight. Further study of hormonal effects in the gerbil should bring us one step closer to an understanding of hormonal actions as well as providing information that will assist in separating adaptive mechanisms from more universal mechanisms of hormone action.
SECTION 1a

Dose and Temporal Parameters of Ovarian Steroid Effects on Sexual Receptivity

Estrogen and progesterone are the primary regulators of sexual receptivity in the female rodent. Ovariectomy abolishes receptivity whereas estrogen and progesterone restore receptivity in ovariectomized animals if the appropriate hormone doses are administered in a specific temporal sequence. The minimum doses of estrogen and progesterone required, the frequency of estrogen administration, and the temporal sequence of the two steroids for the elicitation of maximal receptivity varies somewhat among rodent species (Beach, 1976; Feder, 1978). For example, female rats and hamsters will exhibit high levels of receptivity when given 5-6 μg EB 24-48 hr before testing plus 500 μg P 3-4 hr prior to testing (Carter, Landauer, Tierney, & Jones, 1976; Hardy & de Bold, 1971) while some strains of female mice require more prolonged estrogen treatment for the elicitation of maximal receptivity (Gorzalka & Whalen, 1974). Thus, treatments that elicit high levels of receptivity in one species do not necessarily elicit high levels of receptivity in a different species since dose and/or temporal patterning requirements may differ.

Few researchers have investigated the dose and temporal parameters of ovarian steroid effects on receptivity in the female gerbil. McDermott & coworkers (1981) have determined that in the gerbil, as in other rodents, chronic P administration exerts an initial facilitatory effect on
receptivity but later exerts an inhibitory effect. Kuehn & Zucker (1968) found that the administration of 6 μg EB 24-48 hr before testing plus the administration of 400 μg P 9 hr prior to testing would elicit high levels of receptivity in ovariectomized female gerbils. However, their choice of that specific regimen appears arbitrary. It is possible that levels of receptivity similar to those observed by Kuehn & Zucker (1968) might be obtained with considerably lower doses which would approximate endogenous steroid levels. In addition, when examining the effects of experimental variables on sexual receptivity, it is frequently desirable to use doses that do not elicit maximal receptivity. If an animal is already responding maximally, any facilitatory effect of the independent variable on receptivity will be masked. The use of doses which induce moderate levels of receptivity overcome this problem while still allowing room for the observation of inhibiting effects as well.

Thus, it would appear that before any non-parametric research can be performed on the sexual behaviour of the female gerbil, dose and temporal thresholds must first be established.

**GENERAL METHODS**

All experimental animals were purchased from Tumblebrook Farms (West Brookfield, MA) at 90 days of age, housed in pairs, and kept on a reversed, 12:12 hr light-dark cycle with food and water available ad libitum. To eliminate individual differences in circulating hormone levels caused by experimental conditions, all animals were bilaterally ovariectomized. All EB-primed animals received 10 μg EB 48 and 24 hr before testing unless
otherwise noted. Also, when tests of sexual receptivity occurred more than one week after surgery, animals received a priming dose of 10 μg EB one week prior to testing. EB and P were dissolved in peanut oil and all injections were given subcutaneously. Plexiglas chambers containing about 3 cm San-i-cel bedding material and measuring 30 cm (l) x 30 cm (w) x 45.5 cm (h) were used for sexual testing. All tests occurred between 2 and 6 hr after the onset of the dark portion of the light-dark cycle. Tests of sexual receptivity lasted for 20 min or for an interval of 10 mounts with pelvic thrusting by a sexually vigorous male, whichever came first. The elicitation of lordosis (the elevation of the hindquarters and dorsoflexion of the tail, exposing the perineal region and facilitating male intromission) was recorded and the lordosis quotient (LQ, lordosis/mount x 100) was used as the primary indicator of sexual receptivity. The author has observed that unreceptive female gerbils are quite aggressive towards males attempting to mount. Thus, because the absence of mounts reflects a lack of receptivity rather than a lack of male sexual activity, an LQ of 0 was assigned to the subject.

Most experiments required that testing be carried out over several hours with only a portion of the animals being tested at any given time. Thus, whenever possible, counterbalancing was incorporated into the experimental design with animals from each group being tested at the various subset times.

For the purposes of the present work, the term "maximal receptivity" will be used to refer to LQs near 100 while the term "high levels of receptivity" will be used to refer to LQs
of 70 or above. "Moderate receptivity" will be used to refer to LQs between 41 and 69 and "low receptivity" will be used to refer to LQs of 40 and below.

EXPERIMENT 1

Because animals cannot all be tested at the same time, knowledge of the duration of the synergistic effects of P with EB on receptivity is essential before carrying out other tests of sexual receptivity. Lisk (1960) found that maximal receptivity in EB-primed female rats occurred about 3 hr after P administration. Data on the duration of receptivity in rats after estrogen and progesterone treatment is relatively sparse, with most researchers (e.g., Quadagno, McCullough, & Langan, 1972; Whalen & Gorzalka, 1972; Whalen, 1974) testing 3-6 hr after P administration. Kuehn & Zucker's (1968) study suggested that female gerbils would be receptive for more than 9 hr after P treatment. Thus, in the present study, the decision was made to begin testing 3 hr after P injection and continue beyond the 9 hr period examined by Kuehn & Zucker (1968). Then, if necessary, the time delay between P administration and sexual testing could be reduced or extended.

Method

After a one week recovery period, 36 animals were primed with EB and randomly assigned to one of four groups (n=9/group), with testing occurring either 3, 6, 9, or 12 hr after the administration of 500 ug P. Since receptivity appeared equal in all of these groups, additional groups were tested 0, 1, 2, 3 (n=9), 12, 15, 18 (n=8), and 18, 21, and 24 (n=13) hr after P
administration. Animals served in only one group with each group being represented during a given subtest period.

Results and Discussion

The results suggested that maximal receptivity occurred 2 hr after the administration of P and continued for about 16 hr (Figure 1). After determining that the pairs of overlapping data points at 3, 12, and 18 hr were not significantly different, data for each of these points were combined, giving a total of 11 groups (i.e., 0, 1, 2, 3, 6, 9, 12, 15, 18, 21, and 24 hr). A one-way analysis of variance performed on the data revealed a significant effect of time between P administration and testing, $F(10,128) = 24.43$, $p < 0.0001$. A subsequent Newman-Keul's analysis indicated that the mean LQs 0, 21, and 24 hr after P administration were significantly lower than the mean LQs for all other test times, $p < 0.05$. Furthermore, although the mean LQ at 1 hr was significantly greater than the mean LQs at 0, 21, and 24 hr, $p < 0.05$, it was also significantly less than the mean LQs at 2, 3, 6, 9, and 12 hr, $p < 0.05$, and did not differ from the mean LQs at 15 and 18 hr.

These results indicate that the onset of receptivity begins about 1 hr after P administration, reaches a maximum 2 hr after P administration, and continues for about 16 hr. Sometime between 18 and 21 hr after the administration of P, receptivity sharply declines and, by 24 hr after the administration of P, receptivity is once again at a minimum. Thus, if 10 µg EB is administered 48 and 24 hr prior to testing, the administration of 500 µg P will provide a large time window in which animals can be tested and maximal receptivity insured.
FIGURE 1. Sexual receptivity in female gerbils at various times after progesterone administration. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Animals received 10 µg estradiol benzoate 48 and 24 hr before testing and 500 µg progesterone 0-24 hr prior to testing. Breaks in graph represent tests with different sets of animals.
EXPERIMENT 2

It has been found that the dose of P administered influences the degree and duration of receptivity in the hamster (Ciaccio & Lisk, 1971) and the rat (Fadem, Barfield, & Whalen, 1979). It is possible that the 500 µg P used in Experiment 1 was much greater than required to elicit maximal receptivity in EB-primed gerbils. This experiment was designed to investigate the possibility that a lower dose of P might elicit maximal receptivity and to determine an appropriate dose of P for situations in which only moderate levels of receptivity would be desired (i.e., when a facilitation effect might occur).

Method

After a one week recovery period, 60 EB-primed animals were randomly assigned to one of six groups (n=10/group) receiving either 0, 10, 50, 100, 250, or 500 µg P 3 hr prior to testing. This time delay was chosen because Experiment 1 indicated that all animals receiving 500 µg P would be maximally receptive at this time.

Results and Discussion

The results suggested that 10 µg P elicited minimal sexual responding (LQ ≤ 20), that 50 µg P elicited moderate responding (LQ ≤ 60), and that 100 µg or more of P elicited high levels of sexual receptivity (LQ ≤ 100)(Figure 2). A one-way analysis of vari performed on the data confirmed a significant effect of dose, F(5,54) = 22.12, p < 0.0001. A Newman-Keul's comparison revealed that receptivity in animals given 10 µg P did not differ from that observed in controls, p < 0.05. In addition, although 50 µg P did significantly increase receptivity when
FIGURE 2. Sexual receptivity in female gerbils administered various doses of progesterone. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Animals received 10 \( \mu \)g estradiol benzoate 48 and 24 hr before testing and 0-500 \( \mu \)g progesterone 3-4 hr prior to testing.
compared to oil controls, \( p < 0.05 \), doses of 100 \( \mu g \) P and
greater elicited significantly higher scores than did the 50 \( \mu g \)
dose, \( p < 0.05 \). Thus, it would appear that a dose of 50 \( \mu g \) P
should be used when the elicitation of moderate levels of
receptivity is desired while 100 \( \mu g \) P or more should be used to
elicit high levels of receptivity in EB-primed animals. In
addition, the present results suggest that the prolonged effect
of P observed in Experiment 1 was probably due, to some extent,
to the use of a greater than necessary dose of P.

EXPERIMENT 3

It has been found in the rat (Quadagno et al., 1972) and
hamster (Carter & Porges, 1974) that the degree and duration of
receptivity is also influenced by EB dose. Thus, the effects
observed in Experiment 1 may have also been due, in part, to the
dose of EB administered. To investigate this possibility, and
to determine the minimum dose of EB required to elicit maximal
receptivity, the P dose was held constant while the dose of EB
was varied.

Method

Twenty-eight animals were randomly assigned to one of four
groups \((n=7/group)\). All animals received a priming dose of
10 \( \mu g \) EB one week prior to testing and then received either 0.5,
1.0, 5.0, or 10.0 \( \mu g \) EB 48 and 24 hr before testing. A dose of
100 \( \mu g \) P was administered 3 hr prior to testing as Experiment 2
indicated that this dose of P was the lowest that would elicit
maximal receptivity in animals receiving 10 \( \mu g \) EB.
Results and Discussion

Results of the present experiment suggested that only the 10 μg dose of EB was sufficient to elicit high levels of receptivity (LQ>80) when a dose of 100 μg P was administered (Figure 3). A one-way analysis of variance performed on the data indicated a significant effect of EB dose, F(3,24) = 12.94, p < 0.0001. A Newman-Keul's test revealed that the 10 μg EB dose was significantly more effective in eliciting high levels of receptivity than were the other EB doses, p < 0.05. In addition, the 0.5 μg EB dose was as effective as the 1.0 μg dose, p > 0.05, but less effective than other doses, p < 0.05.

EXPERIMENT 4

Experiment 3 indicated that 10 μg EB, but not lower doses of EB, lead to high levels of receptivity when given in conjunction with 100 μg P. However, in the rat, chronic administration of EB can also result in high levels of receptivity (Davidson, Rogers, Smith, & Bloch, 1968; Gorzalka & Raible, 1981; Gray & Gorzalka, 1980). This experiment was designed: (1) to determine the dose-dependent effects of chronically administered EB on sexual receptivity in the gerbil. and (2) to determine if estrogen, in the absence of progesterone, is capable of eliciting sexual receptivity.

Method

Twenty-seven animals were randomly assigned to one of three groups (n=9/group) and received daily injections of 1, 10, or 100 μg EB. Because previous research in rats indicated that EB-
FIGURE 3. Sexual receptivity in female gerbils administered various doses of estrogen. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Animals received 0.5 - 10.0 μg estradiol benzoate (EB) 48 and 24 hr before testing and 100 μg progesterone 3-4 hr prior to testing.
induced receptivity does not occur until Day 3 of EB administration (Bermant & Davidson, 1974, p. 136), this interval between first injection and first test was employed in the present experiment. Testing continued until animals in each group reached asymptotic levels of sexual responding (Day 12 of EB). It should be noted that, during sexual testing, subjects were simultaneously involved in a food intake and body weight study (Experiment 9) and were weighed daily throughout sexual testing.

Results and Discussion

The results of the present experiment suggested that, although 1 and 10 µg of EB were capable of eliciting some degree of sexual receptivity when administered chronically, only the 100 µg dose of EB appeared able to elicit full sexual receptivity (Figure 4). In addition, the effects of chronic EB on sexual receptivity were not observed until Day 6 of EB administration. A 3 x 10 repeated measures analysis of variance performed on the data revealed a significant effect of estrogen dose, $F(2,24) = 10.11$, $p = 0.0007$, a significant effect of test day, $F(9,216) = 46.83$, $p < 0.0001$, and a significant dose by test interaction, $F(18, 216) = 4.70$, $p < 0.0001$. To determine more precisely the nature of the effects, a Newman-Keul's test was performed on each of the significant effects ($\alpha = 0.05$). It was found that the levels of receptivity exhibited by animals receiving 1 and 10 µg EB did not differ significantly from each other, although they were significantly lower than the levels of receptivity exhibited by animals receiving 100 µg EB. In addition, tests occurring on Days 3-6 of EB administration
FIGURE 4. Sexual receptivity in female gerbils tested daily and chronically administered various doses of estrogen. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Animals received daily injections of 1, 10, or 100 μg estradiol benzoate (EB) beginning on Day 1. Testing occurred on Days 3-12.
produced significantly lower receptivity scores overall than tests conducted on Days 7-12. Finally, it was found that the 1 and 10 µg EB groups did not differ from each other on any of the test days while scores of the 100 µg EB group were found to be significantly greater than the other groups from Day 7 of EB onward.

It would appear that only the 100 µg EB dose is sufficient to elicit maximal receptivity when administered chronically while either the 1 or 10 µg EB dose will elicit lower levels of responding. It is of interest that, although 100 µg EB leads to high levels of responding, its influence becomes apparent at about the same time as the lower doses. This suggests that there is a minimal required period of chronic exposure to EB and that this period is independent of EB dose.

GENERAL DISCUSSION

The results of Experiment 1 indicate that maximal receptivity in EB-primed gerbils occurs approximately 2 hr after the administration of 500 µg P and lasts for about 7 hr, with high levels of receptivity being observable until 18 hr after P administration (Figure 1). The duration of high levels of sexual receptivity was somewhat longer than expected and it is possible that the dose of P administered was, to some extent, responsible for this effect. Experiment 2 indicated that 100 µg P elicited maximal receptivity in EB-primed animals (LQ=100), suggesting that the 500 µg P dose used previously was in excess of the required amount (Figure 2). However, the results of Experiment 3 for animals given 10 µg EB plus 100 µg P (X LQ=83)
indicate that, to insure maximal receptivity, a dose of 250 μg P or more should be administered.

The results of Experiment 2 also imply that a dose of 50 μg P elicits moderate levels of receptivity in EB-primed animals. This is somewhat misleading, however, as the effect of P on receptivity appears to be an all-or-none phenomenon. For example, while six animals given 50 μg P exhibited maximal receptivity, the other four exhibited no receptivity. No animals receiving the 50 μg P dose actually exhibited an LQ within the moderately receptive range. Rather, half of the subjects exhibited high levels of receptivity while the other half exhibited low levels of receptivity, resulting in a mean LQ in the moderate range. This bimodal distribution suggests that the threshold dose of P required to elicit receptivity in EB-primed gerbils is between 50 and 100 μg for about one half of the animals tested. The remaining animals require a threshold dose of P somewhere between 0 and 50 μg. A bimodal distribution was also observed in Experiment 1, where the mean LQs at 1 hr and at 18-21 hr reflect the number of receptive and non-receptive animals rather than the actual modal LQs of the animals within the group. This type of bimodal distribution is also suggested by the size of the standard errors.

A bimodal distribution was not observed in Experiment 3, where P dose was held constant and EB dose varied. In this situation, a more even distribution of LQs was observed, as suggested by the smaller standard errors (Figure 3) when compared to the first experiment. For example, LQs for the 5 μg dose ranged from 30 to 80, with the mode at 50. Thus, if
moderate levels of receptivity are desired, it would be most appropriate to use a dose of 5 µg EB plus 100 µg P rather than 10 µg EB plus 50 µg P.

Experiments 1-3 suggest that the synergistic action of P with EB on receptivity is all-or-none while the effect of EB is graded. This type of information could enhance the understanding of molecular mechanisms of estrogen and progesterone action.

The results of Experiment 4 indicate that only the 100 µg EB dose, when administered chronically, was capable of eliciting maximal receptivity while lower doses induced low to moderate levels of receptivity (Figure 4). This is in contrast to rats, where doses of EB as small as 0.8 µg per day can elicit moderate to high levels of receptivity (Bermant & Davidson, 1974; Gorzalka & Raible, 1981). This also suggests that the gerbil is more dependent than the rat on the synergistic action of P with EB for the induction of maximal receptivity. Although 100 µg EB did produce high levels of receptivity, it was later found that more prolonged chronic administration of 100 µg EB led to bladder stones, hemorrhaging, and death, with initial symptoms occurring as early as Day 30 of EB treatment. This indicates that 100 µg EB is a supraphysiological dose, suggesting that EB-induced receptivity in female gerbils given this dose may be due to a pharmacological effect. In any case, it certainly is not advisable to use this dose chronically for a prolonged period of time and results obtained by this method should not be viewed as representative of normal processes within the gerbil.

The failure to observe receptivity in any of the EB treated
animals until Day 6 of EB treatment indicates that the gerbil requires more prolonged EB treatment than the rat (Bermant & Davidson, 1974) for the induction of receptivity in the absence of P. Furthermore, the finding that raising the dose of EB did not reduce the minimal interval for initial receptivity suggests that there may be a minimum time requirement before the effects of chronic EB can be observed. This finding, plus the finding that lower doses of EB given chronically will induce moderate levels of receptivity suggests that a chronic EB paradigm may prove valuable in the examination of experimental effects when daily testing is desirable.

The results of the present experiments indicate that the influence of ovarian steroids on sexual receptivity in the gerbil is very similar to that observed in other rodents. However, the results of Experiments 1-4 do suggest that, like the hamster (Feder, Siegel, & Wade, 1974), the gerbil is more dependent than the rat (Davidson et al., 1968) on the synergistic action of P with EB for the elicitation of maximal receptivity.
SECTION Ib

Effects of Housing Condition, Adrenal Steroids, and Hormone Dose on Sexual Receptivity

Gorzalka & Raible (1981) have found that housing conditions (i.e., social isolation versus grouping) influence lordosis in female rats, the first demonstration of a differential housing effect on sexual receptivity. This raises the possibility that female gerbils as well are influenced by housing conditions. Due to the space limitations in our colony, it is frequently preferable to house gerbils in pairs. However, occasionally one animal will die and the aggressive nature of the gerbil makes it difficult to form a new pair by introducing a novel cagemate (Anisko, Christenson, & Buehler, 1973; Marston & Chang, 1965; Rieder & Reynierse, 1971). Since the survivor would become socially isolated, it is important to determine if differential housing conditions alter the sexual responding of the female gerbil.

Gorzalka & Raible (1981) also found that the isolation induced facilitation observed in the female rat was apparently mediated by the adrenal gland, since adrenalectomy eliminated the effect. Presumably, social isolation acts as a stressor which, via the pituitary-adrenal axis, results in alterations of adrenal secretions (Hatch, Wiberg, Balazs & Grice, 1963), one or more of which then acts upon the system regulating sexual receptivity. For example, stress can increase the secretion of adrenocorticotrophic hormone (ACTH) by the pituitary which results in increased secretion of adrenal P,
deoxycorticosterone, and corticosterone. All of these steroids are known to alter receptivity in the female rat (deCatanzaro, Knipping, & Gorzalka, 1981; Gorzalka & Whalen, 1977). Cullen & Scarborough (1970) found that the gerbil was highly dependent on adrenal secretions for survival (the rat is not), suggesting that the adrenal may play an important role in mediating the effects of environmental stress in this species as well.

It is also possible that factors other than social isolation (i.e., repeated injections, handling, exposure to unfamiliar animals) may be stressful, resulting in increased adrenal steroid secretion. Testing for these factors would prove time consuming and difficult. However, testing the effects of adrenal steroids on receptivity is relatively simple. The administration of adrenal steroids to ovariectomized and ovariectomized-adrenalectomized animals would reveal any facilitatory or inhibitory effects of adrenal steroids. Once the effects of adrenal steroids on receptivity are determined, possible experimental confounds can be avoided by alterations in experimental design.

Finally, although ovarian steroids may be the primary regulators of sexual receptivity in other rodents (Beach, 1976; Kelley & Pfaff, 1978), the possibility that adrenal steroids are involved in the modulation of sexual receptivity in the gerbil can not be excluded. In fact, there is evidence that, under certain conditions, adrenal steroids do influence the level of sexual receptivity in female rats (deCatanzaro et al., 1981; Gray & Gorzalka, 1980; Gorzalka & Whalen, 1977).

The following experiments were designed to determine (1)
the effects of differential housing on receptivity, (2) a potential adrenal mediation of any observed effects, (3) any effects of adrenalectomy itself on sexual receptivity, and (4) the effects of various adrenal steroids on sexual receptivity in female gerbils.

**GENERAL METHODS**

The methods outlined in Section Ia were followed with the addition of the following procedures. Ovariectomized animals not receiving adrenalectomies were sham adrenalectomized to control for possible stress due to the surgical procedure itself, all animals were given a two and one half week recovery period before testing began. In addition, all adrenalectomized animals were given access to a 0.9% saline solution. Upon termination of an experiment, all adrenalectomized subjects were autopsied to verify the success of the surgical procedure.

**EXPERIMENT 5**

Comparing isolated and grouped animals, Gorzalka & Raible (1981) found that isolation facilitated sexual receptivity in ovariectomized, EB-primed rats. The effect appears to be mediated by the adrenal gland and, because the gerbil is dependent upon adrenal secretions for survival (Cullen & Scarborough, 1970), it is possible that the gerbil may also be influenced by housing conditions. At least one study has found that social isolation in the gerbil is stressful, as indicated by an increase in ascorbic acid content of the adrenals (Hughes & Nicholas, 1971). However, ascorbic acid content provides only
an indirect measure of adrenal secretion and therefore it remains an open question whether social isolation is stressful in the gerbil.

The following experiment was designed to determine (1) the effects of differential housing on receptivity, (2) potential adrenal mediation of any observed effects, and (3) any effects of adrenalectomy itself on sexual receptivity.

**Method**

Cullen & Scarborough (1970) found that adrenalectomized gerbils require injections of 1 mg hydrocortisone acetate (HCA) in addition to access to a 0.9% saline drinking solution for survival. HCA is an ester of hydrocortisone, a major steroid of the gerbil adrenal (Oliver & Péron, 1964). A pilot study in our laboratory determined that the HCA injections did not significantly alter receptivity in adrenalectomized gerbils.

Immediately after surgery, subjects were housed either one animal per single cage (isolated condition) or three animals per triple cage (grouped condition), keeping housing density constant. This housing procedure was similar to that used by Gorzalka & Raible (1981) and would thus allow comparison with that study. Half of the subjects in each housing condition received bilateral ovariectomies and adrenalectomies while the remainder received ovariectomies and sham adrenalectomies. Injections of 1 mg HCA (adrenalectomized animals) and the vehicle, Tween 80 and 0.9% saline (ovariectomized animals) were initiated on the day of surgery and given every other day for the duration of the experiment.

Adrenalectomy facilitates sexual behaviour in the rat.
(Gray & Gorzalka, 1980) and has no effect in the hamster (Carter, 1972). In a species as adrenal dependent as the gerbil, adrenalectomy could be either facilitatory, inhibitory, or have no effect on receptivity. Therefore, a dose of EB which would allow detection of increased or decreased sexual responding was desired. Since estrogen was to be administered chronically, the results of Experiment 4 indicated that the 10 µg EB dose would be an appropriate initial dose. If animals reached asymptote at low levels of receptivity, subsequent injections of 100 µg EB could be administered while asymptote at high levels of receptivity would indicate that 1 µg EB injections should instead be administered.

Injections of 10 µg EB were administered daily for 12 days, with testing occurring on Days 5-12 of 10 µg EB treatment. On Days 13-20, injections of 100 µg EB were administered in an attempt to increase the low levels of responding exhibited by the adrenalectomized animals. Finally, on Day 21, 500 µg P was administered 3-4 hr prior to testing in a final attempt to increase the level of sexual responding in adrenalectomized animals.

**Results and Discussion**

Data from animals that died during the course of the experiment were eliminated from the analysis. The resulting n's were 12 for grouped ovariectomized, 17 for isolated ovariectomized, and 11 each for grouped and isolated adrenalectomized animals. An examination of the data suggested that, although differential housing did not appear to have an effect, adrenalectomy did reduce receptivity (Figure 5).
FIGURE 5. Effects of adrenalectomy, housing condition, and dose of chronically administered estrogen on sexual receptivity in female gerbils. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Ovariectomized (ovex) and ovariectomized-adrenalectomized (ovex-adrex) animals were housed one per single cage (isolated) or three per triple cage (grouped). Animals received daily injections of 10 μg estradiol benzoate (EB) beginning on Day 1 and 100 μg EB beginning on Day 13. 500 μg progesterone (P) was administered 3-4 hr prior to testing on Day 22. Testing occurred on Days 5-22.
10ug EB

100ug EB

● = grouped, ovex
● = isolated, ovex
△ = grouped, ovex-adrenergic
△ = isolated, ovex-adrenergic

Lordosis/Mount x 100

EB Injection Day

5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 21 22 P

0 10 20 30 40 50 60 70 80 90 100
Neither the 10 μg nor the 100 μg dose of EB were sufficient to restore control levels of receptivity although the 100 μg EB dose did appear to raise levels of receptivity somewhat. In addition, although P did facilitate sexual responding in adrenalectomized animals, levels of receptivity still appeared somewhat low for the doses of hormones administered.

A 2x2x2x8 repeated measures with nested factors analysis of variance performed on the EB data revealed significant effects of surgery, $F(1,47) = 102.64$, $p < 0.0001$, and surgery by test block (10 μg vs 100 μg EB), $F(1,47) = 12.06$, $p = 0.0012$. Other significant effects revealed by the analysis of variance were test block, $F(1,47) = 86.00$, $p < 0.0001$, housing by test, $F(14,658) = 2.18$, $p = 0.0074$, and surgery by test, $F(14,658) = 6.44$, $p < 0.0001$. A t-test was performed on the P data for ovariectomized versus adrenalectomized animals and revealed that adrenalectomized animals exhibited significantly lower receptivity scores than ovariectomized animals, $p < 0.05$. Subsequent Newman-Keul's analyses on the effect of surgery and of surgery by test block revealed that, overall, ovariectomized animals were significantly more receptive than adrenalectomized animals, $p < 0.05$. In addition, although adrenalectomized animals receiving 100 μg EB were significantly more receptive than adrenalectomized animals receiving 10 μg EB, $p < 0.05$, both of these groups were significantly less receptive than ovariectomized animals receiving 10 μg EB, $p < 0.05$.

The present results indicate that adrenalectomy decreases sexual behaviour in ovariectomized animals chronically administered EB. This effect does not appear to be due solely
to a decreased sensitivity to EB since increasing the dose of EB administered to adrenalectomized animals did not result in levels of receptivity equivalent to those of ovariectomized controls. In addition, although P increased receptivity, it did not eliminate the difference between ovariectomized and adrenalectomized animals. The reduction in receptivity observed in adrenalectomized animals could be due to one or more of the following factors: (1) adrenalectomized gerbils may be more dependent upon the synergistic actions of P with EB in the induction of maximal receptivity, (2) adrenalectomy may decrease sensitivity to P or to a metabolite of P, (3) gerbils may require one or more adrenal steroids in addition to EB and P for the elicitation of maximal receptivity, (4) adrenalectomy may have a non-specific debilitating effect. However, since substantially increasing the dose of EB administered did not significantly alter receptivity in adrenalectomized animals, it is unlikely that the decrease in receptivity observed in these animals was due to a decreased sensitivity to EB.

EXPERIMENT 6

Experiment 5 indicated that adrenalectomy significantly reduced receptivity in gerbils given chronic EB treatment. It is possible that this effect is be due to an increased dependence or a decreased sensitivity to some action of P in the elicitation of maximal receptivity in adrenalectomized gerbils. This experiment was designed to investigate the sensitivity of adrenalectomized gerbils to P and to a metabolite of P known to induce receptivity in female rats (Gorzalka & Whalen, 1977;
Whalen & Gorzalka, 1972) and in some mouse strains (Gorzalka & Whalen, 1974).

**Method**

Doses of 0.0, 0.1, 0.5, or 1.0 mg P or 1.0 mg dihydroprogesterone (DHP) were administered to ovariectomized and ovariectomized-adrenalectomized animals, creating a total of ten treatment groups. One week before the first test, animals received a priming dose of 10 μg EB in addition to the EB administered 48 and 24 hr prior to testing. Due to the size of the experiment and limited colony space, a counterbalanced, repeated-measures design was employed. Animals were tested once every seven days with a P vehicle (EB only) test occurring between each P test. Previous work indicates that the repeated measures design is not confounded by residual hormonal effects provided tests are separated by a week (Whalen & Gorzalka, 1972). Each animal thus received each of the three P doses and the DHP plus four tests with EB only, resulting in a total of eight tests. HCA and vehicle (peanut oil) injections were carried out as stated in Experiment 5.

**Results and Discussion**

Data from animals dying during the course of the experiment were eliminated from the analysis, resulting in n's of 12 for all ovariectomized groups and 20 for all adrenalectomized groups. An examination of the data indicated that adrenalectomy again produced a decrease in sexual receptivity. Furthermore, even the 1 mg dose of P did not raise the level of receptivity in adrenalectomized animals to that exhibited by ovariectomized controls (Figure 6). In addition, relative to the effect
FIGURE 6. Sexual receptivity in ovariectomized and ovariectomized-adrenalectomized female gerbils administered various doses of progesterone. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Ovariectomized (ovex) and ovariectomized-adrenalectomized (ovex-adrex) animals were administered 10 μg estradiol benzoate 48 and 24 hr before testing and 0.0, 0.1, 0.5, or 1.0 mg progesterone or 1 mg dihydroprogesterone (DHP) 3-4 hr prior to testing.
produced by even 0.1 mg P in ovariectomized animals, 1.0 mg DHP was relatively ineffective in stimulating receptivity in ovariectomized and ovariectomized-adrenalectomized animals.

A 2x5 repeated measures analysis of variance performed on the data indicated significant effects of surgery, $F(1,30) = 65.78, p < 0.0001$, dose, $F(4,120) = 30.85, p < 0.0001$, and surgery by dose, $F(4,120) = 6.89, p < 0.0001$. Subsequent Newman-Keul's analyses performed on the significant interaction effect indicated that the only conditions in which ovariectomized animals and adrenalectomized animals did not differ significantly were the oil and DHP conditions. Furthermore, although adrenalectomized animals given 0.5 and 1.0 mg P were significantly more receptive than oil treated ovariectomized animals, $p < 0.05$, they were significantly less receptive than ovariectomized animals receiving as little as 0.1 mg P, $p < 0.05$. The results of the present experiment indicate that even relatively high doses of P given in conjunction with EB are not sufficient to raise levels of receptivity in adrenalectomized animals to those exhibited by ovariectomized controls. It seems improbable that the reduced levels of receptivity observed in adrenalectomized animals are due solely to a reduced sensitivity to P or an increased dependence on the synergistic action of P with EB. Even when given 10 times the amount received by ovariectomized animals (0.1 vs 1.0 mg), adrenalectomized animals remained significantly lower in their receptivity. Thus, it seems more plausible that ovarian steroids alone may not be sufficient to induce maximal receptivity and that adrenal steroids play some role in the
mediation of sexual receptivity in the female gerbil.

EXPERIMENT 7

Experiments 5 and 6 indicated that the reduced levels of receptivity observed in adrenalectomized animals were not due solely to reduced sensitivity to ovarian hormones, though this may be one factor. This suggests that either adrenalectomy has a general debilitating effect or that perhaps adrenal steroids are in some way normally involved in the regulation of sexual receptivity in the female gerbil.

The health of adrenalectomized gerbils may be related to the sodium retention and/or glycogen deposition properties of the adrenal steroid administered to maintain life. In adrenalectomized rats, it has been found that hydrocortisone (cortisol) is about 50% more effective than cortisone for sodium retention and glycogen deposition, though both are considered to be primarily glucocorticoids. Deoxycorticosterone, on the other hand, is about 300 times as effective as cortisone in its sodium retention abilities but will not maintain glycogen deposition (Liddle & Melmon, 1974).

Cullen & Scarborough (1970) found that HCA was fairly effective in maintaining adrenalectomized gerbils while deoxycorticosterone acetate (DOCA) was not. Hydrocortisone is a major adrenal steroid of the gerbil and, given its mineralcorticoid and glucocorticoid properties, it is perhaps not surprising that it is the most effective adrenal steroid for maintenance of adrenalectomized gerbils. DOCA, on the other hand, has no glucocorticoid properties and would not be expected
to be as effective as HCA. Cortisone acetate (CA) has both mineralcorticoid and glucocorticoid properties but is less effective than HCA in its action and thus may not be very effective in maintaining adrenalectomized gerbils. Thus, if the decreased receptivity observed in adrenalectomized gerbils is a result of poor health due to lack of sufficient glucocorticoids and mineralcorticoids, receptivity in adrenalectomized animals receiving DOCA or even CA should be lower than receptivity in adrenalectomized animals receiving HCA since the former steroids are less effective than the latter.

It is also possible, however, that a given adrenal steroid may play a role in sexual receptivity, either by facilitating receptivity, inhibiting receptivity, or by being a requirement, in addition to EB and P, for maximal receptivity. Adrenal steroids have been found to influence receptivity in female rats under certain conditions. For example, corticosterone eliminates the adrenalectomy-facilitation effect observed in female rats (deCatanzaro et al., 1981). On the other hand, DOCA facilitates sexual receptivity in ovariectomized, EB-primed rats (Gorzalka & Whalen, 1977). A facilitating or inhibiting effect of a given adrenal steroid could mask, or be masked by, the health promoting effects of that steroid in adrenalectomized animals. For example, if DOCA eliminated the adrenalectomy-induced decrease in receptivity seen in gerbils, it could be due to a direct facilitating effect of DOCA on receptivity, a direct effect of DOCA on health which indirectly increased receptivity, or both. The use of ovariectomized as well as adrenalectomized animals would reduce or eliminate this difficulty by allowing
the effects of adrenal steroids on receptivity to be determined in adrenally-intact animals.

The present experiment was designed to investigate the possibility that the reduction in receptivity observed in adrenalectomized gerbils could be due to a non-specific debilitating effect of adrenalectomy or an extraovarian dependence on some adrenal steroid for the elicitation of maximal receptivity. In addition, the general effects of the steroids on sexual receptivity in ovariectomized animals will be investigated to help control for the possibility of facilitatory or inhibitory effects.

Method

All adrenal steroid injections (HCA, DOCA, or CA) were given every other day in doses of 1 mg, starting on the day of surgery. Ovariectomized and ovariectomized-adrenalectomized animals received injections of one of the steroids. In addition, one group of ovariectomized animals received injections of the vehicle (Tween 80 and 0.9% saline). After a postoperative recovery period, animals began receiving daily subcutaneous injections of 10 μg EB. The first test of sexual receptivity occurred on Day 9 of EB treatment when, according to the results of Experiment 4, ovariectomized animals would be near their maximal level of responding for the 10 μg dose. On the following day, animals were given 100 μg P 3-4 hr prior to testing.

Results and Discussion

CA was found to be incapable of sustaining life in adrenalectomized gerbils and thus, that group was eliminated
from the data analysis. The final number of animals per group was 10 for ovariectomized animals receiving HCA, CA, and vehicle injections and for adrenalectomized animals receiving HCA injections, 22 for ovariectomized animals receiving DOCA injections, and 32 for adrenalectomized animals receiving DOCA injections.

The results of the present experiment suggest that there was an overall decrease in receptivity in adrenalectomized animals. Furthermore, no adrenal steroid given alone appeared capable of raising receptivity in adrenalectomized animals to levels exhibited by controls, regardless of the presence or absence of P (Figure 7).

Due to the nested nature of the design and the necessarily missing blocks (adrenalectomized vehicle and CA), a two-way analysis of variance (group and test) was performed on the data. The analysis revealed significant effects of group, $F(5,88) = 21.63, p < 0.0001$, test (pre-progesterone versus post-progesterone), $F(1,88) = 177.87, p < 0.0001$, and group by test, $F(5,88) = 8.85, p < 0.0001$. Subsequent Newman-Keul's analyses ($\alpha = 0.05$) were performed on each of these effects. It was found that, overall (i.e. independent of test), both adrenalectomized groups (HCA and DOCA) had significantly lower receptivity scores than all ovariectomized groups. None of the ovariectomized groups receiving adrenal steroids differed from the vehicle group. The group by test analysis indicated that, although none of the groups differed significantly from the vehicle group during the EB test, scores of both of the adrenalectomized groups were significantly lower than those of
FIGURE 7. Sexual receptivity in ovariectomized and ovariectomized-adrenalectomized female gerbils administered adrenal steroids. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Ovariectomized (ovex) and ovariectomized-adrenalectomized (ovex-adrex) animals were chronically administered hydrocortisone acetate (HCA), deoxycorticosterone acetate (DOCA), cortisone acetate (CA), or the vehicle (Ve). 10 µg estradiol benzoate (EB) was administered for 9 days, with testing occurring on Day 9. On the following day, animals received 100 µg progesterone (P) 3-4 hr prior to testing.
the ovariectomized animals receiving adrenal steroids on the post-progesterone test. This suggests that adrenalectomized gerbils are less sensitive than ovariectomized animals to the synergistic action of P with EB. Furthermore, although adrenalectomized animals receiving HCA did not differ from ovariectomized animals receiving the same treatment in the pre-progesterone test, adrenalectomized animals receiving DOCA were significantly less receptive than ovariectomized animals receiving similar treatment. This effect would appear to be due to the non-significant facilitation of receptivity observed in ovariectomized animals rather than an inhibiting effect of DOCA on receptivity in adrenalectomized animals.

The present results indicate that, overall, neither HCA nor DOCA, when administered alone, is sufficient to restore receptivity in adrenalectomized gerbils to levels exhibited by ovariectomized controls. However, in pre-progesterone tests, adrenalectomized animals exhibit lower, but not significantly lower, levels of receptivity. The scores of the adrenalectomized animals during post-progesterone tests appear to be the primary contributors to the overall significance of the adrenalectomy effect. These results suggest that at least part of the decrease in responding in adrenalectomized animals may be due to a decreased sensitivity to the synergistic action of P with EB. It is possible that some combination of adrenal steroids may enhance the action of P with EB or adrenal steroids per se may facilitate receptivity in animals treated with either EB or EB plus P.
EXPERIMENT 8

Experiment 7 indicated that no single adrenal steroid was capable of restoring receptivity of adrenalectomized animals to those levels exhibited by controls. However, because each adrenal steroid acts primarily as a glucocorticoid or a mineralcorticoid, it is possible that a combination of steroids is necessary. If a combination of an adrenal mineralcorticoid and a glucocorticoid does not restore receptivity to control levels in adrenalectomized animals, one could assume reduced receptivity is either due to (1) a lack of some other adrenal secretion involved in receptivity, (2) an excess of some pituitary secretion, or (3) a nonspecific debilitating effect of adrenalectomy. However, if a combination of adrenal steroids does restore overall levels of receptivity in adrenalectomized animals, it would suggest that the female gerbil, unlike other female rodents, is dependent upon both adrenal steroids and EB and P for the induction of maximal receptivity.

Method

The procedures outlined in Experiment 7 were followed with the exception of the adrenal steroids administered. Ovariectomized and ovariectomized-adrenalectomized animals received either 1 mg HCA + 1 mg DOCA, 1 mg HCA + 1 mg CA, or 1 mg DOCA + 1 mg CA. Thus, the HCA + CA group was administered two mineralcorticoids while the others received both a mineralcorticoid and a glucocorticoid. As in Experiment 7, there was also an ovariectomized control group which received injections of the vehicle, resulting in a total of seven groups (four ovariectomized, three ovariectomized-adrenalectomized).
Results and Discussion

Data from animals not completing both tests were eliminated from the analysis. The resulting number of animals per group was 10 for ovariectomized animals receiving HCA + DOCA, DOCA + CA, and vehicle injections, 16 for ovariectomized animals receiving HCA + CA, 12 for adrenalectomized animals receiving HCA + CA, 14 for adrenalectomized animals receiving HCA + DOCA, and 17 for adrenalectomized animals receiving DOCA + CA.

An examination of the data suggests that, once again, adrenalectomy produced an overall decrease in sexual responding. Yet, at least during the pre-progesterone test, all steroid treatments appeared effective in restoring receptivity in adrenalectomized animals to control levels (Figure 8). However, during the post-progesterone test, adrenalectomized animals given HCA + CA remained less receptive than ovariectomized animals receiving the same treatment. This did not appear to be the case in groups where animals were receiving both a glucocorticoid and a mineralcorticoid. Finally, it appeared that, at least in the pre-progesterone test, ovariectomized animals receiving DOCA + HCA exhibited an increase in receptivity beyond that exhibited by vehicle controls.

To be consistent with the analysis performed in Experiment 7, a two-way analysis of variance was performed on the data. This analysis revealed significant effects of group, $F(6,82) = 7.92, p < 0.0001$, test, $F(1,82) = 83.50, p < 0.0001$, and group by test, $F(6,82) = 2.57, p = 0.025$. Newman-Keul's procedures were subsequently performed on each of these effects ($\alpha = 0.05$). An analysis of the overall effect of group revealed
FIGURE 8. Sexual receptivity in ovariectomized and ovariectomized-adrenalectomized female gerbils administered various combinations of adrenal steroids. Receptivity is expressed as the ratio of lordosis responses to mounts with pelvic thrusting. Ovariectomized (ovex) and ovariectomized-adrenalectomized (ovex-adrex) animals were chronically administered hydrocortisone acetate and cortisone acetate (HCA+CA), deoxycorticosterone acetate and cortisone acetate (DOCA+CA), deoxycorticosterone acetate and hydrocortisone acetate (DOCA+HCA), or the vehicle (Ve). 10 μg estradiol benzoate (EB) was administered for 9 days, with testing occurring on Day 9. On the following day, animals received 100 μg progesterone (P) 3-4 hr prior to testing.
EB

- ovex
- ovex-adrex

Lordosis/Mount x 100

Ve  HCA + CA  DOCA + CA  DOCA + HCA  Ve  HCA + CA  DOCA + CA  DOCA + HCA
that only the adrenalectomized animals receiving HCA + CA, two mineralcorticoids, were significantly less receptive than ovariectomized animals receiving vehicle injections. Examination of the groups by test interaction revealed that, for the pre-progesterone test, ovariectomized animals receiving DOCA + HCA were significantly more receptive than control animals. This effect was not observed in the post-progesterone test, presumably because of a ceiling effect (i.e., DOCA + HCA animals neared the maximum LQ in the pre-progesterone test). The only other group found to differ significantly from ovariectomized animals receiving vehicle injections was the adrenalectomized HCA + CA group, which was significantly less receptive than vehicle controls during the post-progesterone test.

Due to the observed facilitation effect of HCA + DOCA and the necessary lack of a vehicle control group in adrenalectomized animals, it also seemed appropriate to compare the effectiveness of various steroid combinations in adrenalectomized animals and also with those of ovariectomized animals. Overall, it was found that adrenalectomized animals receiving DOCA + HCA were significantly more receptive than the remaining two adrenalectomized groups which did not differ from each other. In addition, neither of the adrenalectomized groups receiving DOCA differed significantly from the ovariectomized groups receiving a similar treatment while adrenalectomized animals receiving the two mineralcorticoids remained significantly less receptive than the corresponding ovariectomized group.
These results suggest that the administration of a glucocorticoid plus a mineralcorticoid is sufficient, and perhaps necessary, to raise the level of sexual responding observed in adrenalectomized gerbils to that exhibited by control animals. Furthermore, although DOCA + CA restored receptivity to levels exhibited by controls, DOCA + HCA was significantly more effective, perhaps because of a direct facilitatory effect on sexual responding.

If one compares the performance of a given group during the pre- and post-progesterone tests, it becomes apparent that, for all ovariectomized groups (except the DOCA + HCA group which was already at a high level of sexual responding), scores during the post-test test were significantly greater than during the pre-progesterone test. This is not the case for any of the three adrenalectomized groups. To determine the exact nature of this effect, a 2x2x4 analysis of variance with nested factors was performed on the data. This analysis revealed a significant surgery by test interaction, $F(1,82) = 6.54, p = 0.012$. A Newman-Keul's analysis indicated that, overall, levels of receptivity exhibited by ovariectomized animals were similar to those exhibited by adrenalectomized animals during the pre-progesterone test but were significantly greater during the post-progesterone test ($\alpha = 0.05$). Thus, for any given treatment, adrenalectomized animals given EB plus P do not differ from ovariectomized animals. However, overall, adrenalectomized animals were significantly less receptive than ovariectomized animals when given EB plus P. This suggests that at least part of the adrenalectomy effect is due to a decreased
level of responding to P or to a reduced synergism with EB rather than being due solely to poor health. Thus, it would appear that, even when given adrenal glucocorticoids and mineralcorticoids, adrenalectomized animals still exhibit some differences in responding when compared to ovariectomized animals. This difference appears to be due to a reduced responding to the effects of P when given in conjunction with EB rather than to a general reduction in responsivity to estrogen. In addition, a generalized debilitating effect of adrenalectomy does not appear to be responsible for the decreased responding in adrenalectomized animals since they did not differ from ovariectomized animals during the pre-progesterone test.

GENERAL DISCUSSION

The results of Experiment 5 indicate that, although housing has no significant effect on sexual receptivity in the female gerbil, adrenalectomy significantly decreased receptivity. Initially, it was suggested that this decline could be due to one or more of the following effects of adrenalectomy: (1) a decreased sensitivity to EB, (2) a decreased sensitivity to P, (3) an increased dependence on the synergistic action of P with EB, (4) a dependence on one or more adrenal steroids in addition to EB and P for maximal receptivity, (5) a non-specific debilitating effect of adrenalectomy.

Although the possibility that adrenalectomy produces a decrease in sensitivity to EB in the gerbil can not be entirely eliminated, the fact that a ten-fold increase in the dose of EB administered fails to restore receptivity in adrenalectomized
gerbils to that exhibited by controls (Figure 5) makes it unlikely. In addition, adrenalectomized animals receiving appropriate adrenal steroids exhibited normal sensitivity to EB, suggesting that a lack of appropriate adrenal steroids may have been, to some extent, responsible for reduced responding to EB. Furthermore, the results of Experiment 4 indicated that the gerbil was dependent on the synergistic action of P with EB for the elicitation of maximal receptivity unless exceedingly high doses of EB were administered. Thus, a reduction in sensitivity to P in adrenalectomized animals could easily result in lowered receptivity during testing with EB plus P. In support of this, the results of Experiments 6-8 indicated that adrenalectomized animals were less sensitive than ovariectomized animals to the action of P when administered in conjunction with EB. Experiment 6 indicated that EB-primed, adrenalectomized gerbils given varying doses of P were consistently less receptive than ovariectomized controls. In addition, Experiments 7-8 indicated that adrenalectomized gerbils receiving adrenal glucocorticoids and mineralcorticoids still exhibited an overall reduction in responding when tested with EB plus P although not when tested with EB alone.

The finding that the combination of a glucocorticoid and a mineralcorticoid was most effective in restoring control levels of receptivity in adrenalectomized animals suggests that a portion of the effect observed in Experiments 5 and 6 may have been due to poor health. However, the results of Experiments 7 and 8 indicate that the reduced receptivity in adrenalectomized animals is also, in part, due to a decreased sensitivity to the
effects of P. The gerbil is the first known rodent to require adrenal steroids in addition to EB and P for the induction of maximal receptivity.

The finding that adrenalectomy does not facilitate receptivity in the female gerbil is similar to the results obtained for the hamster (Carter, 1972) but is in contrast to the results obtained for the rat, where adrenalectomy facilitates receptivity (de Catanzaro et al., 1981; Gray & Gorzalka, 1980). Recently it has been found that the chronic administration of corticosterone (the major adrenal steroid of the rat) eliminates the adrenalectomy facilitation effect in EB and P treated rats (de Catanzaro et al., 1981), suggesting that it might normally exert an inhibitory effect in the ovariectomized animal. It is interesting to note that both rodent species which do not exhibit an increase in sexual receptivity after adrenalectomy (hamster and gerbil) have cortisol rather than corticosterone as their major adrenal steroid, suggesting that knowledge of the major adrenal steroid may allow prediction of the effects of adrenalectomy on sexual receptivity in a given species.

The finding that housing conditions did not alter receptivity in the female gerbil is in contrast with results obtained for the rat, where isolation facilitates sexual responding (Gorzalka & Raible, 1981). Adrenalectomy eliminates the facilitating effect of isolation on receptivity in female rats, suggesting that the adrenal mediates the effect. Initially, there appeared to be two likely explanations of this effect in the rat. First, social isolation could have led to
increases in adrenal progesterone which then acted synergistically with estrogen to facilitate receptivity (Gorzalka & Raible, 1981). Second, social isolation may have increased adrenal secretion of deoxycorticosterone, which facilitates receptivity in female rats. Deoxycorticosterone is not secreted by the gerbil adrenal. Given the gerbil's increased dependence on the synergistic action of P with EB, one might have expected that, if social isolation was stressful to the gerbil, a similar effect would be observed. It was not, despite indications, using the adrenal ascorbic acid content technique, that social isolation is stressful to the gerbil (Hughes & Nicholas, 1971). Perhaps adrenal progesterone is not the steroid involved, or at least is not the only steroid involved in the effect observed in the rat.

No major adrenal steroids are shared between the rat and the gerbil. It seems possible that an effect of housing is not observed in the gerbil because the steroid responsible for the effect in the rat is not secreted by the gerbil adrenal. Moreover, even if identical quantities of identical steroids were secreted, rat and gerbil brains could still differ in relative sensitivities to the relevant steroids. For example, while DHP mimics progesterone in the ability to facilitate EB action in the rat (Gorzalka & Whalen, 1977), it was completely ineffective in a similar gerbil paradigm (Experiment 6). Thus, interspecific differences in either the adrenal steroids secreted, sensitivity to the relevant steroids, or both, could account for the failure of housing manipulations to alter receptivity in the gerbil. Experiment 6 indicated that, in
contrast to results obtained for the rat (Gorzalka & Whalen, 1977), guinea pig (Wade & Feder, 1972) and some mouse strains (Gorzalka & Whalen, 1974), DHP does not facilitate receptivity in the EB-primed gerbil. Thus, $5\alpha$-reduction, the process by which progesterone is normally converted to dihydroprogesterone, inactivates progesterone in the gerbil but not in other species.

Experiments 5-8 indicated that adrenalectomized animals receiving only HCA were less responsive to EB while those receiving combinations of adrenal steroids were only less responsive to the synergistic effects of P. Experiments 7 and 8 indicated that DOCA and DOCA + HCA may facilitate sexual behaviour in the EB and P treated gerbil. This result is somewhat similar to that obtained for the rat (Gorzalka & Whalen, 1977) although it is unclear whether or not DOCA would facilitate receptivity in EB plus P treated rats. However, DOCA is structurally similar to P and is thought to act via P receptors (Gorzalka & Whalen, 1977). If so, one would expect that the facilitatory action of DOCA would decrease as the dose of P increases since the two would be competing for receptor sites. To the best of my knowledge, this study has not yet been done.

Further investigation into the apparently unique role of adrenal steroids in the induction of maximal receptivity in the female gerbil would greatly increase our understanding of the role of adrenal steroids in the display of sexual behaviour. For many years it was believed that ovarian steroids were the sole regulators of sexual receptivity in the female rodent. More recent evidence in the rat has suggested that adrenal
steroids also alter receptivity although receptivity is not dependent upon their presence. Ovarian steroids then became the primary regulators of sexual receptivity in female rodents and adrenal steroids became secondary influences. The present results suggest that, in the gerbil, ovarian steroids and adrenal steroids regulate sexual responding. Thus, although ovarian steroids are the primary regulators of sexual behaviour, there is reason to doubt that this generalization holds for all rodents. Relatively little work has been done on the effects of adrenal steroids on receptivity in rodents. Yet research on the effects of adrenal steroids on receptivity does seem to be on the increase. The author feels this is a positive step towards developing a more global model of the hormonal regulation of sexual receptivity in rodents.
SECTION II
Hormonal Regulation and Sexual Differentiation of Food Intake Mechanisms and Mating Activity

The research of Maass & Wade (1977) and Roy et al. (1977) indicates that female gerbils may be unlike other female rodents in terms of the hormonal regulation of food intake and body weight. Whereas rats (Tarttelin & Gorski, 1973; Wade, 1975; Wade & Zucker, 1970), mice (Wright & Turner, 1973), and, to a much lesser degree, hamsters (Gerall & Thiel, 1975; Morin & Flemming, 1978), exhibit a decrease in body weight and food intake when EB is administered, female gerbils appear to increase food intake and body weight in response to EB administration (Maass & Wade, 1977). The results of Maass & Wade (1977) and Roy et al. (1977) are difficult to interpret however since these authors did not investigate the effect of endogenous steroid removal (by ovariectomy). Furthermore, the relative functions of estrogen and progesterone in restoring presurgical levels of food intake and body weight were not determined.

The potential difference in the response of the female gerbil to EB administration in terms of food intake and body weight is also of interest in light of evidence suggesting that in rodent species where the male is heavier than the female (rats, mice, and guinea pigs), the greatest alterations in food intake and body weight result from the administration of ovarian steroids. In the species where the female is heavier than the male (hamsters), greater alterations are observed after the
administration of androgens (Kowalewski, 1969; Wade, 1976). Apparently, in the smaller of the two genders, the endogenous level of the more effective sex hormone is responsible for the lesser weight of that gender. To be consistent with this pattern, food intake in the gerbil should be regulated by ovarian steroids as females are lighter than males (Marston & Chang, 1965) but one would expect food intake and body weight to be reduced by EB as estrogen is assumed to be related to the female's reduced weight. Maass & Wade's (1977) study suggests that the female gerbil may not fit the pattern exhibited by other rodents. If so, this information may assist in refining hormonal models of food intake and body weight regulation in rodents.

In addition to the direction of the difference in male and female body weights in hamsters, they differ from other laboratory rodents in another respect. Unlike male rats (Dunlop, Gerall, & Hendricks, 1972; Goldman, Baker, Chen, & Wieland, 1972), mice (Edwards & Burge, 1971), and guinea pigs (Brown-Grant & Sherwood, 1971; Pheonix, Goy, Gerall, & Young, 1959), in which behaviour is naturally defeminized at birth, male hamsters are not naturally defeminized at birth and may exhibit male and female sexual behaviour in adulthood (Johnson, 1975; Tiefer & Johnson, 1971, 1975; Whitsett & Vandenbergh, 1975). The administration of androgens to neonatal female rodents may also induce male patterns of sexual behaviour in adulthood. Pre- and/or neonatally administered TP masculinizes and defeminizes female rats (Gorski, 1980; Ward, 1969), mice (Edwards & Burge, 1971), and guinea pigs (Brown-Grant &
Sherwood, 1971; Pheonix et al., 1959; Slob et al., 1973), and masculinizes but only partially defeminizes female hamsters (Johnson, 1975; Tiefer & Johnson, 1975). Studies in the rat, guinea pig, and hamster indicate that the sex difference in food intake and body weight in adulthood is related to the presence or absence of sex steroids around the time of birth (Gentry & Wade, 1976a,b; Slob, Goy, & Van der Werff ten Bosch, 1973; Swanson, 1967; Wade & Zucker, 1970). TP administration during sexual differentiation produces masculine food intake and body weight patterns while neonatal castration produces food intake and body weight patterns similar to those observed in the female. Since hamsters differ from rats in the degree of androgen-induced defeminization and in the sex steroid producing the greatest alterations in food intake and body weight, it is possible that the differences in the hormonal regulation of adult food intake and body weight seen between hamsters and other laboratory rodents are related to the degree of perinatal androgen induced sexual differentiation occurring in the species.

Although, to the best of my knowledge, there are no studies indicating that differential effects of perinatal androgens are responsible for between species differences in the hormonal regulation of behaviour, there are studies indicating they are responsible for within species differences. In fact, a great deal of evidence exists indicating that androgens modify brain morphology (Gorski, Gordon, Shryne, and Southam, 1978; Ross, Glick, and Meibach, 1982), neural structure and dendritic synapses (Dörner and Staudt, 1968, 1969; Field and Raisman, 1971; Greenough, Carter, Steerman, and De Voogd, 1977; Loy and
Milner, 1980; Nishizuka and Arai, 1981; Toran-Allerand, 1976), and steroid binding and retention capacities (De Bold, 1978; Green, Luttge, and Whalen, 1969; Maurer and Woolkey, 1971; Tuohimaa and Johansson, 1971; Whalen & Massicci, 1975) in various areas of the brain in rats, mice, and hamsters. Since androgens are normally present in fetal and neonatal males and absent in females, this results in a sexual dimorphism of the brain and presumably is responsible for sexual dimorphisms in hormonally mediated behaviours such as food intake.

Investigation into the degree of defeminization at birth and the later hormonal control of food intake and body weight in the gerbil should provide some interesting information. This information combined with that obtained in other species will improve the present understanding of the interrelation of early hormonal environment and adult food intake and body weight patterns. If ovarian steroids do appear to be more effective than androgens in altering food intake and body weight in the gerbil, and if the administration of androgen around the time of birth does defeminize the gerbil, it would further support the present hypothesis that early hormonal environment may determine which sex steroid will later regulate food intake and body weight in rodents. A failure to observe defeminization after androgen treatment in the female gerbil would suggest that the extent of sexual differentiation at birth may not be closely related to which sex steroid will mediate adult food intake and body weight. To the best of my knowledge, this is the first time that the possible relationship between early hormonal environment and interspecific differences in the hormonal
regulation of adult food intake and body weight has been investigated. Thus, the purpose of the present series of experiments is to (1) determine the effects of ovarian steroids on adult food intake and body weight patterns in male and female gerbils and (2) test the hypothesis that the extent of androgen induced defeminization during the period of sexual differentiation determines which sex steroid has the greatest influence on food intake and body weight regulation in adulthood.

IIa - Food Intake, Body Weight, and Lordosis

Ovarian steroids have been reported to influence food intake and body weight in a variety of species (see Wade, 1976, for a review). For example, ovariectomy results in a weight gain while estrogen treatment reduces body weight in female rats (Tarttelin & Gorski, 1973; Wade, 1975; Wade & Zucker, 1970), mice (Wright & Turner, 1973), and guinea pigs (Slob et al., 1973). In male rats, castration leads to slight reductions in body weight and food intake (Gentry & Wade, 1976b; Kakolewski, Cox, & Valenstein, 1968; Wade, 1976). Additional studies have indicated that P not only attenuates the effects of EB on food intake and body weight in ovariectomized rats (Roberts, Kenny, & Mook, 1972; Ross & Zucker, 1974; Wade, 1975) but also attenuates the effects of high doses of TP on food intake and body weight in castrated male rats (Gentry & Wade, 1976b). Following a review of the literature, Wade (1976) suggested that, because ovarian hormones are considerably more effective than androgens in altering food intake and body weight in the rat, they are the
primary hormonal regulators of food intake and body weight in that species.

Ovariectomy and EB treatment have also been found to lead to weight gain and loss, respectively, in the female hamster (Gerall & Thiel, 1975; Morin & Fleming, 1978). The effects are less pronounced than those observed in the rat and may, according to Morin & Fleming (1978), explain why some researchers (e.g., Kowaleski et al., 1969; Swanson, 1967; Zucker, Wade, & Zeigler, 1972) have failed to observe significant effects. Unlike male rats, male hamsters exhibit increases in food intake and body weight after castration (Swanson, 1967; Kowaleski et al., 1969; Zucker, Wade, & Ziegler, 1972). Furthermore, EB appears to have no effect on body weight or food intake in the castrated male hamster (Kowaleski, 1969; Zucker et al., 1972) whereas TP treatment reduces both food intake and body weight in the male hamster (Zucker et al., 1972). This led Wade (1976) to suggest that, in the hamster, androgens are the important activating hormones for the regulation of food intake and body weight in that species.

Maass & Wade (1977) examined food intake and body weight changes in response to castration and androgen treatment in male Mongolian gerbils and in response to the administration of ovarian hormones in ovariectomized female gerbils. They found that castration led to increases in body weight without altering food intake. This effect is somewhat similar to that observed in the male hamster, which also exhibits a weight increase after castration (Swanson, 1967; Zucker et al., 1972). Unlike the male hamster, the male gerbil exhibits no change in food intake.
or body weight in response to TP administration (Maass & Wade, 1977). Maass & Wade (1977) also found that ovariectomized, EB-treated gerbils gained significantly more weight and ate more than ovariectomized gerbils given vehicle injections. Even more intriguing was the finding that P, given in conjunction with EB, resulted in a further increase in food intake, to levels significantly above those exhibited by animals given EB alone (Maass & Wade, 1977). Thus, unlike other female rodents which demonstrate decreased weight and intake in response to EB and an attenuation of this effect when also given P, female gerbils appear to exhibit an increase in weight and intake in response to EB, with P eliciting a further increase in food intake. The results of Maass & Wade (1977) are of considerable interest as they suggest that female gerbils are unlike other female rodents studied to date in their hormonal regulation of food intake and body weight. However, the physiological significance of these results remains to be determined.

Experiment 9 will examine the effects of ovariectomy, EB dose, and the effects of P, alone and in conjunction with EB, on food intake and body weight. Moreover, lordosis behaviour will also be examined in order that the relative effectiveness of ovarian hormones in producing alterations in food intake and sexual receptivity can be compared.

GENERAL METHODS

Male and female Mongolian gerbils were obtained from Tumblebrook Farms at 90 days of age and housed individually in standard laboratory single cages with wire bottoms. A 12:12 hr
light-dark cycle was maintained throughout the experiment with food and water available ad libitum.

To prevent food pellets from dropping through the cage bottoms before they were consumed, only pellets weighing more than 4.5 g were used. Food was placed inside the cages to insure that food which did drop through the cage would fall onto the paper below each cage where it could be easily collected. Food intake was determined every 24 hr by subtracting the weight of the food remaining inside and below each cage from the weight of the food supplied 24 hr before. Food was weighed to the nearest 0.01 g on an analytical balance (Mettler, model AC88). Immediately after consumption data were collected, animals were weighed to the nearest 0.5 g on a triple beam balance (Ohaus, model 700) and, if appropriate, received subcutaneous injections.

Three days after the animals were introduced to the colony, daily measurements of food intake and body weight commenced and continued throughout the experiment. The first four days of measurements provided baseline data that were used to subdivide the animals into four matched groups. Over the next two days, animals were either gonadectomized while under anaesthesia grade ether (experimental groups) or left intact (control groups). During the phases of hormone administration, intact control animals (IC) received daily injections of the vehicle, 0.01 cc peanut oil. The doses of EB employed were 1 μg (group E1), 10 μg (group E10), or 100 μg (group E100) administered daily. Because Experiment 2 indicated that 100 μg P is the minimal effective dose for the elicitation of high levels of receptivity
in EB-primed gerbils, this dose was used to test the potential of P administration on food intake and body weight in EB-primed gerbils. Tests of lordosis occurred on Days 3-12 of EB treatment (for details, see experiment 4).

During the latter portion of phase 2 (EB) and the start of phase 3 (EB+P), some animals in groups E10 and E100 began losing weight and eventually died. Autopsy suggested that death was probably due to internal hemorrhage, presumably related to the chronic EB treatment. After two animals in a given group had died, the injections were terminated in that group. E10 males and E10 and E100 females were terminated from the study during the EB + P phase and E100 males were terminated during the EB phase and were eliminated from the analysis.

A 4 (or 3 for males) x2x5 (groups, treatment phase, data points) analysis of variance for repeated measures and nested factors was performed on the data provided by females and males in groups IC, E1, and E10, and, for females, group E100 during the first two phases of each experiment (gonadectomy and EB treatment). A 2x5x5 analysis of variance for repeated measures and nested factors was performed on data generated by groups IC and E1 throughout each experiment. All subsidiary analyses used the Newman-Keuls's procedure with \( \alpha \) set at 0.05.

Due to the nested factors and the quantity of information, the initial 3 weeks after surgery were eliminated. The remaining data were condensed and the number of data points per treatment phase were equalized. Thus, each treatment consisted of five points with each point representing the groups mean of the averages of the individual animals over 3 - 6 day periods.
EXPERIMENT 9

Work in our laboratory on the sexual behaviour of female gerbils has indicated that ovariectomized gerbils may be less sensitive to EB and/or more dependent on the synergistic action of P with EB than ovariectomized rats (Section I). In rats, doses of EB that alter food intake and body weight also induce sexual receptivity (Davidson et al., 1968; Wade, 1976). This suggests that in the rat, the system regulating food intake and body weight is about as sensitive to EB as the system regulating receptivity. The decreased sensitivity to EB in the female gerbil may or may not be restricted to sexual behaviour. Thus, when determining the EB doses to be used in the present experiment, an attempt was made to approximate doses which would represent low, moderate, and high levels of EB. In this manner, we attempted to observe any differential effects of EB dose on sexual behaviour, food intake and body weight.

Methods

Once body weights had stabilized after surgery (Phase 1), EB treatments (Phase 2) were initiated and continued until the average body weight of E1 animals was similar to that of the IC animals. This allowed a more accurate comparison of the effects of the next treatment phase (EB + P) with control animals. During the EB +P phase, animals in groups E1, E10, and E100 received 100 μg P daily in conjunction with their respective EB doses while IC animals continued to receive injections of the vehicle. When body weight had again stabilized, EB was withdrawn (Phase 4) and E1 animals continued to receive 100 μg P daily (groups E10 and E100 had been terminated by this phase).
Finally, P was withdrawn (Phase 5) and the effects of no hormone treatment observed. All E1, E10, and E100 animals dying within one week of the termination of any treatment phase were eliminated from the analysis. The resultant numbers of subjects for food intake and body weight data were 8 for groups IC and E10, and 7 for groups E1 and E100. As no animals had died within a week of the last day of sexual testing, the number of animals for groups E1, E10, and E100 used in the analysis was 9. IC animals did not receive EB treatment and were thus not tested for sexual receptivity.

Results and Discussion

Three day averages for body weight and food intake are presented for all groups in Figure 9. An examination of the data suggested that E1, E10, and E100 animals exhibited a decrease in weight and intake after ovariectomy while EB treatment increased weight and intake. Analysis of the body weight data indicated a significant group by point interaction, \( F(24,208) = 4.01, p < 0.0001 \). Further analysis of this interaction revealed that IC animals weighed significantly more than E1 animals on the last three points (14 days) of Phase 1 (ovariectomy) and the first two points of Phase 2 (EB treatment), \( p < 0.05 \). By the last two points of Phase 2, E1, E10, and E100 animals had body weights similar to those of IC animals but E100 animals weighed significantly more than E1 and E10 animals, \( p < 0.05 \). Thus, ovariectomy decreased body weight while EB restored it to control levels in a manner that varied slightly with dose. A significant group by Phase interaction was found for food intake data, \( F(3,26) = 6.11, p = 0.003 \), with
Figure 9. Effects of ovariectomy and ovarian steroid administration on food intake and body weight in female gerbils. Food intake and body weight were measured daily and three day averages were recorded. Data for intact controls (IC) and animals receiving 1 µg estradiol benzoate (E1) during estradiol benzoate (EB) phases are presented for all phases of the experiment. For progesterone (P) phases, a dose of 100 µg was used. The phase sequence is from top to bottom in the figure. Food intake and body weight means for groups E10 (10 µg EB) and E100 (100 µg EB) are presented for the first two phases of the experiment. These groups were later discontinued when detrimental effects of high doses of chronic EB were observed.
further analysis indicating that E100 animals ate more than all other groups during the EB phase, \( p < 0.05 \), while E1 and E10 animals were found to consume about as much as control animals. Finally, there was a significant effect of Phase for body weight, \( F(1,26) = 170.79, p < 0.0001 \), and food intake \( F(1,26) = 42.43, p < 0.0001 \).

The ratio of means for E1:IC animals, expressed as a percentage, is presented in Figure 10. Data obtained from groups IC and E1 throughout the experiment suggest that treatment in Phase 3 (EB + P) increased food intake without altering body weight. Withdrawal of EB (Phase 4) decreased body weight without altering food intake and withdrawal of P (Phase 5) appeared to cause no further weight loss and an eventual return of normal food consumption. Analysis of the intake and weight data of groups IC and E1 for all phases of the experiment indicated a significant group by Phase interaction for body weight, \( F(4,52) = 6.17, p = 0.0004 \), and food intake, \( F(4,52) = 2.96, p = 0.03 \) (Table 1). Further analysis of these interactions revealed that during Phase 1 (ovariectomy), IC animals weighed more and consumed more food than E1 animals, \( p < 0.05 \). However, during Phase 2 (EB), there were no significant differences between the two groups with respect to food intake and body weight, suggesting that 1 ug EB was sufficient to restore normal weight and intake in ovariectomized gerbils. IC and E1 animals were also found to be of similar weight during Phase 3 (EB + P) although E1 animals ate significantly more food than IC animals during that phase, \( p < 0.05 \). The finding that E1 animals also ate more during
Figure 10. Effects of ovariectomy and ovarian steroid administration on food intake and body weight in female gerbils relative to levels exhibited by intact control animals. Daily measures of food intake and body weight were averaged to create five data points per treatment phase, presented on the abscissa. The ordinate is the ratio of experimental animal scores to intact control animal scores, expressed as a percentage. Estradiol benzoate (EB) and progesterone (P) doses were 1 and 100 µg, respectively.
Table 1. Mean food intake and body weight in female gerbils as a function of ovariectomy and ovarian steroid treatment.

<table>
<thead>
<tr>
<th>PHASE</th>
<th>BODY WEIGHT</th>
<th>FOOD INTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\overline{X} \pm SE$</td>
<td>$\overline{X} \pm SE$</td>
</tr>
<tr>
<td>OVX</td>
<td>68.87 1.66</td>
<td>65.88 0.93</td>
</tr>
<tr>
<td>EB</td>
<td>74.29 1.82</td>
<td>71.91 1.38</td>
</tr>
<tr>
<td>EB+P</td>
<td>77.89 1.81</td>
<td>75.51 1.23</td>
</tr>
<tr>
<td>P</td>
<td>77.47 1.84</td>
<td>69.89 1.18</td>
</tr>
<tr>
<td>NH</td>
<td>75.89 1.83</td>
<td>67.75 0.70</td>
</tr>
</tbody>
</table>

IC = intact control animals which received no hormone treatments. E1 = animals received 1 µg estradiol benzoate (EB) during EB phases and 100 µg progesterone (P) during P phases. NH = no hormone treatment.
EB + P treatment than they had when given EB alone, \( p < 0.05 \), suggests that P acts in a synergistic or additive manner with EB in regulating food intake but not body weight. The analysis of the group by Phase interaction also revealed that E1 animals weighed less than IC animals for Phases 4 and 5 (P and no hormone treatment, \( p < 0.05 \)) although the E1 animals continued to eat more than the IC animals during those phases, \( p < 0.05 \). The finding that the body weight of E1 animals for Phases 4 and 5 did not differ suggests that the weight decrease observed in Phase 4 was, in part, due to the withdrawal of EB treatment rather than an effect of P. However, it is also possible that P was exerting a metabolic effect which required elevated food intake to counteract an increase in metabolic rate. The present experiment does not allow the differentiation of these possibilities.

Other effects found to be significant for body weight data were Phase, \( F(4,52) = 33.73, \ p < 0.0001 \), points, \( F(20,260) = 15.91, \ p < 0.0001 \), and group by points, \( F(20, 260) = 4.77, \ p < 0.0001 \). For food intake, significant effects were found for Phase, \( F(4,52) = 10.56, \ p < 0.0001 \), and points, \( F(20, 260) = 1.83, \ p = 0.02 \).

Overall, these results suggest that ovariectomy decreases body weight and food intake whereas estrogen serves to increase body weight and food intake, effects opposite to those found in other laboratory rodents. Furthermore, rather than attenuating the effects of EB on food intake, P appears to act in an additive and/or synergistic manner with EB to increase food intake without altering body weight. Because P may exert
effects that are independent of its action with EB, one cannot
determine whether its effect with EB is additive or synergistic.

Mean lordosis quotients for the chronic estrogen treatment
were presented in Figure 4. The analysis performed (see
Experiment 4 for details) indicated that neither the 1 μg nor
the 10 μg dose led to levels of receptivity observed in animals
given 100 μg EB. The 1 μg and 10 μg EB doses did not differ
significantly from each other. Furthermore, only the 100 μg EB
dose was sufficient to induce high levels of receptivity. This
contrasts with the ability of 1 and 10 μg EB to restore food
intake and body weight in ovariectomized animals to levels
exhibited by intact controls. Thus, the dose of EB which
adequately restores food intake and body weight after
ovariectomy (1 μg) does not fully restore sexual receptivity.
This suggests that the system regulating sexual behaviour in the
gerbil is less sensitive to EB than the system regulating
feeding behaviour.

EXPERIMENT 10

Because ovarian hormones did exert an effect on food intake
and body weight in the female gerbil (Experiment 9), it is
possible that they may also exert effects on consummatory
behaviour and body weight in the male gerbil. Evidence
indicates that, while castration increases body weight in the
male gerbil, androgen replacement has little influence on food
However, it may be that both male and female gerbils are more
sensitive to ovarian steroids with respect to food intake and
body weight regulation, as appears to be the case in the rat (Roberts et al., 1972; Ross & Zucker, 1974; Wade, 1975, 1976). Thus, the present experiment was carried out in order to investigate the possible effects of ovarian steroids on these responses in the male gerbil. In addition, the effects of castration were examined in an attempt to replicate the finding of Maass & Wade (1977) that castration increases body weight, a result seemingly inconsistent with the failure of androgen treatment to influence food intake and body weight. Finally, in an attempt to gain some indication of the extent of natural defeminization occurring in the male gerbil, tests of lordosis behaviour in the male were carried out as well.

**Method**

In order that any effects observed in the male could be compared to effects seen in the female, initiation of the five treatment phases followed the time course originally set during Experiment 9. For example, EB was administered the same number of days after surgery in males as for females and continued the same length of time before Phase 3 started. However, in males, the detrimental effects of the 100 μg EB dose were observed sooner than in the females and thus, group E100 was terminated before the completion of the EB phase and was eliminated from all experimental analyses. All analyses performed were otherwise identical to those done for Experiment 9. The resulting numbers of animals per group were 10 for group IC and E10 and 7 for group E1. Due to exceedingly low levels of receptivity exhibited by the male gerbils given 1 or 10 μg EB, no analysis was performed on these data.
Results and Discussion

Three day averages for food intake and body weight are presented in Figure 11. An examination of the data for groups IC, E1, and E10 suggests that castration had a transient facilitatory effect on body weight and that EB administration appeared to increase food intake only slightly. Although castrates and castrates given EB treatment appeared to eat more than intact animals, an analysis of variance indicated no significant effect of castration on food intake. This may have been due, in part, to the within subjects nature of the design. This design could have greatly increased the variance when different treatment phases were collapsed to determine an overall castration effect. Indeed, a Kruskal-Wallis test performed on IC, E1, and E10 data for Phases 1 and 2 indicated a significant overall effect of castration on food intake \( Z = 4.68, p < 0.05 \). The analysis of variance performed on IC, E1, and E10 data for Phases 1 and 2 indicated a significant effect of points, \( F(8,192) = 7.79, p < 0.0001 \). The group by points interaction approached significance, \( F(16,192) = 1.66, p < 0.06 \). Analysis of body weight data revealed a significant effect of Phase, \( F(1,24) = 19.65, p = 0.0002 \), and a significant effect of points, \( F(8,192) = 2.98, p = 0.0004 \).

Figure 12 shows the ratio of food intake and body weight means for group E1 relative to group IC expressed as a percentage. Examination of the data for groups IC and E1 in all phases of the experiment indicate that castration had only a transient effect on body weight and that EB, EB + P, and perhaps P treatment had no effect on body weight. Food intake levels
Figure 11. Effects of castration and ovarian steroid administration on food intake and body weight in male gerbils. Food intake and body weight were measured daily and three day averages were recorded. Data for intact controls (IC) and animals receiving 1 μg estradiol benzoate (E1) during estradiol benzoate (EB) phases are presented for all phases of the experiment. For progesterone (P) phases, a dose of 100 μg was used. The phase sequence is from top to bottom in the figure. Food intake and body weight means for group E10 (10 μg EB) is presented for the first two phases of the experiment. This group was later discontinued when detrimental effects of high doses of chronic EB were observed.
Figure 12. Effects of castration and ovarian steroid administration on food intake and body weight in male gerbils relative to levels exhibited by intact control animals. Daily measures of food intake and body weight were averaged to create five data points per treatment phase, presented on the abscissa. The ordinate is the ratio of experimental animal scores to intact control animal scores, expressed as a percentage. Estradiol benzoate (EB) and progesterone (P) doses were 1 and 100 μg, respectively.
for E1 animals were elevated throughout the experiment. An analysis of variance revealed a significant group by point interaction for weight, $F(20,300) = 3.37, p < 0.0001$, and a significant Phase by group interaction for food intake, $F(4,60) = 2.77, p = 0.04$, (Table 2). Further analysis indicated that E1 animals weighed more than IC animals during the first point of the castration phase, $p < 0.05$. Weights of IC and E1 animals did not differ during the EB and EB + P phases. However, beginning with the fourth point in the P only phase and continuing through the second point of the P withdrawal (no hormone) phase, IC animals weighed significantly more than E1 animals, $p < 0.05$. By the final point in the P withdrawal phase, this had reversed, with IC animals now weighing less than E1 animals, $p < 0.05$. It was further found that food intake for E1 animals was significantly greater than that of controls during the final phase of the experiment, $p < 0.05$, whereas the analysis of variance revealed no significant differences in food intake during the other four phases. Finally, there were significant effects of Phase for body weight, $F(4,60) = 28.03, p < 0.0001$, and food intake, $F(4,60) = 6.05, p = 0.0004$, and of points for food intake, $F(20,300) = 4.69, p < 0.0001$.

Overall, these results suggest that castration may have only a temporary effect on body weight. Ovarian steroids appeared to exert little effect on food intake and body weight while withdrawal of P may have served to increase food intake above postcastration levels.
Table 2. Mean food intake and body weight in male gerbils as a function of castration and ovarian steroid treatment.

<table>
<thead>
<tr>
<th>PHASE</th>
<th>BODY WEIGHT</th>
<th>FOOD INTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC</td>
<td>E1</td>
</tr>
<tr>
<td></td>
<td>X ±SE</td>
<td>X ±SE</td>
</tr>
<tr>
<td>CAST</td>
<td>78.34 1.63</td>
<td>79.78 0.95</td>
</tr>
<tr>
<td>EB</td>
<td>80.55 1.83</td>
<td>81.62 1.01</td>
</tr>
<tr>
<td>EB+P</td>
<td>76.33 1.81</td>
<td>77.20 0.95</td>
</tr>
<tr>
<td>P</td>
<td>74.74 1.82</td>
<td>72.60 1.14</td>
</tr>
<tr>
<td>NH</td>
<td>73.46 1.65</td>
<td>72.82 0.98</td>
</tr>
</tbody>
</table>

IC = intact control animals, received no hormone treatments. E1 = received 1 μg estradiol benzoate (EB) during EB phases and 100 μg progesterone (P) during P phases. NH = no hormone treatment.
GENERAL DISCUSSION

The results of the present experiments indicate that female gerbils exhibit a significant decrease in food intake and body weight after ovariectomy while male gerbils exhibit a transient increase in body weight after castration. Maass & Wade (1977) observed a longer lasting increase in body weight after castration. In addition, Maass & Wade (1977) observed no significant effect of castration on food intake while the present results indicate that castration may have a delayed effect on food intake.

A delayed effect of castration on behaviour is not unusual and has been reported for alterations in food intake and body weight in male rats (Gentry & Wade, 1976b) and for sexual behaviour in male rats and gerbils (Yahr, 1981). Moreover, the restorative effects of TP treatment on behaviour in castrates are also often delayed. It is possible that these delays in the behavioural effects of castration and TP treatment are due to a slow decline and increase, respectively, in receptor activity (Gorzalka, personal communication).

Ovariectomized female gerbils given chronic EB treatment exhibited a dose-dependent increase in food intake and body weight, with body weight eventually reaching that of intact controls. The EB-induced increase in food intake and body weight is consistent with the results obtained by Maass & Wade (1977) for ovariectomized gerbils. In addition, the present results also indicate that the decreases in food intake and body weight observed after ovariectomy are due to the removal of endogenous estrogens and that EB treatment serves a restorative
function. Castrated male gerbils, on the other hand, exhibited no significant alterations in food intake or body weight in response to EB treatment.

Although administration of P in conjunction with EB produced no significant alterations in female body weight, it did produce a significant decrease in food intake, a result consistent with that of Maass & Wade (1977). This suggests that progesterone, given in conjunction with EB, may alter metabolic activity, thus necessitating greater food intake for the maintenance of body weight. The present results further extend the findings of Maass & Wade (1977) by indicating that the administration of EB alone is sufficient to restore both food intake and body weight of ovariectomized animals to levels exhibited by intact controls. Indeed, the further increase in food intake observed in animals also receiving progesterone is not necessary for the restoration of body weight to control levels. In the male gerbil, administration of P in conjunction with EB had no significant effect on food intake and body weight.

Treatment of ovariectomized females with P alone resulted in decreased body weight while food intake remained above levels exhibited by control animals. Further analysis suggested that the decrease in body weight might be due, in part, to the prior removal of EB. This possibility was strengthened by the finding that the withdrawal of P did not lead to a significant increase in body weight, which one would expect if P administration was responsible for the decrease in body weight. Females did, however, exhibit a return to normal levels of food intake after
P withdrawal, indicating that progesterone may have been partially responsible for the elevated food intake levels during the P only phase.

Unlike the effect observed in the female gerbil, decreased body weight in the male gerbil during the P only phase did not appear to be due to the removal of EB. Rather, the decrease in body weight appeared to be due solely to an effect of P treatment. This is supported by the finding that, in males, withdrawal of P led to a gradual increase in body weight, eventually to levels above those of controls. An analysis of variance indicated that the withdrawal of progesterone also led to significantly greater food intake in male gerbils, a result opposite of that observed in females. However, the general elevation of food intake in male castrates throughout the experiment makes this result difficult to interpret. There is no indication that P administration was having any more effect on food intake and body weight than EB or EB + P, treatments which themselves appeared to have little effect. Yet, the withdrawal of P apparently led to further increases in food intake even though its administration did not appear to decrease food intake. It is possible that ovarian steroids were exerting a slight inhibitory effect that was masked by (or was masking) a delayed effect of castration. If so, it would not be the withdrawal of P *per se* that led to alterations in food intake and body weight. Rather, it would be the complete withdrawal of all ovarian steroids that produced the effects. This type of inhibition might also help explain the rather unusual result obtained by Maass & Wade (1977). They found that the
administration of TP to castrated male gerbils produced no observable alteration in food intake or body weight. However, when Maass & Wade (1977) ceased TP treatment, body weight increased. Presumably, the delayed effects of castration in either experiment would have been an increase in food intake and/or body weight if steroid treatments had not been initiated.

The finding that ovariectomy decreased and EB treatment increased food intake and body weight in the female gerbil is of great interest in relation to results obtained for other rodent species. In other female rodents, ovariectomy has been found to increase food intake and body weight (Gerall & Thiel, 1975; Morin & Flemming, 1978; Slob et al., 1973; Tarttelin & Gorski, 1973; Wade, 1975; Wade & Zucker, 1970; Wright & Turner, 1973). In addition, progesterone has been found to attenuate rather than facilitate the effects of EB on food intake and body weight (see Wade, 1976, for a review). Thus, the female gerbil is quite different from other female rodents studied to date with respect to the hormonal mediation of food intake and body weight. The study of these differences may aid in refining present models of the hormonal mediation of food intake and body weight in rodents.

Male gerbils exhibited a temporary increase in body weight after castration. This is somewhat similar to the effect observed in the male hamster, which also exhibits a weight increase after castration (Kowalewski, 1969). However, in the castrated male hamster, TP treatment reduces both food intake and body weight (Zucker et al., 1972) while in the castrated male gerbil, it does not (Maass & Wade, 1977). Thus, although
androgens may be the primary activating hormones for the regulation of food intake and body weight in the hamster (as suggested by Wade, 1976), this is not the case in the male gerbil. The findings of Maass & Wade (1977) resulted in the present suggestion that perhaps the hormonal mediation of food intake and body weight in the gerbil is similar to that observed in the rat, where ovarian steroids, rather than androgens, are the primary hormonal regulators of food intake and body weight (Wade, 1976). Recall that, in the rat, the following evidence supports the conclusion that ovarian steroids are the primary hormonal regulators of food intake and body weight. First, low doses of TP increase, while high doses of TP decrease food intake and body weight in male castrates. This is consistent with the suggestion that higher doses of TP would result in more aromatization to estrogen, assuming aromatizing enzyme sufficiency. Second, P attenuates the effects of high TP doses in male castrates, suggesting that P may be attenuating the effect of estrogen derived from TP. Finally, EB produces decreases in food intake and body weight in ovariectomized females while P attenuates this effect. The present results indicate that ovarian steroids may have some effect in the male gerbil but it is uncertain as to which sex steroids are the primary regulators of food intake and body weight in the male gerbil. In the female gerbil, ovarian steroids do exert a clear and significant effect and it seems likely that they play a major role in the hormonal mediation of food intake and body weight.

Research on the hormonal mediation of food intake in the
rat indicates that the effects of ovariectomy and EB treatment on food intake and body weight may be due, in part, to estrogen-induced alterations in brain catecholamine levels (Bapna, Neff, & Costa, 1971; Crowley, O'Donohue, Waschslicht & Jacobowits, 1978; Hoebel, 1971; Simpson & Dicara, 1973; van der Gugten & Slangen, 1975). In general, it has been found that the administration of dopamine (DA) or low doses of norepinephrine (NE) produce increases in food intake while DA and NE antagonists block or reverse this effect (Clineschmidt, McGuffin, & Werner, 1974; Hoebel, 1971, 1977). Ovariectomy has been found to increase synthesis of NE in the hypothalamus (Anton-Tay & Wurtman, 1968; Bapna et al., 1971) while EB administration has been found to decrease hypothalamic NE synthesis (Bapna et al., 1971). Simpson & Dicara (1973) found that DA increased food intake only when EB levels were low while NE increased food intake levels independent of EB levels. This led them to suggest that EB might interfere with dopamine-β-hydroxylase, the enzyme which converts DA to NE. This would lead to decreased brain NE and a corresponding decrease in food intake (Simpson & Dicara, 1973).

In the female gerbil, the increase in food intake and body weight in response to EB administration is the opposite of the effect observed in the female rat. If, in the female rat, EB decreases food intake by interfering with the conversion of DA to NE, it would suggest that the opposite effect in the female gerbil could be due to at least one of three mechanisms. First, EB may facilitate rather than inhibit DA to NE conversion, resulting in higher NE levels that would lead to increased
levels of food intake. Second, NE may exert an inhibitory rather than facilitatory effect on food intake in the gerbil. Thus, if EB inhibited DA to NE conversion, NE levels would decrease and food intake would increase. Third, EB may be exerting its effects on other neurotransmitters. To the best of my knowledge, relevant data to separate these potential mechanisms is not available.

Relatively little work has been done on the effects of castration and EB treatment on brain neurotransmitter levels in the castrated male rat. Donoso, Stefano, Biscardi, & Cukier (1967) found that castration in male rats led to increased hypothalamic DA concentrations, producing a higher NE:DA ratio. Thus, increased NE, decreased DA, and/or an altered NE:DA ratio could be responsible for the observed decrease in food intake and body weight in the castrated male rat.

Although it is difficult to determine the exact nature of the effects of castration in the male gerbil, it seems plausible that at least some of the effects would be mediated by hormone-induced alterations in catecholamine levels. Since the male gerbil appears to exhibit a food intake and body weight increase after castration while the male rat exhibits a decrease, one might again suspect that the effects of castration on neurotransmitter levels, or the effects of neurotransmitters, differ between these species. Castration in the male gerbil may decrease, rather than increase NE levels, and/or increase, rather than decrease, DA levels. Either would produce a lower rather than higher NE:DA ratio and might be responsible for the increases in food intake and body weight seen in the male gerbil
after castration. Alternatively, the effects of castration on NE and DA levels in the male gerbil may be similar to those observed in the male rat while the action of the neurotransmitters may differ between these species. Finally, castration may alter the level of some other neurotransmitter.

Experiments 4, 11, and 12 indicated that, while castrated male gerbils exhibited essentially no female sexual behaviour during chronic EB administration, female gerbils exhibited high levels of receptivity when given 100 μg EB/day. Doses of 1 and 10 μg EB/day led to moderate receptivity. Thus, although both 1 and 10 μg EB/day were sufficient for the restoration of food intake and body weight to control levels, these doses were not capable of eliciting high levels of receptivity. This is in contrast to the female rat where chronic administration of less than 1 μg EB/day can elicit high levels of receptivity (Davidson et al., 1968; Gorzalka & Raible, 1981; Gray & Gorzalka, 1980) as well as significantly alter food intake and body weight (Landau & Zucker, 1976; Wade, 1975). It would appear that the neural substrate regulating sexual responding in the female gerbil is less sensitive to EB than that of the female rat. In addition, the marked differences in sensitivity to estrogen seen between the system regulating food intake and body weight and the system regulating sexual responding in the gerbil is not apparent in the female rat. The differential EB sensitivities of the two systems in the gerbil and rat may merely reflect the fact that sexual receptivity is normally more progesterone-dependent in the gerbil (Experiments 2-4).

Male gerbils did not exhibit significant female sexual
behaviour when given chronic EB treatment. This is in contrast to the male rat which will show low levels of female sexual behaviour when given chronic EB treatment in doses such as 20 µg/kg/day (Eriksson & Södersten, 1973). The 10 µg EB dose used in the present experiments represents a dose of about 120 µg/kg/day. Although this dose is substantially larger than that used by Eriksson & Södersten (1973), it failed to elicit any appreciable level of sexual receptivity in male gerbils. This indicates that male gerbils are naturally defeminized during the period of sexual differentiation. Furthermore, endogenous androgens appear to defeminize the male gerbil to a greater extent than they do the male rat. It also appears that both male and female gerbils are less sensitive than male and female rats to the effects of EB on sexual responding. This again indicates that the hormonal regulation of sexual receptivity in the gerbil may be more like that observed in the hamster, which also exhibits less sensitivity to EB and is more progesterone-dependent than the rat (Feder et al., 1974).

In summary, ovarian steroids were found to exert a major effect on food intake and body weight in the female gerbil whereas the effects of ovarian steroids on food intake and body weight in the male gerbil remain unclear. In the female gerbil, EB treatment appears to serve a restorative function in the ovariectomized animal, bringing food intake and body weight back to presurgical levels. It is possible that the effects of EB on food intake and body weight in the gerbil are produced by hormone-induced alterations in brain catecholamine levels, as may be the case in the rat. The present results also indicate
that the system regulating food intake and body weight in the gerbil is more sensitive to estrogen than the system regulating female sexual behaviour. Finally, the results suggest that exogenous quantities of estrogen which induce receptivity in male and female rats are insufficient to induce receptivity in male and female gerbils.

It seems likely that ovarian steroids play a major role in the hormonal mediation of food intake and body weight in the female gerbil. Although the results of Experiment 10 and those obtained by Maass & Wade (1977) suggest that the hormonal regulation of food intake and body weight in the male gerbil may differ from that observed in other male rodents examined so far, a great deal of further study will be required before the mechanisms underlying these differences can be determined.

IIb - Pre- and Neonatal Testosterone Propionate Exposure: Effects on Male and Female Sexual Behaviour in Adulthood

Since Phoenix et al., (1959) first suggested that sex steroids could exert both an organizational as well as an activational effect on the brain, a great wealth of evidence has accumulated in support of this hypothesis. Activational effects are seen not only in the hormonal regulation of sexual behaviour but also in the mediation of eating behaviour, aggression, scent marking, and a wide variety of other behaviours, all of which are altered by gonadectomy in adulthood (Gorski, 1979). Much of the support for the organizational effects of sex steroids has been more recent and it is now clear that adult sexual behaviour, as well as a variety of other behaviours, is
influenced by the hormonal environment during the period of sexual differentiation (for reviews, see Feder & Wade, 1974; Gorski, 1971, 1979, 1980; Goy, 1970; McEwen, Denef, Gerlach, & Plapinger, 1974).

In the genetic male, androgen secretions (primarily testosterone) have been found to: (1) guide sexual differentiation of male reproductive organs and increase the sensitivity of these tissues and the brain to androgens in adulthood, (2) act on the hypothalamus to produce a tonic, rather than cyclic, pattern of gonadotropin release in adulthood, and (3) decrease the responsiveness of tissues mediating sexual behaviour in adulthood to estradiol and progesterone (Feder & Wade, 1974; Gorski, 1980; Goy, 1970). In genetic females, the absence of androgens permits female reproductive organs to develop, gonadotropins to be released cyclically (the estrous cycle), and the tissues mediating sexual behaviour to be highly sensitive to estrogen and progesterone (Feder & Wade, 1974; Flerko, Mess & Illei-Donhoffer, 1969; Gorski, 1980).

The term 'defeminization' was initially used by Whalen in reference to the profound decrease in estrogen sensitivity that occurs when androgens are present during the period of sexual differentiation (Whalen & Edwards, 1967; Whalen, Luttge, & Gorzalka, 1971). The term 'masculinization' ideally, but not always, refers to the presence of all three components of male sexual responding (mounts with pelvic thrusting, intromission, and ejaculations).

When reviewing the literature on the effects of early
hormonal environment on adult sexual behaviour, it became clear to the author that the terms 'masculinized' and 'defeminized' were frequently confused with each other and used inappropriately. An animal which exhibits a decline in sexual receptivity is only defeminized if the reduction in sexual receptivity is pronounced, such that only very low levels of receptivity are observed. Although the term 'defeminized' is generally used appropriately in the rat, mouse, and guinea pig literature, it is frequently misused in the hamster literature. The male hamster exhibits fairly high levels of female sexual behaviour in adulthood and is considered, properly, to be the one rodent examined so far that is not defeminized by androgens during sexual differentiation (Goldman, 1978; Tiefer & Johnson, 1975). Despite this, several authors (e.g. Baum, 1979; Carter Clemens, & Hoekema, 1972; Tiefer & Johnson, 1975), upon finding that neonatal androgen treatment results in a male pattern of sexual behaviour in the female hamster, conclude that the female hamster is defeminized by neonatal androgens. This seems to be an unreasonable conclusion since the TP treated female hamster exhibits the same degree of female sexual behaviour as does the male hamster, and that level is sufficient to indicate the hamster is not defeminized although it is masculinized. This also clearly indicates that masculinization and defeminization need not be concurrent.

As stated earlier, the term 'masculinization' is best used to refer only to an animal which exhibits all components of male sexual behaviour. There are several good reasons for this restriction. For example, normal female rats will exhibit
mounting behaviour in the absence of exogenous androgens (Baum, 1979; Plapinger & McEwen, 1978). Furthermore, neonatal castration of male rats does not eliminate mounting behaviour in adulthood (Goldman, 1978). Thus, mounting behaviour is not a gender-specific response, does not seem highly dependent upon the influence of perinatal androgens and therefore is not an adequate indicator of behavioural masculinization. On the other hand, intromissions and ejaculations are rarely exhibited by genetic females and neonatal castration of males does eliminate intromitting and ejaculatory behaviour (Goldman, 1978), suggesting that they are gender-specific responses that are dependent upon early androgens. Furthermore, although a TP treated genetic female may be physiologically incapable of intromitting and ejaculating, she is behaviourally capable of exhibiting these responses (Coniglio & Clemens, 1976; Gerall & Ward, 1966; Nadler, 1969). Male rats exhibit very stereotypical responses when intromitting and ejaculating. These stereotyped responses are also exhibited by masculinized genetic females although there is generally no insertion or emission. Thus, it does not seem unreasonable to require all components of male sexual behaviour to be present before the term 'masculinization' is used, especially since the absence of intromitting and ejaculatory behaviour in male rats is viewed as evidence for abnormal male sexual behaviour (Whalen, 1974). Unfortunately, a large number of reports on the effects of neonatal castration and early androgen treatment report only results obtained for mounting behaviour (e.g., Edwards & Burge, 1971; Goy, Bridson, & Young, 1964; Paup, Coniglio, & Clemens, 1972; Phoenix et al.,
1959) although they often state that they recorded other components of male sexual behaviour (e.g., Edwards & Burge, 1971; Phoenix et al., 1959). This makes it very difficult to draw any firm conclusions on the masculinizing effects of various treatments and raises the possibility that many of the procedures reported to induce masculinization do not. They may, however, induce partial masculinization.

There is one additional problem frequently encountered in the literature which also bears directly on the present paper. Some researchers (e.g., Edwards & Burge, 1971; Nucci & Beach, 1971) have apparently failed to consider the possibility that the lack of an effect (or the nature of an effect) could be due entirely to the fact that their treatments did not coincide with any or all of the appropriate sensitive period. Many erroneous conclusions occur in the literature because of an apparent failure to consider this possibility. For example, Edwards & Burge (1971) concluded that there is "little reliable evidence in our data to indicate neonatal androgenic stimulation facilitates adult expression of masculine sexual behavior.". This is despite the fact that they used only one dose of TP (100 µg) administered only on Day 1 after birth. More prolonged treatment and/or a larger dose may have produced different effects.

The findings of Nucci & Beach (1971) may have had more significant consequences on the literature. Nucci & Beach (1971) found that prenatal TP treatment in the hamster had no effect on any aspect of sexual behaviour or physiology in adulthood. Since that time, little, if any, work has been
published on the effects of prenatal TP on sexual development in the hamster. This suggests that subsequent researchers have assumed that Nucci & Beach's (1971) results preclude the possibility that prenatal TP may exert an effect when followed by postnatal treatment. This is a dangerous conclusion for several reasons. First, Nucci & Beach (1971) used only one dose of TP whereas different doses might have produced different effects. Second, the dose employed by Nucci & Beach (1971) was quite high (2 mg/day for three days). That it was either detrimental to the pregnant females, the pups, or both is indicated by the fact that although 18 pregnant females were given TP, only 18 pups survived to testing (an average of one per litter). It is not clear whether the low number of subjects was a result of spontaneous abortion or pup mortality, but either is indicative of a detrimental (and probably stressful) situation. Thus, one begins to wonder what made the surviving pups the exception to the rule and whether this may have affected their behaviour. Third, although Nucci & Beach (1971) administered injections over either Days 11-13, 12-14, or 13-15 of gestation, they do not indicate the number of pups surviving under each condition. Although they do state that there were no differences between pups in different conditions, it is possible that the majority of survivors were from a group in which prenatal treatments did not exert any effect. Finally, the failure of prenatal TP treatment to exert effects on sexual behaviour does not preclude the possibility that it is a prerequisite for the masculinizing action of postnatal TP. For example, prenatal TP might exert a priming or organizing effect
on neural tissues that is essential if postnatal TP is to exert its full effect. Prenatal exposure might exert no visible behavioural or physiological effects, yet the absence of perinatal androgen exposure could preclude the full development of other behaviours influenced by androgens. Thus, it would seem unwise to conclude that prenatal androgen treatments exert no effect on adult sexual behaviour in the hamster solely on the basis of the results obtained by Nucci & Beach (1971). Fortunately, there does seem to be a trend in the recent literature to carefully consider the possibility that the effects observed may reflect the nature of the treatment as well as the process of sexual differentiation.

The effects of early hormonal environment on adult sexual behaviour have most frequently been examined following the perinatal administration of androgens to females and following the neonatal castration of males. Experimental manipulations such as these have indicated that, in all laboratory rodents studied to date, the presence of androgens during the period of sexual differentiation alters adult sexual behaviour in a manner that varies with dose, interval of steroid administration relative to parturition, and duration of treatment (Carter & Landauer, 1975; Coniglio & Clemens, 1976; Edwards, 1971; Gerall & Ward, 1966; Goy et al., 1964; Nadler, 1969; Paup et al., 1972; Phoenix et al., 1959).

The dose, interval and duration dependent effects of perinatal androgen treatment on female sexual behaviour in adulthood are fairly clear and uncomplicated. In the two laboratory rodent species with altricial young (no fur, eyes and
ears closed at birth, not very motile) and males that are naturally defeminized (rat and mouse), the administration of 100 μg to 500 μg TP within 48 hr of birth is sufficient to defeminize the animal (Clemens, Shryne, & Gorski, 1970; Edwards & Burge, 1971). This treatment does not necessarily fully masculinize the animal, indicating that defeminization is not dependent upon masculinization. It is also possible to produce behavioural masculinization without producing physiological masculinization (as determined by anogenital distance). For example, Ward (1969) found that female rats given TP every other day from birth to Day 40 did not differ from normal females with respect to anogenital distance although they did exhibit all three components of male sexual behaviour at levels comparable to normal males. Thus, the period of physiological masculinization and the period of behavioural masculinization do not necessarily coincide.

The hamster also gives birth to altricial young but males are not naturally defeminized (Baum, 1979; Goldman, 1978; Tiefer & Johnson, 1975). In this species, the administration of a single injection of 100 μg to 500 μg TP does not defeminize the female hamster although it does produce levels of sexual receptivity similar to those observed in the male (Alleva, Alleva, & Umberger, 1969; Johnson, 1975; Paup et al., 1972).

The guinea pig differs from the rat, mouse and hamster in that it gives birth to more precocial young (fur present, eyes and ears open, motile at birth). For TP administration to exert any major effects in this species, it must be given prenatally, via maternal injections, indicating that full differentiation
occurs before birth (Goy et al., 1964; Phoenix et al., 1959). To insure the sensitive period for defeminization is not missed, researchers (e.g., Goy et al., 1964; Goy, Phoenix, & Meidinger, 1967; Phoenix et al., 1959) generally employ a repeated injection procedure over different periods of gestation. This makes it difficult to determine if a single injection of TP at the appropriate time would defeminize the female guinea pig. In addition, the actual amount of TP reaching the pups cannot be determined. It is clear, however, that the administration of TP from Day 30-55 of gestation (which is about 68 days in length) produces a permanent and severe deficit in female receptivity (Goy et al., 1964; Goy et al., 1967).

In females which can be defeminized by a single injection of TP around the time of birth (i.e., the rat and mouse), the exact effects of TP on sexual receptivity in adulthood depend on the dose of TP administered. The higher the dose, the lower the receptivity (Gerall & Kenny, 1970; Goy et al., 1964; Whitsett & Vandenbergh, 1975). Behavioural studies indicate that the decreased receptivity caused by perinatal TP is due to reduced responding to EB and to EB given in conjunction with P (Clemens et al., 1970; Edwards & Thompson, 1970; Gerall & Kenny, 1970; Goy et al., 1967; Whalen et al., 1971). Furthermore, it now appears that the decreased responsiveness to EB is due, at least in part, to a perinatal androgen-induced decrease in the rate of estradiol receptors are replenished (Cidlowski & Muldoon, 1976). Thus, the dose-dependent effects of perinatal androgens on female receptivity in adulthood may reflect dose-dependent effects on the replenishment of estrogen-binding proteins.
The dose, interval, and duration effects of perinatal TP on the masculinization of behaviour are more complex than the effects on defeminization. Because there are several components to male sexual behaviour, it is possible that perinatal androgen treatment could induce some or all components of male sexual behaviour. Unfortunately, few researchers have been ambitious enough to carefully investigate the relative importance of dose, duration, and interval of TP administration on the masculinization of sexual behaviour. Most often, two factors (i.e., dose, treatment interval) are held constant while the third (i.e., duration) is varied. This can make it difficult, if not impossible, to determine the relative importance of all factors. For example, if varying the duration of treatment exerted only a slight behavioural effect, it could be that the dose was too low, the interval was incorrect, or the duration too short (or some combination). Although there are few experiments that use similar doses, intervals, or durations, comparisons between experiments do shed some light on the importance of various factors. It appears that interval and duration are more important factors than dose in rodent species studied so far. For example, Ward (1969) found that, in female rats, either prenatal plus postnatal or postnatal TP treatment led to ejaculatory responses while prenatal TP treatment did not, indicating that postnatal TP treatment is essential for the ejaculatory response in the rat. Goy et al. (1964) found that female guinea pigs receiving a total of 50 mg TP over days 15-40 of gestation gave birth to young which exhibited significantly less mounting behaviour than the young of females given the same
amount of TP over Days 30-55 of gestation.

In the hamster, the relative importance of the dose, interval, and duration of TP administration for masculinization is less clear. Nucci & Beach (1971) found that prenatal TP treatment in the hamster did not increase any aspect of male sexual behaviour, suggesting that masculinization might take place postnatally or at least requires postnatal androgen exposure. Coniglio & Clemens (1976) found that the administration of 40 μg or 100 μg TP to female hamsters from birth to Day 5 led to a greater degree of masculinization than treatment from Day 6-10. However, no ejaculations were observed in any of the TP treated females. Tiefer & Johnson (1975) found that the administration of 10 μg TP from birth to Day 20 also produced mounts and intromissions although, again, no ejaculatory behaviour was observed. This suggests that perhaps pre- and postnatal TP treatment is required to elicit the ejaculatory response in the hamster.

To summarize, in female rats and mice a single neonatal injection of TP will decrease lordosis responding in a dose-dependent manner. The effect of neonatal TP on receptivity in the hamster differs from that observed in the rat and mouse. Although TP does decrease female sexual responding in the hamster, the effect is not nearly as pronounced as that observed in other species. However, it seems clear that, in those species examined, a single neonatal TP injection can result in a level of receptivity similar to that observed in the male of that species (Alleva et al., 1969; Clemens et al., 1970; Edwards & Burge, 1971). Note that this does not necessarily indicate
that the female is defeminized. The sexual behaviour of the female hamster is masculinized by a single neonatal TP injection but the female, like the male, remains capable of displaying female sexual behaviour in adulthood (Alleva et al., 1969; Johnson, 1975). In contrast, all components of male sexual behaviour can not be induced by a single neonatal TP injection (Edwards & Burge, 1971; Paup et al., 1972). Rather, multiple injections over the appropriate interval appear to be required for full behavioural masculinization (Coniglio & Clemens, 1976; Goy et al., 1964; Ward, 1969).

Aside from the effects of perinatal androgen on reproductive behaviour and physiology, effects are observed in other hormonally mediated behaviours as well. TP administration during differentiation produces masculine food intake and body weight patterns in adult female rats, guinea pigs, and hamsters (see Wade, 1976, for a review). In addition, neonatal castration of male rats and hamsters produces a food intake and body weight pattern in adulthood which resembles that observed in the female of the species. Perinatal TP also alters aggressive behaviour in mice (Goldman, 1978) and scent marking behaviour in gerbils (Turner, 1975). Both of these behaviours are influenced by sex hormones in adulthood, with gonadectomy decreasing behaviour and sex steroid replacement therapy restoring behaviour to presurgical levels (Brain, 1977; Yahr, 1981).

There is now substantial evidence that perinatal androgens modify neural connections in various areas of the brain in the rat, mouse, and hamster (Dörner & Staudt, 1968, 1969; Field &
Raisman, 1976; Greenough et al., 1978; Kolata, 1979; Loy & Milner, 1980; Raisman & Field, 1976; Toran-Allerand, 1976). This indicates that the effects of perinatal androgens go beyond the simple alteration of steroid binding capacity and replenishment of receptors and into the more complex realm of neurocytological modifications. Thus, the behavioural alterations observed within a species after perinatal androgen exposure probably reflect both changes in neural structure and alterations in receptor replenishment and ability of neurons to bind various hormones.

Given the capacity of perinatal androgens to alter a variety of hormonally mediated behaviours within a species of rodent, it seems plausible that interspecific differences in the organizational effects of perinatal androgens may be responsible for some of the interspecific differences observed in hormonally mediated behaviours. The organizational effect of perinatal androgens could differ between species in the effects on neural synapses, steroid binding capacity, receptor replenishment, or some combination of these factors. If so, one might expect to find this difference to be reflected by several hormonally mediated behaviours since the organizing effects of perinatal androgens are not confined to sexual behaviour. We have already seen that rats, mice and guinea pigs are masculinized and defeminized by perinatal androgens (Edwards & Burge, 1971; Phoenix et al., 1959; Slob et al., 1973; Ward, 1969) while the hamster is masculinized but not defeminized by perinatal androgens (Tiefer & Johnson, 1971, 1975; Goldman, 1978). It has also been noted earlier that the hamster is the only rodent
studied so far in which the female eats and weighs more than the male (Kowalewski, 1969, Wade, 1976). Furthermore, the hamster is the only known rodent where the female displays more aggression than the male (Hart, 1974; Moyer, 1974). Thus, we find that the hormonal regulation of a variety of hormonally mediated behaviours in the hamster differs from other laboratory rodents. Between species differences in the organizing effects of perinatal androgen on the neural substrates regulating these behaviours provides one explanation of these differences (although it is not necessarily the only explanation).

Regardless of the reason for the difference between hamsters and other laboratory rodents, the differences in the effects of perinatal androgen on sexual behaviour and the difference in the hormonal regulation of food intake and body weight in adulthood suggest that it is at least possible that the extent of defeminization by androgens may determine which sex steroid will produce the greatest alterations in food intake and body weight in adulthood. Rodents not defeminized by physiological levels of androgens during the period of sexual differentiation may be most influenced by androgens while rodents that are defeminized by androgens would respond more to ovarian steroids. Thus, the gerbil, being most responsive to ovarian steroids in regards to food intake and body weight regulation should, like the rat, mouse, and guinea pig, be defeminized by the presence of perinatal androgens.

The objectives of the research in this section are to: (1) determine the effects of perinatal androgens on adult sexual behaviour and (2) evaluate the present hypothesis that knowledge
of the extent of perinatal androgen-induced defeminization allows the prediction of which gonadal steroids will exert the primary influence on food intake and body weight in adulthood (and vice versa).

GENERAL METHODS

Mated pairs of gerbils were obtained from Tumblebrook Farms. All had given birth to at least one, but no more than two, litters. Upon parturition, animals were supplied with two 27 cm x 23.5 cm paper towels to use as nesting material. Fresh bedding material leads to heightened digging activity which may temporarily disrupt pup care (personal observation). Therefore, no cages were changed until the pups were at least 20 days of age, when their eyes open and motility is greatly increased.

Experimental animals were weaned at approximately 30 days of age and housed with littermates until about 55 days of age when they were then separated into same sex pairs. At this time, all TP treated males were removed. Beginning at approximately 90 days of age, animals received five tests for male sexual behaviour, with tests occurring every other day. Stimulus females were brought into maximal receptivity by the administration of 10 µg EB 48 and 24 hr before testing plus an injection of 500 µg P 3-5 hr prior to testing. Mounts with pelvic thrusting, intromissions (mounts with thrusting and penetration), and ejaculations were recorded using an Esterline-Angus event recorder. This method of recording also allows the determination of temporal parameters during testing. Immediately after the final test for male sexual behaviour in
intact animals, all subjects were gonadectomized while under anesthesia grade ether. After recovery, daily injections of 10 μg EB were initiated with tests for female sexual behaviour beginning on Day 6 of EB treatment. Testing continued daily through Day 13 of EB treatment with a final test on the following day which occurred 3-4 hr after the administration of 500 μg P. After a period of no hormone treatment, allowing dissipation of residual hormones, daily injections of 100 μg TP were initiated and continued until the termination of the experiment. Tests of male sexual behaviour occurred on Days 15, 22, 29, and 36 of TP treatment. Upon termination of testing, animals were weighed, anogenital distances recorded and scent glands measured (length and width).

Lighting cycles, general maintenance, and female sexual testing were as described previously.

EXPERIMENT 11

The endocrine glands are not functional throughout the entire developmental period. Thus, the period in which perinatal androgens influence sexual differentiation may be missed if exogenous steroids are administered at a time different from the endogenous increase in steroid secretions (Goy, 1970). In addition, the influence of perinatal androgens on sexual development depends upon the level of circulating androgens during differentiation (Gorski, 1971, 1979). Thus, either a low dose of TP or the administration of TP during only a portion of the period of differentiation can produce degrees of behavioural masculinization and behavioural defeminization.
Furthermore, the sensitive periods for physiological masculinization, behavioural masculinization, and behavioural defeminization do not necessarily coincide. However, it does appear to be the case that, in rodents with altricial young (hamster, rat, and mouse), the period of susceptibility to androgen-induced defeminization occurs around the time of birth (Feder & Wade, 1974; Gorski, 1979; Goy, 1970).

It was previously hypothesized that the extent of androgen-induced defeminization can be used to predict which sex steroid is the primary regulator of food intake and body weight in a given species of rodent (and vice versa). Little is known about the effects of perinatal androgens on sexual behaviour in the gerbil. However, the finding that male gerbils do not exhibit female sexual behaviour during chronic EB treatment (Experiment 10) suggests that the male gerbil is defeminized by perinatal androgens. There remains the possibility that the female gerbil is not fully defeminized by perinatal androgens. Furthermore, although the rat and mouse can be defeminized by a single neonatal injection of 100 to 500 μg TP, this is not necessarily the case in the female gerbil. However, gerbils, like rats, mice, and hamsters, have a short gestation period and give birth to altricial young. It seems reasonable to assume that, if the differentiation process is similar to that observed in the rat and mouse, or in the hamster, a single neonatal injection of TP should result in some degree of defeminization in the female gerbil as well. Thus, a paradigm which would allow comparisons between species of the effects of a single neonatal injection of TP on female sexual behaviour would be
The present experiment was designed to determine: (1) the extent of neonatal TP-induced defeminization in the female gerbil, (2) between species differences in the effects of a single neonatal TP injection, and (3) if the effects of neonatal TP administration on the exhibition of male and female sexual behaviour in adulthood can be predicted on the basis of which sex steroid exerts the greatest influence on adult food intake and body weight regulation. Based on the results of Experiments 9 and 10 plus the results of Maass & Wade (1977), the present hypothesis predicts that the presence of androgens during the period of sexual differentiation will defeminize male and female gerbils.

**Method**

Pups received a single injection of either 100 μg TP (low dose), 1 mg TP (high dose), or the vehicle (0.01 cc peanut oil) within 24 hr of birth. Because the skin of newborn gerbils does not readily close after needle puncture, a small amount of Collodion (Fisher Scientific) was applied to the puncture to prevent leakage. In addition, success of the injection was verified by examination of the injection site for a slight but distinct bulge under the skin which is created by the fluid. Immediately after injection, pups were returned to the home cage. A total of four groups was created, vehicle treated females (VF), vehicle treated males (VM), 100 μg TP treated females (LTP), and 1 mg TP treated females (HTP). The final n's at the time of testing were 21, 15, 19, and 14 for the VM, VF, LTP and HTP groups, respectively.
Results and Discussion

An examination of the sexual behaviour data indicated that none of the groups during intact testing for male behaviour exhibited even moderate levels of behavioural masculinization (Table 3). This was expected for the female subjects since they lack a significant source of endogenous androgens. However, the relative lack of male sexual behaviour in male controls is somewhat puzzling. The relative lack of male sexual activity was also evident during the gonadectomy plus TP testing, although VM animals did appear to exhibit somewhat greater sexual activity (Table 4, 4a). The data for the female sexual behaviour tests suggested that only the VF animals exhibited an appreciable level of receptivity (Table 5). No analyses were performed on the male sexual behaviour data since all groups exhibited a very low level of activity. The data do suggest that neither the 100 μg nor the 1 mg dose of TP were adequate to produce the full complement of male sexual behaviour, and thus were not sufficient to fully behaviourally masculinize the animals. However, the exact nature of the masculinizing action of TP is difficult to assess since the control animals also exhibited fairly low levels of male sexual behaviour.

A 4x8 (group by test day) repeated measures analysis of variance with a nested factor was performed on the female sexual behaviour data and revealed a significant effect of group, $F(3,66) = 7.77$, $p = 0.0002$. A subsequent Newman-Keul's analysis on this effect indicated that, overall, VF animals were significantly more receptive than LTP, HTP, and VM animals. Other significant effects were test, $F(7,441) = 2.86$, $p = 0.006$. 
Table 3. Male sexual behaviour in intact male gerbils and in female gerbils treated neonatally with testosterone propionate.

<table>
<thead>
<tr>
<th>SEXUAL RESPONSE</th>
<th>PERCENT RESPONDING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VM (n=21)</td>
</tr>
<tr>
<td>M T1 I</td>
<td>4.8</td>
</tr>
<tr>
<td>E</td>
<td>4.8</td>
</tr>
<tr>
<td>M T2 I</td>
<td>4.8</td>
</tr>
<tr>
<td>E</td>
<td>4.8</td>
</tr>
<tr>
<td>M T3 I</td>
<td>9.5</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
</tr>
<tr>
<td>M T4 I</td>
<td>4.8</td>
</tr>
<tr>
<td>E</td>
<td>4.8</td>
</tr>
<tr>
<td>M T5 I</td>
<td>10.0</td>
</tr>
<tr>
<td>E</td>
<td>4.8</td>
</tr>
</tbody>
</table>

VF = vehicle injected females, LTP = 100 µg testosterone propionate (TP) injections, HTP = 1 mg TP injection, VM = vehicle injected males. M = mounts, I = intromissions, E = ejaculations. T1-5 = consecutive tests every 2 days.
Table 4. Male sexual behaviour in castrated male gerbils and in ovariectomized female gerbils treated neonatally with testosterone propionate.

<table>
<thead>
<tr>
<th>SENSUAL RESPONSE</th>
<th>PERCENT RESPONDING</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VM (n=21)</td>
<td>HTP (n=14)</td>
<td>LTP (n=19)</td>
<td>VF (n=15)</td>
</tr>
<tr>
<td>M</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>T1 I</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>M</td>
<td>4.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>T2 I</td>
<td>4.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>M</td>
<td>9.5</td>
<td>7.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>T3 I</td>
<td>4.8</td>
<td>7.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>M</td>
<td>76.2</td>
<td>7.1</td>
<td>15.3</td>
<td>0.0</td>
</tr>
<tr>
<td>T4 I</td>
<td>33.3</td>
<td>7.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>E</td>
<td>23.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

VF = vehicle injected females, LTP = 100 μg testosterone propionate (TP) injection, HTP = 1 mg TP injection, VM = vehicle injected males. M = mounts, I = intromissions, E = ejaculations. T1-4 = consecutive weekly tests.
Table 4a. Additional measures of sexual behaviour for T4 data of Table 4*.

<table>
<thead>
<tr>
<th>MEASURE</th>
<th>n</th>
<th>VM</th>
<th>±SE</th>
<th>HTP</th>
<th>±SE</th>
<th>LTP</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML(sec)</td>
<td>16</td>
<td>1057.6</td>
<td>210.0</td>
<td>11076.0</td>
<td>000.0</td>
<td>31028.7</td>
<td>497.7</td>
</tr>
<tr>
<td>IL(sec)</td>
<td>8</td>
<td>877.4</td>
<td>144.3</td>
<td>11561.0</td>
<td>000.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EL(sec)</td>
<td>5</td>
<td>565.4</td>
<td>154.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PEI(sec)</td>
<td>4</td>
<td>234.5</td>
<td>30.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EJ(freq)</td>
<td>5</td>
<td>1.4</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Animals that did not exhibit male sexual activity were excluded from the table.

VM = vehicle injected males, HTP = females injected with 1 mg testosterone propionate (TP) within 24 hr of birth, LTP = females injected with 100 μg TP within 24 hr of birth. ML = mount latency, IL = intromission latency, EL = ejaculation latency, PEI = post-ejaculatory interval, the time interval between the first intromission and ejaculation, EJ = mean number of ejaculations.
Table 5. Female sexual behaviour in castrated male gerbils and in ovariectomized female gerbils treated neonatally with testosterone propionate and administered ovarian steroids in adulthood.

<table>
<thead>
<tr>
<th>TEST (EB)</th>
<th>VF X ±SE</th>
<th>LTP X ±SE</th>
<th>HTP X ±SE</th>
<th>VM X ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.7 2.7</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T2</td>
<td>4.0 4.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T3</td>
<td>10.0 6.8</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T4</td>
<td>4.7 4.7</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T5</td>
<td>11.3 7.2</td>
<td>5.3 5.3</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T6</td>
<td>25.3 11.3</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T7</td>
<td>34.0 12.2</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>T8</td>
<td>26.7 10.7</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>(EB+P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T9</td>
<td>86.6 7.1</td>
<td>2.1 1.6</td>
<td>0.0 0.0</td>
<td>2.9 2.9</td>
</tr>
</tbody>
</table>

VF = vehicle injected females, LTP = 100 μg testosterone propionate (TP) injection, HTP = 1 mg TP injection, VM = vehicle injected male. X = mean sexual receptivity, number of lordosis postures exhibited by the female divided by the number of male mounts with pelvic thrusting and multiplied by 100. Animals received 13 consecutive injections of 10 μg estradiol benzoate (EB) and were tested on Days 6-13. On Day 14, 100 μg progesterone (P) was administered 3-4 hr prior to testing.
and group by test, $F(21,44) = 3.43, p < 0.0001$. Thus, both the 100 ug and the 1 mg TP doses defeminized the female gerbil. In addition, male gerbils appeared to be defeminized by endogenous androgens during the period of sexual differentiation.

An examination of the data for the physiological measures (scent gland length and width, body weight, and anogenital distance) suggested that some degree of physiological masculinization had occurred and that, for all measures, the degree of masculinization was related to the dose of TP administered at birth (Figure 13a-d). Separate one-way analyses of variance were performed on the data for each of the physiological measures and revealed significant group differences, beyond the 0.0001 level, for body weight, $F(3,62) = 9.28$, scent gland length, $F(3,62) = 64.80$, scent gland width, $F(3,62) = 30.38$, and anogenital distance $F(3,62) = 620.66$. Newman-Keul's analyses, with $\alpha$ set at 0.05 indicated that, for body weight, groups VF and LTP did not differ and that groups HTP and VM did not differ but that animals in groups VF and LTP weighed significantly less than animals in groups HTP and VM. The scent gland length of VF and LTP animals was also found to be similar while scent gland length of HTP animals was greater than for VF and LTP animals and less than for VM animals. For both scent gland width and anogenital distance, VF animal measures were significantly lower than LTP which were significantly lower than HTP, which were significantly lower than VM measures. Thus, both scent gland width and anogenital distance appear to be quite sensitive to the effects of early androgens.
Figure 13. Comparison of indices of physiological masculinization in male gerbils and in female gerbils receiving a single neonatal injection of testosterone propionate. Measures of scent gland length (a), scent gland width (b), body weight (c), and anogenital distance (d) were taken after 36 days of treatment with 100 μg testosterone propionate (TP). Within 24 hr of birth, females received a single injection of the oil vehicle (VF), 100 μg TP (LTP), or 1 mg TP (HTP) while males received a single injection of the vehicle (VM).
The results of the present experiment indicate that a single injection of TP at birth is capable of defeminizing the female gerbil. However, the effects of a single TP injection on the development of male sexual behaviour is difficult to assess since all animals exhibited low levels of male sexual activity. It is possible that the low levels of male copulatory behaviour exhibited by control males during the intact testing were due to an extraneous variable (i.e., stress) which indirectly resulted in decreased levels of endogenous androgens. A large decrease in endogenous androgens could result in decreased sexual behaviour. If this is the case, sexual behaviour during TP administration should have been greater than during intact testing because the level of circulating androgens is controlled. This is indeed the case (see Tables 3, 4 and 4a) although the difference is not as great as one might expect. The results for tests of male sexual activity during TP administration also suggest that TP treated females were not fully masculinized by a single neonatal TP injection. On the final test of male sexual activity during TP treatment, 76% of VM animals, versus 12% of TP-treated females exhibited mounting behaviour. Furthermore, while 33% of VM animals exhibited behavioural intromission, only 3% of TP-treated females did so. Finally, 24% of VM animals ejaculated at least once during the final TP test while none of the TP-treated females exhibited the behavioural equivalent of this response (see Table 4). Although the results are not definitive, there is a suggestion that the female gerbil, like other female rodents, requires more than a single neonatal TP injection for full behavioural
masculinization to occur. Note, however, that this does not appear to be the case for defeminization, which does appear to be achieved by a single neonatal injection of TP. Thus, the effects of neonatal androgen treatment in the gerbil on the development of sexual behaviour is, at least in some respects, similar to that observed in other rodents.

Based on the food intake data, the prediction was made that androgens during the period of sexual differentiation would defeminize the gerbil. The results of the present experiment support the present hypothesis that the extent of defeminization during sexual differentiation determines which sex steroid will exert the greatest influence on adult food intake and body weight. However, it remains to be determined if perinatal androgens will fully masculinize sexual behaviour in the gerbil.

**EXPERIMENT 12**

The results of Experiment 11 indicate that some degree of physiological masculinization as well as behavioural defeminization can be achieved by a single injection of 1 mg TP administered with 24 hr of birth. Furthermore, it would appear that physiological masculinization can occur in the absence of behavioural masculinization. However, full behavioural masculinization does not appear to be achieved by such an injection. In the female hamster, prenatal androgen administration appears to exert no masculinizing effect on physiological measures or the capacity to display male sexual behaviour in adulthood (Nucci & Beach, 1971). Postnatal TP
administration does alter the capacity of the female hamster to exhibit male sexual activity in adulthood (Johnson, 1975; Tiefer & Johnson, 1975). The rat, on the other hand, shows the greatest degree of behavioural masculinization when given TP pre- and postnatally (Goy, 1970; Gerall & Ward, 1966; Ward, 1969). Although the masculinizing effect of perinatal androgens is not a major issue in the hypothesis being tested, it is of interest that neonatal TP may not lead to a significant increase in mounting behaviour in the female gerbil. It is possible that prenatal or pre- plus postnatal TP treatment will increase the extent of behavioural masculinization in the gerbil. Thus, the present experiment was designed to determine the effects of pre- plus postnatal androgen treatment on physiological and behavioural masculinization in the female gerbil.

Method

Pregnant female gerbils were given injections of either 100 μg or 1 mg of TP per day for approximately 6 days prior to birth. Pups received injections of either 100 μg TP or oil within 24 hr of birth. Thus, a total of four groups was created, 100 μg + oil (G1), 100 μg + 100 μg (G2), 1 mg + oil (G4), and 1 mg +100 μg (G4). All weaning, housing, surgical, injection, and testing procedures were identical to those used in Experiment 11 with one exception. To insure that lack of male sexual activity was not due to the dose of TP administered, 100 μg TP tests were followed by 4 (one per week) tests of male sexual behaviour with animals receiving 500 μg TP. Physiological measures were taken after the final 100 μg TP test to allow comparison with the results obtained in Experiment 11.
Originally, vehicle control animals from Experiment 11 were to be the only control animals employed. However, the observation of low levels of sexual activity in this study as well as in Experiment 11 suggested that, although unlikely, there may have been an effect of injection stress which was sufficient to alter behaviour. Thus, an additional set of control animals was tested to determine if injections *per se* were influencing sexual development. For control purposes, pregnant females either received daily injections of oil for approximately the final 6 days of gestation or they received no injections. In addition, half of the pups in each of the prenatal conditions received a single injection of oil within 24 hr of birth while the remainder did not, resulting in a total of four groups (prenatal with or without injection by postnatal with or without injection).

**Results and Discussion**

None of the TP treated animals in the present experiment exhibited male or female sexual behaviour, indicating that they were indeed behaviourally defeminized although they apparently were not behaviourally masculinized. Additional control animals did, however, display male and female sexual behaviour.

Results obtained for the additional control animals indicated that neither pre-, post-, nor pre- plus postnatal injections *per se* produced a decline in the sexual activity of intact males. As in Experiment 11, the level of male sexual activity was rather low but it did not appear to differ between groups (Table 6). However, some differences did become apparent during the TP testing. Data presented in Tables 7 and 7a
Table 6. Male sexual behaviour in intact male gerbils exposed to vehicle injections perinatally.

<table>
<thead>
<tr>
<th>SEXUAL RESPONSE</th>
<th>PERCENT RESPONDING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1 (n=12)</td>
</tr>
<tr>
<td>M T1 I E</td>
<td>8.3 9.1 0.0 14.1</td>
</tr>
<tr>
<td>M T2 I E</td>
<td>8.3 9.1 0.0 14.3</td>
</tr>
<tr>
<td>M T3 I E</td>
<td>8.3 9.1 8.3 21.4</td>
</tr>
<tr>
<td>M T4 I E</td>
<td>25.0 9.1 0.0 7.1</td>
</tr>
<tr>
<td>M T5 I E</td>
<td>25.0 9.1 8.3 21.4</td>
</tr>
</tbody>
</table>

Prenatal vehicle injections were achieved by injecting one half of the gerbils during the last 6 days of pregnancy with the vehicle. Postnatal administration consisted of injecting one half of the pups in each prenatal condition within 24 hr of birth. G1 = no pre- or neonatal treatment, G2 = neonatal treatment only, G3 = prenatal treatment only, G4 = pre- and neonatal treatment. M = mounts, I = intromission, E = ejaculations. T1-5 = consecutive tests every 2 days.
indicate that males exposed to vehicle injections pre- and postnatally exhibited more sexual activity while receiving TP than while intact. This suggests that perinatal injections may produce alterations in steroid sensitivities. The finding that all females exhibited equivalent and normal sexual receptivity when given chronic EB and EB plus P (Table 8), suggests that any alterations in steroid sensitivities may be restricted to androgens.

There is a physiological indication that perinatal injections may have been stressful. Testicular weights were recorded at the time of gonadectomy and were found to be lower, although not significantly lower, in all animals exposed to pre- and/or postnatal injections. This suggests that levels of endogenous androgens may have been reduced in these animals. The observation that TP administration did increase sexual activity in animals given the vehicle pre- and postnatally is consistent with this idea. However, the failure to observe intergroup differences in male sexual activity during intact testing suggests that, unless there was another factor operating to reduce sexual activity in adulthood, the alteration in testes weight was not sufficient to result in behavioural change.

There is at least one explanation of these apparently contradictory results. It is possible that perinatal injections actually increased the sensitivity of the animals to androgens. If an extraneous factor was operating in adulthood to reduce androgen levels and thus sexual behaviour, the effect might not be apparent because the levels of sexual responding in all animals would be limited by the low androgen levels. The
Table 7. Male sexual behaviour in castrated male gerbils exposed to perinatal vehicle injections.

<table>
<thead>
<tr>
<th>SEXUAL RESPONSE</th>
<th>PERCENT RESPONDING</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1 (n=12)</td>
<td>G2 (n=9)</td>
<td>G3 (n=12)</td>
<td>G4 (n=11)</td>
</tr>
<tr>
<td>M</td>
<td>8.3</td>
<td>11.1</td>
<td>0.0</td>
<td>18.2</td>
</tr>
<tr>
<td>T1 I</td>
<td>0.0</td>
<td>11.1</td>
<td>0.0</td>
<td>18.2</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>11.1</td>
<td>0.0</td>
<td>9.2</td>
</tr>
<tr>
<td>M</td>
<td>16.7</td>
<td>22.2</td>
<td>0.0</td>
<td>63.6</td>
</tr>
<tr>
<td>T2 I</td>
<td>8.3</td>
<td>11.1</td>
<td>0.0</td>
<td>54.5</td>
</tr>
<tr>
<td>E</td>
<td>8.3</td>
<td>0.0</td>
<td>0.0</td>
<td>45.5</td>
</tr>
<tr>
<td>M</td>
<td>16.7</td>
<td>11.1</td>
<td>0.0</td>
<td>45.5</td>
</tr>
<tr>
<td>T3 I</td>
<td>8.3</td>
<td>11.1</td>
<td>0.0</td>
<td>45.5</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>11.1</td>
<td>0.0</td>
<td>45.5</td>
</tr>
<tr>
<td>M</td>
<td>8.3</td>
<td>11.1</td>
<td>0.0</td>
<td>54.5</td>
</tr>
<tr>
<td>T4 I</td>
<td>8.3</td>
<td>11.1</td>
<td>0.0</td>
<td>54.5</td>
</tr>
<tr>
<td>E</td>
<td>8.3</td>
<td>11.1</td>
<td>0.0</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Prenatal administration was achieved by injecting one half of the gerbils with the vehicle (oil) during the last 6 days of pregnancy. Neonatal administration consisted of injecting one half of the pups from each of the prenatal conditions within 24 hr of birth. G1 = no pre- or neonatal injection, G2 = neonatal injection only, G3 = prenatal injection only, G4 = pre- and neonatal injection. M = mounts, I = intromissions, E = ejaculations. T1-4 = consecutive weekly tests.
Table 7a. Additional measures of sexual behavior for T4 data of Table 7*.

<table>
<thead>
<tr>
<th>MEASURE</th>
<th>G1</th>
<th>G2</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>X</td>
<td>±SE</td>
</tr>
<tr>
<td>ML(sec)</td>
<td>1</td>
<td>460.0</td>
<td>000.0</td>
</tr>
<tr>
<td>IL(sec)</td>
<td>1</td>
<td>468.0</td>
<td>000.0</td>
</tr>
<tr>
<td>EL(sec)</td>
<td>1</td>
<td>577.7</td>
<td>000.0</td>
</tr>
<tr>
<td>PEI(sec)</td>
<td>1</td>
<td>220.0</td>
<td>000.0</td>
</tr>
<tr>
<td>EJ(freq)</td>
<td>1</td>
<td>3.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Animals that did not exhibit male sexual activity were excluded from the table.

Prenatal administration was achieved by injecting one half of the gerbils with the vehicle (oil) during the last 6 days of pregnancy. Neonatal administration consisted of injecting one half of the pups from each prenatal condition with the vehicle within 24 hr of birth. G1 = no pre- or neonatal injection, G2 = neonatal injection only, G4 = pre- and neonatal injection. ML = mount latency, IL = intromission latency, EL = ejaculation latency, PEI = post-ejaculatory interval, the time interval between the first intromission and ejaculation, EJ = mean number of ejaculations.
Table 8. Female sexual behaviour in ovariectomized female gerbils exposed to perinatal vehicle injections.

<table>
<thead>
<tr>
<th>TEST (EB)</th>
<th>GROUP</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G_1$</td>
<td>$G_2$</td>
<td>$G_3$</td>
<td>$G_4$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\bar{X} \pm SE$</td>
<td>$\bar{X} \pm SE$</td>
<td>$\bar{X} \pm SE$</td>
<td>$\bar{X} \pm SE$</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>5.6 5.6</td>
<td>0.0 0.0</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>3.6 3.6</td>
<td>0.0 0.0</td>
<td>8.9 8.9</td>
<td>14.0 9.5</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>23.6 12.6</td>
<td>0.0 0.0</td>
<td>17.8 12.2</td>
<td>0.0 0.0</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>14.5 10.1</td>
<td>0.0 0.0</td>
<td>15.6 9.1</td>
<td>20.0 13.3</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>12.7 8.5</td>
<td>0.0 0.0</td>
<td>17.8 9.1</td>
<td>19.0 12.7</td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>15.5 10.6</td>
<td>0.0 0.0</td>
<td>18.9 11.1</td>
<td>19.0 12.7</td>
<td></td>
</tr>
<tr>
<td>(EB+P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T9</td>
<td>74.5 11.8</td>
<td>72.1 7.9</td>
<td>80.0 4.7</td>
<td>79.0 8.5</td>
<td></td>
</tr>
</tbody>
</table>

Prenatal administration was achieved by injecting one half of the gerbils with the vehicle (oil) during the last 6 days of pregnancy. Neonatal administration consisted of injecting one half of the pups from each of the prenatal conditions within 24 hr of birth. $G_1$ = no pre- or neonatal injection, $G_2$ = neonatal injection only, $G_3$ = prenatal injection only, $G_4$ = pre- and neonatal injection. $\bar{X}$ = mean sexual receptivity, number of lordosis postures exhibited by the female divided by the number of male mounts with pelvic thrusting and multiplied by 100. Animals received 13 consecutive injections of 10 µg estradiol benzoate (EB) and were tested on Days 6-13. On Day 14, 100 µg progesterone (P) was administered 3-4 hr prior to testing.
administration of TP would increase androgen levels and could remove the ceiling effect due to low androgen levels. This would allow differences to be observed. It should be noted that, in animals receiving perinatal TP, TP itself may have exerted effects which could have been quite different from those observed in vehicle treated animals.

The results for the physiological measures indicate that, as in Experiment 11, some degree of physiological masculinization occurred in all TP treated animals for all measures. In addition, it appeared that prenatal exposure to 1 mg TP was more effective in its masculinizing effects on scent gland length and width and on anogenital distance than was the 100 μg prenatal TP treatment (Figure 14a-c). Furthermore, when comparing the means of these measures for each of the four experimental groups with the means for the additional control animals, it appears that animals in all TP groups exhibit a masculine trend and, in addition, G4 animals are quite similar to male controls on several measures (Table 9). Finally, it also appears that prenatal exposure to vehicle injections exerted some masculinizing effects while neonatal exposure did not.

Data for the physiological measurements of the TP treated animals were analyzed separately using a 2x2 analysis of variance for nested factors. A 2x2x2 (gender, prenatal exposure, postnatal exposure), nested factor analysis of variance was used to analyze the additional control data. All subsidiary analyses used the Newman-Keul's procedure with α set at 0.05. The analyses of variance for TP treated animals
Figure 14. Comparison of indices of physiological masculinization in female gerbils treated perinatally with testosterone propionate. Prenatal administration was achieved by injecting gerbils during the last 6 days of pregnancy with either 100 μg or 1 mg testosterone propionate (TP). Postnatal administration consisted of injecting pups within 24 hr of birth with either the vehicle or 100 μg TP. The effects of prenatal TP dose, independent of postnatal treatment, are presented in panels a-c. The effects of postnatal treatment, independent of prenatal TP dose, are presented in panel d.
Table 9. Comparison of indices of physiological masculinization in untreated male and female gerbils, male and female gerbils exposed to vehicle injections perinatally, and female gerbils exposed to testosterone propionate perinatally.

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>SCENT GLAND LENGTH mm ±SE</th>
<th>SCENT GLAND WIDTH mm ±SE</th>
<th>BODY WEIGHT g ±SE</th>
<th>ANOGENITAL DISTANCE mm ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>VF1</td>
<td>14.45 0.74</td>
<td>3.36 0.20</td>
<td>63.16 2.06</td>
<td>2.18 0.12</td>
</tr>
<tr>
<td>VF2</td>
<td>15.50 0.52</td>
<td>3.75 0.16</td>
<td>59.50 1.34</td>
<td>2.46 0.17</td>
</tr>
<tr>
<td>VF3</td>
<td>16.33 0.53</td>
<td>4.11 0.26</td>
<td>61.80 1.56</td>
<td>2.33 0.17</td>
</tr>
<tr>
<td>VF4</td>
<td>15.67 0.57</td>
<td>3.80 0.22</td>
<td>60.49 1.11</td>
<td>2.40 0.24</td>
</tr>
<tr>
<td>G1</td>
<td>18.88 1.12</td>
<td>5.13 0.35</td>
<td>64.38 1.54</td>
<td>6.25 0.77</td>
</tr>
<tr>
<td>G2</td>
<td>20.09 0.58</td>
<td>5.46 0.21</td>
<td>66.77 0.62</td>
<td>8.45 0.36</td>
</tr>
<tr>
<td>G3</td>
<td>20.70 1.27</td>
<td>5.90 0.31</td>
<td>67.10 1.80</td>
<td>8.50 0.40</td>
</tr>
<tr>
<td>G4</td>
<td>22.40 0.98</td>
<td>6.20 0.29</td>
<td>64.45 1.60</td>
<td>9.40 0.40</td>
</tr>
<tr>
<td>VM1</td>
<td>23.83 0.49</td>
<td>6.17 0.17</td>
<td>70.59 1.44</td>
<td>11.83 0.27</td>
</tr>
<tr>
<td>VM2</td>
<td>24.67 0.55</td>
<td>6.33 0.17</td>
<td>63.99 1.63</td>
<td>11.67 0.50</td>
</tr>
<tr>
<td>VM3</td>
<td>26.08 0.48</td>
<td>6.25 0.18</td>
<td>70.39 2.03</td>
<td>12.82 0.48</td>
</tr>
<tr>
<td>VM4</td>
<td>25.00 0.51</td>
<td>6.45 0.28</td>
<td>69.64 1.64</td>
<td>12.82 0.48</td>
</tr>
</tbody>
</table>

Prenatal testosterone propionate (TP) administration was achieved by injecting gerbils during the last 6 days of pregnancy with either 100 μg or 1 mg TP. Postnatal administration consisted of injecting pups within 24 hr of birth with either the vehicle or 100 μg TP. Vehicle administration followed a similar procedure with one half of the pregnant females receiving injections and one half of the pups from each condition receiving an injection at birth. VF = vehicle treated females, VF1 = no pre- or neonatal vehicle injection, VF2 = neonatal vehicle injection only, VF3 = prenatal vehicle injection only, VF4 = pre- and neonatal vehicle injection. G1 = 100 μg TP prenatally and oil postnatally, G2 = 100 μg TP prenatally and neonatally, G3 = 1 mg TP prenatally and oil neonatally, G4 = 1 mg TP prenatally and 100 μg TP neonatally. VM1-4 treatments were similar to the VF1-4 groups except that the subjects were male.
indicated a significant effect of prenatal hormone dose (100 μg vs 1 mg) for scent gland length, $F(1,35) = 3.92, p < 0.05$, scent gland width, $F(1,35) = 6.54, p = 0.01$, and anogenital distance, $F(1,35) = 8.74, p = 0.006$. The subsidiary analyses indicated that, for each measure, the 1 mg dose of TP led to significantly higher scores than the 100 μg dose. Postnatal hormone exposure (oil vs 100 μg) had a significant effect on anogenital distance, $F(1,35) = 8.46, p = 0.006$, although it did not exert a significant effect on other measures. No effects of TP were found to be significant for body weight measures, possibly because all four groups exhibited body weights similar to those observed in group VM animals in Experiment 11.

The analysis of variance performed on the data from the additional control animals indicated a significant effect of prenatal treatment for scent gland length, $F(1,79) = 29.73, p < 0.0001$, scent gland width, $F(1,79) = 9.71, p = 0.0003$, and anogenital distance, $F(1,79) = 48.77, p < 0.0001$. Further analysis of these effects indicated that, in all cases, animals exposed to prenatal vehicle injections exhibited greater physiological measurements than animals not exposed to prenatal injections, regardless of postnatal treatment. In addition, a significant gender by prenatal treatment by postnatal treatment interaction was found for scent gland length, $F(1,79) = 4.46, p = 0.04$, and scent gland width, $F(1,79) = 4.42, p = 0.04$. Further analysis of these effects indicated that males receiving only prenatal vehicle injections had significantly greater scent gland lengths than other males. All males had significantly greater scent gland lengths than females. On the other hand,
scent gland width did not differ between males but females receiving any injection exposure had significantly greater scent gland widths than non-injected females. Although these results indicate that injections *per se* can exert a masculinizing effect, an examination of Table 9 reveals that all vehicle injected females exhibited physiological measures below those observed in TP treated females. This indicates that a perinatal injection effect did not, in itself, produce the masculinizing effect observed in TP treated animals, although it may have contributed to it.

The results of the present experiment indicate that pre-plus postnatal TP treatment (G4) can lead to high levels of physiological masculinization without necessarily producing behavioural masculinization. It should be noted, however, that the lack of male sexual activity may be due to a factor (i.e., stress) that is not directly related to the organizing action of perinatal androgens. Physiological masculinization appears most influenced by prenatal hormone exposure, as indicated by greater scent gland width, length, and greater anogenital distances than observed in Experiment 11. Furthermore, as in Experiment 11, dose dependent effects of TP were observed on physiological measures. The present experiment indicates the presence of prenatal TP is generally more important than the presence or absence of TP on the day of birth. However, it is not possible to determine whether this effect is due to the total amount of TP administered, the duration of hormone treatment, an increased sensitivity to TP during part or all of prenatal treatment, or to some combination of these factors.
GENERAL DISCUSSION

It was originally predicted that, because ovarian steroids appear to be the primary hormonal regulators of food intake and body weight in the gerbil, the presence of androgens during the period of sexual differentiation would defeminize the gerbil. The results of Experiment 11 and 12 indicate that both pre- plus postnatal and neonatal TP treatment do defeminize the gerbil, a result consistent with the hypothesis. These results are similar to those obtained for the female rat and mouse which are also defeminized by perinatal androgens (Clemens et al., 1970; Edwards & Burge, 1971; Edwards & Thompson, 1970; Whalen et al., 1971) and are in contrast with those obtained for the female hamster which is not fully defeminized by perinatal androgens (Alleva et al., 1969; Paup et al., 1972; Tiefer & Johnson, 1971, 1975).

Experiment 11 further indicated that 100 μg and 1 mg TP were essentially equal in their ability to defeminize the female gerbil, suggesting that if dose-dependent effects on lordosis behaviour are to be observed, lower neonatal doses of TP must be employed. This contrasts with results obtained in the rat where 100 μg only partially defeminizes behaviour (mean LQ = 40, Clemens et al., 1970). It is possible that this difference is due to the lesser body weight and blood volume of the gerbil which could result in a higher concentration of testosterone reaching the brain in the gerbil. However, mice, which are smaller than gerbils, also exhibit some receptivity after a single neonatal injection of 100 μg TP (mean LQ = 20, Edwards & Burge, 1971). This suggests that the gerbil may be more
sensitive to the defeminizing action of exogenous TP than are the rat and mouse. It seems unlikely that the failure to observe significant levels of receptivity in TP-treated females was due solely to the dose of estrogen administered since even the administration of P after prolonged EB treatment did not greatly enhance sexual responding in these animals. This would suggest that the animals' ability to respond to estrogen was reduced. Thus, it seems likely that in the gerbil, as in the rat (Green et al., 1969; Maurer & Woolley, 1971; Tuohimaa & Johnson, 1971), TP decreases estrogen binding capacity and/or alters neuronal structures in the brain and this results in decreased lordosis responding.

Unlike the rat (Södersten, 1973), mouse (Edwards & Burge, 1971), and hamster (Johnson, 1975) which exhibit significant increases in mounting behaviour after a single injection of TP within 48 hr of birth, the female gerbil failed to exhibit any major increase in mounting behaviour, regardless of the TP dose administered. However, because control males did not exhibit high levels of male sexual behaviour, these results are difficult to interpret. It is conceivable that neonatal TP treatment was provided outside the sensitive period for behavioural masculinization. Another possibility is that more prolonged TP treatment is necessary for the elicitation of male sexual behaviour in TP-treated gerbils. Experiment 12 indicated that pre- plus postnatal TP treatment also failed to elicit male sexual activity. The possibility that the treatment duration in Experiment 12 was also too short or that the sensitive period was again missed can not be eliminated. However, all other
rodents examined to date exhibit at least an increase in mounting behaviour after similar perinatal TP treatment. This plus the low levels of male sexual behaviour exhibited by all animals, including intact males, during most tests of male sexual behaviour suggested that some other factor (e.g., the neonatal injection procedure or the consequences of injection stress in pregnant animals) might have been influencing behaviour. In an attempt to determine if injections *per se* were responsible for the absence of male sexual behaviour, additional control groups were tested. These results indicated that injections *per se* did not decrease male sexual behaviour in adulthood. Furthermore, even non-injected males exhibited fairly low levels of sexual activity, suggesting that some other factor might be responsible.

One factor which has not received a great deal of attention from investigators concerns the effects of housing different species within one colony room. It has been found that the reproductive cycle of the rat is altered by the presence of hamsters in the same colony (Weizenbaum, McClintock, & Adler, 1977). To the best of my knowledge, there is no published study related to the effects of mixed housing on gerbil reproduction. However, it is possible that the presence of rats in the same colony room is stressful to the gerbil. In the present experiments, the presence of rat odour could have theoretically exerted an effect prenatally, prepubertally, or both. However, it is unlikely that effects are exerted postpubertally because normal copulatory behaviour is observed in gerbils brought into the present colony after maturity. Prenatal and/or prepubertal
effects might occur if rat odours were stressful to the gerbil. Long-term stress increases adrenal secretions (Hatch et al., 1963), some of which could act upon the developing fetus (via maternal circulation) or on the prepubertal animal. Although the exact mechanism of action and the accompanying physiological and/or neurological alterations remain to be determined, the finding that only male sexual behaviour appears to be affected suggests at least two possibilities. First, female sexual behaviour is more reflexive than male sexual behaviour. For example, decortication decreases male rat sexual responses but has no effect on female rat sexual responses (Gorzalka & Mogenson, 1977, pp. 179). It is possible that, because female sexual behaviour is more reflexive that male sexual behaviour, it is less likely to be influenced by various factors. Thus, in the present experiments, the decrease in male sexual activity without a decrease in female sexual activity may reflect a greater susceptibility of the mechanisms regulating male sexual behaviour to extraneous influences rather than any change in the hormones regulating the behaviour. Second, the behavioural changes may be related to alterations in androgen levels and/or the ability to respond to androgens. Mildly supportive of this is the finding that a greater number of vehicle control males in Experiment 11 and vehicle males treated pre- and postnatally in Experiment 12 exhibited some component of male sexual behaviour during TP administration than when tested while intact. However, it is also possible that the increase was a function of repeated testing. At present, there is no way to differentiate between these possibilities.
The absence of mounting behaviour, for whatever reason, in the presence of partial and full physiological masculinization in TP-treated females is intriguing. As far as I have been able to determine, physiological masculinization in other rodents has always been accompanied by at least an increase in mounting behaviour. The opposite effect, behavioural masculinization in the absence of physiological masculinization, has been observed in TP-treated female rats (Ward, 1969). The results of Ward (1969) and others (e.g., Alleva et al., 1969; Coniglio & Clemens, 1970) suggest that, in the rat and hamster, the period of physiological masculinization is contained within or overlaps with the sensitive period for behavioural masculinization. The results of the present experiments suggest that the gerbil may differ from other rodents in that the sensitive periods for behavioural and for physiological masculinization might be non-overlapping. However, until the period for behavioural masculinization in the gerbil is clearly determined, this remains speculation.

The presence of partial and full physiological masculinization in the absence of male sexual behaviour suggests that, if some extraneous factor is responsible for the low levels of male sexual behaviour observed, it does not appear to be affecting the ability of peripheral tissues to respond to TP pre- or postpubertally. Experiments 11 and 12 indicated that scent gland size and anogenital distance were affected by TP dose and the time of TP administration. Thus, prepubertal TP was capable of exerting effects on peripheral tissues. Furthermore, the ability of peripheral tissues to respond to
androgens in adulthood is apparently not altered in these animals. Scent gland size in adulthood is determined to a very large extent by circulating gonadal steroids. Castration leads to eventual dissappearance of the scent gland and TP treatment reverses this effect (Blum & Thiessen, 1971; Owen & Thiessen, 1973; Yahr, 1981; Yahr, Newman & Stephens, 1979; Yahr & Thiessen, 1972). All animals in Experiments 11 and 12 exhibited scent glands that were normal in appearance and actively secreting sebum at the end of TP administration, indicating that exogenous TP was capable of exerting effects on peripheral tissues in adulthood. Therefore, if the decrease in male sexual behaviour was due to decreased responsiveness to androgens, the decreased responsiveness is probably limited to the CNS. This does not, however, preclude the possibility that the production of testicular androgens was also reduced since the amount of TP required to restore scent gland appearance is less than that required for the restoration of male sexual activity (Yahr, 1981). Thus, if some extraneous factor was responsible for the low level of male sexual behaviour observed in the present experiments, it may exert its action by decreasing testicular androgen levels as well as by altering the capacity of the CNS to respond to androgens.

The effects of ovarian steroids on food intake and body weight in the male gerbil were difficult to assess because castration may have been exerting delayed effects. However, the finding that androgens (Maass & Wade, 1977) are no more effective than ovarian steroids in altering food intake and body weight in the male gerbil while ovarian steroids clearly alter
food intake and body weight in the female gerbil suggests that ovarian steroids may be the primary hormonal regulators of food intake and body weight in this species.

It was hypothesized that, in species where ovarian steroids are the primary regulators of food intake and body weight, the presence of androgens during the period of sexual differentiation would have a defeminizing effect. The present experiments indicate that ovarian steroids are probably the primary hormonal regulators of food intake and body weight in the gerbil. Furthermore, both male and female gerbils are clearly defeminized by the presence of androgens during the period of sexual differentiation. These results support the hypothesis that the extent of perinatal androgen-induced defeminization can be used to predict which sex steroid will exert the major influence on adult food intake and body weight (and vice versa).

Although the evidence in support of a connection between species differences in the extent of androgen-induced defeminization and in which gonadal steroid is the primary hormonal regulator of intake and weight in adulthood is indirect, there is at least one plausible explanation for a connection. There is now sufficient evidence to conclude that the presence of androgens during differentiation produces definite alterations in neuronal structure and steroid binding capacity of the brain (deBold, 1978; Dörner & Staudt, 1968, 1969; Field & Raisman, 1971; Gorski et al., 1978; Green et al., 1969; Greenough et al., 1977; Maurer & Woolley, 1971; Nishizuka & Arai, 1981; Tuohimaa & Johansson, 1971). It is possible that
species differences in the defeminizing action of perinatal androgens reflect more general interspecific differences in the overall organizational effects of perinatal androgens. For example, perinatal androgens may exert somewhat different effects on steroid binding capacity or steroid sensitivity in different species. The majority of androgen related neuronal changes reported so far occur in an area implicated in the hormonal regulation of food intake, sexual behaviour, and other motivated behaviours, the hypothalamic area. Therefore, if the more general organizational effects of perinatal androgens do differ between species, this may well be reflected by between species differences in behaviours mediated by the hypothalamus. Thus, there is at least some indirect support for the speculation that the difference between the hamster and other rodents in the hormonal regulation of food intake and body weight is related to species differences in the more general organizing actions of perinatal androgens. Unfortunately, data which could reveal the presence or absence of species differences in these areas are relatively sparse. It does appear that the sex differences in the dendritic branching of the preoptic area observed by Greenough et al., (1977) in the hamster are similar to those observed by Raisman & Field (1972) in the rat. However, it is not clear that the extent of the sex difference observed is the same between these two species.

Feder et al. (1974) have observed differential rates of estradiol uptake in rats, hamsters, and guinea pigs. This difference appears related to the sensitivity of each species to estradiol in the induction of receptivity, with the more
sensitive rodents (rats and guinea pigs) exhibiting the greatest uptake (Feder et al., 1974). It is possible that this difference between the hamster, rat and guinea pig reflects differential effects of perinatal androgens on estradiol binding capacity. However, it is clear that a great deal of further research is required before interspecific differences in the organizational effects of androgens can be clearly determined.
SECTION III
Summary and Conclusions

Experiments 1-4 indicated that the female gerbil is similar to other female rodents studied to date in that estrogen and progesterone are the primary regulators of sexual receptivity. However, the gerbil does appear to be more dependent than the rat, guinea pig, and some mouse strains (see Uphouse, Wilson, & Schlesinger, 1970) on the synergistic action of progesterone with estrogen for the induction of high levels of receptivity. In this respect, the gerbil is similar to the hamster, which is also fairly dependent on the action of P for induction of maximal receptivity during acute estrogen administration. Feder et al. (1974) found that the rate of estrogen uptake was slower in the hamster than in the rat and guinea pig and suggested that this was reflected in the lower sensitivity of the hamster to estrogen-induced receptivity. It is plausible that the gerbil, like the hamster, may also have a slower rate of estrogen uptake than do the rat and guinea pig and that this is responsible for the gerbil's greater progesterone-dependence or lower sensitivity to estrogen. Hamsters exhibit greater plasma estradiol levels during the estrous cycle than do rats (Baranczuk & Greenwald, 1973) and, apparently, guinea pigs (Feder et al., 1974). This led Feder et al. (1974) to conclude "it appears that the higher the endogenous level of a steroid, the less avid is the uptake of that steroid (relative to plasma) and the less sensitive is the animal to that steroid.". To the best of my knowledge, the gerbil is the only other rodent
examined so far which is similar to the hamster in the requirement of comparatively high acute estrogen doses for the elicitation of maximal receptivity. Furthermore, the dose of chronic estrogen required to elicit high levels of receptivity in the gerbil is greater than that required by other rodents including the hamster (Carter, Michael, & Morris, 1973). Thus, investigation into the levels of estrogen secreted by the gerbil during the estrous cycle as well as examination of the rate of estrogen uptake would provide further evidence for the validity of the speculation that, as endogenous steroid levels increase, sensitivity and/or uptake decrease. Further information on this could prove valuable since it is possible that the slower uptake of estrogen observed in the hamster is related to the organizing action of perinatal androgens. If this is the case, one would expect to find that the gerbil would have an uptake pattern similar to the rat and guinea pig since all three species are defeminized by perinatal androgens (which alter steroid uptake). However, if the decreased rate of uptake in the hamster reflects an adaptive response to relatively high estrogen titers, then one might expect the gerbil to be similar in that respect to the hamster since both species exhibit lower estrogen sensitivity relative to rats and guinea pigs. Further examination of these differences would aid in determining the relative contributions of perinatal androgens versus genetic factors on steroid sensitivity.

Experiments 5 and 6 indicated that housing conditions did not alter receptivity in the female gerbil. This contrasts with results obtained in the rat, where isolation increases sexual
responding, presumably via adrenal steroids. It was suggested that the absence of a differential housing effect in the gerbil may reflect either species differences in adrenal steroids or differences in brain sensitivity to particular steroids. Thus, even if differential housing did alter adrenal steroid secretion, these changes need not be reflected in sexual responding.

Experiments 5-8 indicated that adrenalectomy significantly decreased receptivity in the gerbil. This effect is not observed in the rat, where adrenalectomy facilitates rather than inhibits receptivity (Gray & Gorzalka, 1980; Gorzalka & Raible, 1981). It was also found that particular combinations of adrenal steroids eliminated any difference in receptivity due to adrenalectomy in tests where animals received EB alone but not in tests where animals received P in conjunction with EB. This suggests that some aspect of adrenalectomy other than loss of adrenal steroids may be, in part, responsible for a reduced sensitivity to the synergistic action of P with EB. Furthermore, it would appear that the gerbil, unlike other female rodents studied to date, requires adrenal steroids in addition to EB and P for the elicitation of maximal receptivity. A great deal of further investigation will be required before the precise nature of this relationship is determined.

The results of Experiments 5-8 add to the growing amount of evidence that adrenal steroids can alter sexual receptivity in the female rodent. Furthermore, at least in the rat, there are indications that stress alters circulating levels of adrenal steroids in an amount sufficient to alter sexual responding. It
is thus somewhat surprising that the possible effects of stress on experimental results are often overlooked. For example, a quick glance through the literature indicates that the majority of researchers do not report housing conditions. Although this would not seem to create problems as long as the housing conditions were the same for all groups, it could create problems for those trying to replicate experimental findings. It is often possible that many of the apparent contradictions in the literature on female receptivity actually reflect procedural differences. For example, procedural differences between experiments could produce different effects on adrenal steroid secretion. This, in turn, could result in different levels of receptivity between experiments.

The results of Experiment 9 indicated that ovariectomy decreased food intake and body weight in the female gerbil, an effect not found in the rat, mouse, guinea pig and hamster. Treatment with estrogen restored food intake and body weight levels to those exhibited by control animals. It was suggested that norepinephrine may be altered by estrogen, as is the case for the rat. However, if estrogen does influence NE levels, either the effects of estrogen on NE or the effects of NE on food intake differ from the effects observed in the rat. One could differentiate between these possibilities by examining the effect of NE on food intake and then determining the effect of EB on NE levels.

The hormonal regulation of food intake and body weight in the gerbil differs from other rodents examined to date in another important respect. In all other rodents examined so
far, it appears that the primary gonadal hormone that regulates food intake and body weight for that species is, to a great extent, responsible for the sex difference in food intake and body weight. For example, in the rat, where females are lighter than males and ovarian steroids are the primary regulators of food intake and body weight, ovariectomy leads to increases in food intake and body weight which substantially reduce the sex differences. In the hamster, the male is lighter than the female, androgens are the primary hormonal regulators of food intake and body weight and castration increases food intake and body weight levels in the male to those observed in the female. In the gerbil, as in the female rat, the female weighs less than the male and ovarian hormones appear to be the primary regulators of food intake and body weight. However, in the female gerbil, unlike the female rat, ovariectomy leads to decreases in food intake and body weight, further increasing the sex differences. To the best of my knowledge, this is the first known rodent in which factors other than circulating hormone levels clearly contribute to the sexual dimorphism observed in food intake and body weight regulation. Perinatal androgens may contribute to this sexual dimorphism. Body weight data collected from Experiments 11 and 12 indicate that neonatal androgenization increased female body weight to that observed in control males. This suggests that part of the dimorphism observed in adulthood is due to an organizational effect of perinatal androgen that is independent of any activational effects of sex steroids later in life. If this is the case, neonatal castration should result in a permanent reduction in
adult body weight which would not be influenced by circulating hormones in adulthood. Previous research has indicated that the organizational action of perinatal androgens influence the activational effects of hormones on food intake and body weight in adulthood. Until now, there has been little evidence that sex steroids exert organizational effects on food intake and body weight that are independent of the later activational effects of sex steroids. The female gerbil could thus provide a useful tool for the examination of these effects.

The effects of ovarian steroids on food intake and body weight in the male gerbil (Experiment 10) were less clear than those obtained for the female. Castration appeared to exert delayed effects on food intake and, perhaps, body weight, which may have masked any effects of ovarian steroid treatment. Although it is possible that neither ovarian steroids nor androgens influence food intake and body weight in the male gerbil, it seems more likely that, as in the female hamster (Morin & Flemming, 1978), the effects are simply not robust.

Experiments 9 and 10 also indicated that, in the gerbil, the system regulating food intake and body weight was more sensitive to EB than the system regulating sexual behaviour. This does not appear to be the case in the rat. One could hypothesize that the differential sensitivity to EB observed in the food intake and sexual behaviour systems of the gerbil reflects a difference in estradiol binding capacity of the two systems. Investigation of the distribution of radiolabeled estradiol in specific hypothalamic areas could provide some interesting information. For example, the lateral hypothalamus
(LH) and ventromedial hypothalamic nuclei (VMH) are considered quite important in the regulation of food intake and body weight (Wade, 1976). Lesions of the VMH lead to hyperphagia while lesions of the LH produce aphagia (Carlson, 1977, pp. 356; Ellison, Sorenson, & Jacobs, 1970). Evidence from lesion and stimulation studies indicates that the preoptic area of the hypothalamus (POA), the VMH, and the anterior hypothalamus are important in the regulation of sexual behaviour (Gorski, 1976; Kelly & Pfaff, 1978; Pfaff, 1980). If, as may be the case in the gerbil, the system regulating food intake has a greater estradiol binding capacity than that regulating sexual receptivity, a greater concentration of estradiol should occur in the areas mediating that behaviour. This type of information could be compared between rodents in which the sensitivity of the food intake and the sexual behaviour systems to EB are known. Information on the brain patterns of estrogen uptake does not exist for the gerbil although it does exist for the rat. Autoradiographic studies of estradiol-concentrating cells in the rat brain suggest that the POA and anterior hypothalamus (sexual behaviour) may contain more estrogen-concentrating cells than the VMH and LH (feeding behaviour and sexual behaviour) but these differences are very slight (Pfaff, 1980). These findings are consistent with the hypothesis. However, no conclusions can be drawn until other species have been examined and the data compared.

Experiments 11 and 12 indicated that neonatal and pre- plus neonatal TP treatment defeminized the female gerbil and produced partial to full physiological masculinization but it could not
be determined unequivocally whether behavioural masculinization occurred. These results, in conjunction with those found for other rodents may shed some light on a present controversy concerning the differentiation of sexual behaviour. Three models of the differentiation of sexual behaviour have been proposed within the last two decades, the linear or unidimensional model (Harris, 1964), the orthogonal model (Whalen, 1974), and the oblique model (Reinisch, 1976).

The unidimensional model argues that male and female sexual behaviour are separate ends of a single continuum (Figure 15a). Thus, the degree of masculinization would be negatively correlated with the degree of feminization. However, as previously discussed, it is possible to achieve full behavioural defeminization without full behavioural masculinization. In fact, a single injection of TP around the time of birth is sufficient to defeminize, but not fully masculinize, the female rat, mouse, and gerbil. Furthermore, adult male and androgenized female hamsters can exhibit both male and female sexual behaviour. Thus, the unidimensional model does not seem to account for some of the behavioural data now available. It does, however, describe the differentiation process for some of the physiological data. For example, anogenital distance is a unidimensional phenomenon. Female rodents have the shortest anogenital distance possible while males have the longest, intermediate distances represent a movement towards one end of the continuum and away from the other.

The orthogonal model views masculinization and feminization as separate continua (Figure 15b). In this model,
Figure 15. Models of sexual differentiation. In the linear or unidimensional model (a), masculinization and feminization are viewed as separate ends of a single continuum. In the orthogonal model (b), masculinization and feminization are viewed as separate, orthogonal continua that are not necessarily correlated. In the oblique model (c), masculinization and feminization are viewed as separate continua but the assumption is made that, in the natural course of development, the two are, to some degree, correlated.
masculinization-demasculinization comprise one continuum that is orthogonal to a feminization-defeminization continuum. Masculinization and feminization are not necessarily correlated. This model creates a total of four quadrants within which sexually dimorphic behaviours may fall, masculinization-defeminization, masculinization-feminization, demasculinization-defeminization, and demasculinization-feminization. When speaking strictly of sexual behaviour, we find examples of each of the four categories in the animal data. The male rat, gerbil, and androgenized female rat fall into the masculinization-defeminization quadrant. The female rat and gerbil and the neonatally castrated male rat fall into the feminization-demasculinization quadrant. The male and androgenized female hamster fall into the masculinization-feminization quadrant. Finally, if one considers that perinatal androgens did not fully masculinize the female gerbil in Experiments 11 and 12, one would conclude that the androgenized female gerbil falls into the defeminization-demasculinization quadrant. Thus, this model covers all possibilities suggested by the available behavioural data on sexual behaviour in rodents. The application of this model to some of the behavioural data and much of the physiological data does not, however, seem appropriate. In fact, the orthogonal model is clearly unnecessary if one is discussing quantitative differences of a single factor which is qualitatively similar in both sexes. For example, scent marking behaviour in male and female gerbils is similar in quality between the sexes although the quantity of scent marking behaviour displayed differs
between males and females. Because quantity is the only dimension in which the sexes can differ, the behaviour is necessarily unidimensional. A gerbil can not exhibit more than one pattern of scent marking. Thus, the level of marking behaviour exhibited by each of the sexes represent opposite ends of a single continuum. Similarly, anogenital distances can not be both masculinized and feminized or defeminized and demasculinized. Thus, the orthogonal model is useful in the description of sexual behaviour but should not be readily applied to other sexually dimorphic factors unless it is clear that there are qualitative sex differences in those factors (e.g., urination posture in dogs).

The oblique model (Figure 15c) was proposed by Reinisch as a modification of the orthogonal model and states that "masculinization and defeminization, as well as feminization and demasculinization, are correlated, but that one is not necessarily determined, under all circumstances, by the other." (Reinisch, 1976). It is difficult to see how this model differs in any important respect from the orthogonal model proposed by Whalen (1974). The rationale presented by Reinisch (1976) for the modifications of the orthogonal model appears to be that the orthogonal model is based on data from short-term perinatal TP administration studies rather than those which used more prolonged treatment. She seems to suggest that the former do not reflect the natural course of events during differentiation. Unfortunately, it is not clear why Reinisch feels that the orthogonal model does not encompass both the natural course of events and the experimental results obtained in this area of
research. Furthermore, it is not clear that the oblique model adequately covers the experimental data. In fact, it is not clear what, exactly, is stated by the oblique model. For Reinisch does not discuss the applicability of her model to the experimental data. In addition, there appears to be an error in the graphic representation of the model. Thus, we are presented with a model which uses masculinization-feminization as one continuum and demasculinization-defeminization as the other continuum, resulting in two quadrants which are impossibilities (masculinization-demascuinelization, feminization-defeminization). However, if one assumes that this is a printing error and that the continua are similar to those in the orthogonal model, it is still not clear that the oblique model provides the 'best fit' for the data. Thus, of the three models proposed, the unidimensional or linear model seems best for describing quantitative differences in qualitatively similar sexually dimorphic behaviours. The orthogonal model, on the other hand, is best applied to qualitative differences in sexually dimorphic behaviours.

While the models discussed above provide a good description of the results obtained in perinatal TP studies, they do little in revealing why these types of results are obtained. With the wealth of evidence that has accumulated over recent years, descriptive models no longer seem sufficient to deal with the present knowledge. Although I know of no model yet generated that deals with the concept of sensitive periods, it is this concept that most researchers now refer to when discussing their data. It appears from a variety of studies (e.g., Alleva et
al., 1969; Coniglio & Clemens, 1970; Ward, 1969) that the sensitive period for physiological masculinization of external genitalia occurs during the early portion of the sensitive period for behavioural masuclinization, at least in the rat, hamster, and guinea pig. Each of these periods appears to require a specific duration of androgen exposure as well. Thus, although several days of pre- plus neonatal TP treatment are required to elicit full behavioural masculinization in the rat, a single exposure to androgens can defeminize the female rat.

If one now returns to the orthogonal model, the circumstances that would result in each of the four quadrants become clear. If an animal is behaviourally masculinized and defeminized, either the sensitive period for defeminization must occur within the sensitive period for behavioural masculinization or androgens must be present during both the sensitive period for feminization and that for masculinization. In the male and androgenized female rat, defeminization can be brought about by a single injection of androgens during the sensitive period for masculinization, suggesting that the period for feminization is contained within the period for masculinization. If an animal is behaviourally masculinized and feminized, either there is no sensitive period for defeminization, the sensitive periods for defeminization and masculinization do not overlap sufficiently for one to greatly effect the other, or androgen levels during differentiation are sufficient to masculinize but not defeminize the animal. The latter would appear to be the case for the male hamster. Feminization is the result of an absence of sex steroids during
the sensitive period for defeminization. Similarly, demasculinization is the result of an absence of appropriate steroids during the sensitive period for masculinization. Feminization and demasculinization would thus occur when sex steroids were absent during the period of sexual differentiation. Examples of this are the female and functionally castrated male rat, mouse, hamster, and guinea pig, the female gerbil, and, I suspect, the neonatally castrated male gerbil. Finally, demasculinization and defeminization could occur if (1) the sensitive periods for masculinization and defeminization did not overlap and (2) the appropriate steroids were absent during the period for masculinization and present for the period of feminization. This might be the case for the androgen treated female gerbils in Experiments 11 and 12 although further study is necessary before this can be determined. Finally, in each of these situations, the presence of androgens during only a portion of one or more sensitive periods would result in degrees of masculinization and/or defeminization. Thus, although the orthogonal model provides a good description of experimental results, the concept of sensitive periods which may overlap provides an explanation of why varying degrees of sexual behaviour may be observed.

It was hypothesized that ovarian steroids would be the primary regulators of food intake and body weight in animals which were defeminized by the presence of androgens during sexual differentiation while androgens would be the primary hormonal regulators of food intake and body weight in animals which were not defeminized by the presence of androgens during
sexual differentiation. On the basis of Experiment 9 and 10 plus the study by Maass & Wade (1977) which indicated that ovarian steroids were probably the primary hormonal regulators of food intake and body weight in the gerbil, it was predicted that gerbils would be defeminized by the presence of androgens during the period of sexual differentiation. Experiments 11 and 12 indicated that this was indeed the case. Thus, the gerbil is similar to the rat, mouse, and guinea pig which are also defeminized by perinatal androgens and have ovarian steroids as the primary regulators of food intake and body weight. These rodents differ from the hamster which is not defeminized by perinatal androgens and where androgens are the primary regulators of food intake and body weight.

The concept of overlapping sensitive periods for the organizational actions of perinatal androgens on sexually dimorphic behaviours can provide at least one explanation of interspecific differences in the hormonal regulation of food intake, body weight, and sexual behaviour. For example, in the rat, perinatal androgens decrease estradiol-binding capacity while increasing androgen sensitivity. In male rats, gerbils, guinea pigs, and mice, androgens may be present during the entire sensitive period for differentiation of sexual behaviour but only a portion of the sensitive period for differentiation of food intake and body weight. This would lead to full masculinization of sexual behaviour. However, the presence of androgens during only a portion of the sensitive period for food intake and body weight could decrease sensitivity to EB without substantially increasing sensitivity to androgen. The female
rat, gerbil, mouse, and guinea pig, lacking perinatal androgens, would be more sensitive than the male to the effects of EB on food intake, body weight, and sexual behaviour. The hamster, on the other hand, is relatively insensitive to estrogen, even in the absence of perinatal androgens, as indicated by the finding that untreated female hamsters are less sensitive than rats to EB in the induction of receptivity.

It is of interest to note that the female gerbil, like the female hamster, is relatively insensitive to estrogen for the induction of sexual receptivity. However, the female gerbil is more sensitive than the hamster to the effects of estrogen on food intake and body weight regulation. This, plus interspecific differences in estrogen sensitivity (e.g., rat versus hamster) suggest that genetic factors, in addition to perinatal androgens, play a role in determining interspecies differences in hormone sensitivities.

It is clear that a great deal of further research will be required before an understanding of the activational effects of perinatal androgens on neural mechanisms underlying sexually dimorphic behaviours within a species, and differences in sexually dimorphic behaviours between species is reached. However, the concept of sensitive periods in conjunction with evidence for androgen-induced neuronal alterations, point towards a promising area of research. Although much data needs to be collected on existing laboratory rodents, the need for new species of rodents to examine the generality of supposed mechanisms of action should not be overlooked. The gerbil, differing in so many ways from other laboratory rodents,
provides one source of comparative information and promises to be an interesting new addition to the present list of laboratory rodents.
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