72-5807

LEVIN, Robert Gary, 1942-
DIFFERENTIAL EFFECTS OF PROGESTERONE ON THE MATERNAL BEHAVIOR OF PRIMIPAROUS AND MULTIPAROUS RATS.

The City University of New York, Ph.D., 1971
Psychology, experimental

University Microfilms, A XEROX Company, Ann Arbor, Michigan

DIFFERENTIAL EFFECTS OF PROGESTERONE ON THE MATERNAL BEHAVIOR OF PRIMIPAROUS AND MULTIPAROUS RATS

by

ROBERT LEVIN

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1971

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

August 26, 1971
date

Barton Meyer
Chairman of Examining Committee

August 27, 1971
date

Norman C. Weinberg
(Deputy) Executive Officer

Donald Pfaff

Howard Moltz

Supervisory Committee

The City University of New York

Table of Contents

INTRODUCTION

All mammalian species exhibit an integrated complex of responses which center around the care and protection which their young require for survival. In the rat, the most salient components of this maternal complex are retrieving, nursing and maternal nest building.

Retrieving is typically displayed by the puerperal rat in response to the stimulus of a pup outside the nest. Once the postpartum female detects the presence of this pup, she proceeds to it with alacrity, grasps the middorsal section of the pup with her teeth, picks it up, carries it at least several inches, and deposits it, usually in the nest (Rosenblatt and Lehrman, 1963; Wiesner and Sheard, 1933). Occasionally the pups are retrieved to a section of the cage other than that containing the nest. When this occurs, the pups are either returned to the nest within a short period of time, or else a new nest is constructed, usually in that area of the cage to which the pups had already been retrieved (Moltz, Unpublished Observations). The fact that the pups are not simply deposited at random distinguishes retrieving from what might be called "carrying." Retrieving is directed and serves to collect the young at a specific location; carrying is apparently aimless and ends by leaving the young scattered.

Retrieving initially occurs in the puerperal female at about the time of parturition. Indeed, it has been observed, both in the primiparous and multiparous rat, that if the first born is delivered out of the nest, it will be retrieved to the nest where the remainder of the pups will be born (Rosenblatt and Lehrman, 1963). After parturition, the puerperal rat rapidly improves her retrieving ability as she gains practice. Within the first four days postpartum, the female has considerably reduced both her latency to begin retrieving and her latency to resume it after depositing a pup in the nest (Moltz and Robbins, 1965; Rosenblatt and Lehrman, 1963). Her ability to locate a pup that has strayed from the nest has increased, and the route she takes from the nest to a pup and back again has become more direct (Rosenblatt and Lehrman, 1963). She also quickly learns that it is most efficient to grasp the pup by the middorsal region rather than attempting to pick it up by an extremity (Carlier and Noirot, 1965). Moreover, before the end of the first week postpartum, the female is usually able to discriminate her own from alien pups. She initiates retrieving more quickly when it is her own rather than alien pups which are scattered on the cage floor, and if offered a choice, she will retrieve her own young first (Beach and Jaynes, 1956).

Considering the increases in efficiency of retrieving described above, it is hardly surprising that a gradual increase in the speed of retrieving the entire litter has been reported to occur between the second and the ninth day after parturition (Moltz and Robbins, 1965). Beginning, however, at about the tenth to twelfth day postpartum, a progressive decline in the incidence of retrieving begins such that by Day 21 postpartum only 20 to 25 percent of the females were seen to retrieve their young (Moltz and Robbins, 1965; Rosenblatt and Lehrman, 1963).

Nursing, another component of the maternal complex, begins almost immediately after parturition, with the mother crouching over the young in such a way as to expose her mammary region. As the positively thermotactic pups being to nuzzle in her fur, she adjusts her posture, allowing attachment first to one nipple and then to another. After the pups are attached and sucking has begun, the mother usually remains either entirely passive or engages in no activity other than that of readjusting her position to sustain attachment (Rosenblatt and Lehrman, 1963; Wiesner and Sheard, 1933; Moltz, Unpublished Observations).

Although simple in appearance, nursing is in fact a complex response. The mother must not only inhibit her own motor activity while adopting a posture that permits sucking, but at the same time must crouch in such a way as to maintain the body temperature of the young, which are poikilothermic for the first few days of life (Wiesner and Sheard, 1933). Both these functions invariably demand subtle positional changes in response to movement of the pups. Just how subtle these adjustments actually are is exemplified occasionally by the female which to all appearances seem to be nursing but whose pups are discovered to be cold and either dead or moribund (Moltz, Unpublished Observations).

At this early stage in the nursing episode, the only active part the pups play in the feeding relationship is to search for and grasp the nipple. Beginning, however, at about twelve to fourteen days after parturition, the increasing mobility of the young results in the instigation of nursing outside the nest and an increase in pup initiated nursing (Rosenblatt and Lehrman, 1963). After about the sixteenth day postpartum, nursing is observed to be almost totally initiated by the pups, much of it occurring outside the nest. Nursing behavior ends some three to four weeks after birth when the female weans her young by refusing to allow them to reach a nipple.

In view of the above results it is hardly surprising that the amount of time the puerperal female remains in the nest decreases towards the end of the breeding episode. Holland (1965) for example, reports that for about the first two weeks postpartum the female spends an average of some seventeen hours a day in the nest. However, beginning at about the fourteenth day after parturition, the amount of time in the nest begins to decline such that by Day 21 postpartum the rat spends an average of only eight hours per day in the section of the cage which was the nesting site. However, this increasing mobility evinced by the puerperal female is not associated with a reduction in the incidence of nursing during the breeding episode. Moltz and Robbins (1965) found that nursing behavior, or more specifically the amount of time a female remained in a nursing posture with her pups positioned under her, did not decrease during the period of their observation (i.e., Day 1 through Day 21 postpartum).

Maternal nest building is also initiated shortly after the completion of the birth process. To construct the maternal nest the female reaches out with her mouth and pulls the material employed--usually paper, hay or excelsior--at first toward and then over her. When completed, the postparturient nest is a compact, four to six inch high mound which often hides both the mother and the pups from view (Kinder, 1927; Moltz, Unpublished Observations; Rosenblatt and Lehrman, 1963). This maternal nest is distinctly different from the nest which the preparturient rat begins to construct several days before term, the preparturient nest being only a loosely constituted circular or semicircular nest, having sides rarely exceeding one or two inches in height (Kinder, 1927; Rosenblatt and Lehrman, 1963; Wiesner and Sheard, 1933). However, both the pre- and post- parturient nests are idential with regard to the site of construction. In the laboratory, both nests are constructed in that corner of the cage which the female has previously selected for sleeping (Eible-Eibesfeldt, 1961).

The birth of the young takes place in the preparturient nest and is heralded by periodic waves of contractions of both the abdominal muscle layer and the myometrium of the uterus. During this contraction phase of birth, the female typically lies in the nest with her body pressed against the floor of the cage and her hind legs stretched out behind her. In the hour before delivery of the first fetus, the contractions become more forceful and more frequent. At this time the female rises from the nest and begins to aid actively in expulsion of the fetus by stretching her body. These stretching movements continue until the fetus enters the lower birth canal. At this time the rat assumes the characteristic "head-between-the-heels" position. In this position she vigorously licks the vaginal orifice, often biting and tearing the surrounding tissue as well. Final expulsion of the fetus is accomplished by a series of forceful uterine contractions, aided by the female who grasps the fetus with her mouth and gently tugs at it. Within seconds after the fetus appears, the placenta is usually expelled. The female then pulls the fetus and the attached placenta forward between her hind legs, and while still crouching over the fetus, she devours the placenta and umbilical cord. Following this, she proceeds to lick the pup vigorously, tearing the amniotic membrane covering it. She then deftly grasps the edge of the membrane and pulls it until it is separated from the pup, after which she consumes the membrane and completes delivery of the pup.

The second and subsequent deliveries occur in essentially the same way as the first, except that during these deliveries the female is less likely to adopt the "head-between-heels position." The interval between deliveries is variable, and in the case of a large litter, the delivery process may take several hours. Once the young are born and each has been cleaned, the female proceeds to rebuild the nest, transforming it from the low and loosely structured preparturient nest to the high and compact postparturient nest (Rosenblatt and Lehrman, 1963).

The nest undergoes little change during the first week postpartum. Beginning, however, at about the middle of the second week postpartum as the pups become larger and more active, the shape of the nest begins to change. The inner hollow of the nest gradually becomes enlarged while the walls of the nest become progressively lower. This trend continues until about the third week postpartum, by which time the nesting material has become scattered over the floor of the cage (Rosenblatt and Lehrman, 1963; Moltz, Unpublished Observations).

It is evident from the above discussion that the puerperal female displays virtually immediate maternal responsiveness to young. However, it has been reported by several authors (Rosenblatt and Lehrman, 1963; Wiesner and Sheard, 1933) that the pregnant rat will not begin such maternal attentiveness until about the time of parturition. Wiesner and Sheard (1933), for example, reported that of 21 pregnant primiparous rats tested for retrieving one to seven days before parturition, only four responded positively. Subsequently, Rosenblatt and Lehrman (1963) confirmed this finding and extended their study to include nursing and maternal nest building as well. In this study seventeen pregnant primiparous rats were tested for nursing, maternal nest building, and retrieving on alternate days, starting eleven days before parturition and continuing up to the day of parturition. The results indicate that the components of the maternal complex were initiated only after the emergence of the young.

LATENCY TO MATERNAL BEHAVIOR IN THE NULLIPAROUS FEMALE

Important in understanding the determinants of maternal behavior is the fact that the nulliparous females, if kept continuously in the presence of young, will begin eventually to display interest in these young (Rosenblatt, 1967). They will begin, in other words, to build a nest, lick, retrieve, and finally crouch in a nursing posture, all in a manner undistinguishable from that of puerperal rats. Moreover, these responses occur with the same reliability in similarly confined nulliparal rats that have been either ovariectomized or hypophysectomized (Rosenblatt, 1967).

But whereas the nulliparous female requires, on the average, some six or seven days of continuous exposure to young before she will begin attending to them, her puerperal counterpart responds immediately upon the emergence of the pups from the birth canal. This difference in latency between the postparturient and nulliparous female raises the question of just what physiological and experiential events occur typically to render the puerperal rat more responsive to young than her nulliparous counterpart.

The Experience of Parturition

Several investigators have examined the hypothesis that the very act of expelling the fetuses from the uterus may be responsible for the immediate initiation of maternal attentiveness in the puerperal female. Klein (1952), for example, ligated the lower end of each cornu during mid-pregnancy. At the expected time of parturition, but of course while still retaining their own fetuses, such females were offered newborn foster young. Despite the continuance of uterine distension, each quickly showed what the experimenter referred to as "quite normal nestling and maternal behavior" (P. 86).

Further evidence indicating that expulsion of the fetuses and the events attendant upon parturition are not essential for the instigation of maternal behavior in the postparturient rat is provided by Moltz, Robbins, and Parks (1966). In this study both primiparous and multiparous rats were subjected to caesarean delivery 21 days ± six hours post coitum. Approximately 24 hours later, the females of each group were presented with foster young and subsequently observed for nursing, nest building, and retrieving. Again, the data left no doubt that the female rat can enter into an effective nurtural relationship despite having been deprived of the experience of parturition. Moreover, this was found to be as true for the primiparous as for the multiparous rat. Obviously, neither the results of Klein (1952) nor those of Moltz et. al. (1966) suggest that the experience of parturition can account for the puerperal-nulliparous differences in maternal latency.

Self-licking

Perhaps some of the physiological or behavioral events which occur during pregnancy are responsible for the rapid initiation of maternal behavior characteristic of the postpartum rat. For example, Birch (1956) hypothesized that development of ventral self-licking behavior in the preparturient rat is necessary for the initiation of maternal licking and that the latter, in turn, is prerequisite for the initiation of other response components of the nurtural complex. Birch (1956) tested this hypothesis by observing the maternal behavior of a group of female rats which had been reared, until several hours before parturition, with large collars which precluded self-licking. He reported that the maternal behavior of these collared females was so severely disrupted that none of the young survived to weaning. Unfortunately, Birch's results have not been replicated although several attempts to confirm his findings have been made (Christophersen and Wagman, 1965; Coomans, as cited by Rosenblatt and Lehrman, 1963; Kirby and Horvath, 1968). The precise reason for this lack of reproducibility of Birch's findings is unclear, since his report is so vague as to render exact replication impossible. However, in view of the results of Christophersen and Wagman (1965) and Kirby and Horvath (1968), it is unlikely that Birch's (1956) results can be attributed to any restriction of the females self-licking behavior. Thus, the question still remains as to just what mechanisms are essential for the rapid initiation of the several components of maternal behavior in the puerperal rat.

Mammary Engorgement

Possibly mammary engorgement or, more precisely, the peripheral tension accompanying that engorgement, plays a critical role in initiating maternal behavior. It is well known, of course, that lactogenesis and the accompanying increase in intramammary pressure occurs typically at about the time of parturition. As Lehrman (1961) and Rosenblatt and Lehrman (1963) have suggested, such stimulation may well be responsible, in some part at least, for the immediate interest in young characteristically displayed by the postparturient female. Just this suggestion was tested by Moltz, Geller, and Levin (1967). These investigators performed a total mammectomy on female rats when each S was approximately 21 days of age. At maturity these same Ss were impregnated and allowed to give birth normally. Their results indicated that preclusion of intramammary tension does not disrupt the typical initiation of maternal behavior: all experimental subjects subsequently adopted a typical nursing posture and did so as frequently and with the same alacrity as their control counterparts. In addition, they built nests and retrieved young, again in a manner comparable in every respect to Ss subjected to a sham mammectomy. Obviously enough, these findings fail to support the suggestion that intramammary pressure is necessary for the immediate initiation of maternal behavior in the puerperal rat.

The Hormones of Parturition

Thus far, none of the experiential or physiological events discussed above has provided a clue to the nature of the events which are responsible for the puerperal female's characteristically short latency of initiation of maternal responsivity to young. But there is still another manner in which the postparturient rat differs from her nulliparous counterpart. That is, the puerperal female has experienced the endocrine events associated with pregnancy and parturition. Unfortunately, our knowledge of these events is incomplete. For example, although it is known that in the rat some prostaglandins elicit a tachyphylatic response from isolated uterus (Adamson, Eliasson, and Wiklund, 1967; Eliasson and Brzdekjewicz, 1969 (a) (b); Paton and Daniel, 1967), decrease ovarian progesterone content (Pharriss and Wyngarden, 1969) and secretion (Behrman, Yoshinaga and Greep, 1971) during pseudopregnancy and terminate pregnancy (Deis, 1971; Gutnecht, Cornette and Pharriss, 1969), it is unclear that these pharmacological activities of prostaglandins are indicative of their physiological role or that, in the rat, there is a near term release of prostaglandins. However, despite the gaps in our understanding of the endocrine events which accompany parturition in the rat, it is known that changing blood concentrations of such hormones as progesterone, 20α-hydroxypregn-4-en-3-one, estrogens, prolactin, FSH and oxytocin typically occur at about the time of the termination of pregnancy.

Progesterone as assayed in both peripheral plasma (Grota and Eik-Nes, 1967; Wiest, Kidwell, and Balogh, 1968) and ovarian venous plasma (Eto, Hsoi, Musudo, and Suzuki, 1962; Fajer, and Barraclough, 1967; Hashimoto, Hendricks, Anderson, and Melampy, 1968), begins to increase on Day 4 of pregnancy, reaching maximal concentration on Day 14. Thereafter, the output of the steroid begins to fall slowly, until, on

Day 20, the decline becomes abrupt. Coincident with the onset of progesterone withdrawal in the rat is the characteristic increase in plasma levels of 20α- hydroxypregn-4-en-3-one (20α-OH). 20α-OH is a metabolite of progesterone synthysized within the ovary by 20α-hydroxysteriod dehydrogenase. Thus, the sharp increase in peripheral blood levels of 20α-OH which occurs between Day 19 and Day 21 of gestation (Wiest et al, 1968) may be without functional significance, 20α-OH being merely a biproduct of the enzymatic process by which progesterone concentration in the peripheral plasma and uterus are lowered (Wiest, 1968).

Also occurring in close temporal association with progesterone withdrawal is an increase in plasma estrogen (Yoshinaga, Hawkins, and Stocker, 1969). Estrogen, which is maintained at low concentrations prior to the time of mid-pregnancy, begins to increase on about Day 15. At first slowly and then more rapidly, estrogen rises to a peak value on or shortly before the day of parturition. Similarly, peak levels of prolactin, FSH and oxytocin also seem to occur on the day of parturition. Prolactin titer, which is low until about Day 20 of pregnancy (Grindeland, McCulloch and Ellis, 1969; Kwa and Verhofstad, 1967), increases sharply on Day 22, the day of parturition. Unfortunately, plasma levels of FSH and oxytocin during pregnancy in the rat have not been assayed. However, Greenwald (1966) does report that significant release of pituitary FSH does not occur until day of delivery, and Fuchs and Saito (1971) have recently observed that the major depletion of pituitary oxytocin does not occur until after the expulsion of the first fetus.

The hypothesis that some of the hormonal changes which accompany the termination of pregnancy underlie the onset of maternal behavior is readily suggested by the observation that such maternal responses as nursing, retrieving, and nest building are typically initiated in the puerperal female in close temporal association with the occurance of these changes. The evidence in support of this hypothesis, although far from conclusive, is nonetheless highly suggestive. Terkel and Rosenblatt (1968), for example, have recently demonstrated that the substances carried in the plasma of the postparturient rat are capable of increasing the readiness of virgins to respond maternally to young. These authors reported that blood plasma removed from a puerperal rat within 48 hours after parturition and injected into a virgin rat could induce maternal behavior in this nulliparous female after only 48 hours of concaveation. A similar reduction in latency was not observed in nulliparous females after injection of either saline or diestrus blood plasma; these nulliparae taking, as is characteristic of such females, some six or seven days to respond.

Following this work, Terkel (1970) developed a method for effecting a continuous cross-transfusion of blood between two freely-moving rats. Using this technique to transfuse "maternal blood" he was able to reduce not only the latency with which his nulliparae responded to foster young but the high variability they had previously exhibited following discrete injections. Of course, these results of Terkel and Rosenblatt (1968) and Terkel (1970) provide some support for the hypothesis that the puerperal females' immediate interest in young is hormonally induced. Obviously, however, these studies do not offer any clues to which of the many endocrine changes occurring at or near the time of parturition might characteristically precipitate nurtural responsivity in the puerperal rat. This more specific question, has, of course, been the subject of considerable investigation. For example, many studies have sought to gain insight into the nature of the endocrine determinants of maternal behavior through manipulation of the blood concentration of various gonadal steriods and pituitary gonadotrophins in the nulliparous female. The aim of these studies was to induce immediate or near-immediate maternal behavior in the non-pregnant, non-lacting rat.

One of the first studies of this kind was performed by Wiesner and Sheard (1933). These authors injected both intact and ovariectomized nullipare with one or another of a battery of crude hormone extracts, prepared, respectively, from mare and human pregnancy urine, bovine corpora lutea, minced human placenta and bovine anterior pituitary and tested daily for retrieving. The authors found that only the anterior pituitary extract was effective in inducing retrieving, although even here only about 33 percent of the nulliparae tested responded maternally. Of course, a frequency of 33 percent is not an impressive finding. Moreover, in the present case interpretation of the results is particularly difficult because of the crude nature of the pituitary extracts employed, making it impossible to ascertain which pituitary hormone is responsible for behavioral effect.

In a later series of studies Riddle and his associates, having previously found that broodiness in fowl "seems to be induced only or chiefly by prolactin," attempted to determine whether prolactin--and a variety of other hormones and chemical agents as well--would induce maternal behavior in the rat (Riddle, 1957; Riddle, Hollander, Miller, Lahr, Smith, and Marvin, 1942; Riddle, Lahr, and Bates, 1935a; Riddle, Lahr, and Bates, 1935b; Riddle, Lahr, and Bates 1942). Nulliparous females between 60 and 70 days were used, some of which remained intact, while others were ovariectomized. After eliminating what Riddle, Lahr, and Bates (1942) called "normal reactors," that is, females which spontaneously showed interest in young during a series of 10-minute tests prior to treatment, each animal was injected daily for 10 days and then tested each day for 10 minutes with a single pup. If the pup was retrieved, the female was considered to have acted maternally.

Riddle, Hollander, Miller, Lahr, Smith, and Marvin (1942) found prolactin, intermedin, and luteinizing hormone to induce retrieving in some 50 to 80 percent of both their intact and ovariectomized females. In contrast, whole anterior pituitary extract was effective only in the intact females, while follicle stimulating hormone (with thyrotrophin), Pregnant Mare's Serum, "andrenotropin," and "Prolan" were each largely without effect. Among the steroid hormones used, progesterone, testosterone, and pellet implants of desoxycorticosterone (but not injections) were followed by retrieving in at least 65 percent of the animals tested. Phenol and thyroxine were also effective in inducing retrieving, but only in ovariectomized females. Esterone proved to have special properties--it terminated retrieving in animals that had begun to respond after injection of one or another of the above-mentioned substances.

Needless to say, these results are difficult to interpret. First, Riddle, et al., used a bewildering variety of compounds, some of which seemed effective in one type of experimental female and some in another. However, these authors do not believe that each of these agents functions to induce retrieving in the puerperal female. On the contrary, they suggest that prolactin excites, either directly or indirectly, the "sensorimotor mechanism" mediating maternal behavior, while the several other agents claimed effective in inducing retrieving in nulliparae may have induced retrieving through their respective release of this hormone. Second, Riddle, et al, failed to control the amount of experience with young each experimental subject received. A female that was unresponsive to young after a series of hormone treatments was given, after an interval of ten or fifteen days, a second, or even a third, exposure to young during a series of injections with another substance or hormone.

Obviously, because of the flaws in the experimental design of their studies, the conclusions of Riddle, et al., with regard to the role played by prolactin in the typical initiation of maternal behavior in the rat, require further confirmation. Unfortunately, however, the attempts to replicate the findings of Riddle, et al., have not met with success. Lott (1962), for example, injected .25 mg of progesterone in exactly the same manner as Riddle et. al. (1942), i.e. once daily for a period of 10 days. On Day 11 each experimental and control (oil-injected) female was tested with a single foster pup for 10 minutes daily. Under these conditions the virgin females did not respond maternally to the presence of young. Lott and Fuchs (1962) injected 40 IU of prolactin daily for 10 days and tested their females in the same manner as Lott (1962), Lott and Fuchs (1962) also failed to induce retrieving behavior in the nulliparous rat. Finally, Beach and Wilson (1963) injected 400 IU of prolactin per day for five days, following which not one but six foster pups were proffered daily for three consecutive days. In a second experiment these authors subjected non-pregnant, non-lactating multiparous rats to an injection regimen of either 20 days of estrogen followed by 10 days of prolactin or 21 days of estrogen followed by seven days of both progesterone and prolactin. Following their hormone treatments, the subjects were tested for their willingness to retrieve young. The results of these tests, Beach and Willson report, indicate that there is no significant difference between the hormone treated females and their control counterparts.

The failure of Lott (1962), Lott and Fuchs (1962) and Beach and Willson (1963) to confirm the findings of Riddle et. al. has several alternative explanations. Lott, Lott and Fuchs and Beach and Willson used strains of rats different from the strain employed by Riddle et. al.; they also used hormones of greater purity. Either of these factors could conceivably be responsible for the failure of these authors to replicate the findings of Riddle et. al. Of course, another explanation is that the dosage, combination or sequence of hormones tested was unable to induce maternal behavior in the nulliparous female. Still another possibility is that the hormones employed by Lott, Lott and Fuchs and Beach and Willson have no effect on the initiation of maternal behavior in the rat. And finally, it is also possible that the authors' procedures were not sufficiently sensitive to detect any reduction in latency of initiation of maternal behavior. That is, since neither Lott, Lott and Fuchs nor Beach and Willson allowed their subjects to have continuous exposure to young, it is possible that the failure to replicate the results of Riddle et. al. may have been the result of an inadequate level of stimulation from the young. However, regardless of the reasons for these contradictory results, it is evident that the attempts to induce maternal behavior in the nulliparous female have provided little clear information as to the nature of the hormones necessary for the induction of immediate maternal responsivity in the rat.

Perhaps a more fruitful approach to the determination of the specific agents responsible for the hormonal induction of maternal behavior would involve the disruption of some of the near term endocrine events in the preparturient rat. That is, if the hormonal events occurring at about the time of parturition are indeed responsible for puerperal females' responsivity to young than preclusion of one or more of these endocrine events should result in the disruption of maternal behavior.

Just this hypothesis has been tested by Obias (1957) with regard to the near term increase in gonadotrophin release. Obias (1957) hypophysectomized six female rats on Day 13 of pregnancy, which perforce, precluded any further secretion of hypophysial prolactin. Obias reported that his procedure, while it did disrupt parturitive behavior, did not exert any significant effect on maternal behavior.

There appear to be two plausable explanations for this result. First, it is certainly possible that the measures used by Obias (1957) were not precise enough, nor the number of subjects large enough, to reliably reveal any deficiencies in maternal behavior. The likelihood of this possibility is increased by the fact that of the six subjects tested, only two females retrieved immediately. Of the remaining four subjects, one took five days to begin retrieving, and it is not clear when the other three females began to carry young back to the nest. And second, it is also possible, if the results of Obias (1957) are confirmed, that the near term changes in pituitary gonadotrophin release are irrelevant to the initiation of maternal behavior in the puerperal rat. Obviously, the question still remains as to the nature of the hormonal events which are responsible for the puerperal females immediate interest in young.

Perhaps some of the hormonal events which underlie parturition may be responsible for the puerperal rat's characteristically short latency of initiation of maternal responsivity to young. Of course, the hormonal events which mediate parturition are complex and at the present time only incompletely understood. However, there is general agreement that associated with the birth process, and at least partly responsible for the occurrence of that process, is a decrease in the level of progesterone and a relative increase in the level of estrogen (Schofield, 1957).

It has been suggested by several authors (e.g., Moltz and Wiener, 1966; Moltz, Lubin, Leon, and Numan, 1970; Zarrow, Farroq, Denedberg, Swain, and Ross, 1963) that it is this shift in the gestational ratio of estrogen to progesterone, in the direction of estrogen dominance, which is responsible (in some part at least) for the virtually immediate instigation of maternal attention in the puerperal female. This suggestion was recently subjected to experimental verification in a study conducted by Moltz and Wiener (1966). These authors manipulated this presumably focal endocrine mechanism by subjecting both primiparous and multiparous rats to a bilateral ovariectomy some twelve to fifteen hours before term. These same animals were also delivered by caesarean section, since it was found that once ovariectomized, they could not give birth normally. About 18-23 hours after the operation, foster pups six to fifteen hours of age were proffered to each female. Measures of nursing, nest building, and retrieving were taken each day for 21 days following the introduction of young. It was found that virtually 100 percent of the multiparous subjects raised their pups to weaning while only 50 percent of the primiparous subjects did so; the remaining 50 percent either cannibalized or simply allowed their young to remain scattered. These results suggest that the hormonal effects of ovariectomy (i.e., a reduction in near-term plasma levels of estrogen, and in view of the positive feed-back-effect of this steroid on gonadotropic discharge [Chen and Meites, 1970; Everett, 1966; Kanematsu and Sawyer, 1963 (a), (b); Meites and Nicoll, 1966; Rothchild, 1965] perhaps prolactin as well) can disrupt, at least in many primiparous animals, the normal initiation of maternal behavior. Moreover, these same results indicate that previous parity may play a role in mitigating the deleterious behavioral effects of an exogenously induced hormone imbalance.

Of course, the presence of this difference in maternal behavior of primiparous and multiparous rats under the experimental condition employed by Moltz and Wiener (1966) quite naturally raises the question of what role, if any, previous parity plays in the expression of maternal behavior under normal breeding conditions. It has been recognized for quite some time that the expression of maternal behavior in the primiparous female is sufficient for the maintenance of the young (Wiesner and Sheard, 1933). But, is the primiparous female as proficient in the execution of nursing, nest building, and retrieving as her multiparous counterpart? To answer this question, Moltz and Robbins (1965) compared the manner in which primiparous and multiparous rats build nests, nursed their young, and retrieved. They found that the primiparous rat was every bit as proficient as her multiparous counterpart in executing each component of the maternal complex. This result confirms the findings of Beach and Jaynes (1956) and Wiesner and Sheard (1933) concerning retrieving in multiparous and primiparous females.

However, the results reported by Beach and Jaynes (1956) were later called into question by Carlier and Noirot (1965). Carlier and Noirot (1965) attempted to correct what they felt to be a flaw in the testing method employed by Beach and Jaynes (1956). Instead of handling the mothers whenever the experimental procedure demanded that the mother be separated from her litter, Carlier and Noirot induced her to enter a side alley of the cage for a food reward. They found that the primiparous female spent more time retrieving than the multiparous female, relating this to the tendency of the primiparous female to pick the pup up by one of the extremities. This method of holding young is less efficient than grasping the pup by the middorsal region and often results in the primiparous female dropping the pup. These results, however, are open to question because of poorly controlled experimental conditions. Since Carlier and Noirot (1965) compared the first and second breeding experiences of the same subjects in the same apparatus, it is possible that the female was more adapted to the maternity cage during the second breeding experience than during the first, and consequently was less likely to engage in behavior irrelevant to retrieving. Considering all the evidence just presented, it would seem that, at least under normal breeding conditions, it is doubtful that previous parity has any effect on maternal behavior. This, however, does not imply that previous parity will also be without effect under breeding conditions which are made to depart from the normal (Moltz and Robbins, 1965; Rosenblatt and Lehrman, 1963). For example, Seitz (1958) reports that the intensity and efficiency of maternal behavior was greater during the second breeding episode if the female's first litter was large (fifteen to sixteen pups). However, if the first litter was small, then no significant change was found in maternal behavior with second litters, even if those subsequent litters were large. Apparently previous breeding experience enables the female to better meet the demands of a large number of pups. And further, as already mentioned, previous parity, under some conditions at least, seems to be a variable of significance in the initiation of maternal behavior. Specifically, it will be recalled that Moltz and Wiener (1966) found that although virtually 100 percent of their ovariectomized multiparous females acted maternally, only 50 percent of their primiparous females did so.

To summarize, it appears that there may be at least two factors which play a determining role in the puerperal female's rapid initiation of maternal responsivity of young. First, there is the change in the ratio of estrogen to progesterone in the direction of estrogen predominance. Second, there is, at least under some breeding conditions, the previous parity of the animal. It is the purpose of the present experiment to test the role of both of these factors in the initiation of maternal behavior in the puerperal female.

As already mentioned, associated with the birth process in the rat is a critical shift in ovarian output resulting, inter alia, in a decrease in progesteron titer. Considering both the time course of this decrease (cf. Grota and Eik-Nes, 1967) and the fact that maternal behavior is initiated typically at a point of low progesterone concentration, it is unlikely that the ovariectomy performed by Moltz and Wiener (1966) could have produced the observed behavioral deficit merely as a result of having effected a still further reduction in progesterone titer. Indeed, not only does the present author consider this particular consequence of the operation to have been irrelevant for the results obtained but he would further hypothesize that if the characteristic near-term decrease in blood progesterone were to be prevented from occurring, then the initiation of maternal behavior in turn would effectively be precluded (cf. Richards, 1967; Rothchild, 1965). Accordingly, the present experiment was designed to determine whether such manipulation of progesterone would in fact interfere with the instigation of maternal behavior and, if so, whether the interference thus exerted would once again be manifested differentially in the primiparous and multiparous female.

METHOD

Subjects

The Ss were female rats of the Wistar strain, most of whom were born and reared in the author's laboratory. They were maintained under a relatively constant ambient temperature of 20° C and under a day-night cycle consisting of 12 hours light and 12 hours darkness. This day-night cycle was reversed to permit convenient observations of the Ss during the peak portion of their activity cycle (i.e. during the hours of darkness).

Maternity Cages

About 24 hours after the discovery of sperm in the vaginal tract, each subject was transferred from her home cage (Wahmann LC 375/N cage) to a specially designed maternity cage identical with those employed by Moltz and Robbins (1965) in their normative study. These cages measured 22 X 20 inches and were equipped a front panel of clear plexiglas to allow unobstructed view of the interior. The cages were also equipped with a side chamber separated from the main compartment by means of a solid partition. A guillotine door in the center of the partition provided a means of exit and re-entry whenever the experiment required separation of a female and her litter. A 7-w bulb, connected through a Variac, was used to illuminate the interior of each cage during the time measurements were taken. The intensity of illumination was just sufficient to afford observation of the occupant of the cage without disturbing her behavior.

Breeding Histories

The multiparous females had raised two litters prior to their inclusion in the present experiment. During the time they were acquiring their breeding experience, these *S*s had remained undisturbed except for three occasions permitting, respectively, the placement of nesting material in their cages shortly before parturition, the reduction of the litter to the standard number of six pups on the day after parturition, and the weaning of the pups 21 days later. The average age of these *S*s at the beginning of the study was comparable to that of the multiparous females studied by Moltz, Robbins and Parks (1966) and Moltz and Wiener (1966), i.e. approximately 250 days old.

The primiparous females included in the present experiment had each been housed in individual cages from the time of weaning. Although actually nulliparous when chosen as *S*s, these females will henceforth be designated as primiparous in accord with their state of parity during the regimen of observations to be described. Part of this group was equated for age with the multiparous group, and part was introduced to the experimental situation at about 150 days of age.

At the beginning of the experiment, each female was exposed to a male for six hours a day. This was continued each day until sperm was found in the vaginal tract. The presence of sperm marked the day of impregnation, the first hour of cohabitation being designated arbitrarily as the first hour of pregnancy.

Surgical Procedure

On the afternoon of Day 21 of pregnancy, all *S*s were subjected to a caesarean section. Caesarean delivery was necessary here since a pilot study had indicated that exogenous progesterone at the dosage used in the present experiment effectively blocked parturition. Clean surgical conditions and ether anesthesia were used during the operation.

The hair of the back was shaved and a dorsal midline incision of about 10 cm. was made. The skin was then separated from the fascia by blunt dissection. A small incision of approximately 2 cm. was then made in the lateral musculature. One cornu of the uterus was withdrawn from the body cavity after which it was incised for approximately 2 cm. along its antimesometrial surface. Through this incision each fetus together with its placenta was expelled. After the cornu was emptied completely, it was sutured and placed back into the body cavity. The same procedure, with the exception of the skin incision, was employed to expel the fetuses and placentas from the contralateral cornu.

Fostering Procedure

A fostering procedure identical to the one employed by Moltz, Robbins, and Parks (1966) was used in this experiment. (Foster pups, rather than surgically-delivered pups, were used because of the depressive effects of ether anesthesia on fetal respiration.) A litter of six normally delivered young, six to twenty hours of age, was given each female approximately 24 hours post-operatively (Day 22 post-coitum). These young, it must be noted, had already been cleaned and nursed by their own mothers prior to the time they were proffered.

Injection Procedure

Five groups of rats were employed. Groups EP-1 and EM consisted respectively, of twenty primiparous females 150 days old and ten multiparous females 250 days old. Group EP-2 consisted of ten primiparous females of the same mean age as the multiparous _Ss_ (250 days). Beginning at nineteen days post-coitum, every _S_ in each of these groups was injected subcutaneously twice daily (10 A.M. and 6 P.M.) with 2 mg. of progesterone in 0.1 cc sesame oil. These injections were continued through Day 23 post-coitum.

The remaining two groups, C-1 and C-2, served as controls, and like their experimental counterparts were subjected to caesarean section on Day 21 and foster young on Day 22. Group C-1 consisted of primiparous females, approximately 150 days of age, injected with sesame oil alone twice daily from Day 19 to Day 23; Group C-2, also primiparous and about 150 days old, was given progesterone twice daily, but _only after_ each animal had been offered and in turn had accepted foster young. This regimen of steroid injection extended from Day 24 through Day 28 and was undertaken to determine whether, in addition to the effect hypothesized above, progesterone would also interfere with maternal behavior once it had been already established. It is worth noting that the females of Group C-2 received their progesterone at a time coincident with the postpartum period reported to encompass the highest endogenous output of the steroid (Grota and Eik-Nes, 1967). Consequently, any deleterious behavioral effects of progesterone, such for example, as might derive from its well-known anesthetic or thermogenic properties, should be most pronounced in these females, the females presumed to have highest total blood concentration of progesterone. Additional control groups were not employed since Moltz, Robbins, and Parks (1966) had already reported that caesarean delivered, 250 day old, primiparous and multiparous rats do not differ from normally delivered animals with regard to either the incidence or efficiency of maternal behavior. In view of these results, it was decided that subjecting 250 day old primiparous and multiparous females to the same regimen as Group C-1 was unnecessary.

Measurements and Observations

The principal measure of maternal behavior in the present study was pup survival. Of course, since rat pups of less than one day of age will inevitably die if left unattended, the survival to weaning of even one member of a litter reliably reflects the presence of at least a minimal level of maternal behavior. However, although maternal behavior is a prerequisite of pup survival, failure to rear a litter does not invariably indicate inadequate nurtural responsivity. Consequently the following procedures were employed to determine whether each S attended to her foster young.

At least ten hours prior to the time each S was scheduled for caesarean delivery, she was given nesting material, which consisted of a packet of some 200 paper strips. Shortly before the operation and again just prior to the time the foster pups were proffered, the presence or absence of a maternal nest was noted. On the following day (Day 23) and again 24 hours later the experimental Ss, as well as the Ss of Group C-1, were observed once again. In each case whether the female had constructed a maternal nest, retrieved young to that nest and was huddling them was recorded. The females of Group C-2 were also observed, but only on the days they received progesterone, Days 24-28.

Immediately after each observation the pups were touched to determine whether they were warm or cold. During this procedure the observer wore a thin plastic glove to prevent the pups from being contaminated by human odor. Since for the first few days of life rat pups are poikilothermic, and are thus warm only if the female maintains an adequate nursing posture, this measure, although crude, does reflect maternal attention. At this time, the pups were also weighed and the number of pups remaining in the litter was recorded.

Histological Analysis

As a control procedure to determine whether or not females of Group EP-2 maintained a level of mammary activity equivalent to that of Group EM, four multiparous and four primiparous females of equivalent age were subjected to the same experimental regimen as Groups EM and EP-2, respectively. However, instead of being given pups, they were sacrificed some twenty hours post-operatively to provide mammary tissue for histological examination.

Mammary tissue was removed from the pectoral and inguinal regions, fixed in Bouin's fluid, embedded in paraffin, sectioned at 10μ and stained with hematoxylin and eosin. The slides prepared in this manner were then examined for mammary activity by rating each slide with respect to the degree to which the aveoli and ducts were distended with secretion products. Four persons independently rated the same set of eight slides. Each slide, which contained several samples of mammary tissue removed from a single subject, was marked with a code number so that all rating would be blind. Every rater focused at random on six different fields and rated the field on a four-point scale, 0 being no secretion and 3 being maximal secretion. The scale values for each slide were averaged across fields and across raters. (See Meites and Nicoll, 1959, for a complete description of the scale.)

RESULTS

Of the twenty primiparous females in Group EP-1 only, nine reared litters to weaning; the remaining eleven females either cannibalized their foster litters on the day they were proffered or allowed them to remain scattered over the floor of the cage. In Group EP-2, the group of primiparous females equated for mean age with the multiparous subjects, only one of ten females successfully raised a litter to weaning. Four of the remaining nine females allowed their litters to remain scattered with the result that the litters died within two to three days. Five of the nine mothers, however, did evince retrieving and nest building, although they failed to adopt a posture which would facilitate nipple attachment. Instead, these females were most often observed to be lying flat against the cage floor with pups lying, not under them, but to one or the other side. These litters were not nursed and died of starvation or were found cannibalized after several days. This difference in behavior between EP-1 and EP-2 is reflected in a significant difference in the number of pups surviving to weaning in each group (40 percent as against 7.7 percent; $X^2 = 20.0$, $P < .01$ [See Table 1]), although the difference in the number of litters reared to weaning did not reach significance ($X^2 = 2.3$, $P > .05$). Perhaps this difference in the number of litters reared to weaning would have been statistically significant had the number of females in Group EP-2 been larger.

In marked contrast to the two experimental primiparous groups just mentioned is the behavior of the experimental multiparous group (Group EM). In this group, nine of ten females raised foster litters to weaning. Moreover, these same nine females were all observed to maintain their nests and nurse their young. The difference between the number of litters reared in Group EM and Groups EP-1 and EP-2, respectively, is significant below the .01 level (X^2 = 3.90 and 9.80) as is the difference between these groups with regard to the proportion of young surviving, 40.0 percent as against 80 percent; X^2 = 24.1 and 7.7 percent as against 80 percent; X^2 = 43.1 (See Table 1). However, among those litters which did survive, there are no significant differences between these groups with regard to the number of pups per litter or the weight of the pups at weaning (See Table 1).

Of the ten females in Group C-1, all but one reared young to weaning; those in Group C-2 despite the administration of progesterone during a time of high endogerous production of this steriod, all continued to nurse, maintain their nests, and retrieve. Among those litters surviving at weaning, there was no significant difference between the control and experimental group with regard to either the number of pups per litter or the average weight of those pups. Evidently progesterone interferes only with the initiation of maternal behavior and then only in the primiparous female.

The results of the histological examination indicated that there was equal production of secretion products in both Groups EP-2 and EM. Group EP-2 had an average Meites-Nicoll scale value of 0.86, and Group EM an average of 0.84 ($t = 0.25$, $P > .05$). This result suggests that the failure to nurse on the part of five members of EP-2 cannot be attributed to a lack of milk production.

TABLE 1

AVERAGE PUP WEIGHT, PROPORTION OF PUPS, & NUMBER OF PUPS PER LITTER SURVIVING AT WEANING.

Group	N	6 Pups	5 Pups	4 Pups	3 Pups	2 Pups	1 Pup	0 Pups	Total No. of Litters Surviving	Average Weight	Proportion of Pups Surviving
EP-1	20	7	1	0	0	0	1	11	9	46.6 gms	48/120 (40.0%)
EP-2	10	0	0	1	0	0	0	9	1	---	4/60 (7.7%)
EM	10	7	1	0	0	0	1	1	9	50.6 gms	48/60 (80.0%)
C-1	10	5	3	1	0	0	0	1	9	49.7 gms	49/60 (81.7%)
C-2	10	8	2	0	0	0	0	0	10	47.6 gms	58/60 (96.7%)

No differences between the weight of the pups in any two groups yields a significant t at the .05 level.

DISCUSSION

The results of the present study indicate that the administration of exogenous progesterone resulted in a failure to initiate maternal behavior in over half of the primiparous females. In contrast, virtually all of the multiparous females were found to respond maternally to the presence of young. These findings raise several questions. First, through what mechanism did progesterone act to preclude the initiation of maternal behavior in the primiparous rat observed in the present study. Second, why did progesterone administration fail to inhibit maternal behavior in some of the primiparous females? Third, in what manner did previous parity function to mitigate the effects of this exogenously induced steroidal imbalance? And fourth, what role, if any, does progesterone play in establishing the level of maternal responsivity to young characteristic of the puerperal female.

An answer to the first question is difficult to propose with any great certainty since there is no research which bears directly on the manner in which progesterone might act to disrupt the instigation of maternal attentiveness in the primiparous female. However, on the basis of the available evidence, it would appear that not all of the possible channels through which progesterone may have acted are equally likely to have been responsible for this behavioral deficit. For example, it is unlikely that the failure of over 50 percent of the females of Group EP-1 to behave maternally was a result of a debilitating effect of progesterone, since the females of Group C-2 all raised their young to weaning. It will be recalled that this group received their progesterone at a time coincident with a high engogenous output of this steriod and thus, presumably, had the highest total blood concentration of progesterone. Consequently, if progesterone was exerting its influence on the initiation of maternal behavior through a depression of general activity then, it is probable that Group C-1 would have exhibited the most profound behavioral deficiency.

It is also improbable that progesterone mediated the disruption of maternal behavior observed in the present study through the preclusion of the near-term rise in circulating levels of estrogen and prolactin, i.e., those hormones which may be at least partially responsible for the immediate responsivity to young characteristic of the puerperal rat. Although, chronic administration of progesterone, in the non-pregnant rat, results in a subsequent reduction in estrogen secretion (Rothchild, 1965), the results of recent studies would tend to indicate that exogenous progesterone does not significantly reduce plasma levels of estrogen in the pregnant rat. Progesterone administration in the cyclic rat inhibits estrogen secretion through suppression of FSH and LH release (Flerko, 1966; Labhsetwar, 1969; and Rothchild, 1965). Since under normal breeding conditions there is neither extensive FSH nor LH (Greenwald, 1966; Rebar, Nakane, and Midgley, 1969) release in the preparturient rat until after the Day 21 increase in estrogen secretion has already begun (Yoshinaga, Hawkins, and Stocker, 1969), it is not likely that exogenous progesterone affected the inhibition of the initiation of maternal behavior reported in the present study by blocking estrogen secretion. However, it must noted, that the possibility of a progesterone induced decrease in estrogen secretion, and of course its concomitant behavioral effects, cannot be entirely eliminated.

Similarly, the ability of large doses of progesterone to partially attenuate the facilitory effects of estrogen on prolactin release (Chen and Meites, 1970) cannot be ignored. However, the likelihood of the exogenous progesterone administered in the present study being consequential to the complete preclusion of the near-term rise in prolactin secretion should not, for several reasons, be regarded as high. First, progesterone even at high dosage does not totally abolish estrogen-stimulated prolactin release (Chen and Meites, 1970). Second, there is considerable evidence that both endogenous and exogenous progesterone can, depending on estrogen level, facilitate rather than inhibit prolactin release (see Rothchild, 1965, for review). And third, there are several studies which have indicated that prolactin secretion can continue unabated in the presence of high concentrations of progesterone. Rothchild (1960) for example, found that in rats, despite progesterone treatment, autotransplanted pituitary glands secreted enough prolactin to maintain and increase the size of the corpora lutea. Moreover, it is well established that in the lactating rat high blood titers of both progesterone and prolactin co-exist. Thus on the basis of the above evidence, it is reasonable to assume that the behavioral deficit observed in the present study should not be attributed to a preclusion of prolactin release. Certainly there must be other channels which are more likely to have mediated the progestogenic disruption of the initiation of maternal behavior observed in the present study.

Perhaps the preclusion of central nervous system (CNS) binding of either prolactin or estrogen by high titers concentration of progesterone is just such a channel. Unfortunately this suggestion, at least with regard to estrogen, is not supported by the available evidence. The selective binding of ^{3}H estradiol to CNS sites, i.e., portions of the septum, preoptic area, and hypothalamus (Eisenfeld and Axelrod, 1965; Kato and Vilee, 1967a, 1967b; McGuire and Lisk, 1969; Pfaff, 1968, Stumpf 1969), is not inhibited by high doses of progesterone (Eisenfeld and Axelrod, 1966). Whether the CNS binding of prolactin is similarly unaffected by high titers of progesterone is uncertain, since even the differential uptake of this hormone by the CNS has not been demonstrated. Without such information, the possibility that progesterone may have prevented the CNS binding of prolactin cannot be excluded, but neither can it be given great weight. Obviously, the question still remains as to the manner in which exogenous progesterone effected the behavioral deficit reported in the present study.

Perhaps progesterone can be conceived of as having the ability to selectively suppress those areas of the brain which typically mediate the initiation of the maternal complex of behaviors. There is some evidence to support this hypothesis, i.e., that progesterone in the present study blocked the instigation of maternal responsivity to young by inhibiting the activity of discrete regions in the CNS but it is only of a suggestive nature. For example, the finding that gestational levels of progesterone in the female rat do not preclude the CNS binding of estrogen (Eisenfeld and Axelrod, 1966) but do inhibit estrogen induced sexual behavior (Powers and Zucker, 1969) would suggest that progesterone is acting by suppressing those neural centers which control sexual behavior. This result, while having no direct bearing on the present study, does raise the possibility that progesterone may inhibit the initiation of maternal behavior in much the same manner as it precludes the occurrence of sexual behavior. Further evidence supporting the suggestion that progesterone can exert a suppressive effect on the CNS is provided by the fact that progesterone, when implanted in the preoptic area of the cat (Heuser, Ling and Kluver, 1967) or injected systemically in the rat (Beyer, Ramirez, Whitmoyer, and Sawyer, 1967; Komisaruk, et al., 1967; Ramirez, et al., 1967), induces a sleep-like EEG and a reduction in single unit activity. However, since Komisaruk, et al., (1967), have also reported that approximately one third of the hypothalamic fibers studied did not reduce their rate of firing after progesterone administration, it is obvious that not all areas of the brain are equally sensitive to progestogenic inhibition.

This observation of Komisaruk, et al., (1967) quite naturally raises the question of whether the neural centers which are responsible for the initiation of maternal behavior are located in those areas of the CNS which are responsive to progesterone's inhibitory effect. Unfortunately, this question cannot be answered at the present time, for several reasons. First, the CNS locus of the maternal mediating system has not yet been adequately determined. Second, although Komisaruk, et al., (1967) recorded from several hypothalamic sites, i.e., anterior, ventromedial, dorsal, and arcuate nuclei, they failed to observe any localized depression of neural activity in response to progesterone administration. And third, the areas of the CNS which are responsive to progesterone are very difficult to determine. In only one study (Smith, Weick, and Davidson, 1969) has a specific area of the rat CNS been shown to be sensitive to implanted progesterone. In this experiment Smith, et al., (1969) reported that the inhibition of LH release by CNS implant of progesterone was restricted to the region of the median eminence. Nor can the pattern of progesterone uptake in the CNS provide any clue to the site of action of this hormone, since no area of the rat CNS has been demonstrated to have a special affinity for radioactively labeled progesterone (Laumas and Farooq, 1966; Raisinghani, Dorfman, Forchielli, Gyermek, and Genther, 1968; Seiki, Higashida, Imanishi, Miyamoto, Kitagawa, and Kotani, 1968; Seiki, Miyamoto, Yamashita, and Kotani, 1969).

Certainly it is obvious from the above discussion that until more information is available, there must remain some doubt as to the manner in which progesterone effected the inhibition of maternal behavior reported in the present study. But despite some reservations, the current evidence appears to favor the conclusion that the major mechanism through which the exogenous progesterone administered in the present study blocked maternal behavior was the suppression of the maternal mediating centers of the CNS. However, the results of the present study suggest that this presumed inhibitory effect of progesterone is influenced by several factors.

One such factor would seem to be the female's parity. This effect of parity is clearly evident in the present study, since virtually all of the progesterone treated multiparous females raised foster young to weaning while only 50 percent or less of the hormone injected primiparous females were successful mothers. However, just why this significantly smaller incidence of immediate acceptance was found among the primiparous as compared with the multiparous females is a question which remains to be answered.

One plausible explanation for this variability in the behavioral effects of exogenous progesterone involves the idea of threshold. More specifically, if, as Moltz and Wiener (1966) suggest, the maternal mediating substrate has threshold characteristics unique, in part at least, to each female, then individual differences in tissue excitability would exist among primiparous animals, with some primiparous females having lower arousal thresholds than others. Moreover, assuming that previous parity increases the level of tissue excitablity, it would be expected that the average level of tissue excitability prevailing among multiparous females would be higher than that prevailing among primiparous animals. The threshold distribution of a multiparous group would then have a lower mean value than that of a primiparous group although the distributions of these two groups would overlap because of the individual differences in tissue excitability which are unrelated to the occurrence of parity.

This kind of primiparous-multiparous difference could not manifest itself in behavior under normal breeding conditions, since the hormonal events which typically occur at about the time of parturition would provide a level of stimulation above that demanded for arousal by even the most elevated threshold. However, if these critical near-term hormonal events were attenuated, then only tissue meiators having low threshold of arousal would respond.

It is possible that just such a limited disruption of key hormonal events occurred in the present study. That is, although progesterone administration did block the abrupt decline in plasma levels of this steriod which typically occurs on Day 20 post-coitum (Hashimoto, et. al., 1968; Wiest et. al., 1968), the dosage of progesterone employed in the present study may not have been sufficient to prevent a partial decrease in progesterone titer. This attenuated decrease in progesterone, assuming it occurred, would have certainly been consequential to a level of activation of the maternal mediating system below that typical of the postparturient rat. Under these conditions, the results of the present study can easily be conceptualized in terms of threshold. That is, the ability of this reduced level of activation of the maternal mediating substrate to disrupt the initiation of maternal behavior in the primiparous but not multiparous group is, presumably, a consequence of the low arousal threshold prevailing among multiparous females.
The immediate initiation of nurtural responsivity evinced by half of the primipare can be attributed to the individual differences in tissue excitability which result in some primiparous animals having thresholds which fall within the lowered threshold distribution of the multiparous population.

Of course, the above explanation of the effects of exogenous progesterone on the initiation of maternal behavior in the rat is only a speculative proposal. More research will be necessary to clearly establish both the existence of individual differences in the sensitivity of the maternal mediating substrate and the effects of previous parity on the ability of progesterone to render this neural substrate refractory to stimulation. Moreover, further research will also be necessary to determine if other factors, such as the age of the animal, can modify the effect of progesterone on the tissue mediating the initiation of maternal behavior.

Certainly, from the behavior, in the present study, of some of the primiparous females equated for mean age with the multiparous subjects (Group EP-2), it would appear that age of female does affect the ability of progesterone to influence the sensitivity of the neural substrate governing the initiation of maternal behavior. As previously noted, it was observed that in Group EP-2 only one out of ten females successfully raised a litter to weaning. This is, of course, in contrast to the survival of nine out of twenty litters reared by the 150 day old primiparous females of Group EP-1. Further, it was also observed that the behavior of some of the females in Group EP-2 differed markedly from that of their EP-1 counterparts. That is, although five of the older primiparous rats did build nests, and were seen to be in these nests with their foster young, they did not assume a posture which would facilitate nipple attachment. These females, rather than adopting a nursing posture would lie with their ventral surfaces pressed against the floor of the cage.

These observations suggest that the lack of pup survival in Group EP-2 was the result of the failure of some of the females in this group to assume a nursing posture. This hypothesis, although attractive, cannot be clearly established since it is not certain that there was equal milk letdown in both Groups EP-1 and EP-2. However, this inability to unequivocally determine the cause of death of the foster young of Group

EP-2 does not have any bearing on the more relevant question of why five females in this group did not huddle their young.

Clearly the disturbance in nursing behavior evinced by these EP-2 females is not likely to be consequential to either the stress of the operation or to their isolation from the time of weaning until their participation in the present study since Moltz et. al. (1966) found these events to be irrelevant to the typical initiation of maternal behavior in the rat. Perhaps, considering these findings of Moltz et. al. (1966), the most probable explanation for the behavior of these five EP-2 females is that age and lack of breeding experience somehow interact to decrease the tissue excitability of at least one portion of the maternal mediating system. This conclusion, although highly speculative, is at least indirectly supported by the observation that sensitivity of the hypothalamic sites regulating ovulation (Clemens, Amenomori, Jenkins, and Meites, 1969) and appetite (Kennedy, 1963) change in old age. Of course, any age dependent decrease in sensitivity of the maternal substrate would only appear in nulliparous rats, since it is probable that previous parity decreases the threshold of those neural centers presumed to be responsible for the initiation of maternal behavior.

In summary then, the results of the present study suggest that the action of progesterone on the initiation of maternal behavior should be conceived of in the following way: While existing at high titers, progesterone elevates the activation threshold of the maternal mediating system, but with the decline of this steroid, the sensitivity of this neural substrate to those hormonal events which are typically responsible for the initiation of maternal behavior increases. Consequently, any near term progesterone administration prevents the activation threshold of these neural centers from reaching the low level typical of the postparturient rat. However, whether this progesterone induced change in threshold is actually evinced in behavior is a function of both the blood concentrations of progesterone and excitability of the neural substrate mediating the initiation of maternal behavior.

The above conceptualization obviously deals only with the inhibitory effects of progesterone even though authors such as Rothchild (1965) have suggested that progesterone can both facilitate and inhibit the initiation of maternal behavior in the rat. Such a biphasic effect of progesterone has been well documented in species other than the rat and for behaviors outside the maternal complex. In the rabbit, although several weeks of progesterone and estrogen injections can induce maternal nest building behavior, maternal behavior will not occur if progesterone administration is not discontinued before estrogen treatment is terminated (Zarrow et. al., 1963). Similarly, in the rat (Lisk, 1969; Nadler, 1970; Powers and Zucker, 1969; Zucker, 1967), guinea pig (Zucker, 1966; Zucker and Goy, 1967), and mouse (Edwards, 1970), progesterone has been demonstrated to possess both facilitatory and inhibitory effects on the occurrence of sexual behavior. But, at the time the present study was conducted, there was no direct evidence to indicate that progesterone also exerts a biphasic influence on the initiation of nurtural responsivity in the rat.

The more recent findings of Moltz, Lubin, Leon, and Numan (1970) however, do suggest that progesterone withdrawal may facilitate the initiation of maternal responsivity in the rat. These authors injected ovariectomized rats with estradiol (once daily from Day 1 through Day 11, 12 ug per injection), progesterone (twice daily on Days 6 through 9, 3 mg per injection), and prolactin (the evening of Day 9 and the morning of Day 10, 50 IU per injection). Each female was given six foster pups on the afternoon of Day 10--approximately twenty hours after the progesterone had been withdrawn. The pups remained until the following morning, at which time a fresh litter was substituted. This procedure continued until maternal behavior was displayed, or failing maternal behavior, until seven days had elapsed. All ten nulliparous females subjected to a hormone schedule showed full maternal behavior at between 35 and 40 hours from the time the pups were first proffered. In contrast, control females given, respectively, only two of the three "inductor hormones" (the vehicle in each case having been substituted for the hormone omitted), or simply injected with all three vehicles, showed marked variability in onset and, of course, a significantly higher median latency.

On the basis of these findings, Moltz et. al. (1970) have suggested that progesterone has a biphasic effect on the neural substrate which mediates maternal responsivity to young in the rat. That is, progesterone, while it exists at high titers, as it does during the larger part of pregnancy, elevates selected activation thresholds in the central nervous system. Being chracteristically refractory to endocrine arousal, the substrate mediating maternal behavior is at this time rendered even more refractory. But on Day 15 of pregnancy plasma progesterone typically begins to decrease (Hashimoto, et al., 1968) and concurrently thresholds within the maternal mediating system start to decline. As this decline continues, the substrate comes to reflect what Kawakami and Sawyer (1959) called the "rebound from progesterone dominance" which is an after-reaction to progesterone that carries activation thresholds to levels lower than normal. For the maternal mediating system, this is the level--reached more or less coincident with the birth process--at which it becomes responsive to hormonal influence, particularly to the influence of estrogen and prolactin. These hormones functioning synergystically and at the concentrations conditioned by the near-term secretory rates, are able to excite the mediating system, making it immediately responsive to the sight, sound, and odor of young. Thus affected, the puerperal female can respond as soon as the young emerge from the birth canal.

Certainly, this proposal of Moltz, et. al. is in accord with the findings of most of the recent research, although there are several studies which are not consistent with this model. For example, Obias (1957) found that midterm hypophysectomy did not disrupt normal postparturient maternal behavior. If this result should be confirmed it would suggest that other factors, such as placental prolactin, may play a role in the initiation of maternal behavior. Under these conditions, the Moltz model would have to be modified. Similarly, the observations of Terkel (1970) cannot be explained by the Moltz model as it is presently constituted. Terkel, it will be recalled, reported that six hours of continuous cross-transfusion of blood between nulliparous and postparturient rats can reduce the latency with which the nulliparae respond to foster young. Considering both the fact that this transfusion began immediately after the puerperal female completed parturition and that progesterone titer is typically low at this time (cf. Grota and Eik-Nes, 1967), it is not possible that these nulliparae experienced a decline in progesterone level. Perhaps, it is not progesterone withdrawal itself, but some of the endocrine consequences of progesterone decline which facilitate nurtural responsivity in the rat.

It is evident from the above discussion that the Moltz model cannot, in its present form be regarded as a definitive explanation of hormonal induction of maternal behavior in the rat. But, because of the many gaps in our knowledge, it is likely that a good deal more research will be required before any model will be able to completely explain just why the postparturient rat rapidly initiates maternal behavior.

REFERENCES

Adamson, U., Eliasson, R., and Wiklund, B. "Tachyphylaxis in Rat Uterus to Some PGs," *Acta Physiologica Scandinavia*, 70 (1967), 451-452.

Amenomori, Y., Chen, C.L., and Meites, J. "Serum Prolactin Levels in Rats during Different Reproductive States," *Endocrinology*, 86 (1970), 506-510.

Beach, F. A. and Jaynes, J. "Studies on Maternal Retrieving in Rats--II. Effects of Practice and Previous Parturition," *American Naturalist*, 90 (1956), 103-9.

Beach, F. A. and Wilson, J. R. "Effects of Prolactin, Progesterone and Estrogen on Reactions of Non-pregnant Rats to Foster Young," *Psychological Reports*, 13 (1963), 321-9.

Behrman, H., Yoshinaga, K., and Greep, R. "Extraluteal Effects of Prostaglandins," *Annals of the New York Academy of Sciences*, 180 (1971), 426-435.

Beyer, C., Ramirez, V. D., Whitmoyer, D. I., and Sawyer, C. H. "Effects of Hormones on the Electrical Activity of the Brain in the Rat and Rabbit," *Experimental Neurology*, 18 (1967), 313-326.

Birch, H. G. "Sources of Order in the Maternal Behavior of Animals," *American Journal of Orthopsychiatry*, 26 (1956), 270-294.

Carlier, C. and Noirot, E. "Effects of Previous Experience on Maternal Retrieving by Rats," *Animal Behavior*, 13 (1965), 423-6.

Chen, C. L. and Meites, J. "Effects of Estrogen and Progesterone on Serum and Pituitary Prolactin Levels in Ovariectomized Rats," *Endocrinology*, 86 (1970), 503-505.

Christophersen, E. R. and Wagman, W. "Maternal Behavior in the Albino Rat as a Function of Self-licking Deprivation," *Journal of Comparative and Physiological Psychology*, 60 (1965), 142-144.

Clemens, J. A., Amenomori, Y., Jenkins, T., and Meites, J. "Effects of Hypothalamic Stimulation, Hormones and Drugs on Ovarian Function in Old Rats," *Proceedings of the Society of Experimental Biology and Medicine*, 132 (1969), 561-563.

Deis, R. P. "Induction of Lactogenesis and Abortion by PG $F_{2\alpha}$ in Pregnant Rats," *Nature*, 229 (1971), 568.

Edwards, D. A. "Induction of Estrus in Female Mice: Estrogen-Progesterone Interaction, "*Hormones and Behavior*," 1 (1970), 299-304.

Eible-Eibesfeldt, I. "The Interactions of Unlearned Behavior Patterns and Learning in Mammals," in *Brain Mechanisms and Learning*. Pp. 53-73. Edited by J. F. Delafresnaye. Oxford: Blackwell Scientific Publications, 1961.

Eisenfeld, A. J. and Axelrod, J. "Effect of Steroid Hormones, Ovariectomy, Estrogen Pretreatment, Sex and Immaturity on the Distribution of ^{3}H-estradiol," *Endocrinology*, 79 (1966), 38-42.

Eisenfeld, A. J. and Axelrod, J. "Selectivity of Estrogen Distribution in Tissues," *Journal of Pharmacology and Experimental Therapeutics*, 150 (1965), 468-475.

Eliasson, R. and Brzdekiewicz, Z. "Tachyphylactic Response of the Isolated Rat Uterus to PGs A," *Pharmacological Research Communications*, 1 (1969), 391-396 (a).

Eliasson, R. and Brzdekiewicz, Z. "Tachyphylactic Response of the Isolated Rat Uterus to PGs F," *Pharmacological Research Communications*, 1 (1969), 397-402 (b).

Eto, T., Hosi, T., Musudo, H., and Suzuki, Y. "Progesterone and preg - 4 - ene - 20 - ol - 3 - one in Rat Ovarian Venous Blood at Different Stages in the Reproductive Cycle," *Japanese Journal of Animal Reproduction*, 8 (1962), 34-40. (title translated.)

Everett, J. W. "The Control and Secretion of Prolactin," in The *Pituitary Gland*. Vol. II, pp. 166-195. Edited by G. Harris and B. Donovan. Berkeley, California: University of California Press, 1966.

Fajer, A. B. and Barraclough, C. A. "Ovarian Secretion of Progesterone and 20 Hydroxypregn 4 en - 3 - one during Pseudopregnancy," *Endocrinology*, 81 (1967), 617-22.

Flerko, B. "Control of Gonadtropin Secretion in the Female," in *Neuroendocrinology*. 1, 613-653. Edited by L. Martini and W. F. Ganong. New York: Academic Press, 1966.

Fuchs, A. R. and Saito, S. "Pituitary Oxytocin and Vasopressin Content of Pregnant Rats Before, During, and After Parturition," *Endocrinology*, 88 (1971), 574-578.

Greenwald, G. S. "Ovarian Follicular Development and Pituitary FSH and LH Content in the Pregnant Rat," *Endocrinology*, 79 (1966), 572-578.

Grindeland, R. E., McCulloch, W. A., and Ellis, S. "Radioimmunoassay of Rat Prolactin. Paper read before the 51st Meeting of the Endocrine Society, 1969.

Grota, L. J. and Eik-Nes, K. B. "Plasma Progesterone Concentration during Pregnancy and Lactation in the Rat," *Journal of Reproduction and Fertility*, 13 (1967), 83-96.

Gutknecht, G. D., Cornette, J. C., and Pharriss, B. B. "Antifertility Properties of PG $F_{2\alpha}$," *Biology of Reproduction*, 1 (1969), 367-371.

Hashimoto, I., Hendricks, D. M., Anderson, L. L., and Melampy, R. M. "Progesterone and preg - 4 - en - 20 - ol - 3 - one in Ovarian Venous Blood during Various Reproductive States in the Rat," *Endocrinology*, 82 (1968), 333-341.

Heuser, G., Ling, G. M., and Kluver, M. "Sleep Induction by Progesterone in the Pre-optic Area in Cats," *Electroencephalography and Clinical Neurophysiology*, 22 (1967), 122-127.

Holland, H. C. "An Apparatus Note on AMBA, Automatic Maternal Behavior Apparatus," *Animal Behavior*, 13 (1965), 201-203.

Kanematsu, S. and Sawyer, C. H. "Effect of Hypothalamic Estrogen Implants on Pituitary LH and Prolactin in Rabbits," *American Journal of Physiology*, 205 (1963), 1073-1079 (a).

Kanematsu, S. and Sawyer, C. H. "Effect of Intrahypothalamic Estrogen Implants on Pituitary Prolactin and Lactation in the Rabbit," *Endocrinology*, 72 (1963), 243-252 (b).

Kato, J. and Villee, C. A. "Factors Affecting Uptake of Estradiol-6, 7-^{3}H by the Hypophysis and Hypothalamus," *Endocrinology*, 80 (1967), 1133-1138 (a).

Kato, J. and Villee, C. A. "Preferential Uptake of Estradiol by the Anterior Hypothalamus of the Rat," *Endocrinology*, 80 (1967), 567-575 (b)

Kawakami, M. and Sawyer, C. H. "Induction of Behavioral and Electroencephalographic Changes in the Rabbit by Hormone Administration or Brain Stimulation," *Endocrinology*, 65 (1959), 631-643.

Kennedy, G. C. "The Role of Depot Fat in the Hypothalamic Control of Food Intake in the Rat," Proceedings of the Royal Society, B 140 (1963), 578-592.

Kinder, E. F. "A Study of the Nest Building Activity of the Albino Rat," Journal of Experimental Zoology, 47 (1927), 117-161.

Kirby, H. W. and Horvath, R. "Self-licking Deprivation and Maternal Behavior in the Primiparous Rat," Canadian Journal of Psychology, 22 (1968), 369-375.

Klein, M. "Uterine Destension, Ovarian Hormones and Maternal Behavior In Rodents," in Ciba Colloquium on Endocrinology, Vol. 3, 84-87. New York: Blakeston, 1952.

Komisaruk, B. R., McDonald, P. G., Whitmoyer, D. I., and Sawyer, C. H. "Effects of Progesterone and Sensory Stimulation on EEG and Neuronal Activity in the Rat," Experimental Neurology, 19 (1967) 494-507.

Kwa, H. G. and Verhofstad, F. "Prolactin Levels in the Plasma of Female Rats," Journal of Endocrinology, 39 (1967), 455-456.

Labhsetwar, A. P. "Influence of Progesterone on Pituitary Content of LH and FSH in the Female Rat," Anatomical Record, 160 (1968), abs.

Laumas, K. R. and Farooq, A. "The Uptake in Vivo of (1, 2 H) Progesterone by the Brain and Genital Tract of the Rat," Journal of Endocrinology, 36 (1966), 95-96.

Lehrman, D. S. "Gonadal Hormones and Parental Behavior in Birds and Infrahuman Mammals," in Sex and Internal Secretions. Vol. 2, 1268-1382. Edited by W. C. Young. Baltimore: William and Wilkins, 1961.

Lisk, R. D. "Progesterone: Biphasic Effect on the Lordosis Response in Adult or Neonatally Gonadectomized Rats," Neuroendocrinology, 5 (1969), 149-160.

Lott, D. F. "The Role of Progesterone in the Maternal Behavior of Rodents," Journal of Comparative and Physiological Psychology, 55 (1962), 610-613.

Lott, D. F. and Fuchs, S. S. "Failure to Induce Retrieving by Sensitization or the Injection of Prolactin," Journal of Comparative and Physiological Psychology, 55 (1962), 1111-1113.

Meites, J. and Nicoll, C. S. "Adenohypophysis: Prolactin," Annual Review of Physiology, 28 (1966), 57-58.

Meites, J. and Nicoll, C. S. "Hormonal Prolongation of Lactation for 75 Days after Litter Withdrawal in Postpartum Rats," Endocrinology, 65 (1959), 572-579.

McGuire, J. L. and Lisk, R. D. "Localization of Estrogen Receptors in the Rat Hypothalamus," Neuroendocrinology, 4 (1969), 289-295.

Moltz, H., Geller, D., and Levin R. "Maternal Behavior in the Totally Mammectomized Rat," Journal of Comparative and Physiological Psychology, 64 (1967), 225-229.

Moltz, H., Lubin, M., Leon, M., and Numan, M. "Hormonal Induction of Maternal Behavior in the Ovariectomized Nulliparous Rat," Physiology and Behavior, 5 (1970), 1373-1377.

Moltz, H. and Robbins, D. "Maternal Behavior of Primiparous and Multiparous Rats," Journal of Comparative and Physiological Psychology, 60 (1965), 417-421.

Moltz, H., Robbins, D., and Parks, M. "Caesarean Delivery and the Maternal Behavior of Primiparous and Multiparous Rats," Journal of Comparative and Physiological Psychology, 61 (1966), 455-460.

Moltz, H. and Wiener, E. "Effects of Ovariectomy on Maternal Behavior of Primiparous and Multiparous Rats," Journal of Comparative and Physiological Psychology, 62 (1966), 382-387.

Nadler, R. D. "A Biphasic Influence of Progesterone on Sexual Receptivity of Spayed Female Rats," Physiology and Behavior, 5 (1970), 95-97.

Obias, M. D. "Maternal Behavior of Hypophysectomized Gravid Albino Rats and the Development and Performance of Their Progeny," Journal of Comparative Physiological Psychology, 50 (1957), 120-124.

Paton, D. M. and Daniel, E. E. "Contractile Response of the Isolated Rat Uterus to PG E_1," Canadian Journal of Physiology and Pharmacology, 45 (1967), 795-804.

Pfaff, D. W. "Autoradiographic Localization of Radioactivity in Rat Brain after Injection of Tritiated Sex Hormones," Science, 161 (1968), 1355-1356.

Pharriss, B. B. and Wyngarden, L. J. "Effect of PG $F_2\alpha$ on the Progestogen Content of Ovaries from Pseudopregnant Rats," Proceedings of the Society of Experimental Biology and Medicine, 130 (1969), 92-94.

Powers, J. B. and Zucker, I. "Sexual Receptivity in Pregnant and Pseudopregnant Rats," Endocrinology, 84 (1969), 820-827.

Raisinghani, J. W., Dorfman, R. I., Forchielli, E., Gyermek, L., and Genther, G. "Uptake of Intravenously Administered Progesterone, Pregnanedione and Pregnaolone by the Rat Brain," Acta Endocrinologica, 57 (1968), 395-404.

Ramirez, V., Komisaruk, B. R., Whitmoyer, D. I., and Sawyer, C. H. "Effects of Hormones and Vaginal Stimulation on EEG and Hypothalamic Unite in Rats," American Journal of Physiology, 212 (1967) 1376-1384.

Rebar, R. W., Nakane, D. K., and Midgley, A. R. "Postpartum Release of Leuteinizing Hormone (LH) in the Rat as Determined by Radioimmunoassay," Endocrinology, 84 (1966), 1352-1358.

Richards, M. P. M. "Maternal Behavior in Rodents and Lagomorphs: A Review," in Advances in Reproductive Physiology. Vol. II, 54-110. Edited by A. McLaren. New York: Academic Press, 1967.

Riddle, O. "Physiological Responses to Prolactin," Cold Spring Harbor Symposium on Quantitative Biology, 5 (1957), 218-228.

Riddle, O., Hollander, W. F., Miller, R. A., Lahr, E. L., Smith, G. C., and Marvin, H. N. "Endocrine Studies," Yearbook of the Carnegie Institute, 41 (1942), 203-211.

Riddle, O., Lahr, E. L., and Bates, R. W. "Effectiveness and Specificity of Prolactin in the Induction of the Maternal Instinct in Virgin Rats," American Journal of Physiology, 113 (1935), 109 (a).

Riddle, O., Lahr, E. L., and Bates, R. W. "Maternal Behavior Induced in Virgin Rats by Prolactin," Proceedings of the Society for Experimental Biology and Medicine, 32 (1935), 730-734 (b).

Riddle, O., Lahr, E. L., and Bates, R. W. "The Role of Hormones in the Initiation of Maternal Behavior in Rats," American Journal of Physiology, 137 (1942), 299-317.

Rosenblatt, J. S. "Nonhormonal Basis of Maternal Behavior in the Rat," Science, 156 (1967), 1512-1514.

Rosenblatt, J. S. and Lehrman, D. S. "Maternal Behavior of the Laboratory Rat," in Maternal Behavior in Mammals. 8-57. Edited by H. L. Rhinegold. New York: Wiley, 1963.

Rothchild, I. "The Corpus Luteum Pituitary Relationship: The Lack of an Inhibiting Effect of Progesterone on the Secretion of Pituitary Liteotropin," *Endocrinology*, 67 (1960), 54-61.

Rothchild, I. "Interrelations between Progesterone and the Ovary, Pituitary and Central Nervous System in the Regulation of Progesterone Secretion," in *Vitamins and Hormones*. Vol. 23, 210-228. Edited by R. Harris, I. Wool, and L. Loraine. New York: Academic Press, 1965.

Schofield, B. M. "The Hormonal Control of Myometrial Functioning during Pregnancy," *Journal of Physiology* (London), 138 (1957), 1-10.

Seiki, K., Higashida, M., Imanishi, Y., Miyamoto, M., Kitagawa, T., and Kotani, M. "Radioactivity in the Rat Hypothalamus and Pituitary after Injection of Labelled Progesterone," *Journal of Endocrinology*, 41 (1968), 109-110.

Seiki, K., Miyamoto, M., Yamishita, A., and Kotani, M. "Further Studies on the Uptake of Labelled Progesterone by the Hypothalamus and Pituitary of Rats," *Journal of Endocrinology*, 43 (1969), 129-130.

Seitz, P. F. D. "The Maternal Instinct in Animal Subjects," *Journal of Psychosomatic Medicine*, 20 (1958), 215-26.

Smith, E. R., Weick, F., and Davidson, J. M. "The Influence of Intracerebral Progesterone on the Reproductive System of Female Rats," *Endocrinology*, 85 (1969), 1129-1136.

Stumpf, W. E. "Too Much Noise in the Autoradiogram?" *Science*, 163 (1969), 958-959.

Terkel, S. "Maternal Behavior in Virgin Rats Induced by Transfusion of Material Blood," Unpublished Doctoral dissertation, Department of Psychology, Rutgers University, 1970.

Terkel, J. and Rosenblatt, J. S. "Maternal Behavior Induced by Maternal Blood Plasma Injected into Virgin Rats," *Journal of Comparative and Physiological Psychology*, 65 (1968), 479-482.

Wiesner, B. P. and Sheard, N. M. *Maternal Behavior in the Rat*. London: Oliver and Boyd, 1933.

Wiest, W. G. "Progesterone and 20α hydroxypregn - 4 - en - 3 - one in Plasma, Ovaries and Uteri during Pregnancy in the Rat," *Endocrinology*, 87 (1970), 43-48.

Wiest, W. G., Kidwell, W. R., and Balogh, Jr., K. "Progesterone catabolism in the Rat Ovary: A Regulatory Mechanism for Progestational Potency during Pregnancy," Endocrinology, 82 (1968), 844-859.

Yoshinaga, K., Hawkins, R. A., and Stocker, J. F. "Estrogen Secretion by the Rat Ovary in Vivo during the Estrous Cycle and Pregnancy," Endocrinology, 85 (1969), 103-112.

Zarrow, M. X., Farooq, A., Denenberg, V. H., Swain, P. B., and Ross, S. "Maternal Behavior in the Rabbit: Endocrine Control of Maternal Nest Building," Journal of Reproduction and Fertility, 6 (1963), 375-383.

Zucker, I. "Facilitory and Inhibitory Effects of Progesterone on Sexual Responses of Spayed Guinea Pigs," Journal of Comparative and Physiological Psychology, 62 (1966), 376-381.

Zucker, I. "Progesterone in the Experimental Control of the Behavioral Sex Cycle in the Female Rat," Journal of Endocrinology, 38 (1967), 269-277.

Zucker, I. and Goy, R. W. "Sexual Receptivity in the Guinea Pig: Inhibitory and Facilitatory Actions of Progesterone and Related Compounds," Journal of Comparative and Physiological Psychology, 64 (1967), 378-383.