0013-7227/79/1045-1247$02.00/0
Endocrinology
Copyright © 1979 by The Endocrine Society
Vol. 104, No. 5
*Printed in U.S.A.*

# Control of the Preovulatory Release of Luteinizing Hormone By Steroids in the Mouse*

F. H. BRONSON AND FREDERICK S. VOM SAAL

*Institute of Reproductive Biology, Department of Zoology, The University of Texas, Austin, Texas 78712*

**ABSTRACT.** The regulation of the preovulatory release of LH by steroids was examined in the mouse, a species in which ovulation is strongly influenced by priming pheromones. Ovariectomized mice were implanted with estradiol in Silastic capsules to invoke negative feedback. Preovulatory-like LH surges then were induced by injections of either estradiol benzoate (EB) or progesterone. LH surges were not observed in the absence of steroid injection. LH surges always occurred at lights out on a 14-h light, 10-h dark cycle on the day after EB injection but occurred on the same day as progesterone injection. The amount of EB or progesterone injected seemed unimportant but, in either case, had to be given within a limited diurnal period of sensitivity. LH surges comparable to those of intact proestrous females were produced either by injecting both EB and progesterone or by manipulating the background dose of encapsulated estradiol. In the latter regard, when ovariectomized females were implanted with a wide range of doses of estradiol (0.1–1000 μg/capsule), a decided window phenomenon became apparent. That is, LH surging could be induced by steroid injections only within a limited range of background doses of encapsulated estradiol. The relationship of the above findings to the pheromonal control of LH secretion and ovulation in mice is as yet unclear. (*Endocrinology* **104:** 1247, 1979)

IT IS WELL established that ovarian steroids exert both positive and negative control over the secretion of LH in female mammals. Negative feedback is assumed to predominate throughout the ovulatory cycle, being interrupted only for a short time when the gonadal steroids elicit the preovulatory release of this hormone. The relationship between preovulatory surging and negative feedback has been studied extensively in the rat (1–5), hamster (6–8), and rhesus monkey (9, 10) and less so in a few other species, such as the dog (11) and the sheep (12, 13). The resulting consensus is that follicular estrogens play the dominant role in both the negative feedback and the surging aspect of LH regulation in most but not all species. Progesterone, ovarian and/or adrenal, typically is accorded only a facilitatory or timing function for the LH surge (14–20) or, depending both upon species and stage of cycle, this steroid also may function to block surging (10, 18, 21–23). Progesterone also may be involved in chronic negative feedback (13, 24).

The primary objective of the present experiments was to characterize the steroidal regulation of the preovulatory release of LH in the mouse. This species is unique among the commonly used laboratory mammals in that its preovulatory processes are strongly influenced by urinary pheromones. Chemical cues in the urine of male mice accelerate ovulation in females perceiving them; female-originating cues decelerate ovulation (25–27). At the level of the recipient female's pituitary, the action of the male's pheromones seems to be exclusively on LH, while female urinary cues act on both LH and PRL (28–30). Thus, during the estrous cycle of the mouse, LH secretion is routinely dependent upon stimulation and inhibition by both exteroceptive and steroidal factors. Such dual control might or might not necessitate accommodation of the steroidal regulation of LH surging for the pheromonal control of the same event. Thus, a secondary objective of these studies was to look for such accommodation by comparing the present results with those generated by past studies with other species whose cycles are not influenced by pheromones.

## Materials and Methods

Several sets of experiments are reported. The methodological details of each will be presented with their respective results. In general, the basic approach used in all of the present experiments was to attempt a duplication in the ovariectomized animal of the most probable sequence of events occurring during the intact female's estrous cycle, *i.e.* first serum LH was chronically depressed with estradiol, administered via Silastic capsules, and then the LH surge was induced by injections of estradiol benzoate (EB) and/or progesterone. Females were housed in 18 × 29 × 13-cm polyethylene cages, four or five per cage, and were used in experiments 7 days after ovariectomy

Received September 18, 1978.

Address requests for reprints to: Dr. F. H. Bronson, Department of Zoology, Institute of Reproductive Biology, Patterson Laboratories Building, University of Texas, Austin, Texas 78712.

* This work was supported by USPHS Research Grant HD-03803 and National Research Service Award HD-05358, both from the NICHHD.

and estradiol implantation. The females were maintained on Purina laboratory chow in male-free rooms which had 12 air exchanges/h. The rooms were maintained at 23 ± 1 C and were on a 14-h light, 10-h dark cycle with lights on at 0600 h. A single 24-watt bulb facilitated work in the animal rooms when needed after lights out at 2000 h.

*Hormone administration*

Estradiol implants were made by mixing 17β-estradiol with Silastic adhesive (type A, no. 891, Dow Corning). The resulting suspension was packed in a 10-mm Silastic capsule (id, 0.04; od, 0.085; Dow Corning). Such dilution is necessary because of the small size of the mouse, and suspension in Silastic adhesive previously has been found preferable to mixing with a variety of other compounds and vehicles (30). Some experimental protocols required different dosages of encapsulated estradiol; capsule length was held constant in these cases and varying amounts of estradiol were suspended to obtain the different doses. Unless otherwise stated, EB was administered by sc injection at a dose of 1 μg/0.02 ml corn oil. Also, unless otherwise stated, progesterone was injected sc at a dosage of 400 μg/0.02 ml corn oil.

*RIAs*

Blood was collected by decapitation. Serum LH concentrations were determined using the NIAMDD rat RIA kit (31). Results are expressed in terms of nanogram equivalents of RP-1. All assays were done on 100-μl aliquots of serum, with a minimal detectable amount of 1–2.5 ng LH.

## Results

*The preovulatory release of LH in the intact CF-1 mouse*

The normal proestrous LH surge was characterized in intact CF-1 females to provide a reference for comparison with the results of later experiments with ovariectomized females. The protocol for this experiment took advantage of the pheromonal synchronization of proestrus that is possible in this species (25). The estrous cycles of 100 females, 60–80 days of age, were first suppressed by housing them 6 per cage in a male-free room. The cycles of most of these females then were synchronized by simultaneously and individually pairing them with males. Typically in this laboratory, we expect about 60% of the CF-1 females treated in this manner to be in proestrus on the third day of cohabitation. In the present experiment, 30 females mated during the first two nights. The remaining 70 females were killed between 1400–2400 h on the third day after pairing. Vaginal smears obtained after death, confirmed by uterine observation, indicated that 10 of these females were not in proestrus. Thus, the bloods of 60 proestrous females, 9–11 for every 2-h interval, were available for hormone assay. Figure 1 indicates a normal LH surge in this stock of mice that is centered on lights out at 2000 h and which averages about 1500 ng LH/ml at its peak.

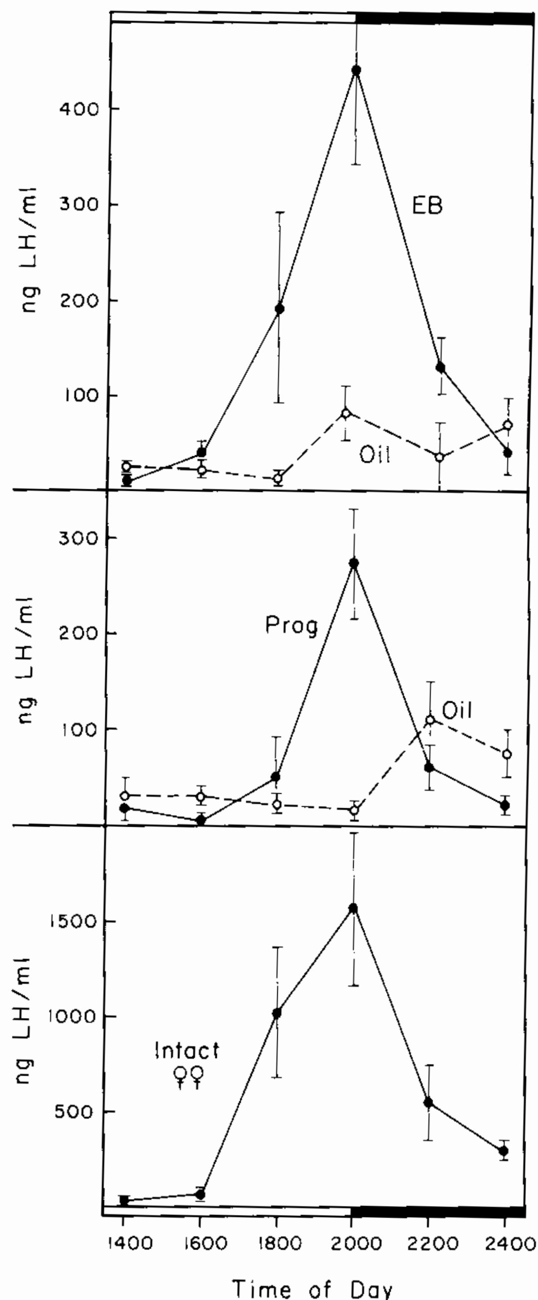


FIG. 1. Serum LH [nanograms per ml (±SE)] at different times of the afternoon or evening in ovariectomized females implanted with Silastic capsules containing 5 μg estradiol, injected with 1 μg EB or oil at 1000 h on day 6 postsurgery, and then killed on day 7 (*top panel*), or injected with 400 μg progesterone or oil at 1000 h on day 7 and killed that same day (*middle panel*). *Lower panel*, Normal preovulatory change in serum LH in intact proestrous adults. The light and dark bars at the top and bottom of the figure indicate the light cycle.

*Induction of preovulatory-like LH surging in ovariectomized, estrogenized mice*

Three sets of experiments examined the induction of preovulatory-like LH surges by injections of EB or progesterone in ovariectomized mice bearing Silastic im-

plants of estradiol. In all three experiments, the protocols for steroid manipulation and times for blood collection were based either upon the results of pilot experiments or upon the known or suspected sequence of events in the intact female mouse (28, 32). Verification of all such choices will follow later in this paper.

The first set of experiments examined the effect of estradiol injection on the LH surge. In the first experiment in this set, 216 females were ovariectomized and immediately implanted with capsules containing 5 $\mu$g estradiol. Six days later, 120 of these females were injected at 1000 h with 1 $\mu$g EB in 0.02 ml oil; 10 were killed every 2 h between 1400–2400 h that evening, another 10 were killed at the same times on the following evening (day 7). Ninety-six females were injected with oil as controls and killed (eight per time) at the same time periods. RIA revealed a well synchronized, preovulatory-like peak in serum LH titers that was centered on lights out (2000 h) of the day after EB injection (day 7). Figure 1 shows only the data collected on day 7. No group killed on day 6 averaged over 30 ng LH/ml regardless of the material injected or the time of killing. More specifically, 1400 *vs.* 2000 h values [nanograms of LH per ml (±SE)] on day 6 were 21 ± 7 *vs.* 24 ± 6 for oil-injected controls and 31 ± 1 *vs.* 14 ± 3 for EB-injected females. In a partial replication of this experiment, another group of 80 females was given 0.1, 1, or 10 $\mu$g EB or oil at 1000 h on day 6 postsurgery and killed only during the evening of day 7. Mean values at 1400 *vs.* 2000 h on day 7 in this second experiment were 26 ± 6 *vs.* 41 ± 23 for oil-injected controls, 31 ± 5 *vs.* 271 ± 119 in response to the 0.1-$\mu$g EB dose, 30 ± 4 *vs.* 375 ± 102 for the 1-$\mu$g dose, and 11 ± 2 *vs.* 440 ± 104 for the 10-$\mu$g dose (all based on 10 females per average).

The effect of progesterone injection was examined in the second set of experiments. Again, 216 females were ovariectomized and implanted with 5-$\mu$g capsules of estradiol. These females were injected at 1000 h on day 7 postsurgery with either oil (96 females) or 400 $\mu$g progesterone in 0.02 ml oil (120 females). Blood collection was accomplished every 2 h between 1400–2400 h either on day 7 or 8 (10 progesterone-injected females at each time on each day as opposed to 8 oil-injected controls at each time on each day). Well synchronized peaks in serum LH were observed among progesterone-injected females during the evening of day 7 but not on day 8. Figure 1 shows only the data obtained on day 7. No averages greater than 50 ng LH/ml were observed in any group killed on day 8 regardless of the material injected or the time of killing. The 1400 *vs.* 2000 h differences on day 8, the day after injection, were 29 ± 7 *vs.* 49 ± 17 for oil-injected controls and 8 ± 2 *vs.* 8 ± 2 for progesterone-injected females. The day 7 aspect of this experiment was repeated with 200-, 350-, 500-$\mu$g doses of progesterone and killing times of 1500 *vs.* 2000 h (16 females/dose/time, no oil controls). The 1500 *vs.* 2000 h values [nanograms of LH per ml (±SE)] in this experiment were 11 ± 2 *vs.* 233 ± 110 for the 200-$\mu$g dose, 13 ± 5 *vs.* 448 ± 149 for the 350-$\mu$g dose, and 12 ± 2 *vs.* 209 ± 88 for the 500-$\mu$g dose of progesterone.

The two sets of experiments that are described above demonstrate that injections of either EB or progesterone will induce a preovulatory-like surge in LH secretion in the mouse when given against a background of chronic release of estradiol from a capsule. However, none of the LH surges observed in these experiments approached the magnitude of the proestrous release of LH in the intact mouse (Fig. 1). Thus, the present experiment examined the possibility of additive or synergistic effects between injections of EB and progesterone. One hundred and five females were ovariectomized and implanted with 5-$\mu$g estradiol capsules. Thirty-five of these females were injected with 1 $\mu$g EB at 1000 h on day 6 postsurgery, 35 females received 400 $\mu$g progesterone at 1000 h on day 7, and 35 received both steroids (EB at 1000 h on day 6 and progesterone at 1000 h on day 7). Control injections of oil were given in all cases where EB or progesterone was not injected. All females were killed between 1400–2400 h on day 7. Five females of each experimental group were killed at each 2-h interval, except at 2000 h when 10 females of each treatment group were killed. Figure 2 indicates either an additive effect or true synergism between EB and progesterone pulses. Progesterone alone yielded a peak LH value of 225 ± 46 ng LH/ml at 2000 h, estradiol alone yielded 881 ± 270 ng LH/ml, while the two steroids together yielded 1674 ± 485 ng LH/ml; all values were significantly different from each other when tested by analysis of variance ($P < 0.05$).

### *Steroid injection and LH surging*

*Time relations.* The previous experiments established that a preovulatory-like surge in the release of LH follows EB injection by more than a day but occurs on the same day as a progesterone injection; in both cases, injections were administered against a background of estradiol released from a capsule. The present experiments more closely examined the time relations involved in the induction of the LH surge by EB or progesterone injection.

In the first experiment, EB was injected at various times during the light or dark phase of the light cycle. Females then were killed at either 1600 or 2000 h (the expected time of the surge) during the following 1.5–2.5 days. Two hundred and ten females were ovariectomized and implanted with Silastic capsules containing 5 $\mu$g estradiol. These females were injected with 1 $\mu$g EB in 0.02 ml oil at either 1200, 1600, 2000, or 2400 h on day 6 postsurgery or at 0400, 0800, or 1000 h of day 7. Fifteen

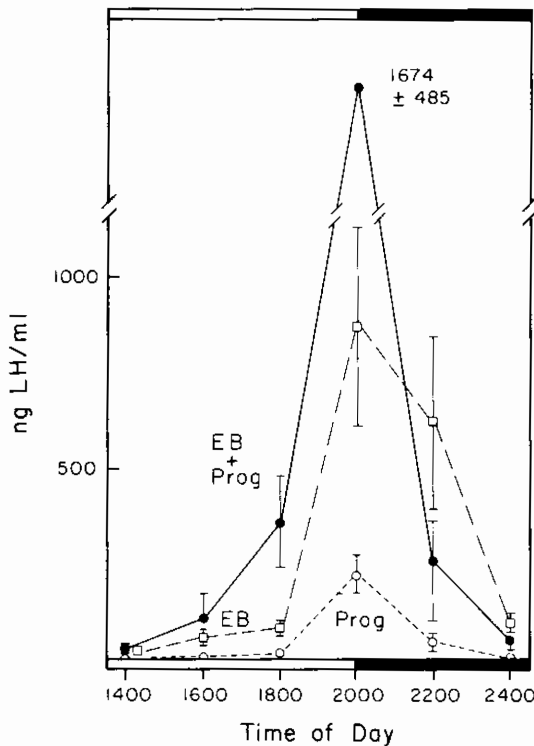


FIG. 2. Serum LH [nanograms per ml (±SE)] in ovariectomized females implanted with Silastic capsules containing 5 μg estradiol and injected with 1 μg EB at 1000 h on day 6 postsurgery or 400 μg progesterone at 1000 h on day 7, or given both hormones at the times indicated. All females were killed on day 7.

females of each group then were killed during the evening of day 7 or 8 (5 females at 1600 h and 10 females at 2000 h on each day). This entire experiment was conducted in one 8-day period utilizing a randomized block design. The results are shown in Fig. 3 and suggest a diurnal period of sensitivity during which EB can induce a well organized LH surge at lights out and outside of which it cannot. Specifically, the data suggest that when EB is given during the light phase of one light cycle it will yield an LH surge at the end of the light phase of the next cycle. Evidence for the reoccurrence of such surges 24 h after the first surge is ambiguous (Fig. 3; injections at 1200 *vs.* 1600 h). Injections of EB given during the dark phase of the light cycle did not yield surges of any reasonable magnitude at the end of either of the two following light periods (Fig. 3; injections at 2400 or 0400 h). It is worth noting that injection of EB at 0800 h yielded an LH surge that was of a magnitude comparable to that observed in intact females (again, however, at the end of the next light cycle).

The second experiment concentrated on the time relations of progesterone injection and differed from the previous design only in that the progesterone-induced surge was expected on the same day as progesterone injection; hence, more animals could be examined efficaciously within the framework on a single 24-h period. A total of 400 females were ovariectomized and implanted with 5-μg estradiol capsules. All females were injected with 400 μg progesterone at one of six times: 2200 h of day 6 postsurgery or 0600, 0800, 1000, 1400, or 1800 h on day 7. Animals were killed at various times, ranging from 0200 h of day 7 to 0400 h of day 8 (10 females/time point). This experiment was also conducted in one 8-day period and within the framework of a single randomized block design. The results, shown in Fig. 4, strongly support the existence of a diurnal period of sensitivity during which progesterone can induce an LH surge and outside of which it cannot. Injections at 2200 h, during the dark phase of the light-dark cycle, yielded no evidence of surging when examined throughout the following 24-h period. Injection at the onset of the light cycle (0600 h) similarly yielded no evidence of surging. On the other hand, progesterone injection during the early to mid part

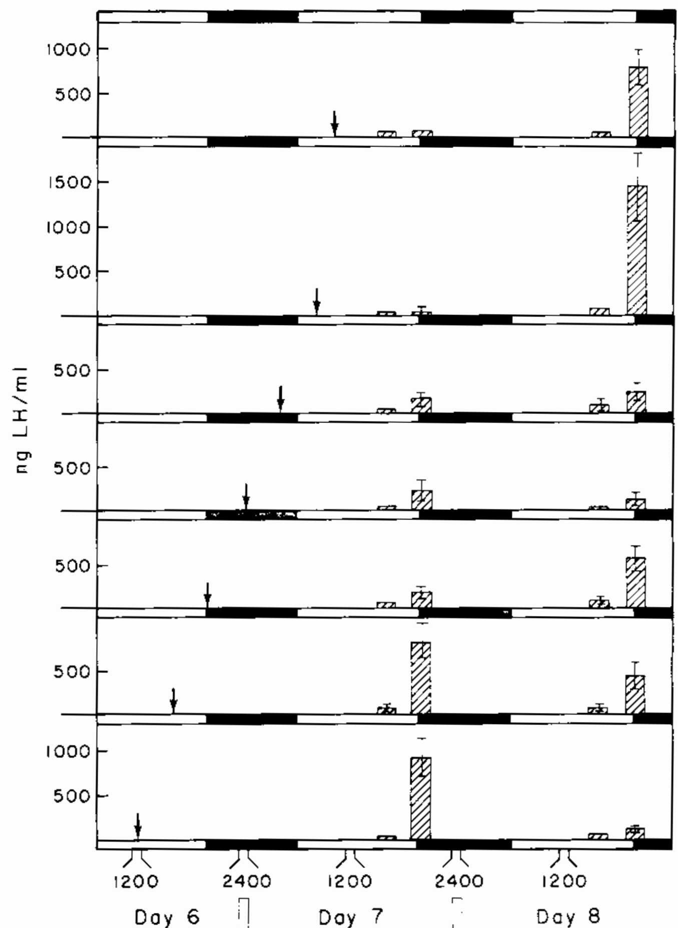


FIG. 3. Serum LH [nanograms per ml (±SE)] in ovariectomized females implanted with 5-μg estradiol capsules, injected with 1 μg EB at various times (↓) on days 6 or 7 postsurgery, and then killed at 1400 or 2000 h of either day 7 or day 8. Specific times of injection were 1200, 1600, 2000, or 2400 h on day 6 or 0400, 0800, or 1000 h on day 7. The light and dark bars indicate the light cycle.

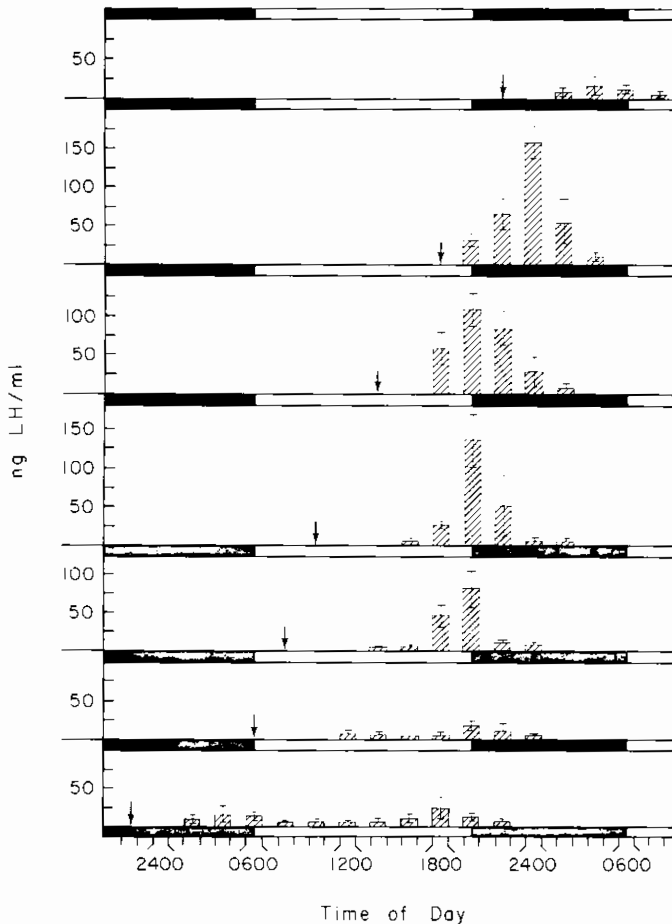


FIG. 4. Serum LH [nanograms per ml (±SE)] in ovariectomized females implanted with Silastic capsules containing 5 μg estradiol, injected with 400 μg progesterone at various times on day 6 or 7 postsurgery (↓), and then killed at various times throughout day 7 until 0400 h of day 8 postsurgery. Specific times of injection were 2200 h on day 6 or 0600, 0800, 1000, 1400, or 1800 h on day 7. The top and bottom panels in this figure involve the same data; the top panel shows only a fraction of the data and is offset by 24 h for illustrative purposes.

of the light phase of the light-dark cycle, *i.e.* at 0800, 1000, or 1400 h, yielded well synchronized LH surges that were centered on the time of lights out (2000 h). Injections administered late in the light phase (at 1800 h), still yielded surges, but these peaked after lights out.

*Estradiol sensitization for steroid-induced, preovulatory-like surging*

All of the preceding experiments utilized a 5-μg estradiol capsule as the background against which EB or progesterone was injected to induce an LH surge. The present experiments more critically examined the dependence of EB- or progesterone-induced surging upon the background dose of encapsulated estradiol.

The first experiment involved EB injections. Specifically, a total of 400 females was ovariectomized and implanted with either blank capsules or capsules containing one of the following doses of estradiol (50 females/dosage): 0.1, 0.5, 1, 5, 10, or 1000 μg/capsule. All females were injected with 1 μg EB at 1000 h on day 6 postsurgery. Ten females per dosage then were killed every 2 h from 1600–2400 h on day 7. Serum LH determinations revealed unambiguous LH surges at 2000 h in response to injected EB only among females bearing capsules containing 1, 5, or 10 μg estradiol (Fig. 5). Females implanted with capsules containing the 0.1- and 0.5-μg doses had serum LH levels similar to females bearing blank capsules. Females exposed to the highest doses of encapsulated estradiol (50 and 1000 μg/capsule) showed nondetectable levels or, sometimes, barely detectable serum LH. Figure 5 shows two different types of response to EB injection against a background of 1 μg estradiol/capsule. In the experiment described here in detail, injection against a 1-μg estradiol background yielded a surge that averaged over 1100 ng LH/ml at lights out, a magnitude not greatly different from that experienced by intact females. This particular area of the dose-response curve (*i.e.* the 0.5- to 10-μg estradiol/capsule doses) was reexamined using similar animals and injection procedures. The results of injecting EB against background doses of 0.5, 5, and 10 μg estradiol/capsule essentially were the same as those shown in Fig. 5. This second time, however, injections of EB against a background of 1 μg estradiol/capsule yielded no negative feedback inhibition and no surging, a result also

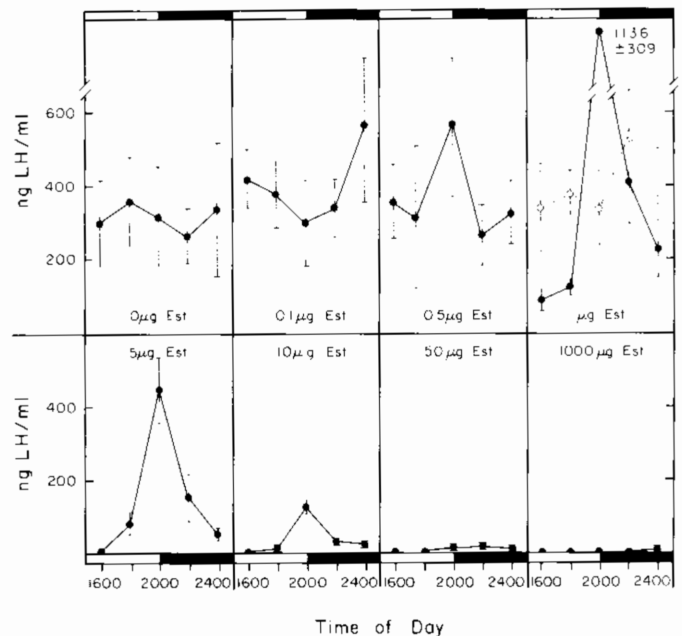


FIG. 5. Serum LH [nanograms per ml (±SE)] in ovariectomized females implanted with various doses of estradiol (Est) in Silastic capsules, injected with 1 μg EB at 1000 h on day 6 postsurgery, and then killed during the evening of day 7. The two different responses (●—● and ○- -○) among those females implanted with 1-μg estradiol capsules involve replicate tests with different batches of capsules (see text).

shown in Fig. 5. It will be argued later that this difference reflects subtle differences in capsule dosage.

The second experiment in this series examined progesterone injection set against a background of various doses of encapsulated estradiol. The methodological details of this experiment were identical to those of the preceding experiment (*e.g.* a total of 400 females), except that progesterone rather than EB was injected; 400 μg in 0.02 ml oil were injected at 1000 h on day 7, and the females were killed that same evening. Serum LH determinations again showed progesterone-induced surges only in the 1- to 10-μg range of doses of encapsulated estradiol (Fig. 6). The 1-μg/capsule dose of estradiol again seemed to be critical; neither negative feedback inhibition nor surging were seen in the first experiment, but a replicate yielded an LH surge averaging over 1300 ng LH/ml at its peak. Again, both types of responses are shown in Fig. 6.

### *Relationships between estradiol sensitization for steroid-inducing surging, negative feedback, and lordosis potential*

The results of the preceding experiments certainly suggest that there are critical levels for the background maintenance of serum estradiol if EB or progesterone injection is to yield an LH surge, *i.e.* too much or too little serum estradiol results in a failure to surge in response to a steroid injection. The present experiments examined two other correlates of these critical background levels of estradiol: uterine weight and the potential for displaying lordosis in response to progesterone (33).

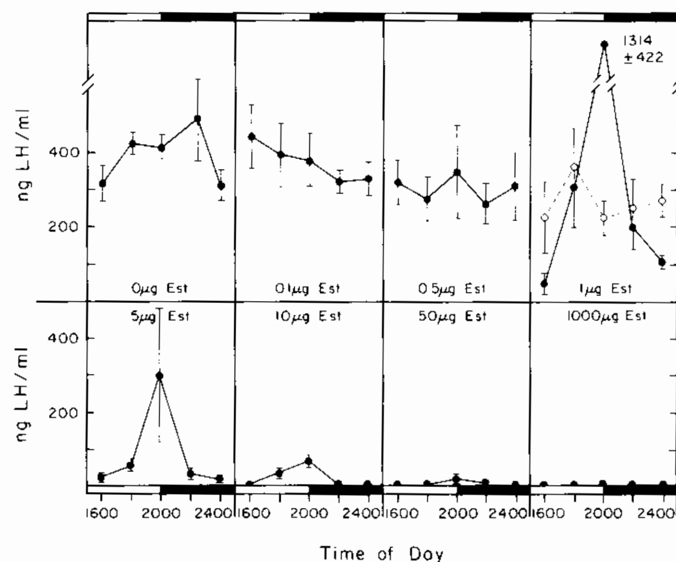


FIG. 6. Serum LH [nanograms per ml (±SE)] in ovariectomized females implanted with various doses of estradiol (Est) in Silastic capsules, injected with 400 μg progesterone at 1000 h on day 7 postsurgery, and then killed at various times on the same day. The two different responses (●—● and ○- -○) among those females implanted with 1-μg estradiol capsules involve replicate tests with different batches of capsules (see text).

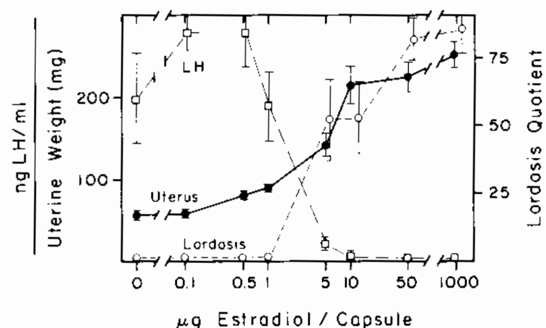


FIG. 7. Serum LH [nanograms per ml (±SE)] and uterine weight [milligrams (±SE)] in ovariectomized females implanted with one of several doses of estradiol in Silastic capsules (graphed on a log scale) and then killed at 1200 h on day 7 postsurgery. Lordosis quotient was calculated for a separate group of females treated in a similar manner, except they were injected with progesterone at 1000 h on day 7 postsurgery, 4 h before behavioral testing.

One purpose of the first experiment in this set was to obtain a negative feedback inhibition curve that was free from any potential interference from EB or progesterone injection for comparison with the data presented in Figs. 5 and 6. Sixty-four females were ovariectomized and implanted with the spectrum of doses of encapsulated estradiol that was used in the preceding two experiments. Eight females for each of the eight doses of estradiol were killed without further treatment at 1200 h on day 7 postsurgery. The results are shown in Fig. 7. Again, sharp suppression of serum LH titers appeared between the 1- and 5-μg/capsule doses of estradiol. Uteri were weighed at sacrifice in this experiment to provide a bioassay of capsule dosage. A significant increase in uterine weight was detected at the 0.5-μg/capsule dosage, an amount lower than the minimum required to depress serum LH even partially (Fig. 7). The largest increase in uterine weight occurred between 1–10 μg/capsule, *i.e.* the general area of the dose-response curve where LH could be induced by a steroid injection.

The second experiment in this series examined the relationship between the estrogen requirement for surging sensitization and that necessary to support the induction of lordosis by progesterone. A group of 64 females was prepared as in the preceding experiment. These females were injected with progesterone (200 μg/0.02 ml oil) on day 7 postovariectomy and capsule implanting, and tested for sexual behavior 4 h later (1200–1500 h). Females were paired individually with one of a large battery of sexually experienced males. A lordosis quotient (number of lordoses per number of mounts × 100) was calculated for each female during a 30-min test. No female bearing a capsule containing less than 5 μg estradiol exhibited lordosis when exposed to a male. However, six of eight, six of eight, six of eight, and four of eight

females did exhibit lordosis for the 5-, 10-, 50-, and 1000μg doses, respectively, sometime during the 30-min test. The zero values recorded at these higher doses resulted from a failure of the males to mount. Figure 7 shows the lordosis quotient for those pairs in which some mounting and lordosis occurred and indicates a general trend for an increasing lordosis quotient from the 5- to 1000-μg doses.

## Discussion

The relationship between chronic negative feedback by estradiol and the capacity of a steroid injection to induce an LH surge in the ovariectomized mouse seems to take the form of a window phenomenon. Doses of encapsulated estradiol that released too little steroid failed to depress serum LH and did not permit injections of either EB or progesterone to induce LH surges (see Ref. 34). Doses of encapsulated estradiol that released too much of this steroid also prevented a surge in response to a steroid injection. Thus, there was only a relatively narrow range of doses of encapsulated estradiol which yielded appropriate sensitization for surging. Importantly, the position of this window on the dose-response curve was correlated with diestrus level uterine weights and with the threshold for the induction of lordosis potential (*c.f.* Figs. 5–7). Such correlates argue for the physiological relevance of this phenomenon. Indeed, the effective window for sensitization of the surging mechanism by estrogen may be quite narrow. Surges of full magnitude were observed in response to an injection of either EB or progesterone, most typically in association with only one critical dosage of encapsulated estradiol (1 μg/capsule; Figs. 5 and 6). The inconsistency noted with this dosage probably reflects our inability to repeatedly make precise suspensions of estradiol in Silastic adhesive and the likelihood that this particular dosage was near a threshold. It is obvious in Figs. 5 and 6 that when a particular set of 1-μg capsules invoked negative feedback inhibition of serum LH (at 1600 h), surges of full magnitude occurred in response to a steroid injection. When this capsule dosage was not sufficient to invoke negative feedback, no surges of any magnitude were observed in response to steroid injections.

Our experiments with the estrogen sensitization aspect of the steroid regulation of LH surging probably relate to that part of the estrous cycle which immediately precedes the late diestrus/proestrus elevation of gonadal activity in the intact mouse. The present results also suggest several characteristics of the control of LH secretion by steroids during the late diestrus/proestrus stage itself. First, as noted above, LH surging could be induced in the mouse by injecting either EB or progesterone if the background release of estradiol from a capsule was appropriate. The dose of steroid being injected seemed unimportant. Second, there appear to be diurnal periods of sensitivity during which EB or progesterone injections are effective in eliciting LH surges and outside of which they are not. This period of sensitivity was well delineated in the experiment with progesterone injection. Progesterone is rapidly metabolized and its injection in a small volume of carrier (0.02 ml) probably yielded a true pulse of this hormone in the blood. Thus, the results shown in Fig. 4 probably accurately indicate a period of sensitivity to progesterone that starts shortly after lights on and ends at or near lights out. The companion experiment with EB, however, involved a form of estrogen that is slowly metabolized. Thus, while the results in Fig. 5 undoubtedly do establish the existence of a diurnal cycle of sensitivity to estrogen, the observed coincidence of this period of sensitivity with the light phase of the light cycle may be misleading.

The present results yield at least two schemes for the induction of the preovulatory release of LH in the intact mouse; one involving only estradiol and the other encompassing actions of both estradiol and progesterone. In the scheme involving only estradiol, the first factor is the relatively low and constant level of circulating estrogen before late diestrus/proestrus. This level would both sensitize for later estradiol action and predetermine the magnitude of the LH surge. The second factor is the late diestrus increase in circulating estradiol, which must coincide with a diurnally cycling period of sensitivity during the day before proestrus. Above a threshold, the amount of estradiol secreted at this time would not be important. The final component of this scheme is a neurally generated signal that would ensure the release of LH at lights out, a temporally different but a long established phenomenon in the rat (35). The high level of serum estrogen, characteristic of the day of proestrus, would play no role in this scheme, perhaps being necessary only to support postovulatory events. The estrogen-progesterone scheme for eliciting the preovulatory release of LH requires only the addition of two factors. First, progesterone could act in concert with estrogen to exert negative feedback before proestrus. This possibility was not examined in these studies but has been found to be of importance in the rat (24) and the sheep (13). Second, the proestrus rise in serum progesterone, set within the framework of a diurnal period of sensitivity on the day of proestrus, could yield the potential for additive or synergistic effects. Regarding the relative support for one or the other of these two schemes, treatment only with encapsulated estradiol and subsequent EB injection twice produced LH surges of full (1500 ng/ml) magnitude (Figs. 3 and 5). Since none of these females was adrenalectomized, interaction with progesterone cannot be ruled out completely. On the other hand, estrogen-progesterone synergism seems to-

tally unnecessary in view of our results with EB injections alone. Indeed, if the proestrus increase in serum progesterone is largely attributable to the release of LH itself (4), the synergism argument becomes untenable. Thus, the LH surging response to steroid injection in the mouse seems to share some of the same estrogen-progesterone redundancy that has been a source of confusion in some but not all other species [*c.f.*, the rat (2–5) *vs.* the rhesus monkey (10)].

A secondary objective of the present experiments was to search for features of the steroidal regulation of LH secretion that might be unique to the mouse and, hence, related to this species's pheromonal regulation. It is difficult to pinpoint uniqueness in the present results, however, since we have followed somewhat different experimental protocols than those typically used in the past by other workers with other species. When all of this voluminous literature is considered, there are suggestions that 1) mice may differ from other species in their absolute requirement for estrogen sensitization before an LH surge can be induced with a pulse of steroid, and 2) mice may differ from other species in their reactions to high levels of estrogen. In the latter regard, the window phenomenon is a case in point. We can find no indication that too much circulating estrogen blocks surging in any other species. Furthermore, while not fully explored in the present studies, the mouse may lack the repetitive daily surging seen in ovariectomized females of other short cycling species when they are exposed to high levels of estrogen (oil controls in Fig. 1 and high doses of estradiol in Figs. 5 and 6) (8, 36). Again, these differences may be real or, conversely, they may reflect only differences in experimental procedures. In any case, it is difficult to relate either of the two possibilities noted above to the pheromonal regulation of LH secretion. Thus, the present results actually seem to raise more questions than are answered about the relationship between the pheromonal and steroidal regulation of ovulation.

## References


1. Everett, J. W., Progesterone and estrogen in the experimental control of ovulation time and other features of the estrous cycle in the rat, *Endocrinology* **43:** 389, 1948.
2. Schwartz, N. B., and C. E. McCormack, Reproduction: gonadal function and its regulation, *Annu Rev Physiol* **34:** 425, 1972.
3. Barraclough, C. A., Sex steroid regulation of reproductive neuroendocrine processes, *In* Greep, R. O., and E. B. Astwood (eds.), Handbook of Physiology, vol. 2, sect. 7, Williams and Wilkins, Baltimore, 1973, p. 29.
4. Neill, J. D., and M. S. Smith, Pituitary-ovarian interrelationships in the rat, *In* James, V. H. T., and L. Martini (eds.), Current Topics in Experimental Endocrinology, vol. 2, Academic Press, New York, 1974, p. 73.
5. Campbell, C. S., and N. B. Schwartz, Steroid feedback regulation of luteinizing hormone and follicle-stimulating hormone secretion rates in male and female rats, *J Toxicol Environ Health* **3:** 61, 1977.
6. Goldman, B. E., V. B. Mahesh, and J. C. Porter, The role of the ovary in control of cyclic LH release in the hamster, *Biol Reprod* **4:** 57, 1971.
7. Turgeon, J., and G. S. Greenwald, Preovulatory levels of plasma LH in the cyclic hamster, *Endocrinology* **90:** 657, 1972.
8. Norman, R. L., C. A. Blake, and C. H. Sawyer, Estrogen-dependent twenty-four hour periodicity in pituitary LH release in the female hamster, *Endocrinology* **93:** 965, 1973.
9. Karsch, F. J., D. J. Dierschke, R. F. Weick, T. Yamaji, J. Hotchkiss, and E. Knobil, Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey, *Endocrinology* **92:** 799, 1973.
10. Knobil, E., On the control of gonadotropin secretion in the rhesus monkey, *Recent Prog Horm Res* **30:** 1, 1974.
11. Concannon, P. W., and R. G. Cowan, The role of estrogen withdrawal in the surge release of LH in the bitch and synergistic effects of progesterone, Abstracts of the 10th Annual Meeting of the Society for the Study of Reproduction, Austin, TX, 1977, p. 33.
12. Goding, J. R., K. J. Catt, J. M. Brown, C. C. Kaltenbach, I. A. Cumming, and B. J. Mole, Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep, *Endocrinology* **85:** 133, 1969.
13. Hauger, R. L., F. J. Karsch, and D. L. Foster, A new concept for control of the estrous cycle of the ewe based on the temporal relationship between luteinizing hormone, estradiol, and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion, *Endocrinology* **101:** 807, 1977.
14. Nequin, L. L., and N. B. Schwartz, Adrenal participation in the timing of mating and LH release in the rat, *Endocrinology* **88:** 325, 1971.
15. Caligaris, L., J. J. Astrada, and S. Taleisnik, Effect of oestrogen and progesterone on the release of luteinizing hormone in psuedopregnant rats, *Acta Endocrinol (Kbh)* **70:** 163, 1972.
16. Swerdloff, R. S., H. S. Jacobs, and W. D. Odell, Synergistic role of progestogens in estrogen induction of LH and FSH surge, *Endocrinology* **90:** 1529, 1972.
17. Mann, D. R., and C. A. Barraclough, Role of estrogen and progesterone in facilitating LH release in 4-day cyclic rats, *Endocrinology* **93:** 694, 1973.
18. Norman, R. L., Estrogen and progesterone effects on the neural control of the preovulatory LH release in the golden hamster, *Biol Reprod* **13:** 218, 1975.
19. Mann, D. R., C. D. Korowitz, L. A. MacFarland, and M. G. Cost, Interactions of the light-dark cycle, adrenal glands and time of steroid administration in determining the temporal sequence of LH and prolactin release in female rats, *Endocrinology* **99:** 1252, 1976.
20. Campbell, C. S., N. B. Schwartz, and M. G. Firlit, The role of adrenal and ovarian steroids in the control of serum LH and FSH, *Endocrinology* **101:** 162, 1977.
21. Scaramuzzi, R. J., S. A. Tillson, I. H. Thorneycroft, and B. V. Caldwell, Action of exogenous progesterone and estrogen on behavioral estrus and luteinizing hormone levels in the ovariectomized ewe, *Endocrinology* **88:** 1184, 1971.
22. Beattie, C. W., and A. Corbin, The differential effects of diestrus progestogen administration on protestrus gonadotropin levels, *Endocrinology* **97:** 885, 1975.
23. Freeman, M. C., K. C. Dupke, and C. M. Croteau, Extinction of the estrogen-induced daily signal for LH release in the rat: a role for the proestrus surge of progesterone, *Endocrinology* **99:** 223, 1976.
24. Goodman, R. L., A quantitative analysis of the physiological role of estradiol and progesterone in the control of the tonic and surge secretions of luteinizing hormone in the rat, *Endocrinology* **102:** 142, 1978.
25. Whitten, W. K., Pheromones and mammalian reproduction, *In* McLaren, A. (ed.), Advances in Reproductive Physiology, vol. 1, Academic Press, New York, 1966, p. 155.
26. Vandenbergh, J. G., Acceleration and inhibition of puberty in female mice by pheromones, *J Reprod Fertil (Suppl)* **19:** 411, 1973.
27. Bronson, F. H., Pheromonal influences on reproductive activities in rodents, *In* Birch, M. (ed.), Pheromones, North Holland, Amsterdam, 1974, p. 344.
28. Bronson, F. H., and C. Desjardins, Circulating concentrations of

FSH, LH estradiol, and progesterone associated with acute, male-induced puberty in female mice, *Endocrinology* **94:** 1658, 1974.
29. Bronson, F. H., and J. A. Maruniak, Differential effects of male stimuli on FSH, LH, and prolactin secretion in prepubertal female mice, *Endocrinology* **98:** 1101, 1976.
30. Bronson, F. H., Serum FSH, LH, and prolactin in adult ovariectomized mice bearing silastic implants of estradiol: responses to social cures, *Biol Reprod* **15:** 147, 1976.
31. Beamer, W. G., S. M. Murr, and I. I. Geschwind, Radioimmunoassay of mouse luteinizing and follicle stimulating hormone, *Endocrinology* **90:** 823, 1972.
32. Bronson, F. H., and M. H. Stetson, Gonadotropin release in prepubertal female mice following male exposure: a comparison with the adult cycle, *Biol Reprod* **9:** 449, 1973.
33. Ring, J. R., The estrogen-progesterone induction of sexual receptivity in the spayed female mouse, *Endocrinology* **34:** 369, 1944.
34. Caligaris, L. J. J. Astrada, and S. Taleisnik, Release of luteinizing hormone induced by estrogen injection into ovariectomized rats, *Endocrinology* **88:** 810, 1971.
35. Everett, J. W., and C. H. Sawyer, A 24-hour periodicity in the "LH-release apparatus" of female rats, disclosed by barbiturate sedation, *Endocrinology* **47:** 198, 1950.
36. Legan, S. J., G. A. Coon, and F. J. Karsch, Role of estrogen as initiator of daily LH surges in the ovariectomized rat, *Endocrinology* **96:** 50, 1975.