1-1-1977

Progesterone : inhibition of female rat sexual behavior and investigations of its mechanism of action.

Jeffrey D. Blaustein
University of Massachusetts Amherst

PROGESTERONE: INHIBITION OF FEMALE RAT SEXUAL BEHAVIOR AND INVESTIGATIONS OF ITS MECHANISM OF ACTION

A Dissertation Presented

By

JEFFREY D. BLAUSTEIN

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY
September 1977
Psychology

PROGESTERONE: INHIBITION OF FEMALE RAT SEXUAL BEHAVIOR AND INVESTIGATIONS OF ITS MECHANISM OF ACTION

A Dissertation Presented

By

JEFFREY D. BLAUSTEIN

Approved as to style and content by:

George N. Wade, Chairperson of Committee

Donald L. Black, Member

Mark I. Friedman, Member

Harvey H. Feder, Consultant

Bonnie R. Strickland, Department Head
Psychology Department

ACKNOWLEDGEMENTS

I would like to thank the members of our lab who have established the environment which enabled the experiments in this dissertation to be carried out... Ed Roy and Tom Gentry for their contribution in initiating me into the field, Janet Gray for giving me the opportunity to help in her initiation, Susan Dudley, my most outspoken office-mate, Jane Soukup and John Ieni, technicians who often did more than their part to keep things running smoothly.

The members of my committee, Mark Friedman, Harvey Feder and Donald Black are to be commended for making my interactions with them (including orals) an enjoyable experience. Each personally assisted in making the writing of this dissertation a challenging and rewarding experience.

I am grateful to my advisor and chairperson of my committees, George Wade. His constant support, encouragement, stimulation and guidance (and technical assistance) provided the atmosphere which has been such an integral part of my education.

I am also grateful to Marilyn Blaustein. Her constant support, encouragement, stimulation (and typing) were equally important in my education.

I also thank Millie for being such a good listener in her own way.

I thank J.P. Raynaud of Roussel-Uclaf for supplying the R 5020, I. Tabachnick of Schering Corp. for supplying the estradiol benzoate and progesterone, and Parke-Davis for supplying the CI-628, α-(4-pyrrolidinoethoxy)phenyl-4-methoxy-α'-nitrostilbene monocitrate.

ABSTRACT

Progesterone: Inhibition of Female Rat Sexual Behavior

And Investigations of Its Mechanism of Action

(September 1977)

Jeffrey D. Blaustein, B.S., University of Massachusetts
M.S., University of Massachusetts
Ph.D., University of Massachusetts

Directed by: Professor George N. Wade

Chronic injections of high doses of progesterone (5 mg) and low doses of estradiol benzoate (EB; 2 µg) resulted in less sexual behavior than low doses of progesterone (0.5 mg) and low doses of EB. In a typical procedure for inducing sexual behavior, EB and progesterone were given sequentially, separated by 42 hours. High levels of progesterone (2.5 and 5 mg) administered concurrently with EB inhibited the induction of sexual receptivity (concurrent inhibition). Increasing the dose of EB from 2 µg to 6 µg or 10 µg offset this inhibition. High doses of progesterone (5 mg) administered simultaneously, or 2 to 16 hours prior to EB, inhibited the induction of sexual behavior, but the inhibition was less if progesterone was administered 48 hours prior to EB. A single injection of progesterone (1 mg) that does not inhibit the induction of sexual behavior when administered concurrently with EB inhibited lordosis if distributed into five injections (0.2 mg) every 4 hours.

When a large dose of progesterone was administered to ovariectomized rats 24 hours after a 2 µg injection of estradiol benzoate, sexual receptivity was inhibited 30 hours later (sequential inhibition). Larger doses of progesterone (1 mg) were required to

inhibit the induction of sexual receptivity when tested 54 hours after EB administration than were necessary to facilitate sexual behavior 30 hours after EB. This inhibition was not due to copulatory stimuli from the first test, because inhibition occurred even if the first test was omitted. The degree of inhibition of sexual behavior produced by progesterone was dose dependent on estradiol; increasing the EB priming dose offset the inhibition caused by 1 mg of progesterone.

The results of two experiments in which progesterone did not inhibit the uptake or retention of ^{3}H-estradiol by brain cell nuclei suggest that the antiestrogenic action of progesterone in the central nervous system is not due to interference with the binding of estradiol. The results of an experiment which dissociated behaviorally the antiestrogenic action of progesterone from that of a synthetic antiestrogen, CI-628, are consistent with the notion that progesterone and synthetic estrogen antagonists inhibit the behavioral effects of estradiol by separate mechanisms.

A synthetic progestin, 17α, 21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R5020), was 50-100 times as effective as progesterone in facilitating (5 μg vs. 250 μg), sequentially inhibiting (10 μg vs. 1,000 μg) and concurrently inhibiting (50 μg vs. 2,500 μg) female sexual behavior in ovariectomized rats. This progestin, which binds to mammalian uterine progestin receptors with higher affinity than progesterone, is bound <u>in vivo</u> by cell nuclei from uterus, pituitary, hypothalamus, preoptic area - septum and cortex of ovariectomized-adrenalectomized rats. Binding is dependent on estrogen pretreatment; it is saturable (suppressed more by pretreatment with

R 5020 or progesterone than by corticosterone or testosterone). The lack of binding after ^{3}H-progesterone injection is attributed to the more rapid dissociation of progesterone from its receptor. These studies support the hypothesis that binding of progestins by brain cell nuclei may be a prerequisite for at least some behavioral responses to progestins.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Progestins antagonize and facilitate the effects of estradiol on various behaviors. Progestins have no apparent behavioral effects that do not require an interaction with estrogens (Feder & Marrone, 1977; Morin, 1977). The facilitatory effect of progesterone after estrogen priming on female sexual behavior has been observed in guinea pigs (Collins, Boling Dempsey & Young, 1938), rats (Beach, 1942; Boling & Blandau, 1939) and hamsters (Carter & Porges, 1974, Frank & Fraps, 1945).

Although sexual behavior can be induced in ovariectomized (OVX) rodents by estrogens alone (guinea pigs: Boling, Young & Dempsey, 1938; Dempsey, Hertz & Young, 1936, rats: Davidson, Smith, Rodgers & Block, 1968; Edwards, Whalen & Nadler, 1968; Green, Luttge & Whalen, 1970; hamsters: Carter, Michael & Morris, 1973), progestins' involvement in the induction of sexual behavior during the estrous cycle is obligatory. By ovariectomizing at a time which prevents the preovulatory progesterone surge (Croix & Franchimont, 1975; Feder, Resko & Goy, 1968; Butcher, Collins & Fugo, 1974; Lukaszewska & Greenwald, 1970), it has been demonstrated that endogenous estrogen is not sufficient to induce sexual receptivity in guinea pigs (Joslyn, Feder, & Goy, 1971), rats (Powers, 1970) or hamsters (Ciaccio & Lisk, 1971). In estrous cycling rodents, the induction of sexual behavior requires not only estrogen priming, but also a subsequent surge of progesterone from either an exogenous or endogenous source.

The use of ovariectomized rodents with sexual behavior induced by controlled doses of hormone enables more complex interactions

of estrogens and progestins to be investigated and parcelled out. For example, when progesterone is administered at various intervals after a sufficient dose of an estrogen, heat occurs with shorter latency with a more discrete duration and in a larger percentage of guinea pigs than with estrogen-induced heat (Collins et al., 1938; Young, 1969; Zucker & Goy, 1967). The addition of progesterone also results in a more consistent latency to heat and in an increase in the duration that the lordosis posture is held (Zucker & Goy, 1967). Increasing doses of progesterone may increase the duration of heat in guinea pigs (Joslyn et al., 1971; but cf. Dempsey et al., 1936). Heat terminates despite high plasma levels of progesterone (Morin & Feder, 1973). Thus, progesterone seems to increase heat duration, but it also causes its termination.

In experiments with rats, measurements do not usually include latency to onset of sexual receptivity or its duration, since sexual receptivity changes as a result of repeated testing (Hardy & DeBold, 1972). Nevertheless, the percentage of rats that become sexually receptive when estrogen is followed by progesterone is greater than with estrogen alone (Beach, 1942; Boling & Blandau, 1939).

The role of progesterone in the induction of sexual behavior in the golden hamster appears to be no different than in guinea pigs or rats. Whereas estradiol alone is followed by estrous behavior in only a few percent of ovariectomized hamsters, if sufficient quantities of progesterone are administered 24-48 hours after estradiol, all or nearly all of the females become sexually receptive (Frank & Fraps, 1945). As with guinea pigs, a progesterone injection increases lordosis duration and decreases the latent period in

hamsters administered daily injections of estradiol. When a single estrogen injection is followed by a single progesterone injection, increasing doses of progesterone result in an increase in the percentage of hamsters in heat and in the lordosis duration during a test (Carter & Porges, 1974).

Besides facilitating the actions of estrogens in the induction of sexual receptivity, progesterone is the most potent naturally-occurring antiestrogen. In 1936, Dempsey, Hertz & Young first suggested "the presence of a functional corpus luteum...might counteract the effect of (estradiol)" on the induction of sexual behavior in female guinea pigs. When sufficient levels of progesterone are present during the time of estrogen conditioning[1], the induction of sexual behavior is inhibited (concurrent inhibition, Powers & Moreines, 1976). Concurrent inhibition has been distinguished from sequential inhibition which occurs subsequent to the completion of estrogen conditioning of sexual receptivity.

The role of progesterone in the inhibition of sexual behavior has been well-documented for guinea pigs (Goy & Phoenix, 1965; Wallen, Goy & Phoenix, 1975; Zucker, 1966; Zucker & Goy, 1967). In intact guinea pigs, a substance of ovarian origin, presumably progesterone, has a transient concurrent inhibitiory influence on the induction of sexual receptivity. Goy et al., (1966) have shown that estradiol benzoate progesterone induction of sexual receptivity

---

[1] Estrogen "conditioning" is shorthand and refers to the changing state of the central nervous system substrate. It is considered to be complete when a progesterone injection will induce sexual behavior.

is inhibited during the luteal phase of the guinea pig estrous cycle. If they attempted to induce heat during most stages of pregnancy, no lordosis was observed (Goy et al., 1966). When guinea pigs were administered EB + progesterone beginning on Day 9 of the estrous cycle, generally none or few of the animals became sexually receptive (Goy et al., 1966; Zucker, 1968). If, however, the animals were ovariectomized at either the time of, 12 hours, or 18 hours after the EB injection, nearly all of the animals became receptive. When ovariectomy was performed 24 or 36 hours after EB, sexual receptivity was inhibited (Zucker, 1968).

When ovariectomized guinea pigs received an injection of progesterone two hours prior to 6 μg of estradiol benzoate, the percentage of animals becoming sexually receptive following a second injection of progesterone 36 hours later was inversely related to the dose of the first (inhibitory) injection; 0.5 mg was adequate to inhibit sexual receptivity in all animals (Goy & Phoenix, 1965; Wallen et al., 1975). One mg of progesterone administered either six hours after estradiol benzoate or up to 24 hours prior to the estradiol benzoate, inhibited the induction of sexual behavior when tested after an additional progesterone injection 36 hours later (Wallen et al., Zucker, 1966). The progesterone injection decreased the percentage of guinea pigs becoming sexually receptive, slightly increased the latency to lordosis and also decreased the duration of heat when compared with animals that either received no concurrent progesterone or received it 48 or 72 hours prior to estradiol.

The results of Wallen et al., (1975) clearly demonstrate that the interval between progesterone and estradiol is critical in

inhibiting sexual behavior. If the progesterone injection precedes the estradiol injection by too long a duration, inhibition is not observed. In fact, using supporting radioimmunoassay data of blood progesterone levels following subcutaneous progesterone injections, they suggest that the inhibition is directly related to blood plasma progesterone concentrations. A similar temporal dependence of inhibition on progesterone has also been demonstrated in intact guinea pigs by Zucker (1966).

Relatively little is known of progesterone's inhibitory effects on sexual behavior in rats. In retrospect, the originally reported failure to inhibit sexual behavior in ovariectomized rats with injections of either 0.16 mg or 1 mg of progesterone given simultaneously with, or twelve hours subsequent to injections of 6 µg of estradiol benzoate is not surprising (Zucker, 1967a). Edwards, Whalen & Nadler (1968) found that 5 µg EB + 0.5 mg progesterone daily for ten days induced high levels of receptivity as measured on the tenth day of treatment. On the contrary, 5 µg EB + 5 mg of progesterone induced levels approximately 50% that of the group that received low levels (0.5 mg) of progesterone. Thus, inhibition in rats seems to depend on high doses of progesterone.

When Powers and Zucker (1969) attempted to induce sexual behavior during pregnancy and pseudopregnancy with estradiol benzoate, they obtained indirect evidence that suggested that high levels of progesterone may be required to concurrently inhibit sexual behavior. During pregnancy, EB was injected and followed 24 or 48 hours later by testing for sexual behavior (Powers & Zucker, 1969). Two µg of EB induced sexual behavior if administered on Day 0, but not if

injected on Days 3-15 of pregnancy. On the other hand, 6 µg of EB induced low levels if administered on Day 5, moderately high levels if administered on Day 10 but did not induce heat if administered on Day 15. Since it is known that plasma progesterone levels first exceed estrous-cycle levels on Days 2-5 of pregnancy and peak at around Day 15 (Fajer & Barraclough, 1967; Hashimoto, Henricks, Anderson & Melampy, 1968), this suggested that progesterone may be causing inhibition of sexual behavior induced by EB. Even more striking, however, is the demonstration that 6 µg of EB can induce sexual behavior at times at which 2 µg of EB cannot, demonstrating a dose dependency on estradiol as well as the indirect suggestion of dose dependency on progesterone. This latter dependency is, of course, purely correlational and is simply based on the fact that inhibition correlates with progesterone levels. The same relationships were obtained on the corresponding days of pseudopregnancy. Thus, assuming that progesterone is responsible for the inhibition during pregnancy, relatively low levels of progesterone can inhibit the induction of heat with low doses of EB, but higher doses of EB can offset this inhibition; high levels of progesterone can inhibit even higher doses of EB, at least up to the 6 µg of EB used in this experiment. These studies therefore, raise the possibility that inhibition of sexual receptivity in rats by progesterone may be critically dependent on the relative doses of progesterone and estradiol benzoate administered.

Following the period of sexual receptivity, a refractory period ensues in guinea pigs during which a subsequent injection of progesterone does not facilitate another episode of sexual behavior

(Boling, Young & Dempsey, 1938). This period, which is of variable duration, can be influenced by the progesterone dose (Wallen, Goy & Phoenix, 1975; Zucker, 1966; Zucker & Goy, 1967), additional estradiol administered (Zucker, 1966) and the interval between the first and second progesterone injection (Zucker, 1966). This type of inhibition has been referred to as sequential inhibition. Boling et al. (1938) first demonstrated that following the induction of heat by estradiol benzoate + progesterone, a second injection of progesterone does not reinduce sexual receptivity, although sexual receptivity can be induced if the first progesterone injection is omitted (Collins, Boling, Dempsey & Young, 1938). Following termination of estrogen-induced heat, however, progesterone did induce a second period of sexual receptivity, thus demonstrating that it is not sexual receptivity per se, but progesterone that inhibits reinduction of sexual behavior.

In subsequent work with ovariectomized guinea pigs (Zucker, 1966) sexual receptivity was first induced by estrogen + progesterone. At various time intervals after this progesterone injection, a second progesterone injection was administered. As the latency to the second progesterone injection increased from 12 to 171 hours, there was a tendency for more frequent recurrence of heat, but even at 171 hours, only one-third of the guinea pigs became receptive.

It has recently been demonstrated that inhibition of sexual behavior can occur without prior facilitation. When guinea pigs were primed with 3.3 µg of estradiol benzoate and tested for sexual receptivity with only 15 µg of progesterone at 36 hours, the lordosis of 65% of the guinea pigs was inhibitied when tested with 0.6 mg of

progesterone at 60 hours (Morin & Feder, 1974a). In a further analysis it was shown that of the animals that were not receptive in the first test, only 20% of them responded on the second; of the animals which were receptive on the first test, 50% responded on the second test. This is accepted as evidence that a lower dose of progesterone is required for inhibition than facilitation with this procedure. It is obvious that this particular set of results is probably critically dependent on the interval between progesterone injections (cf. Zucker, 1966). Presumably, if Morin and Feder (1974) had waited longer than 60 hours for the second progesterone injection and test, a greater quantity of progesterone would have been required for inhibition.

Boling et al. (1938) had initially demonstrated that following EB + progesterone induction of heat, guinea pigs can readily be induced to exhibit a second episode of receptivity if EB is administered prior to the second progesterone injection. Actually most of their guinea pigs could be brought into four or five separate episodes in fifteen days if the sexual behavior was induced by EB + progesterone. Using different doses of hormones than in previous work, Zucker (1966; 6 µg EB + 0.4 mg progesterone) was capable of reinducing sexual receptivity if the latency from the first progesterone injection to the second estradiol injection was of long enough duration. As the latency increased from 6 hours to 15 hours to 58 hours, increasing numbers of the guinea pigs became sexually receptive, and maximum lordosis duration increased.

The refractory period depends on adequate dose of progesterone for the first progestin injection. As the dose of the first

progesterone injection increases, fewer animals become receptive following a second progesterone injection (Zucker & Goy, 1968; Goy & Phoenix, 1965; Wallen et al., 1975).

The results of Boling et al. (1938) that had shown that guinea pigs could be repeatedly brought into heat by EB + progesterone are consistent with those of Zucker (1966) and Zucker & Goy (1967) that had shown that under some circumstances, guinea pigs could be successively brought into heat and under some circumstances they could not. Taking into consideration the dose dependency of the inhibition on progesterone (Goy & Phoenix, 1965; Wallen et al., 1975; Zucker & Goy, 1967) and the temporal dependence of the progesterone and estradiol injections (Zucker, 1966), it becomes obvious that if a wide range of hormone dosages are not tried, contradictory results can readily be obtained. This fact may well account for the early conflicting findings in rats.

As was the case with concurrent inhibition, first attempts to obtain sequential inhibition in ovariectomized rats were unsuccessful (Zucker, 1967a). Ovariectomized rats were injected with 6 µg EB followed at 36 hours with 0.4 mg of progesterone. Most of the rats were sexually receptive when they were tested $42\frac{1}{2}$ hours after EB. Sixty hours after the initial EB injection progesterone was again administered. This resulted in lordosis quotients which were not significantly lower than those obtained on the first test. The results were different from those obtained in guinea pigs and were unexpected in light of the fact that exogenously administered progesterone inhibits sexual receptivity during the estrous cycle in rats (Zucker, 1967b). Progesterone administered on any of the first

three days of the four-day estrous cycle delayed the occurrence of sexual behavior (Zucker, 1967b).

In Zucker's attempt to obtain progesterone-induced sequential inhibition in ovariectomized rats, doses of estradiol and progesterone were chosen which were known to be effective in guinea pigs (Zucker, 1967a). However, rats and guinea pigs differ in their responsiveness to progesterone (Wade et al., 1973). Specifically, rats are less sensitive to progesterone than guinea pigs in the facilitation of sexual behavior (Powers & Valenstein, 1972; Wade & Feder, 1972), and perhaps more sensitive to estradiol than guinea pigs (Boling & Blandau, 1939; Dempsey et al., 1936). Nadler (1970) attempted to induce a refractory period in ovariectomized rats using smaller quantities of estradiol benzoate than had been previously used. One microgram of estradiol benzoate was injected at both 0 and 24 hours. At 48 hours, oil or 0.5 mg of progesterone was administered and rats tested for receptivity. A day later (72 hours) 0.5 mg of progesterone was administered and rats were tested again for receptivity. In this situation, the second progesterone injection was ineffective in reinducing sexual receptivity in rats that had received progesterone at 48 hours. Thus, with these particular dosages of hormones, a progesterone-induced refractory period was observed in rats. That this refractory period was due to progesterone and not to copulatory stimuli (Hardy & Debold, 1970) was demonstrated by omitting testing in one group after the first progesterone injection. The next day's progesterone injection then failed to facilitate sexual behavior. If EB injection was added at the time of the first progesterone injection, no refractory period

was evident. This suggests that progesterone may induce a refractory period under appropriate circumstances and that this inhibition is in part dependent on low levels of estrogen; increasing titres of estrogen apparently overcome this inhibition.

Lisk (1969a, 1969b) used another approach to obtain a progesterone-induced refractory period. Cannulae filled with estradiol were implanted into the anterior-hypothalamus-preoptic area of ovariectomized female rats. After 72 hours, progesterone was implanted subcutaneously and rats tested frequently over a period of 72 hours for receptivity. Progesterone first facilitated sexual behavior, but by 72 hours the facilitation was absent. If the subcutaneous progesterone implant was then removed but reimplanted four days later, the progesterone once again facilitated the induction of sexual behavior. This experiment, however, was confounded by the fact that the same animals were tested several times over the testing period. Lisk, unfortunately, did not take into account the possibility that prior copulatory stimuli may have contributed to his results (Hardy & Debold, 1972).

In an investigation of the refractory period as it relates to the estrous cycle, Powers (1970) attempted to determine if progesterone acts to inhibit receptivity during the estrous cycle. When rats were ovariectomized either before or during the progesterone surge, a progesterone injection 12 to 18 hours after an initial testing for receptivity on the night of proestrus was ineffective in inducing a second episode of receptivity. Since one µg of EB injected at the time of or prior to ovariectomy overcame this inhibition, the data suggest that during the estrous cycle the

refractoriness may be due to the lack of estradiol rather than to the presence of progesterone. It should, however, be emphasized that there is no time during the estrous cycle when ovariectomy can be performed so that the progesterone surge is blocked (to demonstrate that progesterone is necessary for the refractoriness), yet estradiol secretion remains normal (to demonstrate that diminished estradiol levels are not responsible for the refractoriness). In fact, Powers' (1970) early ovariectomies occurred during the estradiol surge (Butcher, Collins & Fugo, 1974) so that both estradiol and progesterone levels may have been diminished.

Barfield and Lisk (1974) combined timed ovariectomies with exogenous hormones. The results of their work suggest that the endogenous progesterone surge induces a refractory period to induction of receptivity by estradiol + progesterone. This, of course, contradicts Powers' (1970) interpretation in that a prior progesterone surge inhibited the induction of receptivity in rats when estradiol levels were otherwise high enough to induce heat.

Using a similar procedure, Powers and Moreines (1976) found however, the presence or absence of a sustained elevation in progesterone levels was <u>without</u> effect on estradiol + progesterone induction of sexual behavior during the estrous cycle. As the authors point out, the procedure was one of <u>concurrent</u> inhibition, not <u>sequential</u> inhibition. During the naturally occurring estrous cycle inhibition should be of the sequential type, simply because the peak in plasma progesterone concentration occurs after the peak in estradiol concentration. In addition, it should not be surprising if the endogenous progesterone surge does not inhibit the induction

of sexual behavior by the dose of estradiol that they used (26.7 µg/kg body weight).

Using a sequential inhibition procedure rather than a concurrent inhibition procedure, Powers and Moreines (1976) obtained what appears to be a progesterone-induced refractory period to subsequent stimulation by progesterone. With this procedure in which the rats' endogenous estradiol was used to condition sexual behavior, its own progesterone used for inhibition and an exogenous progesterone injection used for subsequent facilitation, Powers and Moreines (1976) report some inhibition by the endogenous progesterone.

In order to predict how progesterone might act on the brain to exert its effects on behavior that have been outlined, a prior understanding of progesterone's mechanism of action in more thoroughly-studied tissues is helpful. The model of the initial interaction of a steroid hormone with reproductive tissues has been described and recently reviewed by several authors (Gorski & Cannon, 1976; Jensen & DeSombre, 1973; O'Malley & Means, 1974; Yamamoto & Alberts, 1976). Basically, this model postulates that a steroid hormone enters a cell, binds to a cytoplasmic receptor permitting translocation of the steroid-receptor complex to the cell nucleus where it initiates an alteration in gene expression. Although initially proposed for estrogen's interaction with uterine cells (Gorski, Toft, Shyamala, Smith & Notides, 1968; Jensen, Suzuki, Kawashima, Stumpf, Jungblut & DeSombre, 1968), the "two-step" model[2] has

---

[2] Although admittedly an oversimplification, for convenience this model will be referred to as the "two-step" model (Jensen et al., 1968) to emphasize its dependence on cytoplasmic and nuclear binding.

since been extended in entirety or in part to the interaction of all steroid hormones with their respective target tissues (Gorski & Gannon, 1976; Jensen & DeSombre, 1973; O'Malley & Means, 1974) including the central nervous system (McEwen & Pfaff, 1973; McEwen, Denef, Gerlach & Plapinger, 1974; McEwen, 1975; McEwen, 1976). The utility of such a model for progesterone's mechanism of action is indisputable. It has led to tremendous advances in delineating progesterone's mechanism of action in the chick oviduct, and recently to advances in our understanding of its action in the mammalian uterus.

The model target tissue for progesterone's mechanism of action in modulating gene expression is the chick oviduct because of its well-documented response of synthesis of the egg-white proteins, avidin and ovalbumin under discrete hormonal conditions (Schimke, McKnight, Shapiro, Sullivan & Palacios, 1975; O'Malley, McGuire, Kohler & Korenmann, 1969). Progesterone in either estrogen-stimulated or estrogen-withdrawn chicks induces the synthesis of avidin (O'Malley et al., 1969; Korenmann & O'Malley, 1968; Means & O'Malley, 1971); ovalbumin synthesis is induced by either estrogens or estrogens and progesterone (O'Malley et al., 1969; Palmiter, 1972). The intermediary mechanism of progesterone's action on avidin synthesis is believed to be as follows. High affinity progestin-specific binding proteins are present in the cytoplasm of oviduct cells (Sherman, Corvol & O'Malley, 1970; O'Malley, Sherman & Toft, 1970). Administration of progesterone results in binding to these cytoplasmic receptors (O'Malley, Toft & Sherman, 1971) and subsequent translocation to the nuclear compartment (O'Malley

et al., 1971) where the hormone-receptor complex binds to chromatin acceptor sites (Spelsberg, Steggles & O'Malley, 1971). One subunit of the hormone-receptor protein is believed to bind with high affinity to a limited number of sites on DNA (O'Malley & Schrader, 1972), and one subunit to nonhistone proteins (Spelsberg, Steggles, Chytil & O'Malley, 1972; Schrader, Toft & O'Malley, 1972). At this site it may increase the number of RNA chain initiation sites available to RNA polymerase (Schwartz, Kuhn, Buller, Schrader & O'Malley, 1976), thus stimulating synthesis of specific species of mRNA (O'Malley & McGuire, 1969). The transcriptional products are transported to the cytoplasm where the endpoint of translation into avidin may take place (O'Malley & Means, 1974). In the estrogen-stimulated chick oviduct, this specific response may occur despite the fact that progesterone may also cause a transient decrease in total protein synthesis (Means & O'Malley, 1971).

In mammals, the model system for progesterone's mechanism of action is of course, that organ in which progesterone exerts some of its most obvious morphological effects, the uterus. In the rodent uterus, progesterone both synergizes with and antagonizes estrogens' stimulation of various biochemical and physiological events. Progesterone administered by itself has little (Bronson & Hamilton, 1972; Harris, Lerner & Hilf, 1968) or no (Harris et al., 1968 ; Wade & Feder, 1974) effect on most uterine responses, althugh the fact that it can, by itself, induce deciduoma formation (Madjerek, 1972; Madjerek & Smit-vis, 1974; O'Malley & Strott, 1973) and corrugation of the luminal epithelium (Martin, Finn & Carter, 1970) cannot be overlooked. Progesterone facilitates estrogens' effects on responses

such as increases in uterine weight (Bronson & Hamilton, 1972), RNA Synthesis (Bronson & Hamilton, 1972; Miller, 1975), protein synthesis (Bronson & Hamilton, 1972; Wade & Feder, 1974), RNA:DNA ratios (Bronson & Hamilton, 1972), stromal mitosis (Clark, 1971; Clark, 1974) and endometrial proliferation (Zarrow, Yochim & McCarthy, 1964). Progesterone can induce decidualization (Yochim & DeFeo, 1962) and stimulate the change of the endometrium from a proliferative phase to a secretory one (Finn & Porter, 1975; McPhail, 1934).

Within prescribed dosage and temporal parameters, progesterone also antagonizes most of estrogens' effects including many of the same responses which it facilitates. Progesterone inhibits estrogen-induced increases in uterine wet weight (Harris et al., 1968; Hsueh, Peck & Clark , 1975; Bo, Poteat, Krueger & McAlister, 1971; Martin & Finn, 1970), RNA synthesis (Bronson & Hamilton, 1972), protein synthesis (Bronson & Hamilton, 1972), DNA synthesis (Bronson & Hamilton, 1972; Krueger, Bo & Garrison, 1974), RNA:DNA ratios (Harris et al., 1968), luminal and glandular epithelium mitosis (Clark, 1971, 1974; Martin & Finn, 1970), cAMP levels (Rinard & Chew, 1975), phosphorylase a levels (Rinard & Chew, 1975), glucose-6-phosphate dehydrogenase activity (Harris et al., 1968), lipid content (Harris et al., 1968), retention of luminal fluid (Bo et al., 1971; Kennedy & Armstrong, 1975; Clemetson Verma & DeCarlo, 1977; Armstrong, 1968), glycogen concentrations (Zarrow et al., 1964; Bo et al., 1971), and of course myometrial contractions (progesterone block: O'Malley & Strott, 1973; Davies & Ryan, 1972; but possibly not in the guinea pig, Porter, 1970).

Whether progesterone facilitates or antagonizes estrogens'

action on a particular response seems to be critically dependent on both the injection regimen and dosage parameters for both hormones. In rats and mice, combined treatment with estrogen + progesterone for one (Bo et al., 1971), three (Hsueh et al., 1975), four (harris et al, 1968) or seven (Huggins & Jensen, 1955) days antagonizes uterine growth. Conversely, Bronson & Hamilton (1972) demonstrated the progesterone synergizes with estradiol to increase uterine wet weight in some circumstances. When estradiol was administered on Days 1-3 and 7, the addition of progesterone on Days 4-7 resulted in more growth than when progesterone was omitted.

Early attempts to demonstrate specific progestin binding in the rodent uterus failed. With autoradiographic techniques, progesterone was not found to concentrate in any subcellular fraction (Rogers, Thomas & Yates, 1966; Taylor & Wright, 1971). Likewise, other early attempts to measure in vivo uptake of radioactively labeled progesterone into uterine tissue revealed little or no concentration relative to nontarget tissues (Riegel, Hartop & Kittinger, 1950; Berliner & Wiest, 1956; Wiest, 1963; Lawson & Pearlman, 1964; Laumas & Farooq,1966). In 1970, Falk and Bardin (1970) reported in vivo uptake of ^{3}H-progesterone in the guinea pig uterus, and in that year Milgrom and Baulieu (1970) reported progesterone binding by the cytosol of rats' uterus.

The major obstacle to characterizing progesterone binding has been the rapid dissociation of progesterone from its cytoplasmic binder (Feil, Glasser, Toft & O'Malley, 1972). Several procedural adaptations have been used recently which circumvent this inherent technical problem by decreasing the rate of dissociation. Thio-

glycerol is often used in buffers because it stabilizes some forms of the chick oviduct progesteone receptor (Schrader, 1975; Schrader, Smith & Coty, 1976). Addition of glycerol to buffers markedly slows down the dissociation rate of progesterone from its mammalian uterine receptor (Feil et al., 1972). With a charcoal adsorption assay, Feil, Glasser, Toft & O'Malley (1972) have shown that glycerol may actually increase the half-life of the hormone-receptor complex by as much as 10-15 times. Lastly, a highly potent synthetic progestin, 17α, 21-dimethyl-19-nor pregna-4,9-diene-3,20-dione, has been used to dissociate progestin-specific binding from less-specific binding (Philibert & Raynaud, 1973; Philibert & Raynaud, 1974).

Part of the early ambiguity in the understanding of progesterone binding in mammalian uterus may have been due to the fact that the high affinity binding component is dependent on estrogen priming (Chen & Leavitt, 1975). Estrogen pretreatment seems to be a prerequisite for high levels of progesterone binding, a fact consistent with progesterone's dependence on estrogen priming for most physiological responses. Estrogen injections dramatically increase the amount of in vivo binding in the uterus but not nontarget tissues of rodents (Corvol, Falk, Freifeld & Bardin, 1972; Falk & Bardin, 1970; Leavitt & Blaha, 1972). This increase is also seen with in vitro assay of progesterone binding (Chen & Leavitt, 1975; Leavitt et al.,,1974; Milgrom, Atger & Baulieu, 1970; Freifeld, Feil & Bardin, 1974; Luu Thi, Baulieu & Milgrom, 1975; Faber, Sandmann & Stavely, 1972a,b). In an in vitro assay using hamster uterine strips incubated with estradiol, the increase in progesterone binding was found to be protein-synthesis-dependent since it is inhibited by

cycloheximide (Faber, Saffran, Chen & Leavitt, 1976), and RNA-synthesis-dependent since it is inbhibited when actinomycin-D is present prior to, but not after, the sixth hour of a 12 hour incubation (Faber et al., 1976.

A central requirement of the two-step model for progesterone's action is that the hormone bound to its receptor is translocated to the cell nucleus. Using autoradiography, concentration of radioactivity has recently been found over cell nuclei of the uterus after injection of ^{3}H-progesterone (Warmebourg, 1974; Stumpf & Sar, 1973). With liquid scintillation techniques tritium has been recovered from cell nuclei of the uterus after ^{3}H-progesterone injection (Atger, Baulieu & Milgrom, 1974; Feil, Miljkovic & Bardin, 1976).

A progestin-specific receptor has been reported in the nuclei of rat uterine tissue by an exchange assay (Walters & Clark, 1976; Hsueh, Peck & Clark, 1974). Following a progesterone injection, cytoplasmic progesterone receptors are depleted and progesterone receptors accumulate in the nuclear fraction. This presumably represents translocation of the receptor from the cytoplasm to the nucleus (Saffran, Loeser, Bohnett & Faber, 1976 ; Walters & Clark, 1975; Hseuh et al., 1974). These reports are consistent with the notion that binding which appears in the nucleus is actually due to transformed and translocated cytoplasmic receptors (Feil & Bardin, 1975). In experiments of in vivo binding of ^{3}H-progesterone, however, only slightly more radioactivity accumulates in the nuclear fraction than in the cytosol (Atger et al., 1974, Feil et al., 1976). Although this is not strong evidence for a nuclear site of action for progesterone, the date are not inconsistent with the "two-step" model of steroid action

(Gorski & Gannon, 1976; Jensen & DeSombre, 1973; O'Malley & Means, 1974).

There have been numerous reports of failure to extend the "two-step" model of progesterone action to the pituitary gland and the central nervous system. Many reports have demonstrated that *in vivo* uptake into whole homogenates of these tissues is nonsaturable (Wade & Feder, 1972a; Luttge, Wallis & Hall, 1974; Iramain & Strott, 1973; Whalen & Gorzalka, 1974) and seems to be nonspecific. Its only selectivity seems to obey a rule similar to the "polarity rule" by which steroids bind to plasma proteins (Westphal, 1973). Less polar steroids are taken up in greater concentrations and retained more than steroids with greater polarity (Wade & Feder, 1972b).

In this nonsaturable system, uptake in midbrain is greater than in hypothalamus which is greater than cortex (Wade & Feder 1972a; Luttge *et al.*, 1974; Wade & Feder, 1972b; Seiki, Miyamato, Yameshita & Kitani, 1969; Luttge, Chronister, & Hall, 1973; Wade, Harding & Feder, 1973; Whalen & Luttge, 1971a,b). Highest uptake is sometimes observed in the pituitary gland (Whalen & Gorzalka, 1974; Luttge *et al.*, 1973; Whalen & Luttge, 1971; Presl, Figarova, Herzmann & Rohling, 1975). There are minor differences between experimenters in the ordering of other tissues, most of which may reflect variations in dissection procedures.

Reports of indirect evidence for saturability of this whole homogenate uptake system exist. Whalen's group (Whalen & Gorzalka, 1974; Whalen & Luttge, 1971a,b) reports that adrenalectomy increases the absolute concentration of radioactivity in most regions. However, others have pointed out that when tissue/plasma ratios are

computed, which take into account the higher levels of radioactivity in the blood plasma of adrenalectomized animals, this difference is eliminated(Wade & Feder, 1972a; Zigmond, 1975). Subsequent experiments in mice have failed to replicate the initial effects of adrenalectomy (Luttge et al., 1973).

In vitro cytoplasmic receptor assays have had mixed results. Sucrose density gradient centrifugation experiments have failed to detect progestin binding in the hypothalamus or pituitary of rats (Davies, Naftolin & Ryan, 1974; Davies, Siu, Naftolin & Ryan, 1975; Kato, 1975), guinea pigs (Atger et al., 1974; Iramain, Danzo, Strott & Toft, 1973) and hamsters (Reel & Shih, 1975). Using gel filtration both Seiki and Hattori (1973) in rats, and Iramain, Danzo, Strott and Toft (1973) in guinea pigs have observed binders for progesterone in the hypothalamic area and pituitary; other experimenters have failed to confirm these findings (Atger et al., 1974). Luttge and Wallis (1973) demonstrated saturable binding in whole tissue of the interpeduncular region and pituitary, but no attempt was made to demonstrate steroid specificity.

With the exception of one series of experiments, attempts to observe cell nuclear localization of radioactively labelled progesterone after in vivo injection have all failed (Atger et al., 1974; Marrone & Feder, 1977; McEwen, deKloet & Wallach, 1976). Although Karavolas reported concentration of ^{3}H-progestins in crude hypothalamic and pituitary cell nuclei (Karavolas & Herf, 1971; Cheng & Karavolas, 1973; Robinson & Karavolas, 1973), this binding: 1) is not diminished by boiling, which indicates that it is not protein-bound, and 2) is not found in purified nuclei (Cheng & Karavolas, 1975a,b).

In the one study which isolated cytosol after in vivo injection of ^{3}H-progesterone, high initial concentration and retention were found in the median eminence and pituitary which is difficult to reconcile with the hypothesized nuclear site of action. They used a very crude cytosol fraction (800 x g for 10 minutes), so their results may be attributable to contamination (Seiki & Hattori, 1973).

With autoradiography, Sar & Stumpf (1973) found nuclear concentration of tritium after an injection of ^{3}H-progesterone in a circumscribed area which included the arcuate nucleus, preoptic periventricular nucleus and preoptic suprachiasmatic nucleus after an injection of ^{3}H-progesterone. They also observed enhancement of accumulation by estradiol priming and competition by unlabeled progesterone. Warembourg, however, failed to replicate these findings.

The latter steps in progesterone's central mechanism have been studied less extensively and lend support to an action on protein synthesis and perhaps on transcription. Protein synthesis in rats' neural tissues varies cyclically over the estrous cycle (Litteria, 1973; Moguilevsky, Sacchi, Christot, 1971). This effect is likely due in part to estradiol's effects on translation (Wade & Feder, 1974). Progesterone injection increases overall protein synthesis in all areas of the brain that have been studied as well as in the uterus; it has no effect in non-target tissues such as the diaphragm (Wade & Feder, 1974). In addition, hypothalamic implants of the protein synthesis inhibitor , cycloheximide have been shown to prevent progesterone's inhibition of sexual behavior in guinea pigs (Wallen, Goldfoot, Joslyn & Paris, 1972). Progesterone's positive feedback on LH release is inhibited by systemic injections (Jackson, 1972) or

hypothalamic implants (Jackson, 1975) of the RNA synthesis inhibitor, actinomycin-D.

The purposes of this dissertation are three-fold. In Section I, progesterone's inhibitory effect on female sexual behavior in rats is partially characterized. Section II tests the hypothesis that progesterone's antiestrogenic effects are mediated by a mechanism such as that proposed for the synthetic estrogen antagonists. In Section III, the synthetic progestin, R 5020 is used to investigate the possibility that progesterone's effects on sexual behavior are mediated by a "two-step" mechanism in the brain.

GENERAL PROCEDURE

Behavioral testing. Female Sprague-Dawley rats were purchased from a commercial supplier (Charles River Breeding Laboratories, Wilmington, Massachusetts). They arrived in the laboratory weighing 125-150 g and weighed 250-350 g at the time of testing. All animals were housed in group cages with pine wood shavings as bedding. Environmental illumination was provided from 2400-1200 hours daily and room temperature was maintained at 21-23°C. Purina Laboratory Chow and tap water were available ad lib.

All animals were ovariectomized under methoxyflurane (Metofane) anesthesia through a single midventral incision. After ovariectomy rats were housed singly in Wahmann LC-75SA wire-mesh cages. Two weeks later, rats were given 2 µg of EB followed 42 hours later by 0.5 mg of progesterone and were screened for the presence of lordosis either by response to mounts by a male or by a manual stimulation technique (Zucker, 1967b). Only females that showed lordosis responses under these conditions were included in the experiments. When an animal was used in two studies, approximately 2 weeks were allowed to elapse between tests and subsequent injections. All steroids were injected in 0.1 ml of sesame oil except where otherwise noted.

All tests for lordosis took place in a 76-cm (diameter) round testing arena with walls that were 15 cm high. Three sexually experienced Sprague-Dawley male rats were adapted to the arena for 30 minutes prior to the introduction of the first female on a given day. All tests occurred 1 to 3 hours after the onset of the

dark period under dim red illumination. Testing was done with the experimenter blind to treatment groups. Tests consisted of ten vigorous mounts with thrusting by the males. Quality of each lordosis was rated as 0, 1, 2 or 3 (no, slight, moderate and full dorsiflexion, respectively) after Powers and Valenstein (1972). If an ejaculation occurred during a test, the female was removed from the arena for 10 minutes.

In vivo-^{3}H-estradiol uptake. Rats received injections of 2, 4, 6, 7-^{3}H-estradiol-17β (Specific Activity = 91.3 Ci/mmole, New England Nuclear) dissolved in either oil or ethanol-water (20%), administered through various routes, to be described in each experiment. At a particular time after injection, rats were anesthetized with sodium pentobarbital (Nembutal), a blood sample taken in a heparinized syringe via cardiac puncture, and the rats were perfused with 0.15 M cold saline. The brains were dissected as described in each experiment and the entire pituitary gland removed. All steps were performed at 4°C. Tissues were weighed to the nearest 0.1 mg and homogenized in Teflon-glass coaxial homogenizers. An aliquot of the whole homogenate was taken and a purified nuclear pellet isolated by the method of Zigmond and McEwen (1970) using the following solutions: 1) Nuclear Isolation I (N I) -- 0.32 M sucrose, 1 mM potassium phosphate, 3 mM magnesium chloride, 0.25% Triton X-100 (v/v), pH 6.5; 2) Nuclear Isolation II (N II) -- .32 M sucrose, 1 mM potassium phosphate, 3 mM magnesium chloride, pH 6.5; 3) Nuclear Isolation III (N III) -- 2.39 M sucrose, 1 mM potassium phosphate, 3 mM magnesium chloride, pH 6.5. Tissues were homogenized with 20 slow up-and-down strokes in 2 ml N I.

Two hundred µl of whole homogenate was taken and the remainder centrifuged at 800 x g for 10 minutes. The supernatant was discarded, the pellet resuspended in 2 ml N II; and the mixture centrifuged at 800 x g for 10 minutes. The supernatant was discarded, the pellet resuspended in 0.4 ml N II, and 2.1 ml N III added and mixed thoroughly. The mixture was centrifuged for 90 minutes at 20,000 x g to obtain a nuclear pellet. The supernatant was discarded, and the purified nuclear pellet removed from the centrifuge tubes with 3 x 500 µl 0.01 M citric acid.

Radioactivity was extracted from the whole homogenate and nuclear pellet fraction with 3 x 4 ml of toluene scintillation cocktail (5.0 g 2,5-diphenyloxazole and 0.05 g 1,4-bis-2(5-phenyloxazolyl))-benzene/liter scintillation-grade toluene). After extraction of radioactivity the whole homogenate and nuclear pellet fractions were washed with 5 ml of ethanol, and protein content was analyzed by the method of Lowry, Rosenbrough, Farr and Randall (1951).

Blood samples were centrifuged and 100 µl aliquots of plasma pipetted into scintillation vials. Twelve ml of scintillation cocktail were added and the mixture counted after vigorous shaking. Tissue radioactivity levels were expressed as disintegrations per minute (DPM/mg protein) and corrected for differential plasma radioactivity levels by expression as tissue/plasma ratios (DPM/mg protein: DPM/µl plasma) for reasons that have previously been delineated (McEwen & Pfaff, 1970).

In vivo-^{3}H-progestin uptake. Techniques for investigating the uptake and binding of ^{3}H-progestins are similar to those used for ^{3}H-estradiol with several exceptions. All buffers contained 12 mM

thioglycerol and 10% (v/v) glycerol, and pH was 7.2. These conditions have been optimal for studying the progestin receptor from mammalian uterus (Feil, Glasser, Toft & O'Malley, 1972; Philibert & Raynaud, 1973, 1974; Toft & Sherman, 1975). Also, immediately after dissection, tissues were placed into homogenizers stored at 4°C.

SECTION I: INHIBITION OF SEXUAL BEHAVIOR - THE RAT AS A HYPOSENSITIVE GUINEA PIG

In guinea pigs, unlike rats, inhibition is usually observed within broad ranges of doses of estrogens and progesterone. Following a subcutaneous injection of radioactively labeled progesterone, guinea pig brains take up more progesterone relative to blood plasma levels and retain the progesterone for a longer duration than do rat brains (Wade, Harding & Feder, 1973). If the ambiguity of progesterone's antagonistic effects in rats is related to the lesser neural uptake and/or retention of progesterone, then the progesterone dose necessary to inhibit lordosis should be greater in rats than in guinea pigs. Perhaps sufficient quantities of progesterone must be administered to maintain neural progesterone concentrations at some critical level. In addition, previous work (e.g., Powers & Zucker, 1969) suggests that there may be a critical relationship between the level of estradiol and the level of progesterone administered.

The factors which may influence sequential inhibition have also not been described for rats. However, with very low doses of estrogen priming (Nadler, 1970) or with intrahypothalamic estradiol implants (Lisk, 1969), progesterone may sequentially inhibit in ovariectomized rats.

During the estrous cycle, progesterone has been shown to exert at least a minor sequential inhibitory influence on the facilitation of a subsequent episode of sexual receptivity by progesterone (Powers & Moreines, 1976). Other reports of both success and failure (Barfield & Lisk, 1974; Powers, 1970; Powers & Moreines, 1976) in

finding inhibition by progesterone in estrous-cycling rats have used a concurrent inhibition procedure. In light of the conflicting evidence for sequential inhibition in ovariectomized rats (Zucker, 1967a) and in estrous-cycling rats, it is necessary to first document sequential inhibition by progesterone in OVX rats and then to characterize the conditions under which it occurs. Only after we have characterized progesterone's involvement in a behavior can we make predictions of its mechanism of action. In Section I progesterone's involvement in both concurrent and sequential inhibition in OVX rats will be characterized.

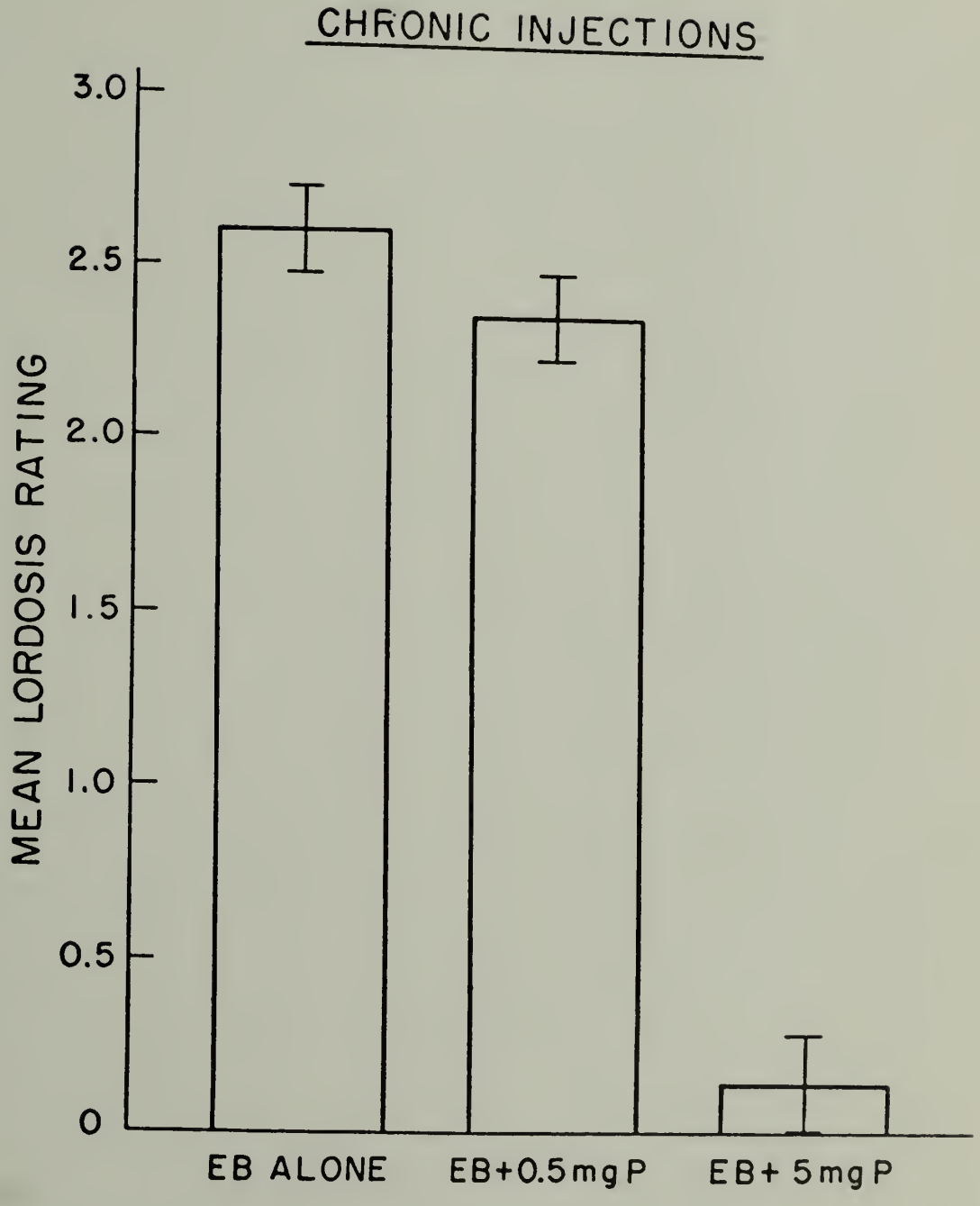
EXPERIMENT 1: DAILY INJECTIONS OF PROGESTERONE AND ESTRADIOL BENZOATE

Chronic daily injections of large doses of progesterone with EB result in lower levels of sexual receptivity than small doses of progesterone with EB when tested after 10 days of treatment (Edwards et al., 1968). However in the previous work, only the effects of chronic injections of the two hormones were investigated. The first experiment is an attempt to replicate these findings and extend them to a lower dose of EB and a more typical testing procedure in which testing occurs 5-7 hours after a progesterone injection.

Procedure. Twenty-two ovariectomized rats were divided into three groups receiving subcutaneous injections daily of either 2 μg of EB at 0600 hours (n=0), 2 μg of EB + 0.5 mg progesterone (n=7) or 2 μg of EB + 5 mg of progesterone (n=6). On Day 10 all rats were injected with 0.5 mg of progesterone (0800 hours) and tested 5-7 hours later for sexual receptivity.

Results. Although high levels of progesterone inhibited the induction of sexual behavior when compared with the group which received EB alone, $U(6,9) = 0$, $p < .005$, or the low progesterone group, $U(6,7) = 0$, $p < .005$, low doses of progesterone did not significantly inhibit sexual behavior, $U(7,9) = 17.5$, $p < .10$, (Figure 1).

Figure 1. Mean lordosis ratings ($\pm$ standard error) of ovariectomized rats given daily injections of 2 µg of estradiol benzoate (EB) alone, 2 µg of EB + 0.5 mg of progesterone, or 2 µg of EB + 5 mg of progesterone for nine days. (Tests occurred on the tenth day and were preceded by a single injection of 0.5 mg of progesterone.)


CHRONIC INJECTIONS
MEAN LORDOSIS RATING
3.0
2.5
2.0
1.5
1.0
0.5
0
EB ALONE
EB+0.5mgP
EB+ 5mgP

EXPERIMENT 2: CONCURRENT INHIBITION - INFLUENCE OF PROGESTERONE DOSE

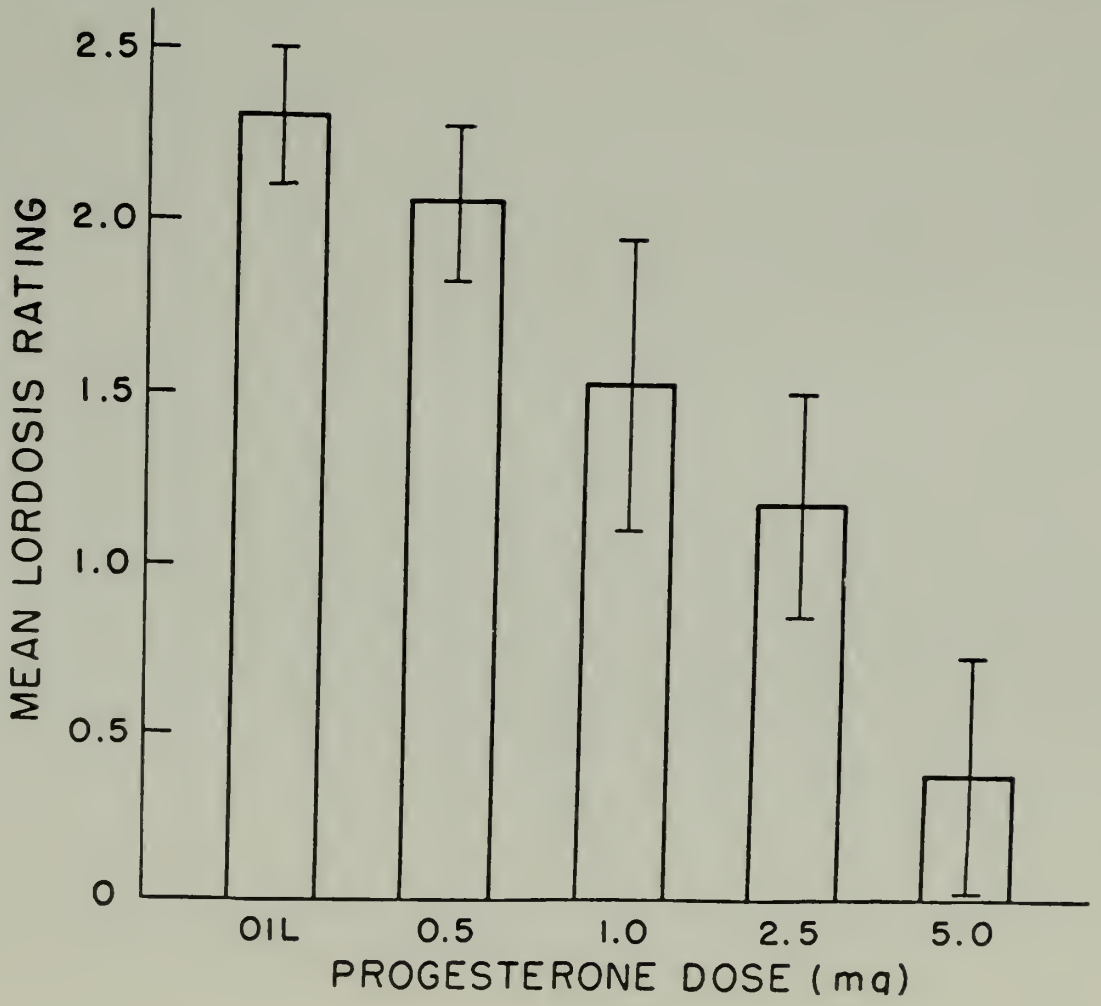
The results of Experiment 1 demonstrated that with chronic daily injections of progesterone and EB, progesterone in a sufficiently high dose can antagonize the actions of estradiol in the induction of sexual receptivity. In Experiment 2 the effects of various doses of progesterone administered simultaneously with EB were studied in a typical induction procedure -- a single injection of EB followed 42 hours later by a single injection of progesterone.

Procedure. Thirty-three ovariectomized rats were divided into five groups receiving various doses of progesterone or oil. All rats were injected subcutaneously with 2 μg of EB at 1400 hours. At this time either oil (n=8), 0.5 mg (n=7), 1 mg (n=6), 2.5 mg (n=6), or 5 mg (n=6) of progesterone was injected at a separate subcutaneous site. Forty-two hours later (0800 hours), 0.5 mg of progesterone was administered, and sexual receptivity was tested 5-7 hours later.

Results. Dosages of 2.5 mg of progesterone and 5 mg of progesterone significantly inhibited the induction of sexual receptivity when compared with rats that received EB + oil, $U(6,8) = 3$, $p < .005$; $U(6,8) = 1$, $p < .005$ (Figure 2). Neither the 0.5 mg progesterone nor the 1 mg progesterone group was significantly different from the controls which received EB and oil.

Figure 2. Mean lordosis ratings (± standard error) of ovariectomized rats treated with various doses of progesterone or sesame oil simultaneously with 2 μg of estradiol benzoate. (All groups received 0.5 mg of progesterone at 42 hours and were tested at approximately 48 hours.)

SIMULTANEOUS 2μg ESTRADIOL BENZOATE + PROGESTERONE



EXPERIMENT 3: CONCURRENT INHIBITION - TEMPORAL DEPENDENCE OF THE PROGESTERONE INJECTION

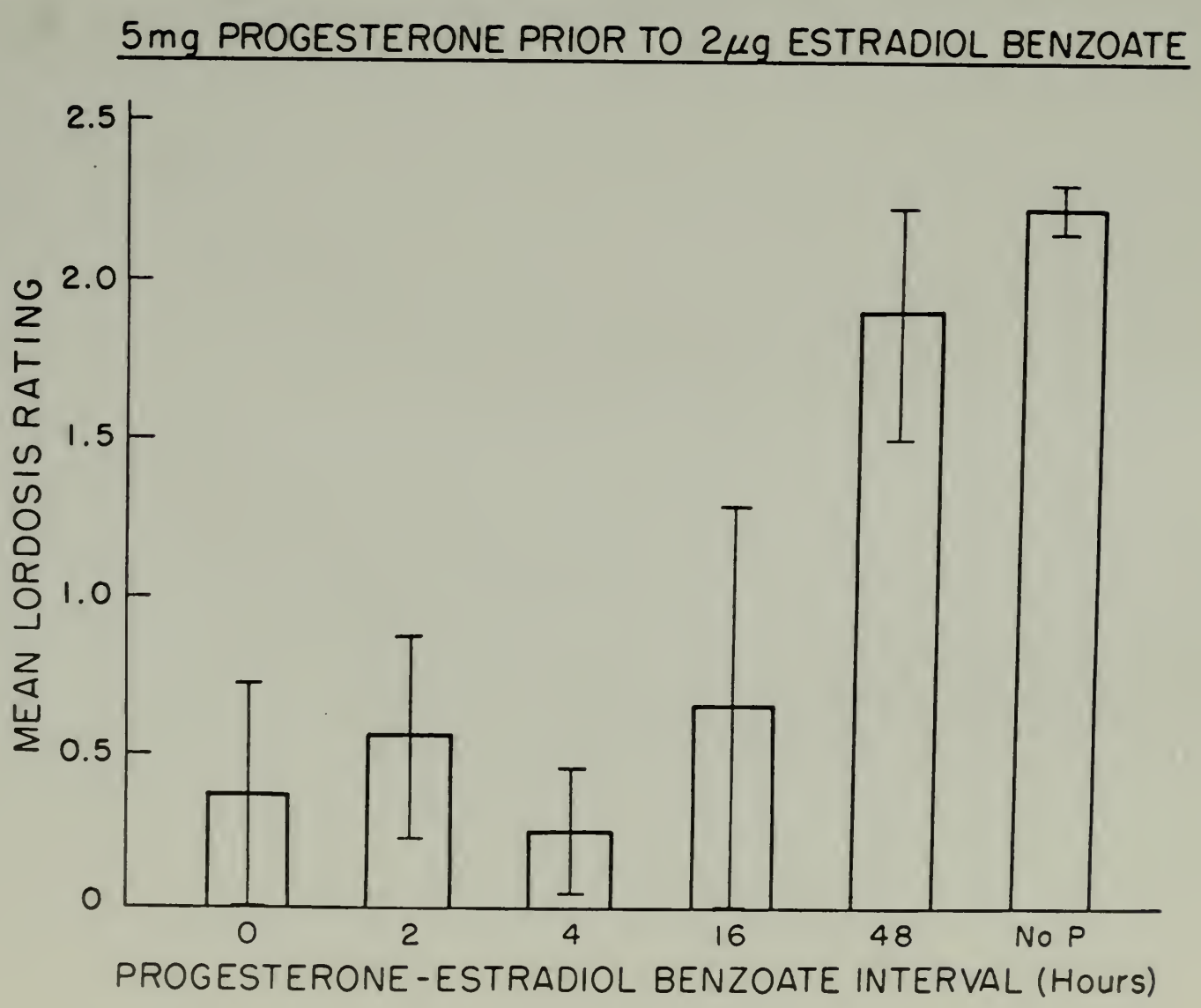
Experiment 2 showed that lordosis can be readily inhibited in a typical induction procedure if a sufficient dose of progesterone is administered simultaneously with the EB injection. The purpose of Experiment 3 was to broaden the basis for comparison of the antagonistic effects of progesterone on sexual behavior in guinea pigs and rats. Progesterone was administered at various intervals prior to the injection of EB in a typical induction procedure to demonstrate that progesterone can inhibit estradiol's action when present temporally close to the EB injection but not if separated by a longer duration. Doses of the two hormones were chosen (2 µg of EB and 5 mg of progesterone) that are approximately behaviorally equivalent to the levels used in a similar experiment with guinea pigs (Wallen et al., 1975).

Procedure. Fifty-four ovariectomized rats were distributed into groups receiving 5 mg of progesterone at various times prior to the EB injection. Progesterone was injected subcutaneously either 2 hours (n=9), 4 hours (n=11), 16 hours (n=8) or 48 hours (n=6) prior to the injection of 2 µg of EB. The control group (No P; n=15) received oil simultaneously with the EB injection. At 42 hours, 0.5 mg of progesterone was injected and animals were tested 5-7 hours later. Data for the 0 hour group that received 5 mg of progesterone simultaneously with the EB injection were taken from Experiment 2.

Results. When compared with the No P controls, progesterone injected either simultaneously with EB, $U(6,15) = 4$, $p < .005$,

2 hours prior to EB, $U(9,15) = 19$, $p < .02$, 4 hours prior to EB, $U(11,15) = 4.5$, $p < .002$, or 16 hours prior to EB, $U(8,15) = 10.5$, $p < .002$, inhibited the action of estradiol in the induction of sexual receptivity (Figure 3). However, if progesterone was given 48 hours prior to EB, there was no evidence of inhibition of lordosis.

Figure 3. Mean lordosis ratings (± standard error) of ovariectomized rats treated with 5 mg of progesterone either simultaneously with or at various intervals prior to 2 μg of estradiol benzoate. The No P control group received only an injection of oil simultaneously with the estradiol benzoate injection. (All groups were injected with 0.5 mg of progesterone at 42 hours and tested at approximately 48 hours.)


5mg PROGESTERONE PRIOR TO 2μg ESTRADIOL BENZOATE
MEAN LORDOSIS RATING
2.5
2.0
1.5
1.0
0.5
0
0
2
4
16
48
No P
PROGESTERONE-ESTRADIOL BENZOATE INTERVAL (Hours)

EXPERIMENT 4: CONCURRENT INHIBITION - INFLUENCE OF ESTRADIOL BENZOATE DOSE

The results of Experiment 3 are remarkably similar to those obtained in guinea pigs with a quite different ratio of the two hormones (Wallen et al., 1975). It is now quite clear that the basis for the original failure to observe antagonism by progesterone of sexual receptivity in ovariectomized rats was due to an insufficient dose of progesterone (Zucker, 1967a; 0.16 or 1 mg of progesterone). Since during some stages of pregnancy and pseudopregnancy 6 μg of EB can overcome the antagonism seen with 2 μg of EB (Powers & Zucker, 1969), it also seems that the dose of estradiol is critical. Experiment 4 tested this hypothesis by varying the dose of EB while holding the progesterone dose constant.

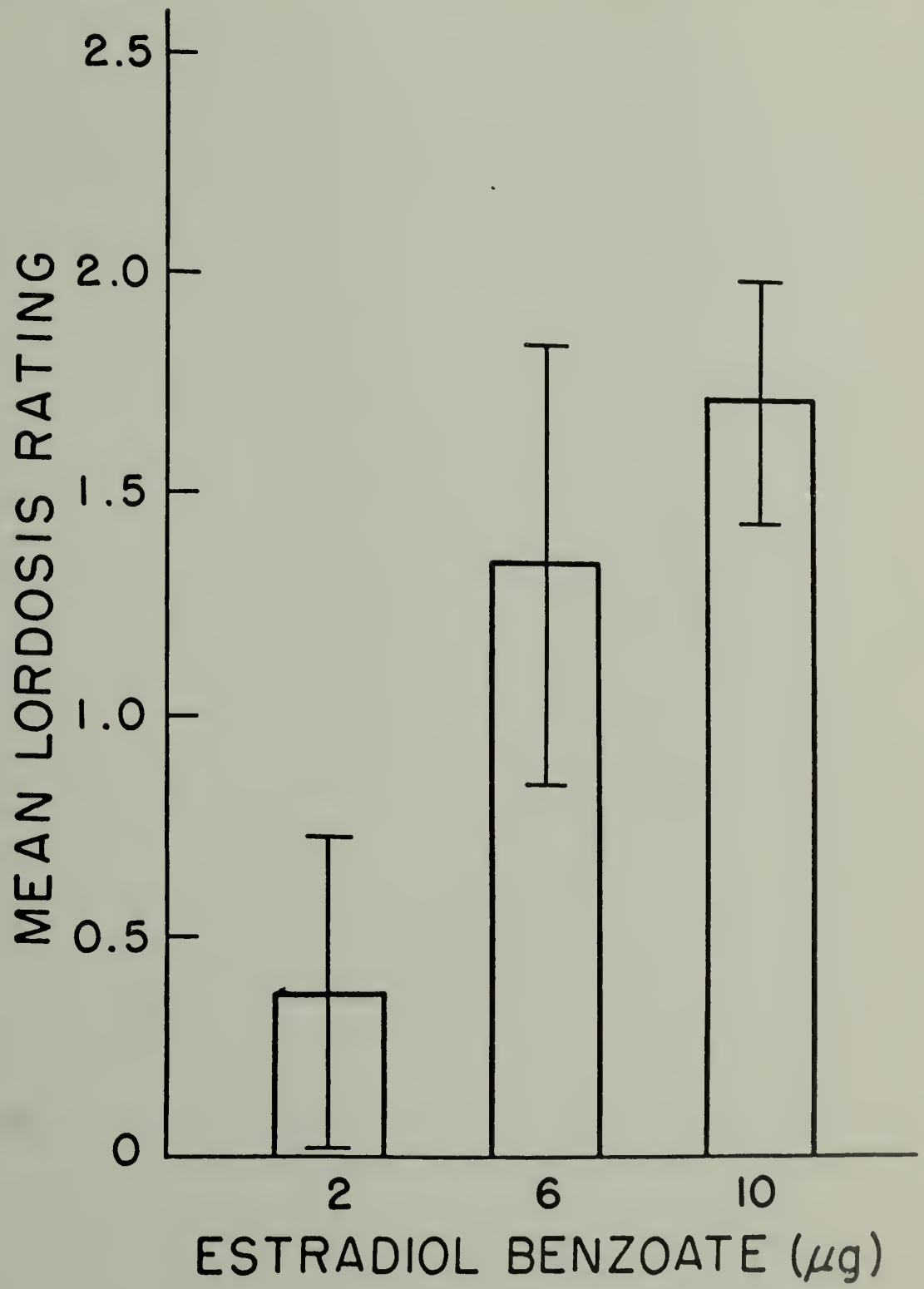
Procedure. Twelve ovariectomized rats were injected with either 6 μg of EB (n=6) or 10 μg of EB (n=6) simultaneously with 5 mg of progesterone. Forty-two hours later, all animals received 0.5 mg of progesterone and were tested for sexual receptivity after 5-7 hours. Data from a group from Experiment 2 that received 2 μg of EB + 5 mg of progesterone (n=6) were included for comparison.

Results and discussion. The 10 μg EB group was significantly different from the 2 μg EB group, $U(6,6) = 4$, $p < .05$, but the 6 μg EB group was not, $U(6,6) = 10$, $p > .20$ (Figure 4).

The results of this experiment demonstrate one more reason for the lack of positive findings on progesterone antagonism of sexual receptivity in rats. Increasing the dose of EB can clearly overcome the inhibition by a particular dose of progesterone (in this case, 5

mg). Some previous research has used 6 μg EB for the induction of sexual receptivity (Zucker, 1967a).

Figure 4. Mean lordosis ratings (± standard error) of ovariectomized rats injected with 5 mg of progesterone (P) + various doses of estradiol benzoate. (Forty-two hours later all groups received 0.5 mg of progesterone followed approximately 6 hours later by testing.)


SIMULTANEOUS EB + 5 mg P
MEAN LORDOSIS RATING
2.5
2.0
1.5
1.0
0.5
0
2
6
10
ESTRADIOL BENZOATE (μg)

EXPERIMENT 5: CONCURRENT INHIBITION - INFLUENCE OF REPEATED PROGESTERONE INJECTIONS

Following a subcutaneous injection of 40 μCi of ^{3}H-progesterone (approximately 0.3 μg), the brains of guinea pigs take up more progesterone and retain it for a longer period than do rat brains. Significant levels of progesterone are retained in neural tissues of guinea pigs in excess of 24 hours after an injection of approximately 0.3 μg; in rats, progesterone is undetectable by 16 hours. As already demonstrated, the dose of progesterone required for concurrent inhibition of sexual receptivity in rats is well in excess of that required in guinea pigs (approximately 2.5 mg of progesterone for 2 μg of EB for rats vs. approximately 0.4 mg of progesterone for 6 μg of EB in guinea pigs; (Wallen et al., 1975; Zucker, 1966). If the basis for less sensitivity in rats is related to the lack of maintenance of significant quantities of progesterone in neural tissues, then the same quantity of progesterone distributed into multiple injections should be more effective than a single injection in inhibiting lordosis. Experiment 5 tests that hypothesis.

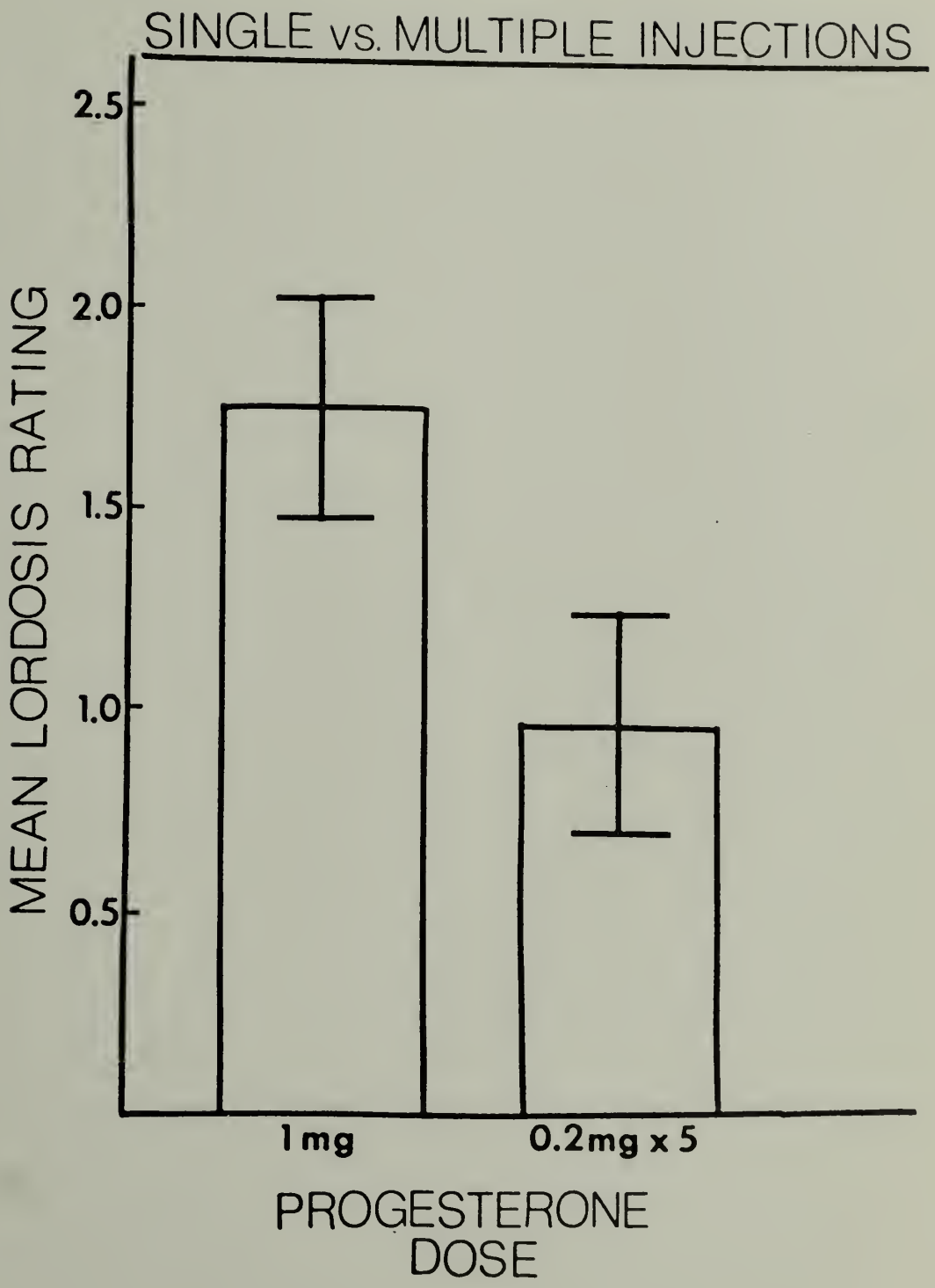
Procedure. Eleven ovariectomized rats were divided into two groups receiving two progesterone treatments in counterbalanced order. One group first received 1 mg of progesterone at the time of the EB (2 μg) injection followed by four oil injections at 4, 8, 12, and 16 hours. The other group first received 0.2 mg of progesterone at each of these five injection times for a total of 1 mg of progesterone. At 42 hours, all rats received 0.5 mg of progesterone followed 5-7 hours later by testing. Eight days later, treatments were reversed so that each animal served as its own control in a within-

subjects desig...

Results and discussion. Treatment with five injections of 0.2 mg of progesterone resulted in significantly lower levels of lordosis than a single injection of 1 mg progesterone, T (11) = 4, $p < .10$, Wilcoxon, (Figure 5).

These results support the hypothesis that the basis of rats' hyposensitivity to progesterone is related to their lack of retention of progesterone in neural tissues. The findings are consistent with the notion that a function of the large single doses of progesterone required to antagonize the induction of sexual behavior in rats might be to maintain neural progesterone concentrations at some particular level.

Figure 5. Mean lordosis ratings (± standard error) of ovariectomized rats injected with 1 mg of progesterone either in one injection simultaneously with 2 µg of estradiol benzoate or in five injections of 0.2 mg each at 4-hour intervals starting at the same time as the initial 2 µg of estradiol benzoate. (Forty-two hours after the estradiol benzoate injection all rats received 0.5 mg of progesterone followed approximately 6 hours later by testing.)


SINGLE vs. MULTIPLE INJECTIONS
MEAN LORDOSIS RATING
2.5
2.0
1.5
1.0
0.5
1 mg
0.2mg x 5
PROGESTERONE DOSE

EXPERIMENT 6: SEQUENTIAL INHIBITION - INFLUENCE OF PROGESTERONE DOSE

It now seems likely that progesterone has some inhibitory influence on sexual receptivity in estrous-cycling rats (Powers & Moreines, 1976) as it does in guinea pigs (Goy, Phoenix & Young, 1966). As discussed, in OVX rats, there are reports of both success (Lisk, 1969; Nadler, 1970) and failure (Zucker, 1967a) to obtain sequential inhibition of sexual receptivity by progesterone. Experiments 1-4 suggest that concurrent inhibition by progesterone is critically dependent on the doses of both progesterone and EB. If concurrent inhibition and sequential inhibition are caused by a common neurochemical event, one would expect similar dose-response relationships for the latter which would explain the previous conflicting observations. That is, sequential inhibition might also require high levels of progesterone with low levels of estradiol. In Experiment 6 I obtain a dose-response relationship for sequential inhibition by progesterone using a low priming dose of EB. Tests occurred 1 and 2 days after EB injections to ensure that the neural substrate would remain "conditioned" by estradiol for the second test. The first test (30 hours) is a test of the facilitatory influence of the particular dose of progesterone and the second (54 hours) is a test for a subsequent inhibitory influence (sequential inhibition).

Procedure. Twenty-nine ovariectomized rats were divided into five groups. All rats received 2 µg of EB at 0 hours. At 24 hours they received either oil (n=6), 0.1 mg (n=6), 0.5 mg (n=5), 1 mg

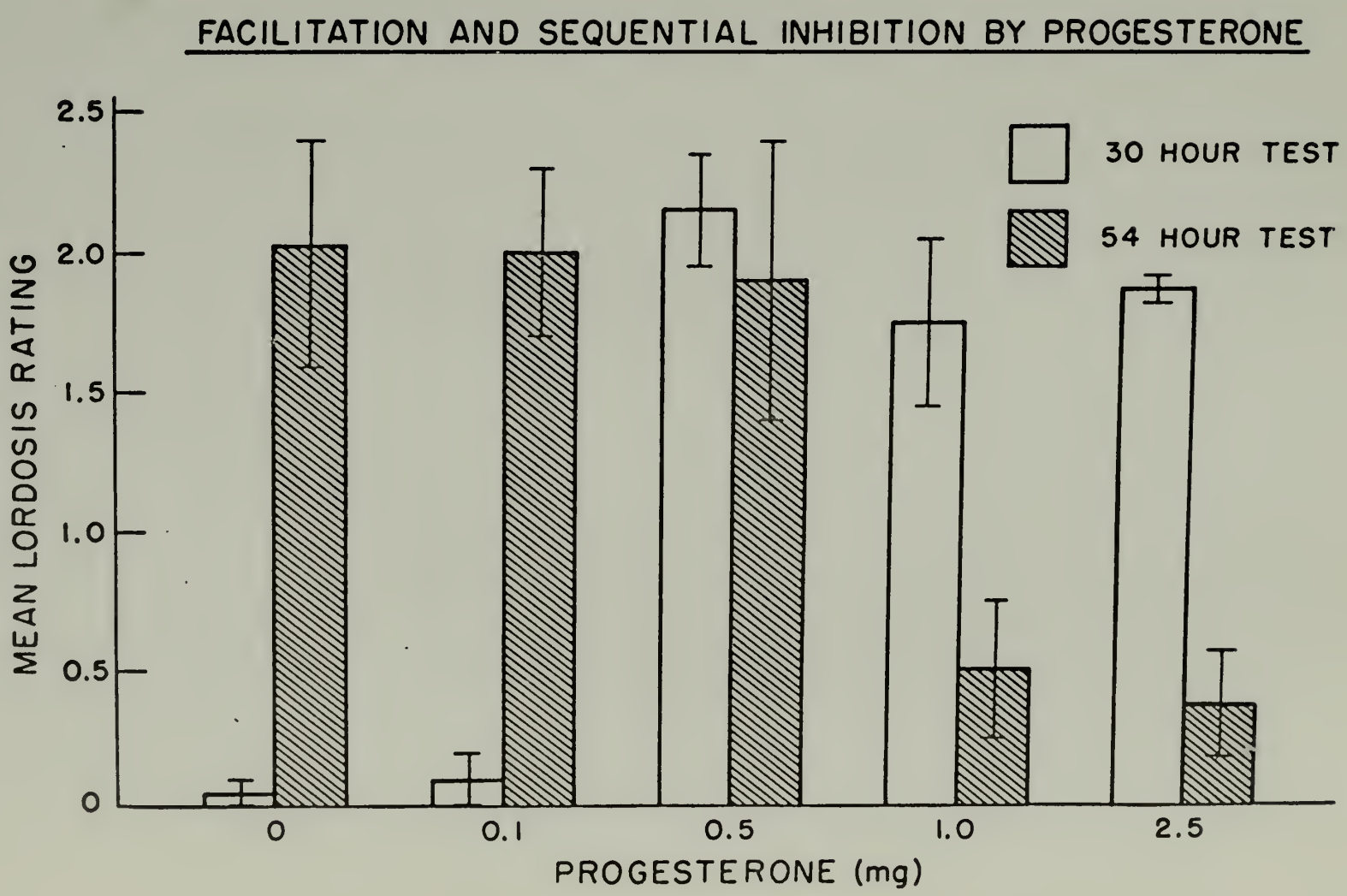
(n=7) or 2.5 mg of progesterone (n=5). Five to 7 hours after progesterone injections (30 hours), rats were tested for sexual receptivity. At 48 hours, all animals were given 0.5 mg of progesterone and tested 5-7 hours later (54 hours).

Results and discussion. Either 0.5 mg, 1 mg or 2.5 mg of progesterone facilitated lordosis at 30 hours, but 0.1 mg of progesterone did not (Figure 6).

One mg of progesterone, $U(6,7) = 4$, $p < .05$, and 2.5 mg of progesterone, $U(4,6) = 2$, $p < .05$, induced a refractoriness to the second progesterone injection, whereas oil, 0.1 mg or 0.5 mg of progesterone did not, (Figure 6). A lordosis rating could not be obtained for the second test of one rat in the 2.5 mg group because it would not allow the males to mount.

The results of this experiment indicated that under conditions of relatively high levels of progesterone, sequential inhibition can be observed in rats. It should be emphasized that although 0.5 mg of progesterone facilitates sexual behavior at 30 hours, it does not subsequently inhibit receptivity measured at 54 hours. Thus, the facilitatory and antagonistic influences of progesterone on sexual receptivity are dissociable in rats. Unlike the situation in guinea pigs (Morin & Feder, 1974) a higher dose of progesterone is required to inhibit sequentially than to facilitate.

Figure 6. Mean lordosis ratings (± standard error) of ovariectomized rats injected with oil or various doses of progesterone 24 hours after 2 μg of estradiol benzoate. Rats were tested 5-7 hours later (30 hour test), administered 0.5 mg of progesterone at 48 hours, and tested again 5-7 hours later (54 hour test).


FACILITATION AND SEQUENTIAL INHIBITION BY PROGESTERONE
MEAN LORDOSIS RATING
2.5
2.0
1.5
1.0
0.5
0
30 HOUR TEST
54 HOUR TEST
0
0.1
0.5
1.0
2.5
PROGESTERONE (mg)

EXPERIMENT 7: SEQUENTIAL INHIBITION - ARE COPULATORY STIMULI NECESSARY?

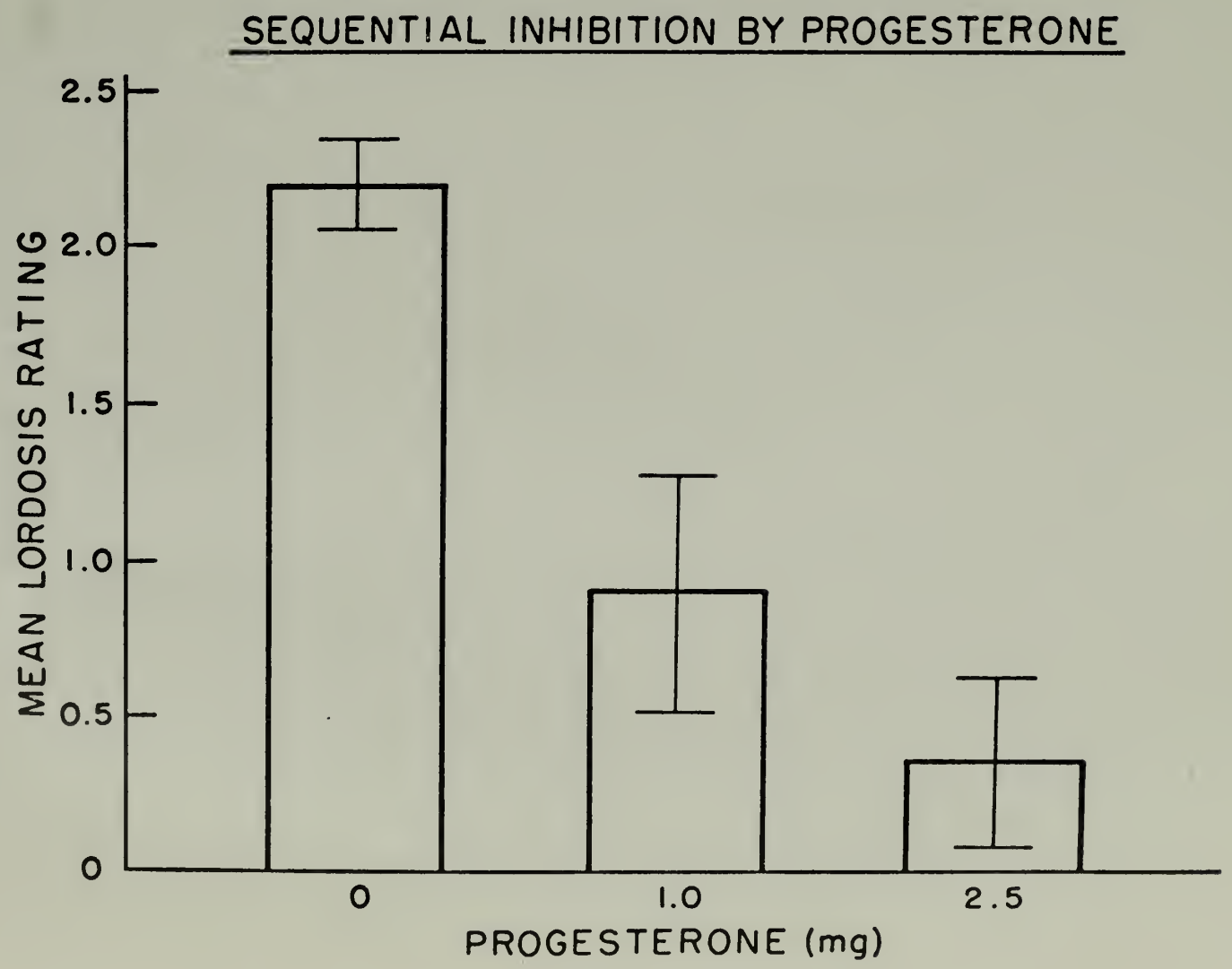
The results of Experiment 6 clearly point out that copulatory stimuli alone are not sufficient to induce the refractoriness to subsequent progesterone because all of the groups that received low doses of progesterone also received copulatory stimuli on the first test, but were not inhibited as measured on the second test. However, since copulatory stimuli can influence lordosis in a repeated testing situation (Hardy & DeBold, 1972), Experiment 7 was performed to determine whether copulatory stimuli are necessary for the sequential inhibition.

Procedure. Nineteen ovariectomized rats were divided into three groups receiving various progesterone treatments 24 hours after a 2 μg EB injection. Either oil (n=5), 1 mg (n=9) or 2.5 mg (n=5) of progesterone was administered at 24 hours, but animals were not tested at 30 hours as they were in Experiment 1. At 48 hours all animals received 0.5 mg of progesterone and were tested 5-7 hours later for lordosis (54 hours).

Results and discussion. Both 1 mg of progesterone $U(5,9) = 7$, $p < .05$ and 2.5 mg of progesterone, $U(5,5) = 0$, $p < .01$, at 24 hours resulted in a decrease in lordosis when tested at 54 hours (Figure 7).

The results of the last two experiments indicate that under conditions of a relatively low dose of EB (2 μg), progesterone can sequentially inhibit the subsequent display of receptive behavior. It is clear that copulatory stimuli are neither necessary (Experiment 7) nor sufficient (Experiment 6) for this inhibition.

Figure 7. Mean lordosis ratings (± standard error) of ovariectomized rats injected with oil, 1 or 2.5 mg of progesterone 24 hours after 2 µg of estradiol benzoate. (Rats were injected with 0.5 mg of progesterone at 48 hours and tested 5-7 hours later.)


SEQUENTIAL INHIBITION BY PROGESTERONE
MEAN LORDOSIS RATING
2.5
2.0
1.5
1.0
0.5
0
0
1.0
2.5
PROGESTERONE (mg)

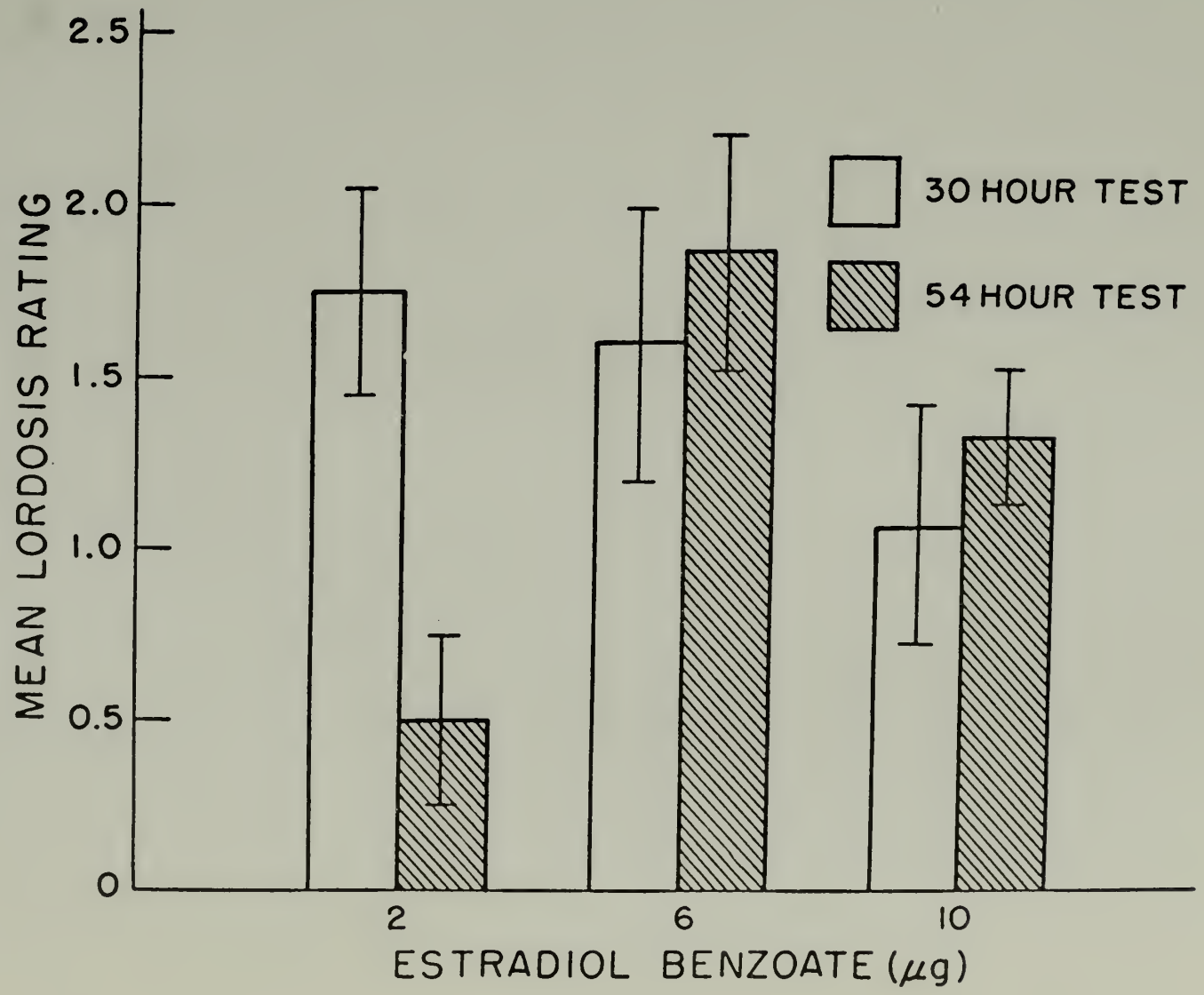
EXPERIMENT 8: SEQUENTIAL INHIBITION - INFLUENCE OF ESTRADIOL BENZOATE DOSE

In Experiment 4 it was shown that concurrent inhibition is critically dependent on the dose of EB administered. In Experiment 8 the effects of various doses of EB on sequential inhibition are compared.

Procedure. Ovariectomized rats were divided into two treatment groups receiving either 6 µg of EB (n=6) or 10 µg of EB (n=6) at 0 hours. At 24 hours all rats were injected with 1 mg of progesterone and tested 5-7 hours later (30 hours) for sexual receptivity. At 48 hours, all animals received a 0.5 mg progesterone injection and were tested 5-7 hours later for sequential inhibition by the first progesterone injection. Data from a group which received 2 µg of EB at 0 hours (Experiment 6) are included for comparison.

Results and discussion. Both the 6 µg EB group, $U\ (6,6) = 5$, $p < .05$, and the 10 µg EB group $U\ (6,7) = 4.5$, $p < .05$, were significantly different from the 2 µg EB group, (Figure 8). These results clearly demonstrate that with a sequential inhibition procedure, increasing doses of EB can offset the antagonism of a particular dose of progesterone just as with concurrent inhibition.

Figure 8. Mean lordosis ratings (± standard error) of ovariectomized rats injected with 1 mg of progesterone 24 hours after 2 µg, 6 µg, or 10 µg of estradiol benzoate. Rats were tested 5-7 hours later (30 hour test), administered 0.5 mg of progesterone at 48 hours and tested again 5-7 hour later (54 hour test).


2.5
2.0
1.5
1.0
0.5
0
MEAN LORDOSIS RATING
30 HOUR TEST
54 HOUR TEST
2
6
10
ESTRADIOL BENZOATE (μg)

EXPERIMENT 9: CONCURRENT INHIBITION - 30 HOUR TEST

In Experiments 6 and 7, 1 mg of progesterone was successful in antagonizing sexual behavior when tested 30 hours later. In Experiment 2 which used a concurrent inhibition procedure, 2.5 mg of progesterone was necessary to inhibit 2 µg of EB. There are several obvious differences between these procedures: 1) In the sequential inhibition procedure, sexual behavior was tested 30 hours after progesterone; in the concurrent procedure, it was tested 48 hours after the inhibitory progesterone injection. 2) Although plasma estradiol levels were not measured in these experiments, in the period after the sequential progesterone injection, estradiol levels were probably lower than after the concurrent progesterone injection (Cheng & Johnson, 1974; Tapper, Greig & Brown-Grant, 1974). 3) At the time of the sequential injection, estradiol had been present in neural tissues for 24 hours; at the time of the concurrent injection, it was not yet present.

The purpose of Experiment 9 was to determine whether 1 mg of progesterone induces a transient inhibition that can be observed when tested 30 hours after the injection but not after 48 hours. This would, perhaps, account for the dose differences in sequential and concurrent inhibition. Steroids were injected in a concurrent procedure and testing occurred at 30 hours rather than at 48 hours as in Experiment 2.

Procedure. Twenty-eight ovariectomized rats were injected with 2 µg of EB at 0 hours and received either an injection of 1 mg of progesterone (n=14) or the oil vehicle (n=14) simultaneously. At 24

hours, all rats received a 0.5 mg progesterone injection and were tested 5-7 hours later for sexual behavior.

Results and discussion. The group which received progesterone concurrently with EB displayed a mean lordosis rating of 1.33 ± 0.23; the oil control exhibited a mean lordosis rating of 1.69 ± 0.20. This difference is not statistically significant, and the difference is actually slightly less than that for rats tested at 48 hours (Experiment 2). Experiment 9 was unsuccessful in demonstrating a transient inhibition with 1 mg of progesterone in a concurrent inhibition procedure when testing occurred at 30 hours. However, in the period after the concurrent injection, estradiol levels were probably considerably higher than after the sequential injections (Experiment 6). Since Experiments 4 and 8 have already demonstrated the importance of EB dose in both concurrent and sequential inhibition, it is perhaps not surprising that this dose of progesterone did not result in inhibition.

DISCUSSION

These studies offer unequivocal evidence that progesterone antagonizes estradiol's induction of sexual behavior in ovariectomized rats using both chronic and single injections. Although there are clearly differences between rats and guinea pigs in the dose of progesterone required for concurrent inhibition, the phenomenon seems to be as robust in rats as in guinea pigs.

There are at least two obvious reasons for the previous negative results of experiments on concurrent inhibition by progesterone in ovariectomized rats (Zucker, 1967a). First, as Experiment 2 demonstrated, with the dose of EB held constant at 2 µg, approximately 2.5 mg of progesterone is required for concurrent inhibition when tested at 48 hours. In addition, the inhibition is dependent on the EB dose as well; six or 10 µg EB can offset the antagonistic influence of 5 mg progesterone. In earlier work (Zucker, 1967a) only 1 mg of progesterone with 6 µg of EB was used.

Perhaps the basis for the species difference in sensitivity to progesterone is the difference in neural retention between rats and guinea pigs. Progesterone is taken up into guinea pig brains in greater concentrations than in rat brains, and it is retained there for a longer period of time (Wade et al., 1973). Experiment 5 was designed to prevent progesterone levels from rapidly waning. One mg progesterone, which does not significantly inhibit the induction of lordosis when administered concurrently with 2 µg of EB, was distributed into 5 injections spaced at 4-hour intervals. This treatment resulted in a 45% decrease in lordosis ratings when tested at

48 hours, confirming the prediction based on the species difference in neural progesterone retention. It suggests that a function of the higher dose of progesterone necessary for concurrent inhibition in rats is to maintain progesterone levels at some critical value. These results also suggest that the initial levels of progesterone are less important for the inhibition than is the maintenance dose. That is, the low initial dose of progesterone (0.2 mg) resulted in greater inhibition than the high dose (1 mg) when the low levels were maintained for 16 hours by frequent injections.

Sequential inhibition is also as robust a phenomenon in rats as in guinea pigs. Just as with concurrent inhibition, species dissimilarities seem to be dose-dependent. Rats seem to be less sensitive to progesterone's sequential inhibitory influence than guinea pigs.

It is clear that the sequential inhibition measured at 54 hours is not a result of copulatory stimuli obtained on the 30-hour test. Copulatory stimuli are obviously not sufficient for the refractoriness, because in Experiment 6 all rats received copulatory stimuli but the sexual behavior of only the 1 mg and 2.5 mg progesterone groups was inhibited. In fact, the 0.5 mg progesterone group showed high levels of receptivity on the30-hour test but was not inhibited when tested at 54 hours. Also, the results of Experiment 7 indicate that copulatory stimuli are not necessary for the inhibition; high doses of progesterone inhibited sexual behavior tested at 54 hours even in the absence of the 30-hour test. Thus, this sequential inhibition is clearly the result of high doses of progesterone.

Increasing the EB priming dose offset the sequential inhibition resulting from a particular dose of progesterone with concurrent inhibition. Thus this inhibition is dose-dependent, both on progesterone and on estradiol. These results help to explain the previous conflicting reports of progesterone-induced refractory period in ovariectomized rats. Previously, Lisk (1969) used hypothalamic implants of estradiol and Nadler (1970) used two daily injections of 1 µg EB. Both of these techniques resulted in moderate inhibition by progesterone. Zucker (1967a), on the other hand, used a large (6µg) EB priming dose which did not result in sequential inhibition. The results of Experiment 8 are consistent with the previous reports and help to explain the basis for the inconsistencies.

The results of these experiments also suggest that concurrent inhibition may not be biochemically distinct from sequential inhibition. Although sequential inhibition has been defined as inhibition that occurs after estrogen conditioning is complete (Powers & Moreines, 1976), there is, as yet no reason to assume that in ovariectomized rats, these are anything but procedural distinctions. Both classes of inhibition are dose-dependent on progesterone and estradiol. The difference seems to be dose-dependent with more progesterone required for concurrent than sequential inhibition. This, as already pointed out, is likely due to differences in plasma levels of estradiol subsequent to the progesterone injection.

In a concurrent inhibition paradigm, a dose of approximately 2.5 mg progesterone is necessary to inhibit the induction of sexual receptivity by 2 µg EB. Experiment 6 demonstrates that as little as 1 mg progesterone injected at 24 hours can inhibit lordosis sequen-

tially when tested at 54 hours. In the concurrent procedure, 48 hours elapse between the inhibitory progesterone injection and testing; in the sequential procedure, only 30 hours elapse. Since Zucker (1966) had shown in guinea pigs that both concurrent and sequential inhibition are transient, I attempted to determine if this dose difference is due merely to the longer delay between the inhibitory progesterone injection and testing in the concurrent procedure. Experiment 9 tested for concurrent inhibition by 1 mg of progesterone at 30 hours rather than at 48 hours. This dose of progesterone actually inhibited slightly less than when tested at 48 hours. In the period following the sequential progesterone injection (24 hours) the plasma levels of estradiol were presumably lower than after the concurrent progesterone injection (0 hours; Cheng & Johnson, 1975; Tapper et al., 1974). We know that estradiol levels are critically involved since increasing doses of EB can offset the inhibition by a particular dose of progesterone in either the concurrent or sequential inhibition procedure.

The physiological role of progesterone's concurrent inhibition in rats is not known. Concurrent inhibition may occur during the estrous cycle of the rat (Barfield & Lisk, 1974; but cf. Powers & Moreines, 1976). However, it has been suggested that progesterone's inhibitory influences during the estrous cycle should be sequential, not concurrent, simply because there are minimal levels of progesterone in the circulation during estrogen priming in the normal estrous cycle (Powers & Moreines, 1976). Although it is instructive to distinguish between procedures that are designed to investigate sequential inhibition, the two classes may not be biochemically distinct.

SECTION II: SYNTHETIC ESTROGEN ANTAGONIST AS A MODEL OF INHIBITION

Although progesterone potentiates estradiol's effects on nest-building in ovariectomized mice (Lisk, 1971) and sexual behavior in rodents (Feder & Marrone, 1977; Morin, 1977), progesterone's influence on other behaviors is limited to antagonism of estradiol's effects. Progesterone inhibits estradiol's induction of sexual behavior (Feder & Marrone, 1977; Morin, 1977), running-wheel activity (Rodier, 1971; Wade, 1976) and maternal behavior (Siegal & Rosenblatt, 1975), and estradiol's suppression of eating behavior (Wade, 1975, 1976).

Recently, evidence has accumulated that the cell nucleus is the site of action for estradiol's effects on behavior (McEwen, 1975; McEwen, Denef, Gerlach & Plapinger, 1974). After an injection of ^{3}H-estradiol the greatest concentration of the radioactivity which accumulates in brain cells is found in purified cell nuclei (Zigmond, 1975; Zigmond & McEwen, 1970). Inhibitors of transcription (Ho, Quadagno, Cooke & Gorski, 1973; Hough, Ho, Cooke & Quadagno, 1974; Quadagno, Shryne & Gorski, 1971; Whalen, Gorzalka, DeBold, Quadagno, Ho & Hough, 1974) or translation (Quadagno & Ho, 1975) reversibly inhibit the induction of sexual behavior when implanted into the preoptic area in temporal proximity to an injection of estradiol.

Compelling evidence that the cell nucleus is a site of action for estradiol derives from work with synthetic estrogen antagonists. These are compounds that prevent the full response of a tissue to an estrogen (Clark, Anderson & Peck, 1973; Katzenellenbogen & Ferguson, 1975). In the rat brain, these compounds deplete cytoplasmic

estrogen receptors (Whalen, Martin & Olsen, 1975), compete with estradiol for estrogen receptors (Whalen et al., 1975), delay replenishment of estrogen receptors (Whalen et al., 1975), inhibit the uptake of ^{3}H-estradiol into brain cell nuclei (Chazal, Faudon, Gogan & Rotsztejn, 1975; Landau, 1977; Luine & McEwen, 1977; Luttge, Gray & Hughes, 1976; Roy & Wade, 1977), and displace tritium, presumably bound ^{3}H-estradiol from cell nuclei (Landau, 1977; Roy & Wade, 1977). Synthetic estrogen antagonists that are effective in inhibiting the induction of sexual behavior by estradiol followed by progesterone also decrease the uptake and retention of radioactively labeled estradiol by cell nuclei of neural target tissues (Landau, 1976, 1977; Roy & Wade, 1977) offering further support for a nuclear site of action.

One possible biochemical mechanism that might account for the inhibitory effects of progesterone could be a decrease in cell nuclear binding of ^{3}H-estradiol in neural estrogen target tissues. Although, using autoradiography, Anderson and Greenwald (1968) have reported a decrease in estrogen uptake in hypothalamic cells, progesterone does not compete with estradiol for estrogen receptors in brain or pituitary in vitro (Chader & Villee, 1971; Davies, Siu, Naftolin & Ryan, 1975; Eisenfeld, 1970; Vertes & King, 1973). Progesterone also does not inhibit the rate of formation of estrogen-receptor complexes in pituitary (Korach & Muldoon, 1975), nor inhibit the replenishment of estrogen receptors in the hypothalamus or preoptic area (DeBold, Martin & Whalen, 1976; Pavlik & Coulson, 1976) as in the uterus (Hsueh, Peck & Clark, 1975, 1976).

The synthetic estrogen antagonist CI-628, is one antagonist that

inhibits the induction of rat sexual behavior (Arai & Gorski, 1968; Powers, 1975; Whalen & Gorzalka, 1973) and decreases the binding of ^{3}H-estradiol by brain cell nuclei (Chazal et al., 1975; Landau, 1977 Luttge, Gray & Hughes, 1976; Roy & Wade, 1977). In Section II, an attempt is made to alter cell nuclear binding of ^{3}H-estradiol through the use of estrogen antagonists, progesterone and CI-628, using sexual behavior as a model.

EXPERIMENT 10: EFFECTS OF PROGESTERONE ON RETENTION OF ^{3}H-ESTRADIOL BY BRAIN CELL NUCLEI

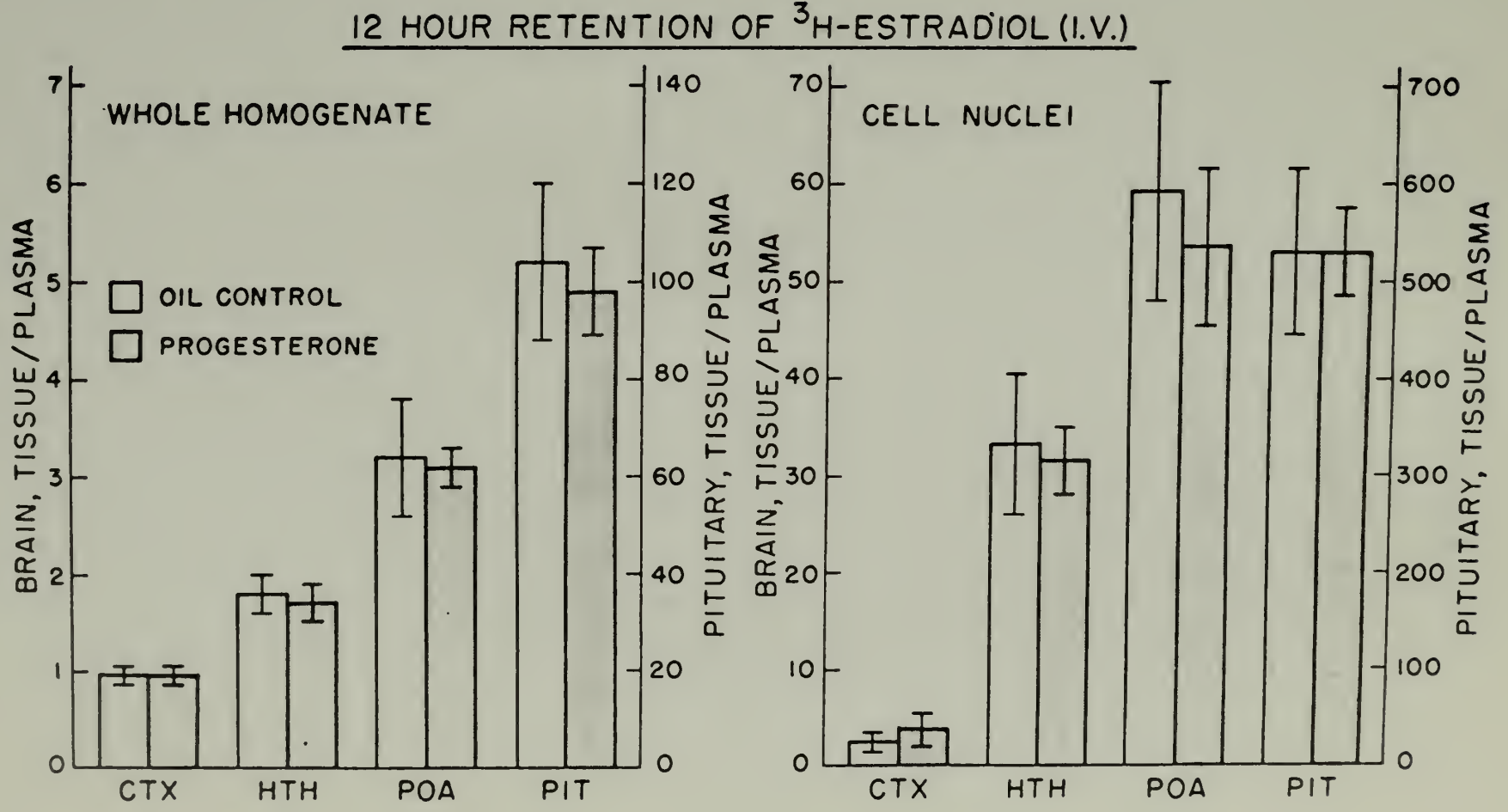
With autoradiographic techniques, it has been reported that uptake of radioactively labeled estradiol into hypothalamic cells can be inhibited by pretreatment with 2.5 mg of progesterone (Anderson & Greenwald, 1969). The present experiment attempted to alter retention of estradiol in cell nuclei of neural areas believed to be involved in sexual behavior in female rats (Lisk, 1969; Powers, 1972; Powers & Valenstein, 1972) by pretreatment with a large quantity of progesterone. The biochemical techniques used are identical to those in which synthetic antiestrogens have been shown to inhibit the uptake and retention of ^{3}H-estradiol in brain cell nuclei (Roy & Wade, 1977).

Procedure. Fourteen female rats ovariectomized at least 2 weeks previously and weighing 250-300 g at the time of sacrifice were used. Six rats received a 10 mg intraperitoneal injection of progesterone (0.2 ml) and eight rats received the sesame oil vehicle, followed 2 hours later by an intravenous injection of 100 μCi (0.3 μg) of 2,4,6,7-^{3}H-estradiol-17β(specific activity: 91.3 Ci/mmole; New England Nuclear, Boston, Mass.) in 20% ethanol-saline. Twelve hours after the ^{3}H-estradiol injection rats were sacrificed as described. The brain was dissected into cerebral cortex (120 mg), preoptic area-septum (60 mg), and hypothalamus (60 mg). The cortex included frontal and parietal cortex without white matter; the preoptic area-septum included the anterior hypothalamus, preoptic area, bed nucleus of the stria terminalis, and septum. The hypothalamus was bounded by the

mammillary bodies, hypothalamic fissures and caudal edge of the optic chiasma, extending dorsally 3 mm. The entire pituitary gland was also taken.

Results. The 10 mg injection of progesterone was without effect on the 12-hour retention of ^{3}H-estradiol, (Figure 9). None of the comparisons of tissue/plasma levels of progesterone vs. oil groups for the whole homogenate or nuclear fractions of the four tissues approached statistical significance. In fact, for all tissues, tissue/plasma ratios are quite similar for the two groups.

Figure 9. Retention of ^{3}H-estradiol in brain areas and pituitary 12 hours after an intravenous injection of 100 μCi ^{3}H-estradiol. Either 10 mg progesterone or oil was injected intraperitoneally 2 hours prior to estradiol. (CTX, cerebral cortex; HTH, hypothalamus; POA, preoptic area-septum; PIT, pituitary gland).


12 HOUR RETENTION OF ^{3}H-ESTRADIOL (I.V.)
WHOLE HOMOGENATE
CELL NUCLEI
OIL CONTROL
PROGESTERONE
BRAIN, TISSUE/PLASMA
PITUITARY, TISSUE/PLASMA
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60
70
0
100
200
300
400
500
600
700
CTX
HTH
POA
PIT

EXPERIMENT 11: EFFECTS OF ESTROGEN ANTAGONISTS ON ^{3}H-ESTRADIOL BINDING

The results of Experiment 10 gave no hint of progesterone inhibition of nuclear retention of ^{3}H-estradiol. However, the ^{3}H-estradiol was administered intravenously and the progesterone intraperitoneally. In an effort to use a procedure with more behavioral relevance, in the next experiment the steroids were administered in a manner that is known to result in behavioral inhibition, that is, subcutaneously (Section I). In addition, to optimize the possibility of observing inhibition if it exists, a very large quantity of progesterone was injected (10^{5}-fold excess). A comparison is also made with inhibition by an injection of CI-628.

Procedure. Fifteen rats ovariectomized approximately two weeks previously and weighing 190-260 g were used. Five rats were injected subcutaneously with 30 mg of progesterone (0.6 ml), five with 4 mg CI-628 (0.6 ml) and five with the sesame oil vehicle (0.6 ml). Within seconds, 100 µCi of ^{3}H-estradiol dissolved in 0.2 ml sesame oil was injected subcutaneously. Due to the large volume of the injections, the progesterone and oil were injected into two separate sites, each consisting of 0.3 ml, and the ^{3}H-estradiol was injected to a third site. Four hours later, animals were sacrificed, tissues dissected, and radioactivity counted as described. In this experiment, the hypothalamus and preoptic area sections were pooled and a sample of midbrain taken (approximately 60 mg), since cannula implants of progesterone in this area affect receptive behavior (Morin & Feder, 1974c; Ross, Claybaugh, Clemens & Gorski, 1971). The midbrain sample

was immediately caudal to the hypothalamus and included the peri-aqueductal region of estradiol-concentrating cells described by Pfaff and Keiner (1973).

Results and discussion. As in the previous experiment, comparisons of the progesterone vs. oil groups for the whole homogenate and nuclear fraction of each tissue revealed that the progesterone treatment was without effect on the uptake of ^{3}H-estradiol, (Table 1). CI-628, however, caused statistically significant inhibition of cell nuclear binding in hypothalamus, (27%) and pituitary gland (88%), but not in cortex or midbrain. CI-628 was effective in the whole homogenates of all areas. These data support the suggestion that progesterone's neural mechanism of antagonism of estradiol's influences is not to inhibit the binding of estradiol by brain cell nuclei.

TABLE I

RADIOACTIVITY[1] IN WHOLE HOMOGENATES AND CELL NUCLEAR FRACTIONS AFTER ^{3}H-ESTRADIOL INJECTION SIMULTANEOUSLY WITH VARIOUS PRETREATMENTS IN OVARIECTOMIZED RATS

	WHOLE HOMOGENATES				NUCLEAR FRACTIONS			
	CEREBRAL CORTEX	HTHPOASEPT[2]	MB[3]	PIT[4]	CEREBRAL CORTEX	HTHPOASEPT	MB	PIT
OIL								
MEAN	8.1	17.9	13.4	410.4	11.2	298.0	89.4	2,064.5
(SEM)	(0.4)	(0.6)	(0.9)	(37.5)	(1.3)	(31.7)	(16.3)	(163.8)
PROGESTERONE								
MEAN	9.3	17.7	16.4	328.8	12.8	252.1	87.2	1,803.7
(SEM)	(0.4)	(0.7)	(1.2)	(38.2)	(1.4)	(21.4)	(16.2)	(181.7)
CI-628								
MEAN	5.6	10.6	8.6	53.3	8.6	218.3	75.4	245.2
(SEM)	(0.4)	(0.4)	(0.6)	(7.7)	(0.7)	(27.4)	(5.5)	(44.0)

[1]Expressed as DPM/mg protein: DPM/ µl plasma

[2]pooled hypothalamus, preoptic area and septum

[3]midbrain [4]pituitary

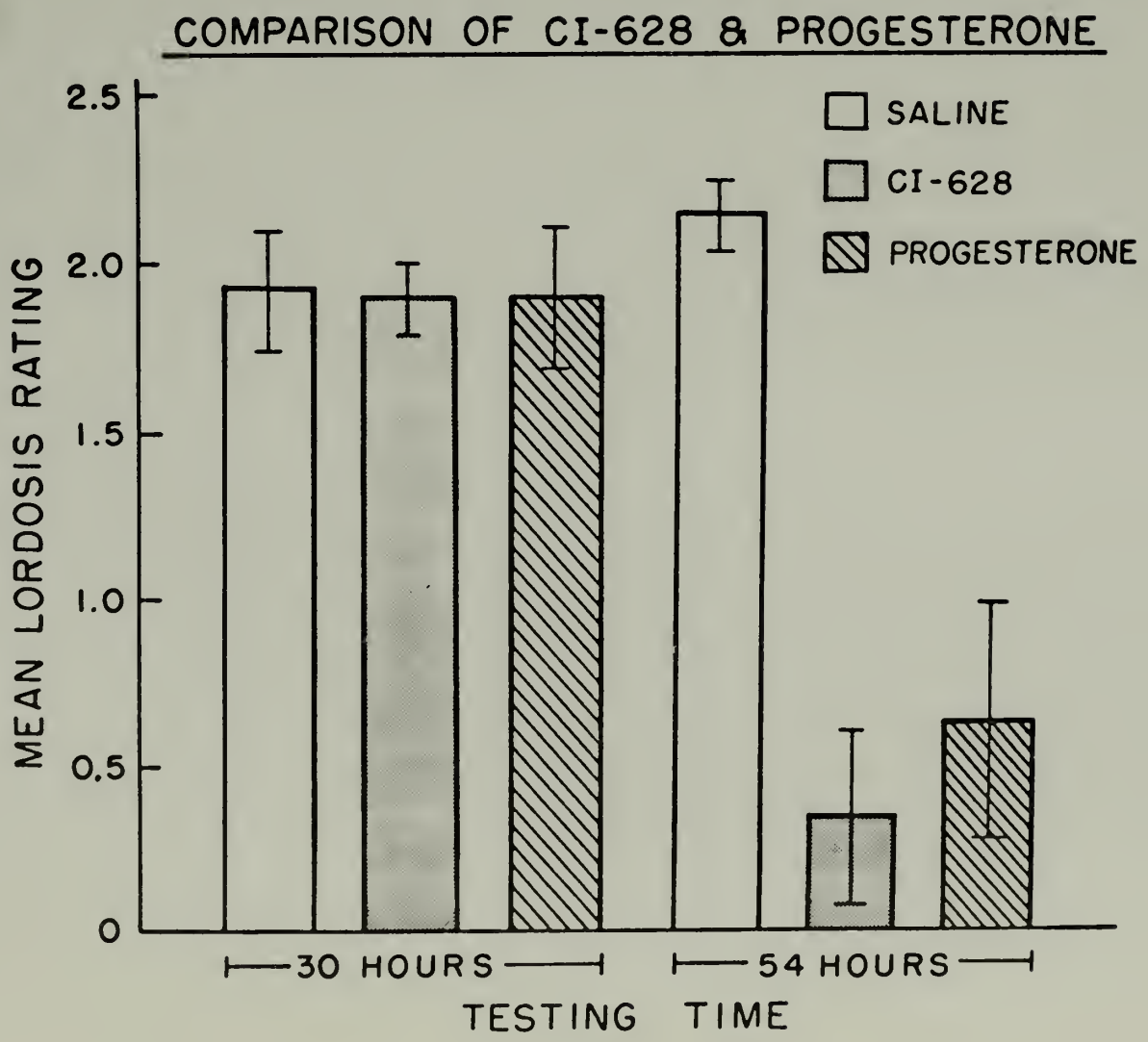
EXPERIMENT 12: SEQUENTIAL INHIBITION BY PROGESTERONE AND CI-628

The synthetic antiestrogen, CI-628 inhibits the uptake and retention of ^{3}H-estradiol in brain cell nuclei (Roy &Wade, 1977; Exp. 11), whereas even unusually large doses of progesterone (10-30 mg) do not (Experiment 11), using identical techniques. Progesterone administered either before or after estrogen conditioning is complete, is capable of inhibiting the subsequent induction of sexual receptivity. The antiestrogen, CI-628 has been shown to significantly inhibit the induction of sexual behavior when injected approximately concurrently with EB (Arai & Gorski, 1968; Landau, 1976; Powers, 1975; Whalen & Gorzalka, 1973). In Experiment 12 the effects of the synthetic estrogen antagonist, CI-628, and the natural estrogen antagonist, progesterone, were compared by administering each in a sequential inhibition procedure. Each compound was administered after rats had been screened for lordosis at 30 hours.

Procedure. Ovariectomized rats were injected with 2 µg of EB followed 24 hours later by 0.5 mg of progesterone and were tested for lordosis at 29-31 hours. At this time, only rats in heat were selected and distributed into three closely matched groups, (Figure 11). One group (n=5) received 4 mg of CI-628 intraperitoneally in 0.4 ml saline. The progesterone group (n=9) received an additional 2 mg of progesterone subcutaneously and the control group (n=5) received 0.5 mg of progesterone and were tested for lordosis 5-7 hours later (54 hours). Since some animals showed a high frequency of rejection of the males, lordosis ratings are based upon five mounts for each rat.

Results. Two mg of progesterone after the first test inhibited the subsequent induction of sexual receptivity by a 0.5 mg progesterone injection at 48 hours, $U\ (5,9) = 7$, $p < .05$. The CI-628 also antagonized the subsequent facilitation by progesterone at 48 hours, $U\ (5,5) = 0$, $p < .01$ (Figure 10).

Figure 10. Mean lordosis ratings (± standard error) of ovariectomized rats injected with 2 μg of estradiol benzoate (0 hours), 0.5 mg of progesterone (24 hours), tested for sexual receptivity at approximately 30 hours and injected with either saline, 4 mg of CI-628 or 2 mg of progesterone after behavioral testing. (All rats received 0.5 mg of progesterone at 48 hours and were retested 5-7 hours later.)


COMPARISON OF CI-628 & PROGESTERONE
SALINE
CI-628
PROGESTERONE
MEAN LORDOSIS RATING
2.5
2.0
1.5
1.0
0.5
0
30 HOURS
54 HOURS
TESTING TIME

EXPERIMENT 13: RAPID ANTAGONISM OF SEXUAL BEHAVIOR BY ESTROGEN ANTAGONISTS

The results of Experiment 12 demonstrate that CI-628 can inhibit sequentially just as progesterone. Thus both synthetic and natural antiestrogens can antagonize receptive behavior not only in a concurrent inhibition paradigm (Powers, 1975) but also after estrogen conditioning has been completed. Feder and Morin (1974) have shown that in guinea pigs, the estrogen antagonist, MER-25 is capable of blocking the induction of sexual receptivity when administered at about the time of the facilitatory progesterone injection. In Experiment 12 the behavioral effects of the two classes of estrogen antagonist were dissociated by administering the CI-628 or a large dose of progesterone near the time of testing. To optimize the possibility of detecting inhibition, a dose of 1 µg of EB was used in this experiment. Since preliminary data had shown that there is no effect on lordosis as long as 10 hours after treatment with CI-628, testing occurred at 13-14 hours after treatment.

Procedure. Fifty ovariectomized rats were divided into three groups receiving various treatments 24 hours after a 1 µg of EB injection. Rats were injected with either 0.5 mg of progesterone + 0.4 ml of saline (n=18), 5 mg of progesterone + 0.4 ml of saline (n=11) or 0.5 mg of progesterone + 4 mg of CI-628 (n=21) and tested 13-14 hours later for lordosis. Progesterone was injected subcutaneously; the CI-628 (dissolved in 0.4 ml of saline) and saline were injected intraperitoneally.

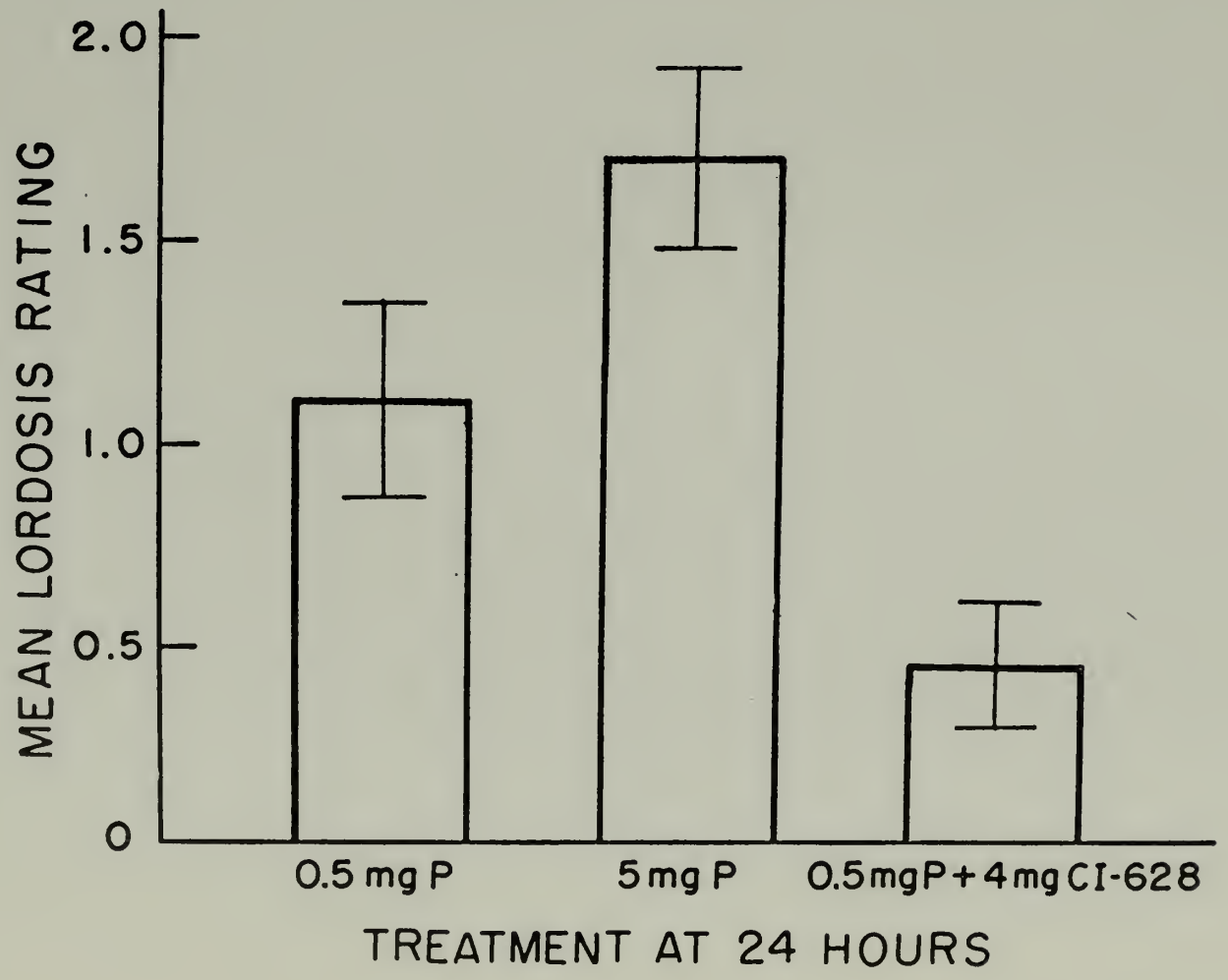
Results and discussion. Addition of the synthetic estrogen

antagonist inhibited sexual receptivity tested 13-14 hours later when compared with the group that received only 0.5 mg of progesterone, $U\ (18,21) = 122.5$, $p < .05$ (Figure 11). The group that received 5 mg of progesterone actually displayed higher levels of lordosis than the group that received 0.5 mg of progesterone.

These results indicate that administration of an estrogen antagonist can decrease receptivity when given at the time of the facilitatory progesterone injection and tested 13-14 hour later, even a high dose of progesterone at this time cannot. These results again suggest a different neural mechanism of antagonism for progesterone than the synthetic estrogen antagonist, CI-628.

Figure 11. Mean lordosis ratings (± standard error) of ovariectomized rats injected with either 0.5 mg of progesterone, 5 mg of progesterone or 0.5 mg of progesterone + 4 mg of CI-628, 24 hours after a 1 µg of estradiol benzoate injection. (Testing occurred 13-14 hours later.)

COMPARISON OF PROGESTERONE AND CI-628



DISCUSSION

Roy and Wade (1977) have reported that synthetic estrogen antagonists that inhibit the induction of sexual behavior also decrease the uptake and retention of ^{3}H-estradiol in brain cell nuclei. The results of Experiment 10 demonstrate that there is no decrease in retention 12 hours after ^{3}H-estradiol injections in rats pretreated with 10 mg of progesterone. This stands in contrast to results using synthetic antagonists. Experiment 11 was performed to examine a more behaviorally-relevant mode of administration of the steroids. An unusually large dose (30 mg) of progesterone or 4 mg CI-628 was administered subcutaneously and concurrently with a low dose of ^{3}H-estradiol in sesame oil. Although CI-628 inhibited nuclear binding in hypothalamus and pituitary, none of the progesterone vs. oil comparisons of 4 hour uptake approached statistical significance. Marrone and Feder (1977) have also failed to inhibit brain ^{3}H-estradiol uptake in guinea pigs with progesterone pretreatment. The lack of striking inhibition by CI-628 is likely referrable to its slow action when administered subcutaneously (Landau, 1977).

Experiments 12 and 13 were designed to contrast behaviorally the inhibition of sexual receptivity by progesterone and the synthetic antiestrogen, CI-628. A potent antagonist of estradiol in the induction of sexual receptivity (Arai & Gorski, 1968; Powers, 1975; Roy & Wade, 1977; Whalen & Gorzalka, 1973), CI-628 also inhibits the uptake (Chazal et al., 1975; Landau, 1977; Luine & McEwen, 1977; Luttge et al., 1976; Roy & Wade, 1977) of ^{3}H-estradiol into cell nuclei (Roy & Wade, 1977). Since large doses of the natural antiestrogen, progesterone, did not interfere with the uptake

or retention of ^{3}H-estradiol in brain cell nuclei with the same procedures that were successful with CI-628 (Roy & Wade, 1977), an attempt was made to dissociate the behavioral effects of the antagonists as well. In Experiment 12, CI-628 or progesterone was administered after a test for sexual receptivity at 30 hours. Both CI-628 and progesterone, when administered at 30 hours, inhibited sexual receptivity tested at 54 hours. In Experiment 13 the two classes of antiestrogen were dissociated behaviorally by administering a high dose of progesterone or CI-628 13-14 hours prior to testing. In this case only the synthetic antiestrogen inhibited the induction of sexual receptivity. Thus, the antiestrogenic influences of progesterone seem to be dissociable from those of the synthetic antiestrogens on the behavioral level as well as on the biochemical level.

The results of Experiment 13 are relevant to an interpretation of estradiol's role in the induction of sexual receptivity. Some reports (Bullock, 1970; McEwen, Pfaff, Chaptal & Luine, 1975) have suggested that estradiol has only a triggering function in the induction of sexual receptivity. That is, it perhaps enters the cell nucleus (McEwen, 1976; McEwen et al., 1975) and stimulates RNA synthesis (Quadagno, Shryne & Gorski, 1971, Terkel, Shryne & Gorski, 1973; Whalen et al., 1974). They have suggested that estradiol need not be retained for the entire duration of estrogen conditioning. In guinea pigs, howerver, the antiestrogen MER-25 is capable of inhibiting sexual behavior when administered at about the time of the progesterone injection (Feder & Morin, 1974). In rats, Whalen and Gorzalka (1973) have demonstrated inhibition when

CI-628 was administered at various intervals following the estrogen injection. Experiment 13, using a dose of CI-628 that is capable of displacing bound ^{3}H-estradiol from hypothalamic cell nuclei (Roy & Wade, 1977), demonstrated that CI-628 injected at 13-14 hours prior to testing can inhibit sexual behavior. Landau (1977) has also demonstrated that CI-628 injected 21 hours after ^{3}H-estradiol benzoate results in diminished nuclear radioactivity 3 hours later. These results offer support for the interpretation that conditioning is an ongoing process which requires sustained estrogen presence (Feder & Morin, 1974; Feder & Silver, 1974).

SECTION III: PROGESTERONE'S MECHANISM OF ACTION: USE OF A SYNTHETIC PROGESTIN

Early experiments that investigated a nuclear site of action for progesterone or binding of progesterone to cytoplasmic receptors in the mammalian uterus were hampered by the technical limitations inherent in studying a relatively weak interaction of a hormone with its receptor. Along with other recent technical advances, the steroid 17α, 21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R 5020) has been synthesized. This synthetic progestin binds specifically to progestin receptors with high affinity and dissociates less rapidly than progesterone itself (Philibert & Raynaud, 1973; 1974). In uterine cytosol it binds to receptors with an association constant 2-5 times that of progesterone. It binds to the same number of sites as progesterone supporting the notion that it binds only to progestin receptors (Philibert & Raynaud, 1973; 1974; Walters & Clark, 1976). R 5020 is physiologically quite active, 200-300 times as active as progesterone in standard uterine bioassays for progestins (Philibert & Raynaud, 1973), a fact consistent with its higher affinity for progestin receptors. The unique binding characteristics of R 5020 have proven useful in characterizing the binding of progestins to cytoplasmic receptors in tumorous and reproductive tissues of various species (McGuire, Raynaud & Baulieu, 1977).

On the basis of experiments which have investigated binding-activity relationships of steroid hormones (Raynaud, 1977), it can be inferred that if binding is involved in behavioral responses, then a progestin that binds to progestin receptors with higher affinity

than progesterone should be more active in affecting sexual behavior. The activity of R 5020 in facilitating and inhibiting sexual behavior is tested in Experiments 14 and 15. In the final two experiments, nuclear binding of ^{3}H-R 5020 is investigated and contrasted with that of ^{3}H-progesterone.

EXPERIMENT 14: FACILITATION AND SEQUENTIAL INHIBITION BY R 5020 - COMPARISON WITH PROGESTERONE

The purpose of Experiment 14 is to determine the relative effectiveness of R 5020 compared with progesterone in the facilitation and sequential inhibition of female sexual behavior.

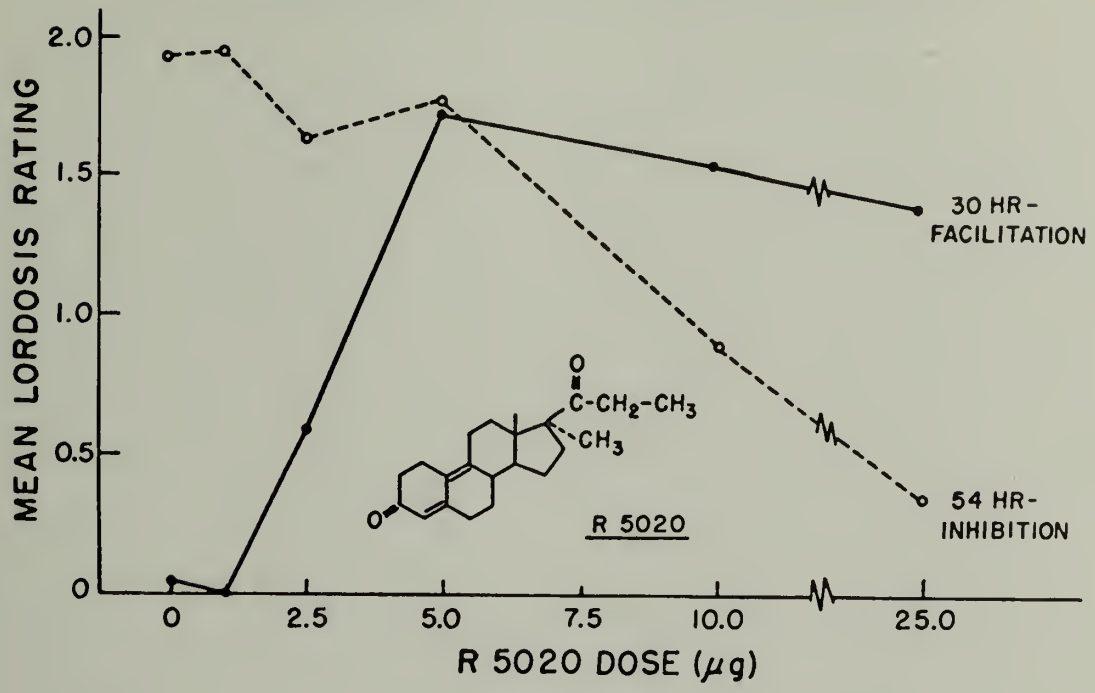
Procedure. Ninety-two ovariectomized rats were divided into ten groups. All rats received 2 μg of EB at 0 hours. At 24 hours, they received either oil (n=13), 1 μg of R 5020 (n=5), 2.5 μg of R 5020 (n=10), 5 μg of R 5020 (n=11), 10 μg of R 5020 (n=8), 25 μg of R 5020 (n=10), 100 μg of progesterone (n=10), 250 μg of progesterone (n=8), 500 μg of progesterone (n=10), or 1,000 μg of progesterone (n=8). Five to seven hours later (30 hours), rats were tested for sexual receptivity. At 48 hours, all rats were administered 0.5 mg of progesterone and tested five to seven hours later (54 hours).

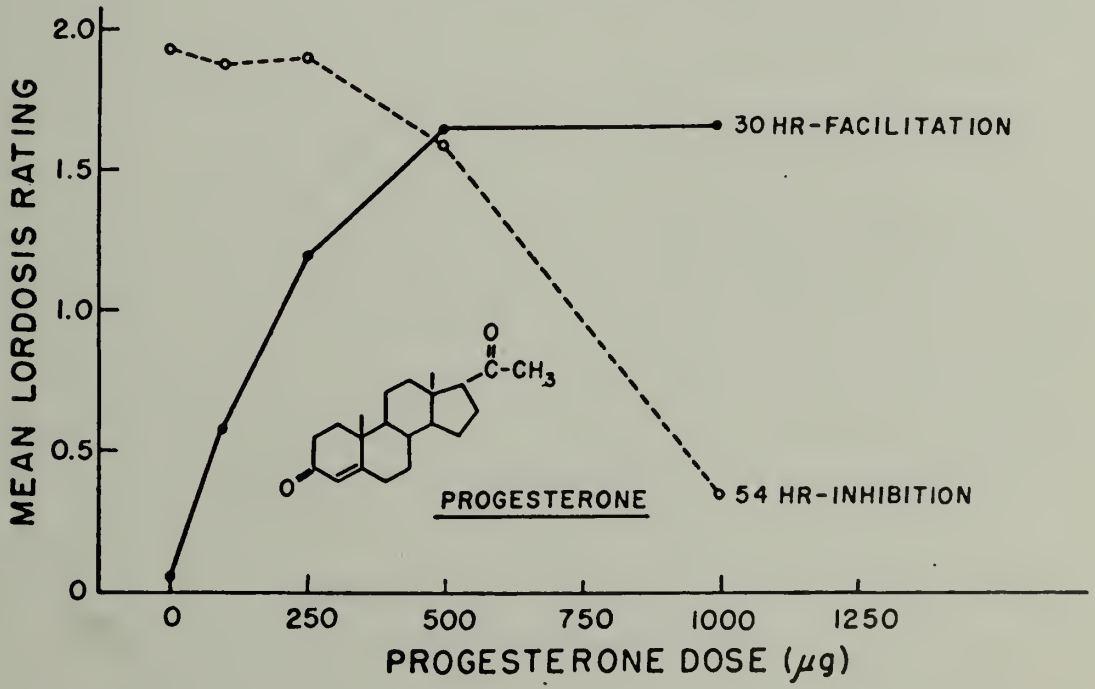
Results. As little as 5 μg of R 5020 significantly facilitated sexual behavior at 30 hours when compared with oil controls, $U(11,13) = 7.5$, $p < .002$, compared with 250 μg needed for progesterone, $U(8,13) = 17$, $p < .02$, (Figure 12).

As low a dose as 10 μg of R 5020 sequentially inhibited sexual behavior at 30 hours, $U(8,13) = 24$, $p < .05$, compared with 1,000 μg needed for progesterone, $U(7,13) = 6.5$, $p < .002$, (Figure 12).

Figure 12. Mean lordosis ratings of ovariectomized rats injected with various doses of R 5020 (top panel) or progesterone (bottom panel), or sesame oil vehicle 24 hours after 2 μg of estradiol benzoate. Rats were tested 5-7 hours later (30 hour test-facilitation). All rats received 0.5 mg of progesterone at 48 hours and were tested again 5-7 hours later (54 hour test-inhibition). (All steroids were dissolved in 0.1 ml of sesame oil and were injected subcutaneously.)

FACILITATION AND SEQUENTIAL INHIBITION OF SEXUAL BEHAVIOR BY R 5020 & PROGESTERONE





EXPERIMENT 15: CONCURRENT INHIBITION BY R 5020 - COMPARISON WITH PROGESTERONE

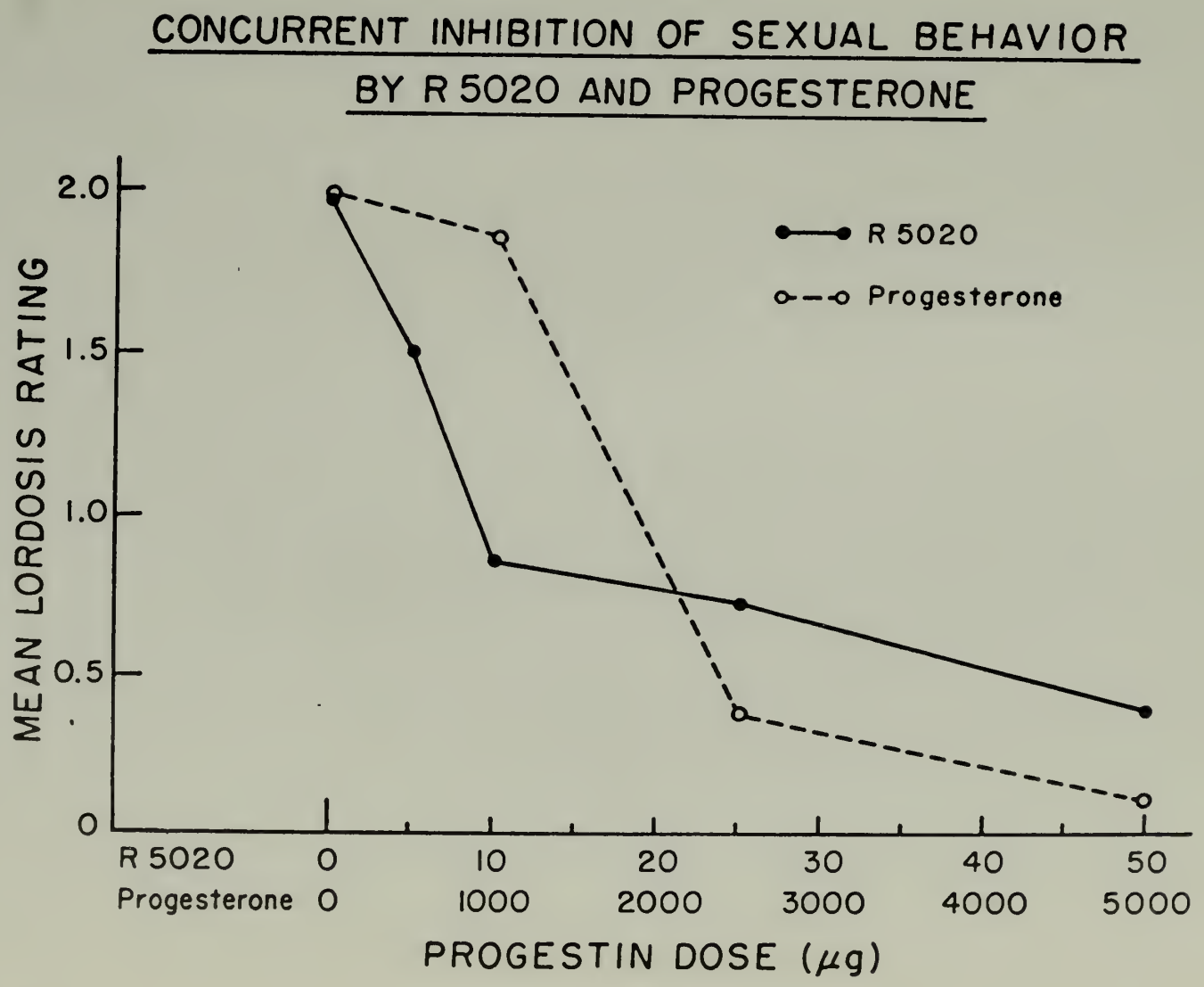
The purpose of Experiment 15 is to further characterize R 5020's inhibitory effects by investigating its relative effectiveness in concurrent inhibition.

Procedure. Forty-eight ovariectomized rats were divided into eight groups receiving various doses of R 5020, progesterone or oil concurrently with EB. All rats were injected with 2 μg of EB at 1400 hours. At this time either oil (n=6), 5 μg of R 5020 (n=6), 10 μg of R 5020 (n=6), 25 μg of R 5020 (n=6), 50 μg of R 5020 (n=6), 1,000 μg of progesterone (n=6), 2,500 μg of progesterone (n=6), or 5,000 μg of progesterone (n=6) were injected at a separate subcutaneous site. Forty-two hours later (0800 hours), 0.5 mg of progesterone were administered, and sexual receptivity was tested five to seven hours later.

Results and discussion. As little as 50 μg of R 5020 inhibited sexual behavior compared with oil controls, $U(6,6) = 4$, $p < .05$, (Figure 13). 2,500 μg of progesterone were required for inhibition, $U(6,6) = 1$, $p < .005$ (Figure 13).

Thus, R 5020 is 50-100 times more effective than progesterone as both a facilitator and inhibitor of sexual behavior. This lends support to the hypothesis that progestins' effects on behavior are mediated by a receptor mechanism as uterine effects may be.

Figure 13. Mean lordosis ratings of ovariectomized rats injected with various doses of R 5020, progesterone or sesame oil vehicle simultaneously with 2 µg of estradiol benzoate. All rats received 0.5 mg of progesterone at 42 hours and were tested at approximately 48 hours. (All steroids were dissolved in 0.1 ml of sesame oil and were injected subcutaneously.)


CONCURRENT INHIBITION OF SEXUAL BEHAVIOR
BY R 5020 AND PROGESTERONE
MEAN LORDOSIS RATING
2.0
1.5
1.0
0.5
0
R 5020
Progesterone
R 5020 0 10 20 30 40 50
Progesterone 0 1000 2000 3000 4000 5000
PROGESTIN DOSE (μg)

EXPERIMENT 16: ^{3}H-R 5020 BINDING IN OVARIECTOMIZED-ADRENALECTOMIZED RATS

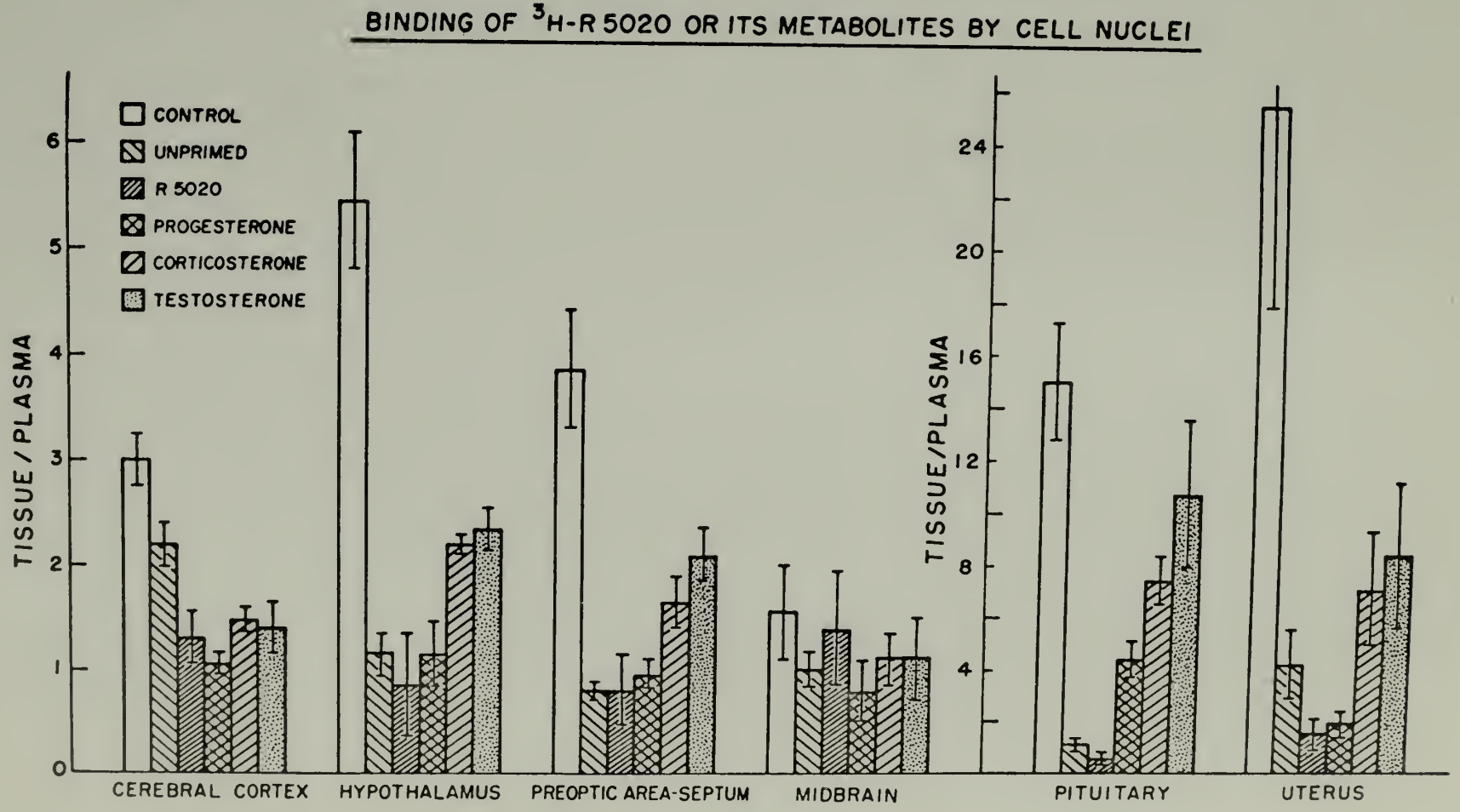
As previously discussed, R 5020's unique binding characteristics have made it a useful tool in the study of uterine progestin receptors. Experiments 14 and 15 are consistent with the interpretation that progestins' behavioral effects are receptor-mediated. In this experiment, an attempt is made to observe binding by brain cell nuclei, using ^{3}H-R 5020.

Procedure. Female Sprague-Dawley rats weighing 150-220 grams were ovariectomized and adrenalectomized and injected daily with 2 µg of EB for six to eight days. To determine the effects of estrogen priming, EB was omitted in one group (unprimed; n=4), and the rats received sesame oil vehicle injections. To demonstrate saturability and progestin-specificity of the binding, primed rats were injected intraperitoneally with 1 mg of unlabelled R 5020 (n=4), progesterone (n=4), corticosterone (n=5), testosterone (n=4), or the ethanol vehicle (n=6), 75 minutes prior to sacrifice. Sixty minutes prior to sacrifice, all rats were injected intravenously with 20 µCi 6,7 ^{3}H-R 5020 (0.1 µg; specific activity = 56.5 Ci/mmole) dissolved in 20% ethanol-saline. Pituitary gland, cerebral cortex, preoptic area-septum and hypothalamus described previously were then dissected. The sample of midbrain (70 mg) which extended 3 mm caudal to the hypothalamic sample was bordered dorsally by the posterior commissure and vertically by the pons. Uterus (120 mg) was minced, homogenized in a ground glass homogenizer, and filtered through two layers of gauze.

Results. The pattern of uptake in whole homogenates of the estrogen-primed rats was uterus > pituitary > midbrain > hypothalamus = preoptic area-septum > cortex. Neither estrogen priming nor competing steroids had any effect on whole homogenate uptake in neural tissues. However, estrogen priming doubled uptake in uterus and quadrupled uptake in pituitary. Either R 5020 or progesterone pretreatment in estrogen-primed rats decreased whole homogenate uptake in uterus and pituitary by 59-89%. Testosterone and corticosterone were less effective competitors than the two progestins.

The pattern of binding in cell nuclei was considerably different from whole homogenates, probably owing to the extensive amount of nonspecific, nonsaturable uptake of progestins in whole homogenates of neural tissues: uterus > pituitary > hypothalamus > preoptic area-septum = cortex > midbrain (Fig. 14). Omission of estrogen priming resulted in an approximately 85% decrease in nuclear binding by hypothalamus, preoptic area-septum, uterus and pituitary, a small decrease in cortex, but no statistically significant change in midbrain. R 5020 pretreatment suppressed nuclear binding in hypothalamus, preoptic area-septum, pituitary and uterus to approximately the levels of unprimed rats. Progesterone was nearly as effective as R 5020 as a competitor. Both progestins were significantly more effective competitors than either testosterone or corticosterone. The competition that was obtained with testosterone and corticosterone is likely due to having used a very large dose of competitor (1 mg) compared with the ^{3}H-R 5020 (0.1 µg).

Figure 14. Binding of ^{3}H-R 5020 or its metabolites in brain, pituitary, and uterine cell nuclei of ovariectomized-adrenalectomized female rats one hour after intravenous injection of 20 μCi of ^{3}H-R 5020. Controls (n=6) received daily injections of 2 ug of estradiol benzoate for 6-8 days as did the groups which received 1 mg of R 5020 (n=4), progesterone (n=4), corticosterone (n=5) or testosterone (n=4) 75 minutes prior to sacrifice. Unprimed rats (n=4) received daily oil injections. Results are expressed as tissue/plasma ratios of DPM/mg protein: DPM/μl plasma.


BINDING OF ^{3}H-R 5020 OR ITS METABOLITES BY CELL NUCLEI
CONTROL
UNPRIMED
R 5020
PROGESTERONE
CORTICOSTERONE
TESTOSTERONE
TISSUE / PLASMA
0
1
2
3
4
5
6
TISSUE/PLASMA
4
8
12
16
20
24
CEREBRAL CORTEX
HYPOTHALAMUS
PREOPTIC AREA-SEPTUM
MIDBRAIN
PITUITARY
UTERUS

EXPERIMENT 17: ^{3}H-PROGESTERONE UPTAKE IN OVARIECTOMIZED-ADRENALECTOMIZED, ESTROGEN-PRIMED RATS

Although previous experiments in guinea pigs (Marrone & Feder, 1977) and rats (McEwen et al., 1976) have failed to detect nuclear binding of ^{3}H-progesterone using liquid scintillation counting techniques, none of these experiments have used identical procedures to those which were used in Experiment 16. Experiment 17 is an attempt to verify either the presence or absence of radioactivity in brain cell nuclei after injection of ^{3}H-progesterone.

Procedure. The procedure is the same as that followed in Experiment 16 except that rats were injected with 1,2-^{3}H-progesterone (Specific activity = 55.7 Ci/mmole; New England Nuclear). One group (n=6) received the ethanol vehicle 75 minutes prior to sacrifice and one group received 1 mg of unlabelled progesterone (n=4).

Results. The small amount of radioactivity that was found in cell nuclear fractions was not saturable as evidenced by the failure of 1 mg of progesterone to compete for binding (Table II). In whole homogenates, only the uterus specifically accumulated radioactivity evidenced by a decrease in uptake after pretreatment with unlabelled progesterone.

TABLE II

RADIOACTIVITY[1] IN WHOLE HOMOGENATES AND CELL NUCLEAR FRACTIONS AFTER INJECTION OF ^{3}H-PROGESTERONE IN OVARIECTOMIZED-ADRENALECTOMIZED, ESTROGEN-PRIMED RATS: EFFECTS OF PROGESTERONE PRETREATMENT

CONTROLS

	WHOLE HOMOGENATES						CELL NUCLEAR FRACTION					
	CEREBRAL CORTEX	HTH[2]	POA-SEPT[3]	MB[4]	PIT[5]	UTR[6]	CEREBRAL CORTEX	HTH	POA-SEPT	MB	PIT	UTR
MEAN	7.7	13.1	11.2	16.8	8.1	27.3	1.3	2.5	1.8	1.9	.9	1.7
(SEM)	(.43)	(1.4)	(.88)	(2.14)	(.84)	(2.83)	(.27)	(.71)	(.51)	(.68)	(.31)	(.29)
PROGESTERONE PRETREATMENT												
MEAN	8.4	12.5	12.2	19.9	9.3	11.4	1.2	2.7	1.4	3.5	.6	1.0
(SEM)	(.42)	(.73)	(1.14)	(1.04)	(.27)	(1.90)	(.33)	(.48)	(.38)	(1.28)	(.13)	(.38)

[1]Expressed as tissue/plasma ratios; DPM/mg protein: DPM/ ul plasma

[2]Hypothalamus [3]Preoptic area-septum [4]Midbrain [5]Pituitary [6]Uterus

DISCUSSION

R 5020 is the first progestin reported to be more effective than progesterone as both a facilitator and inhibitor of sexual behavior. Norprogesterone which is also a 19-norprogestin is 20 times more effective than progesterone in facilitating sexual behavior in female guinea pigs (Kinci, 1964). Medroxyprogesterone (7α-methyl-17α-acetoxypregn-4-ene-3,20-dione) is several times more effective than progesterone in facilitating sexual behavior in female rats (Meyerson, 1967). Neither of these steroids has been tested for inhibitory effects.

The biochemical experiments represent the first report of nuclear binding of a progestin in rat brain, although binding has been reported in guinea pigs' brains using autoradiography (Sar & Stumpf, 1973). Taken as a whole, the behavioral experiments and the biochemical experiments are quite consistent with each other and with the hypothesis that brain cell nuclear binding is involved in behavioral responses to progestins.

There is, of course, an alternative explanation for the effectiveness of R 5020 on sexual behavior. Slowed rate of metabolism of the synthetic progestin, medroxyprogesterone acetate, has already been suggested as the basis of its hypereffectiveness in uterine responses (Feil, Miljkovic & Bardin, 1976). Although this explanation cannot be overlooked, we also cannot overlook the fact that R 5020 is 50 times more effective than progesterone at a very short time after injection (i.e. facilitation of sexual behavior 5-7 hours after injection). It is, however, not unlikely that a combination of factors is involved in R 5020's increased potency.

The high concentration of ^{3}H-R 5020 binding by pituitary cell nuclei, though probably not relevant to behavior, is pertinent to an understanding of progesterone's influences on reproductive physiology. That is, the pituitary is believed to be one site of action for progesterone's effects on gonadotropin release (Barraclough, 1973; Feder & Marrone, 1977; Schally, Arimura & Kastin, 1973). Similarly, the high concentration in the hypothalamus is consistent with the results of cannula implant studies, which have demonstrated that the hypothalamus is one site of action for progesterone's facilition of rat (Powers, 1972; Ross et al., 1971; Ward et al., 1975) and guinea pig (Morin & Feder, 1974b) sexual behavior. The hypothalamus also seems to be a site of action for progesterone's inhibition of rat sexual behavior (Marrone & Feder, personal communication).

The lack of binding in the midbrain is quite puzzling since cannula implant studies have consistently found the midbrain to be one site of action for progesterone's effects on sexual behavior. Unfortunately, the direction of the results are conflicting. In guinea pigs (Morin & Feder, 1974c), hamsters (DeBold et al., 1976) and rats (Yanase & Gorski, 1976), the midbrain is reported to be a site of inhibition of sexual behavior. Conversely, Ross et al., (1971) and Luttge and Gughes (1976) have reported facilitation of rat sexual behavior with progesterone implants in the midbrain reticular formation and interpeduncular nucleus, respectively. Unfortunately, until the gross interlaboratory and potential interspecies differences in the anatomical localization of progesterone's effects are reconciled, it cannot be inferred from implant data that nuclear binding

is involved in only facilitation or only inhibition. Nevertheless, implants of progesterone in the midbrain affect sexual behavior, yet saturable nuclear binding of R 5020 could not be observed in this area. Either the procedures used do not afford sufficient resolution to observe what may be very low concentration of binding or alternatively, progestins may have a different subcellular site of action in this area, perhaps at the neuronal synapse (Janowsky & Davis, 1976).

Finally, the dependence of the progestin binding on estrogen priming is quite exciting. It is of course consistent with the dependence on estrogen priming for the synthesis of progestin receptors (Faber, Sandmann & Stavely, 1972a, 1972b; Freifeld, Feil & Bardin, 1974; Leavitt et al., 1974; Milgrom, Atger & Baulieu, 1970; Luu Thi, Baulieu & Milgrom, 1975) and uptake of ^{3}H-progesterone in peripheral tissues (Chen & Leavitt, 1975; Falk & Bardin, 1970; Leavitt & Blaha, 1972), as well as for progesterone's facilitation of behavior (Feder & Marrone, 1977; Morin, 1977). These results also suggest that estrogen and progesterone may act on the same cells in progestin-responsive neural tissues.

GENERAL DISCUSSION

Section I demonstrated that with appropriate doses, progesterone can inhibit sexual receptivity in rats in both a concurrent and sequential inhibition procedure. These experiments raise a number of interesting questions concerning progesterone's role in the modulation of sexual receptivity.

Inhibition of sexual behavior in rats requires approximately 1 - 2.5 mg of progesterone compared to less than 1 mg required for guinea pigs in similar, but not identical procedures (Wallen et al., 1975; Zucker, 1966). What is the basis for the elevated levels required in rats? Experiment 5 addressed this question by demonstrating that 1 mg of progesterone, which did not inhibit concurrently when injected simultaneously with EB, was effective when divided into five repeated injections over a period of 16 hours. The results are consistent with the notion that maintained neural levels of progesterone may be necessary for inhibition. They are also consistent with an interpretation that blood plasma levels must be maintained at a sufficiently elevated level, or that a sustained supply of unmetabolized progesterone must be maintained. The species difference could be due to shorter retention of progesterone in the brain or plasma or the rapid metabolism in rats.

The fact that the same types of dose relationships held for concurrent inhibition as sequential inhibition suggest that these two classes of inhibition may be essentially the same phenomenon. Nevertheless, we are left to explain the fact that concurrent inhibition requires 2.5 mg of progesterone, but sequential inhibition

requires only 1 mg of progesterone. Experiment 9 demonstrated that the shorter interval between progesterone injection and testing in the sequential inhibition procedure is probably not responsible. It remains to be explained why less progesterone is required to inhibit sequentially than is required to inhibit concurrently. At the time of the progesterone injection in concurrent inhibition, plasma estrogen levels are their highest, but conditioning has just begun. The solution to the problem will likely require a prior understanding of what estrogen conditioning is at a neurochemical level.

Progesterone requires estrogen priming in order to facilitate sexual behavior. The higher the estrogen dose, the lower the dose of progesterone needed to facilitate (Whalen, 1974). Why then, does increasing the priming dose of estradiol decrease responsivity to progesterone's inhibitory influences? This suggests that the dose required for facilitation is dose-dependent on estradiol but the dose required for inhibition is inversely related to the dose of estradiol. Does this imply that progesterone's facilitatory and inhibitory effects are mediated by distinct neurochemical mechanisms with differing dependence on estradiol?

A mechanism of action for progesterone must be able to resolve all of these questions, as well as account for the results of a recent experiment by Marrone, Rodriguez-Sierra and Feder (1977) which suggested that progesterone's inhibitory effects can occur with as short a latency as the facilitatory effects. When the heat duration of rats was extended by injecting a large dose of progester-

one, heat could be terminated within four hours by a second dose of progesterone. Facilitation can occur in less than an hour of administration when injected intravenously (Lisk, 1960; Meyerson, 1972). It has yet to be seen whether inhibition can occur quite this rapidly.

Section II offers evidence that progesterone does not inhibit sexual behavior by the same mechanism as the synthetic estrogen antagonists. With two conflicting reports (Anderson & Greenwald, 1969; Ciaccio & Lisk, 1972) progesterone does not diminish whole homogenate or cell nuclear binding of estradiol in the brain of any rodent species that has been studied. Progesterone does not affect estradiol's interaction with the estrogen receptor, nor affect the replenishment of the estrogen receptor in the hypothalamus. Conversely, synthetic estrogen antagonists are effective in inhibiting each of these steps. The behavioral effects of progesterone and the synthetic estrogen antagonist, CI-628, are dissociable behaviorally as well; CI-628 shortens heat duration, and increasing progesterone doses actually lengthen it.

Recently, the suggestion has been made that lengthy progesterone pretreatment may actually increase *in vivo* ^{3}H-estradiol uptake in preoptic area, hypothalamus and pituitary and *in vitro* nuclear binding of ^{3}H-estradiol in the hypothalamus and pituitary (Reuter & Lisk, 1976). These results are difficult to interpret since, on the basis of current hypotheses of estrogen's mechanism of action, one would predict from these results that progesterone pretreatment would increase responsiveness to estrogen, which it does not.

Although Section II only demonstrated a mechanism by which progesterone probably does *not* affect sexual behavior, Section III

has proposed a testable hypothesis of how it migh affect sexual behavior. As already discussed, the cell nuclear binding of a progestin, ^{3}H-R 5020 is estrogen dependent, lending credence to the hypothesis that the "two-step" mechanism of steroid action may be involved in progesterone's effects on sexual behavior. The specificity with which R 5020 binds to progestin receptors allows the results of ^{3}H-R 5020 binding studies to be generalized to progesterone itself (Raynaud, 1977).

The rapidity with which progesterone's facilitatory andinhibitory actions occur is perhaps the most troublesome argument against a cell nuclear site of action. Although we are accustomed to thinking that an effect of a steroid hormone on sexual behavior involving genetic expression would require many hours to exert itself (McEwen, 1976; McEwen et al., 1975), this need not be the case. Early events of estrogen's action in the uterus occur within a few hours (Anderson, Peck & Clark, 1975). Certainly the "induced protein" is detectable within an hour of estrogen administration (Barnea & Gorski, 1970). We need only postulate that early products of progesterone's effects on genetic expression are involved in progesterone's facilitation and/or inhibition of sexual behavior. It should be emphasized that a proposed nuclear site of action for progesterone in no way precludes effects on the cell membrane (Marrone & Feder, 1977), either independent of, or secondary to the nuclear mechanism.

This dissertation could hardly be considered complete without at least some speculations as to the biological significance, if any, of progesterone's effects on sexual behavior in rats. Joslyn, Feder and Goy (1971) have suggested that the function of the synergistic

action of progesterone in female rodents may be for estrous behavior and ovulation to become "temporally bound to each other in the normal cycle...." An inseparable link between sexual receptivity and ovulation is an association with obvious adaptive significance.

Progesterone sequentially inhibits sexual behavior in ovariectomized rats, as endogenous progesterone may during the estrous cycle. During the estrous cycle, it would seem likely that the progesterone-induced sequential inhibition may more firmly link sexual receptivity with ovulation by opening and then closing a discrete window of time during which both may occur.

Progesterone concurrently inhibits in ovariectomized rats, and may contribute to the absence of sexual behavior during pregnancy (Hardy, 1970). Following copulation, there ensues an immediate postcopulatory period of inhibition (Hardy & DeBold, 1972) and an elevation of progesterone levels in blood plasma within six hours (Adler, Resko & Goy, 1970). Perhaps concurrent inhibition by progesterone is a mechanism that operates as a continuation of the neural inhibition of sexual receptivity that has been described (Lodder & Zeilmaker, 1976).

Lastly, it must be pointed out that progesterone's effects on sexual behavior should not be dismissed as an interesting artifact of laboratory rodents. Progesterone antagonizes estrogen's induction of sexual behavior in guinea pigs, hamsters, rats (Feder & Marrone, 1977; Morin, 1977; Young, 1969), mice (Edwards, 1970), rabbits (Beyer, Vidal & McDonald, 1969), sheep and goats (Phillips, Fraps and Frank, 1945), swine (Day, Anderson, Hazel & Melampy, 1959) and cows (Carrick & Shelton, 1969). Thus, even if it may not be a universal principle, progesterone's inhibition of sexual behavior is certainly widespread.

REFERENCES

Adler, N.R., J.H. Resko and R.W. Goy. Physiol. Behav., 5, 1003, 1970.

Anderson, C.H. and G.S. Greenwald. Endocrinology, 85, 1160, 1969.

Anderson, J. N., E.J. Peck and J.H. Clark. Endocrinology, 96, 160, 1975.

Arai, Y. and R.A. Gorski. Physiol. Behav., 3, 351, 1968.

Armstrong, D.T. Am. J. Physiology, 224, 764, 1968.

Atger, M., E.E. Baulieu and E. Milgrom. Endocrinology, 94, 161, 1974.

Barfield, M.A. and R.D. Lisk. Endocrinology, 94, 571, 1974.

Barnea, A. and J. Gorski. Biochemistry, 9, 1899, 1970.

Barraclough, C.A. In: R.O. Greep and E.B. Astwood (Eds.), Handbook of Physiology, Section 7, Volume II, American Physiological Society, Washington, 1973.

Beach, F.A. Proc. Soc. Exp. Biol. Med., 51, 369, 1942.

Berliner, D.L. and W.G. Wiest, J. Biological Chemistry, 221, 449, 1956.

Beyer, C., N. Vidal, and P.G. McDonald. J. Endocrinology, 45, 407, 1969.

Bo, N.J., W.L. Poteat, W.A. Krueger and F. McAlister. Steroids, 18, 389, 1971.

Boling, J.L. & R.J. Blandau. Endocrinology, 25, 359, 1939.

Boling, J.L., W.C. Young and E.W. Dempsey. Endocrinology, 23, 182, 1938.

Bronson, F.H. & T.H. Hamilton. Biol. Reprod., 6, 160, 1972.

Bullock, D.W. Horms. Behav., 1, 137, 1970.

Butcher, R.L., W.E. Collins and N.W. Fugo. Endocrinology, 94, 1704, 1974.

Carrick, M.J. and J.N. Shelton. J. Endocrinology, 45, 99, 1969.

Carter, C.S., S.J. Michael and A.H. Morris. Horms. Behav., 4, 129, 1973.

Carter, C.S. and S.W. Porges. Horms. Behav., 5, 303, 1974.

Chader, G.J. and C. Villee. In: Influence of Hormones on the Nervous System, Proc. International Society of Psychoneuroendocrinology, Brooklyn, 1970, Karger, Basel, 1971.

Chazal, G., M. Faudon, F. Gogan and I. Rotsztejn. Brain Res., 89, 245, 1975.

Chen, T.J. and W.W. Leavitt. Fed. Proc., (abstract), 1975.

Cheng, Y.J. and H.J. Karavolas. Endocrinology, 93, 1157, 1973.

Cheng, H.C. and D.C. Johnson. Steroids, 24, 656, 1974.

Cheng, Y.J. and H.J. Karavolas. J. Biological Chemistry, 250, 7997, 1975.

Cheng, Y.J. and H.J. Karavolas. Steroids, 26, 57, 1975.

Ciaccio, L.A. and R.D. Lisk. J. Endocrinology, 50, 201, 1971.

Ciaccio, L.A. and R.D. Lisk. Nature, 236, 82, 1972.

Clark, B.F. J. Endocrinology, 63, 343, 1974.

Clark, B.F. J. Endocrinology, 50, 527, 1971.

Clark, J.H., J.N. Anderson and E.J. Peck. Steroids, 22, 707, 1973.

Clementson, C.A.B., V.L. Verma and S.J. DeCarlo. J. Reprod. Fert., 49, 183, 1977.

Collins, V.J., J.L. Boling, E.W. Dempsey and W.C. Young. Endocrinology, 23, 188, 1938.

Corvol, P., R. Falk, M. Freifeld and C.W. Bardin. Endocrinology, 90, 1464, 1972.

Croix, D. and P. Franchimont. Neuroendocrinology, 19, 1, 1975.

Davidson, J.M., C.H. Rodgers, E.R. Smith and G.J. Bloch. Endocrinology, 82, 193, 1968.

Davies, I.J., F. Naftolin, and K.J. Ryan. Presented at the 56th meeting of the Endocrine Society, Atlanta, Georgia, 1974.

Davies, I.J. and K.J. Ryan. Vitamins & Hormones, 30, 223, 1972.

Davies, I.J., J. Siu, F. Naftolin and K.J. Ryan. In: G. Raspe (Ed.), Advances in the Biosciences, Pergamon Press, Oxford, 1975.

Day, B.N., L.L. Anderson, L.N. Hazel and R.M. Melampy. J. Animal Science, 18, 909, 1959.

DeBold, J.F., J.V. Martin and R.E. Whalen. Endocrinology, 99, 1519, 1976.

Dempsey, E.W., R. Hertz and W.C. Young. Am. J. Physiology, 116, 201, 1936.

Edwards, D.A. Horms. Behav., 1, 299, 1970.

Edwards, D.A., R.E. Whalen and R.D. Nadler. Physiol. Behav., 3, 29, 1968.

Eisenfeld, A.J. Endocrinology, 86, 1313, 1970.

Faber, L.E., M.L. Sandmann, and H.E. Stavely. J. Biological Chemistry, 247, 5648., 1972

Faber, L.E., M.L. Sandmann and H.E. Stavely. J. Biological Chemistry, 247, 8000., 1972.

Faber, L.E., J. Saffran, T.J. Chen and W.W. Leavitt. In: K.M.J. Menon and J.R. Reel, Steroid Hormone Action and Cancer, Plenum,

New York, 1976.

Fajer, A.B. and C.A. Barraclough. Endocrinology, 81, 617, 1967.

Falk, R.J. and C.W. Bardin. Endocrinology, 86, 1059, 1970.

Feder, H.H. and B.L. Marrone. Annals N.Y. Acad. Sci., 286, 331, 1977.

Feder, H.H. and L.P. Morin. Horms. Behav., 5, 63, 1974.

Feil, P.D. and C.W. Bardin. Endocrinology, 97, 1398, 1975.

Feil, P.D., S.R. Glasser, D.O. Toft and B.W. O'Malley. Endocrinology, 91, 738, 1972.

Feil, P.D., M. Miljkovic and C.W. Bardin. Endocrinology, 98, 1506, 1976.

Finn, C.A. and D.G. Porter. The Uterus, Elck Science, London, 1975.

Frank, A.H. and R.M. Fraps. Endocrinology, 37, 357, 1945.

Freifeld, M.L., P.D. Feil and C.W. Bardin. Steroids, 23, 93, 1974..

Gorski, J. and F. Cannon. Annual Review of Physiology, 38, 425, 1976.

Gorski, J., D. Toft, G. Shyamala, G. Smith and A. Notides. Recent Progress in Hormone Research, 24, 45, 1968.

Goy, R.W.and C.H. Phoenix. American Zoologist, 5, 725, 1965.

Goy, R.W., C.H. Phoenix and W.C. Young. Gen. and Comp. Endocrinology, 6, 267, 1966.

Green, R., W.G. Luttge and R.E. Whalen. Physiol. Behav., 5, 137, 1970.

Hardy, D.F. Horms. Behav., 1, 235, 1970.

Harris, D.N., L.J. Lerner and R. Hilf. Transactions N.Y. Acad. Sci., 30, 774, 1968.

Hashimoto, I., D.M. Henricks, L.L. Anderson and R.M. Melampy. Endocrinology, 82, 333, 1968.

Ho, G.K.W., D.M. Quadagno, P.H. Cooke and R.A. Gorski. Neuro-endocrinology, 13, 47, 1973/1974.

Hough, J.G., G.K.W. Ho, P.H. Cooke, and D.M. Quadagno. Horms. Behav., 5, 367, 1974.

Hsueh, A.J.W., E.J. Peck and J.H. Clark. Steroids, 24, 599, 1974.

Hsueh, A.J.W., E.J. Peck and J.H. Clark. Nature, 254, 337, 1975.

Hsueh, A.J.W., E.J. Peck and J.H. Clark, Endocrinology, 98, 438, 1976.

Huggins, C. and E.V. Jensen. J. Experimental Medicine, 102, 347, 1955.

Iramain, C.A., B.J. Danzo, C.A. Strott and D.O. Toft. Presented at 45th International Congress of the International Society of Psychoneuroendocrinology, Berkeley, 1973.

Iramain, C.A. and C.A. Strott. Endocrinology, 92, A117, 1973.

Jackson, G.L. Endocrinology, 90, 874, 1972.

Jackson, G.L. Neuroendocrinology, 17, 236, 1975.

Janowsky, D.S. and J.M. Davis. Life Sciences, 9, 525, 1970.

Jensen, E.V. and E.R. DeSombre. Science, 182, 126, 1973.

Jensen, E.V., T. Suzuki, T. Kawashima, W.E. Stumpf, P.W. Jungblut and E.R. DeSombre. Proceedings Nat. Acad. Sci., 59, 632, 1968.

Joslyn, W.D., H.H. Feder and R.W. Goy. Physiol. Behav., 7, 477, 1971.

Karavolas, H.J. and S.M. Herf. Endocrinology, 89, 940, 1971.

Katzenellenbogen, B.S. and E.R. Ferguson. Endocrinology, 97, 1, 1975.

Kato, J. J. Steroid Biochemistry, 6, 979, 1975.

Kennedy, T.G. and D.T. Armstrong. Endocrinology, 97, 1379, 1975.

Kincl, F.A. In: R.I. Dorfman (Eds.), Methods in Hormone Research, Vol. III, Academic Press, New York, 1964.

Korach, K.S. and T.G.Muldoon. Endocrinology, 94, 785, 1974.

Korenmann, S.G. and B.W. O'Malley. Endocrinology, 83, 11, 1968.

Krueger, W.A., W.J. Bo and B.M. Garrison. Anatomical Record, 178, 617, 1974.

Landau, I.T. Pharmacol., Biochem. & Behav., 5, 473, 1976.

Landau, I.T. Brain Res., in press, 1977.

Laumas, K.R. and A. Farooq. J. Endocrinology, 36, 95, 1966.

Lawson, D.E.M. and W.H. Pearlman. J. Biological Chemistry, 239, 3226, 1964.

Leavitt, W.W. and G.C. Blaha. Steroids, 19, 263, 1972.

Leavitt, W.W., D.O. Toft, C.A. Strott and B.W. O'Malley. Endocrinology, 94, 1041, 1974.

Lisk, R.D. Canadian J. Biochem. Physiol., 38, 1381, 1960.

Lisk, R.D. Neuroendocrinology, 5, 149, 1969.

Lisk, R.D. Transactions N.Y. Acad. Sci., 31, 593, 1969.

Lisk, R.D. Animal Behavior, 19, 606, 1971.

Lisk, R.D., L.A. Ciaccio and L.A. Reuter. Gen. Comp. Endocrinology, Supp. 3, 553, 1972.

Litteria, M. Brain. Res., 55, 234, 1973.

Lodder, J. and G.H. Zeilmaker. J. comp. physiol. Psychol., 90, 925, 1976.

Lowry, O.H., J.N. Rosenbrough, A.L. Farr and J.R. Randall. J. Biological Chemistry, 193, 265, 1951.

Luine, V.N. and B.S. McEwen. Endocrinology, 100, 903, 1977.

Lukaszewska, J.H. and G.S. Greenwald. Endocrinology, 86, 1, 1970.

Luu Thi, M.T., E.E. Baulieu and E. Milgrom. J. Endocrinology, 66, 349, 1975.

Luttge, W.G., P.B. Chronister and N.R. Hall, Life Sciences, 12, 419, 1973.

Luttge, W.G. and C.J. Wallis. Steroids, 22, 493, 1973.

Luttge, W.G., C.J. Wallis and N.R. Hall. Brain Res., 71, 105, 1974.

Luttge, W.G., H.E. Gray and J.R. Hughes. Brain Res., 104, 273, 1976.

Luttge, W.G. and J.R. Hughes. Physiol. Behav., 17, 771, 1976.

Madjerek, Z.S. Acta. Morphol. Neerl. - Scand., 10, 259, 1972.

Madjerek, Z.S. and J.H. Smit-vis. Acta. Morphol. Neerl. - Scand., 12, 9, 1974.

Marrone, B.L. and H.H. Feder. Biol. Reprod., in press, 1977.

Marrone, B.L., J.F. Rodriguez-Sierra and H.H. Feder. Horms. Behav., in press, 1977.

Martin. L. and C.A. Finn. J. Endocrinology, 48, 109, 1970.

Martin, L., C.A. Finn and J. Carter. J. Reprod. Fert., 21, 461, 1970.

McEwen, B.S. Presented at International Symposium on Subcellular Mechanisms in Reproductive Neuroendocrinology, Boston, 1975.

McEwen, B.S. In: J.A. Ferrendelli, B.S. McEwen and S.H. Snyder (Eds.), Neuroscience Symposia (Vol. 1), Neurotransmitters, Hormones and Receptors, Society for Neuroscience, Bethesda, 1976.

McEwen, B.S., R. deKloet and G. Wallach. Brain Res., 105, 129, 1976.

McEwen, B.S., C.J. Denef, J.L. Gerlach and L. Plapinger. In: F.O. Schmitt and F.G. Worden (Eds.), The Neurosciences Third Study Program, MIT Press, Cambridge, 1974.

McEwen, B.S. and D.V. Pfaff. Brain Res., 21, 1, 1970.

McEwen, B.S. and D.W. Pfaff. In: W.F. Ganong and L. Martini (Eds.), Frontiers in Neuroendocrinology, Oxford University Press,

New York, 1973.

McEwen, B.S., D.W. Pfaff, G. Chaptal and V.N. Luine. Brain Res., 86, 155, 1976.

McGuire, W.L. Progestin Receptors in Normal and Neoplastic Tissues, Raven Press, New York, 1977.

McPhail, M.K. J. Physiology, 83, 145, 1934.

Means, A.R. and B.W. O'Malley. Biochemistry, 10, 1570, 1971.

Meyerson, B.J. Endocrinology, 81, 369, 1967.

Meyerson, B.J. Horms. Behav., 3, 1, 1972.

Milgrom, E., M. Atger and E.E. Baulieu. Steroids, 16, 741, 1970.

Miller, B.G. Biochim. Biophys. Acta, 299, 568, 1975.

Moguilevsky, J.A., P. Sacchi and J. Christot. Proceedings Soc. Exp. Biol. Med., 137, 653, 1971.

Morin, L.P. Physiol. Behav., 18, 701, 1977.

Morin, L.P. and H.H. Feder. Horms. Behav., 5, 7, 1974. (a)

Morin, L.P. and H.H. Feder. Brain Res., 70, 81, 1974. (b)

Morin, L.P. and H.H. Feder. Brain Res., 70, 71, 1974. (c)

Nadler, R.D. Physiol. Behav., 5, 95, 1970.

O'Malley, B.W. and W.L. McGuire. Endocrinology, 84, 63, 1969.

O'Malley, B.W., W.L. McGuire, P.O. Kohler, and S.G. Korenmann. Rec. Prog. Horm. Res., 25, 105, 1969.

O'Malley, B.W. and A.R. Means. Science, 183, 610, 1974.

O'Malley, B.W. and W.T. Schrader. J. Steroid Biochemistry, 3, 617, 1972.

O'Malley, B.W., M.R. Sherman and D.O. Toft. Proceedings Nat. Acad. Sci., 67, 501, 1970.

O'Malley, B.W. and C.A. Strott. In: R.O. Greep and E.B. Astwood (Eds.), Handbook of Physiology, Section 7, Vol. II, American Physiological Society, Washington, 1973.

O'Malley, B.W., D.O. Toft and M.R. Sherman. J. Biological Chemistry, 246, 1117, 1971.

Palmiter, R.D. J. Bidogical Chemistry, 247, 6450, 1972.

Pavlik, E.J. and P.B. Coulson. J. Steroid Biochemistry, 7, 369, 1976.

Pfaff, D. and M. Keiner. J. Comparative Neurology, 151, 121, 1973.

Philibert, D. and J.P. Raynaud. Steroids, 22, 89, 1973.

Philibert, D. and J.P. Raynaud. Endocrinology, 94, 627, 1974.

Phillips, R.W., R.M. Fraps and A.H. Frank. In: E.T. Engle (Ed.), The Problem of Fertility, Princeton University Press, Princeton, 1946.

Porter, D.C. J. Endocrinology, 46, 425, 1970.

Powers, J.B. Physiol. Behav., 5, 831, 1970.

Powers, J.B. Brain Res., 45, 311, 1972.

Powers, J.B. Horms. Behav., 6, 379, 1975.

Powers, J.B. and J. Moreines. Physiol. Behav., 17, 493, 1976.

Powers, J.B. and E.S. Valenstein. Science, 175, 1003, 1972.

Powers, J.B. and I. Zucker. Endocrinology, 84, 820, 1969.

Presl, J., V. Figarova, J. Herzmann and S. Rohling. Endocrinologica Japonica, 9, 51, 1975.

Quadagno, D.M. and G.K.W. Ho. Horms. Behav., 6, 19, 1975.

Quadagno, D.M., J. Shryne and R.A. Gorski. Horms. Behav., 2, 1, 1971.

Raynaud, J.P. In: W.L. McGuire (Ed.), Progesterone Receptors in Normal and Neoplastic Tissues, Raven Press, New York, 1977.

Reel, J.R. and Y. Shih. Acta Endocrinologica, 80, 344, 1975.

Reuter, L.A. and R.D. Lisk. Nature, 262, 790, 1976.

Riegel, B., W.L. Hartop and G.W. Kittlinger. Endocrinology, 47, 311, 1950.

Rinard, G.A. and C.S. Chew. Life Sciences, 16, 1507, 1975.

Robinson, J.A. and H.J. Karavolas. Endocrinology, 93, 430, 1973.

Rodier, W.I. J. comp. physiol. Psychol., 74, 365, 1971.

Rogers, A.W., G.H. Thomas and K.M. Yates. Experimental Cell Research., 40, 668, 1966.

Ross, J., C. Claybaugh, L.G. Clemens and R.A. Gorski. Endocrinology, 89, 32, 1971

Roy, E.J. and G.N. Wade. Brain Res., 126, 73, 1977.

Saffran, J., B.K. Loeser, S.A. Bohnett and L.E. Faber. J. Biological Chemistry, 251, 5607, 1976.

Sar, M. and W.E. Stumpf. Science, 182, 1266, 1973.

Schally, A.V., A. Arimura and A.J. Kastin. Science, 179, 341, 1973.

Schimke, R.T., G.S. McKnight, D.J. Shapiro, D. Sullivan and R. Palacios. Rec. Prog. Horm. Res., 31, 175, 1975.

Schrader, W.T. In: B.W. O'Malley and J.G. Hardmann (Eds.), Methods in Enzymology, 36, Academic Press, New York, 1975.

Schrader, W.T., R.G. Smith and W.A. Coty. In: B.W. O'Malley and W.T. Schrader (Eds.), Hormone Action and Molecular Endocrinology Workshop, Houston Biological Association, Houston, 1976.

Schrader, W.T., D.O. Toft and B.W. O'Malley. J. Biological Chemistry, 247, 2401, 1972.

Schwartz, R.J., R.W. Kuhn, R.E. Buller, W.T. Schrader and B.W. O'Malley. J. Biological Chemistry, 251, 5166, 1976.

Seiki, K. and M. Hattori. Endocrinologica Japonica, 20, 11, 1973.

Seiki, K., . M. Miyamoto, A. Yamashita and M. Kitani. J. Endocrinology, 43, 129, 1969.

Sherman, M.R., P.L. Corvol and B.W. O'Malley. J. Biological Chemistry, 245, 6085, 1970.

Siegel, H.I. and J.S. Rosenblatt, Horms. Behav., 6, 223, 1975.

Spelsberg, T.C., A.W. Steggles and B.W. O'Malley. J. Biological Chemistry, 247, 4188, 1971.

Stumpf, W.E. and M. Sar. J. Steroid Biochemistry, 4, 477, 1973.

Tapper, C.M., F. Greig and K. Brown-Grant. J. Endocrinology, 62, 511, 1974.

Taylor, W. and D.E. Wright. J. Endocrinology, 51, 727, 1971.

Terkel, A.S., J. Shryne and R.A. Gorski. Horms. Behav., 4, 377, 1973.

Toft, D.O. and M.R. Sherman. In: B.W. O'Malley and J.G. Hardmann, Methods in Enzymology, 36, Academic Press, New York, 1975.

Vertes, M. and R.J.B. King. Recent Devel. Neurobiol. Hung., Vol. IV, Akad. Kiado, Budapest, 1973.

Wade, G.N. J. comp physiol. Psychol., 88, 183, 1975.

Wade, G.N. In: J.S. Rosenblatt, R.A. Hinde, E. Shaw and C.G. Beer, Advances in the Study of Behavior, Vol. 6, Academic Press, New York, 1976.

Wade, G.N. and H.H. Feder. Physiol. Behav., 9, 773, 1972.

Wade, G.N. and H.H. Feder. Brain Res., 45, 525, 1972. (a)

Wade, G.N. and H.H. Feder. Brain Res., 45, 545, 1972. (b)

Wade, G.N. and H.H. Feder. Brain Res., 73, 545, 1974.

Wade, G.N., C.F. Harding and H.H. Feder. Brain Res., 61, 357, 1973.

Wallen, K., D.A. Goldfoot, W.D. Joslyn and C.A. Paris. Physiol. Behav., 8, 221, 1972.

Wallen, K., R.W. Goy and C.H. Phoenix. Horms. Behav., 6, 127, 1975.

Walters, M.R. and J.H. Clark. Presented at Workshop on P gesterone Receptors in Normal and Neoplastic Tissue, Montreal, 1976.

Ward, I.L., W.R. Crowley, F.P. Zemlan and D.L. Margules. J. comp. physiol. Psychol., 88, 53, 1975.

Warembourg, M. Cited in Atger, M., E.E. Baulieu and E. Milgrom, 1974.

Warembourg, M. Endocrinology, 94, 665, 1974.

Westphal, U. In: R.O. Greep and E.B. Astwood (Eds.), Handbook of Physiology, Section 7, Vol. II, American Physiological Society, Washington, 1973.

Whalen, R.E. Horms. Behav., 5, 157, 1974.

Whalen, R.E. and B.B. Gorzalka. Physiol. Behav., 10, 35, 1973.

Whalen, R.E. and B.B. Gorzalka. Endocrinology, 94, 214, 1974.

Whalen, R.E., B.B. Gorzalka, J.F. DeBold, D.M. Quadagno, G.K.W. Ho and J.C. Hough. Horms. Behav., 5, 337, 1974.

Whalen, R.E. and W.G. Luttge. Brain Res., 33, 147, 1971

Whalen, R.E. and W.G. Luttge. Steroids, 18, 141, 1971

Whalen, R.E., J.V. Martin and K.L. Olsen. Nature, 258, 742, 1975.

Wiest, W.G. J. Biological Chemistry, 238, 94, 1963.

Yanase, M. and R.A. Gorski. Biol. Reprod., 15, 544, 1976.

Yochim, J.M. and V.J. De Feo. Endocrinology, 71, 134, 1962.

Young, W.C. In: D.S. Lehrman, R.A. Hinde and E. Shaw (Eds.),

Advances in the Study of Behavior, Vol. 2, Academic Press, New York, 1969.

Zarrow, M.X., J.M. Yochim and J.L. McCarthy. Experimental Endocrinology -- A Sourcebook of Basic Techniques, Academic Press, New York, 1964.

Zigmond, R.E. In: L.L. Iverson, S.D. Iverson and S.H. Snyder (Eds.), Handbook of Phsychopharmacology, Vol. 1, Plenum, New York, 1975.

Zigmond, R.E. and B.S. McEwen. J. Neurochemistry, 17, 889, 1970.

Zucker, I. J. comp. physiol. Psychol., 62, 376, 1966.

Zucker, I. J. comp. physiol. Psychol., 63, 313, 1967. (a)

Zucker, I. J. Endocrinology, 38, 269, 1967. (b)

Zucker, I. J. comp. physiol. Psychol., 65, 472, 1968.

Zucker, I. and R.W. Goy. J. comp. physiol. Psychol., 64, 378, 1967.