bitor, Enovid. No blood prolactin activity was found in a normal male subject during a 31-day period. These observations suggest that prolactin acts in opposition to the other 2 pituitary gonadotropins, FSH and LH, in the normal human menstrual cycle.

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1. Simkin, B., Goodart, D., *J. Clin. Endocrinol. and Metab.*, 1960, v20, 1095.

2. Sulman, F. G., *ibid.*, 1956, v16, 755.

3. McArthur, J. W., Worcester, J., Ingersoll, F. M., *ibid.*, 1958, v18, 1186.

4. Albert, A., *Recent Progr. in Hormone Res.*, 1956, v12, 227.

5. Everett, J. W., in *Sex and Internal Secretions*, W. C. Young, Ed., Baltimore, Williams and Wilkins, 1961, 497.

6. Drill, V. A., *Fed. Proc.*, 1959, v18, 1040.

7. Greep, R. O., in *Sex and Internal Secretions*, W. C. Young, Ed., Baltimore, Williams and Wilkins, 1961, 249.

8. Fried, P. H., Rakoff, A. E., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 321.

9. Riddle, O., Bates, R. W., in *Sex and Internal Secretions*, E. Allen, Ed., Baltimore, Williams and Wilkins, 1939, 1109.

10. Smith, R. A., Albert, A., Randall, L. M., *Am. J. Obstet. Gynecol.*, 1951, v61, 514.

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Delay in Ovulation in the Hen Following Stimulation of the Preoptic Brain. (28405)

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Ovulation in the hen is delayed following electrical stimulation of the anterior median eminence with stainless steel electrodes(1). The response is attributed to the delay in, or blockade of, release of the ovulation-inducing hormone (OIH) resulting from the prolonged irritative action of metallic elements electrolytically discharged from the electrodes. Everett and Radford(2) demonstrated earlier that similarly induced irritative foci in the brain of the rat facilitate ovulation. Stimulation was found to be effective in several brain sites, including areas contiguous with the median eminence, but the most consistent results were obtained by stimulation of the preoptic hypothalamus.

The preoptic region has been shown to participate in the release of OIH in the hen (3,4,5). Destruction of a specific ventral portion of the preoptic paraventricular nucleus by electrolytic lesion blocks both normal and progesterone-induced ovulation(3, 4). Microinjection of progesterone directly into the preoptic region regularly forces ovulation(5). In our previous investigation(1) electrical stimulation of the preoptic brain in a limited number of hens had no immediate effect on ovulation. A more thorough examination, however, has shown that delays in ovulation can be evoked by stimulation at this site.

Methods. All hens were one- or two-year old White Leghorns individually caged in batteries with free access to feed and water and under electric lights from 6:00 A.M. to 8:00 P.M. Hourly records of lay, maintained from 8:00 A.M. to 4:00 P.M., allowed selection of hens ovulating in recurrent sequences of 2 or 3 eggs(1). The stimulus was invoked in the conscious hen at about 14 hours prior to time of ovulation of the first (C_1) follicle of a sequence. Ovulation of the C_1 follicle can be readily advanced or delayed when the appropriate agents are administered at this time. Significant deviations from the time of normal ovulation are easily detected by digital palpation of the oviducal egg at 8:30 A.M. and 2:30 P.M. on the following day. In the present experiments, estimates based on palpation were confirmed by autopsy at 24 hours after stimulation.

Electrodes were placed systematically throughout the preoptic region at 0.5, 1.0, 1.5, and 2.0 mm bilaterally. A single unit

was used to stimulate at the midline. Stainless steel electrodes were of two types. The standard electrode was a concentric bipolar unit* consisting of an external 26 gauge sheath with a 0.1 mm contact area at the tip and an inner core, with contact area on the 90 degree point, extending 0.5 mm beyond the sheath. The core was made the anode. An electrode composed of 2 parallel lengths of 29 gauge wire, each completely insulated except for the last 0.5 mm at the tip, was also used. The cathode was 0.5 mm shorter than the anode. Platinum electrodes of the parallel design were constructed of 0.24 mm wire.

The current was derived from a Grass model S-4C stimulator. Monophasic stimuli of 4-6V, 100 cycles/sec and 1.0 msec in pulse duration were applied at alternate 15 second intervals for 10 minutes. The selected parameters formed an effective stimulus avoiding the use of high open-circuit voltages needed to monitor amperage in the circuitry employed in our earlier investigation of the median eminence. Stimuli were monitored oscilloscopically for voltage.

Electrode positions in the brains of experimental hens were checked by examination of formalin fixed, frozen, Luxol Blue-Cresyl Violet stained sections.

Results and discussion. Ovulation of the C_1 follicle is delayed following stimulation of a limited region of the preoptic hypothalamus of the hen, a finding in general accord with the results of stimulation of the median eminence reported previously. The mode of action of the effective stimulus is not the same in the two sites, however, since a delay in ovulation following stimulation at the preoptic region is not contingent on the use of stainless steel electrodes.

The sensitive region. The responsive area in the preoptic hypothalamus is outlined in Fig. 1. The region encompasses the ventromedial portion of the preoptic hypothalamus from the midline out to about 1 mm bilaterally. Stimulation proved ineffective when electrodes were placed at 1.5 or 2 mm bilaterally. The caudal limits of the area are not established. Stimulation within 0.5 mm of the ventromedial border of the preoptic region produced the most consistent response. The area of greatest sensitivity surrounds the preoptic locus specified by Ralph(3) to participate in normal ovulation, *e.g.*, the ventral portion of the nucleus praeopticus para-

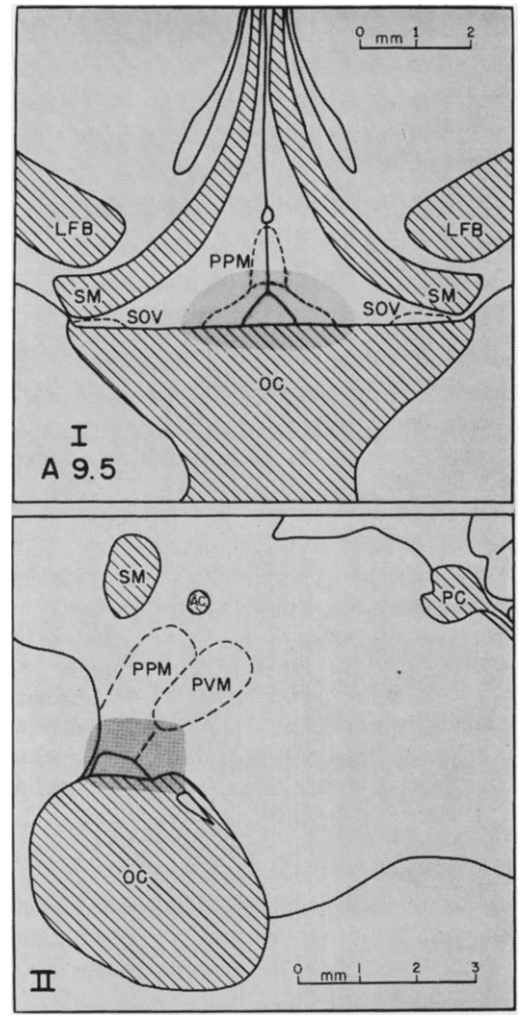

FIG. 1. Tracings of projected sections of the hen's brain indicating reactive region for follicular responses. I — transverse section through anterior-posterior coordinate 9.5. Based on coordinate system of van Tienhoven and Juhasz(7); II — parasagittal section. Shaded areas outline region of positive response. AC—anterior commissure; LFB —lateral forebrain bundle; OC—optic chiasma; PC—posterior commissure; PPM—nucleus praeopticus paraventricularis magnocellularis; PVM—nucleus paraventricularis magnocellularis; SM—tractus septomesencephalicus; SOV—nucleus supraopticus ventralis.

* Obtained from Hamilton Co., Inc., Whittier, Calif.

TABLE I. Follicular Responses Following Stimulation of Preoptic Hypothalamus.

Area	Stimulus*	No. hens	Ovulations: No. normal	Ovulations: No. Delayed P−/−	Ovulations: No. Delayed D−/−	Yolk in body cavity No. hens	Atresia No. hens
Ventromedial	St., C.	89	49	6	20	3	11
	St., No C.	47	39	3	4	0	1
	Pl., C.	32	21	2	3	1	5
Lateral	St., C.	39	37			1	1
	St., No C.	18	18				
	Pl., C.	—	—				

* St., C. steel electrodes, current.
St., No C. " " no current.
Pl., C. platinum " current.

−/− P: presumptive delay.
D: definitive delay.

ventricularis magnocellularis and fiber tracts passing caudally from this site.

Effect on ovulation. The main body of data based on stimulation of 225 hens is summarized in Table I. In the majority of hens hypothalamic stimulation had no effect on the time of C_1 ovulation. There was no evidence for premature ovulation in any hen. Ovulation was delayed in 38 hens following stimulation in the ventromedial preoptic region. As in the earlier study of the median eminence two clearly distinguishable orders of delayed ovulation were observed; (i) a definite delay of at least 10 hours based on the presence at autopsy of an intact C_1 follicle showing no macroscopically visible signs of atresia and (ii) a presumed delay of 3 to 6 hours as judged by palpation records and confirmed by the position of the egg in the oviduct on post-mortem examination. The definitive form of delay was predominant.

In 14 of the 17 hens having atretic follicles (Table I) histological study of the brain revealed that an electrode tip(s) had pierced a wall of the third ventrical or the floor of the hypothalamus. A non-specific effect of operative trauma is inferred. The correlation between specific brain injury and atresia does not always hold, however, since the brains of 4 hens ovulating normally showed equivalent damage.

Delays in ovulation were imposed both in hens given an electrical stimulus and in those in which current was not passed through the electrode. A comparison of the effects of electrode placement with or without current is not permissible in Table I since only electrical stimulation with stainless steel electrodes was used to delimit the reactive region. A consideration of the effects of electrodes placed within 0.5 mm of the ventromedial border of the preoptic paraventricular nucleus (Table II) indicates no advantage in the use of current. Characteristically, insertion of electrodes without current was effective only within this locus.

It was recognized that some proportion of the delays in ovulation might result from palpation procedures, or might in fact reflect the inadvertent use of hens not normally destined to ovulate at the expected time. One hundred and four hens selected by the same criteria as the stimulated birds accordingly were palpated in the same routine. Ovulation occurred within the accepted normal limits in all but one hen.

TABLE II. Follicular Responses Following Stimulation in Region of Preoptic Paraventricular Nucleus.

Stimulus	No. hens	Ovulations: Normal No.	Ovulations: Normal %	Ovulations: Delayed No.	Ovulations: Delayed %	Yolk in body cavity No. hens	Atresia No. hens
St., C.	32	15	46.9	11	34.1	1	5
St., No C.	17	11	64.7	6	35.3	–	–
Pl., C.	14	7	50.0	3	21.4	1	3
Totals	63	33	52.4	20	31.7	2	8

In 5 of the 225 stimulated hens (Table I) the ovulated yolk was found free in the body cavity, so located as to suggest that ovulation had occurred some hours before autopsy. Impaired oviducal function in engulfing the egg is implied and it therefore must be considered as a possible factor in the presumptively delayed ovulation. Results of our previous studies(1) favored the conclusion that ovulations of the presumptively delayed category are in fact delayed to the extent indicated by the position of the egg in the oviduct on autopsy. It is not inconceivable that an occasional ovum was found in the body because ovulation, following a prolonged delay, occurred out of phase with the usual short interval of heightened oviduct motility essential to pick up of the egg.

The delays in ovulation are thought to result from a delay in, or blockade of, neurally mediated release of OIH. We have demonstrated previously that a delay in ovulation following hypothalamic stimulation could be overcome by injection of luteinizing hormone (LH) or progesterone at 6 hours after stimulation, *i.e.*, at about the hour of normal OIH release. The response to either hormone established the integrity of the C_1 follicle. The effectiveness of progesterone affords evidence that stimulation suppressed the neural component of the OIH release mechanism, since the steroid acts on the central nervous system rather than on the anterior pituitary or the follicle(5,6). It was additionally shown that, when follicular atresia did not intervene, the definitively delayed ovulation occurred 24 hours later than would have been expected in the absence of stimulation. This was interpreted to mean that the applied stimulus blocked OIH release through that 6 to 8 hours of the 24 within which the event is normally possible (1). We repeated the experiment here with 20 hens subjected to electrical stimulation of the responsive preoptic region in the usual manner. Seven hens, found not to carry a palpable egg on the following day, were not sacrificed until 48 hours after stimulation. Autopsy findings on 3 birds left no doubt that ovulation of the C_1 follicle had occurred after an approximate delay of 24 hours. Massive follicular atresia was observed in the remaining 4 hens.

Effects on OIH release. The manner in which OIH release is blocked or delayed by preoptic stimulation remains obscure. Current theory calls for the consideration that the extrinsic stimuli may activate, or prolong the action of, mechanisms normally operant in suppression of a participating hypothalamic center. Any plausible explanation of the control of ovulation in the hen necessarily includes the premise that the recurrent release of OIH is under some positive form of inhibition through a prolonged period of each day(6). Under the 14 hour light-day prevailing in the experiments reported here, the episodic discharge of OIH is normally possible only during some 6 to 8 hours of the 24, so-called "open period" beginning at about 10:00 P.M.(1). The restrictions on OIH release are thought by Fraps(6) to be imposed, in part at least, by the feed-back of ovarian steroids. Electrical stimulation or electrode placement may cause secretion of sufficient gonadotrophin(s) to maintain blood levels of the hormone(s) effective in suppressing OIH release. Apart from any mechanism of its action, it is appropriate to note that insertion of an electrode into the hypothalamus of the rat releases LH in amounts detectable by the ovarian ascorbic acid depletion test(8).

Alternately, the stimuli used my directly excite inhibitory neurones modulating activation of the neural release mechanism. Proprioceptive impulses arising from skeletal muscles during heightened activity(9) or from the oviduct during passage of the egg (10) are suggested to inhibit OIH release in the hen. Kurotsu *et al.*(11) have shown electrical stimulation of a lateral parasympathetic zone of the hypothalamus of the rabbit to delay or block ovulation induced by stimulation of the ventromedial hypothalamic nucleus. If the ventromedial preoptic hypothalamus of the hen is, as the work of Ralph(3,4,5) suggests, an integrating center for activation of OIH release, bundles of excitatory and inhibitory neurones may be so closely positioned as to thwart selective stimulation of the excitatory fibers.

The principal objection to either possibility is that the specific inhibiting action of the stimuli applied at 4:00 P.M. must endure for some 9 to 14 hours, *i.e.*, through part or all of the open period for OIH release beginning at 10:00 P.M. This is possible through the use of stimulation with stainless steel electrodes, wherein irritative foci may be set up by electrolytically deposited iron(1,2), but would not seem likely when stimulation by platinum electrodes or insertion of electrodes without current is used.

The inconsistency of the results also argues against a direct inhibition of a central release mechanism. Delays in ovulation were observed in only about one-third of the hens following stimulation in the relatively restricted reactive region. An examination of graph paper reconstructions of projected brain sections bearing electrode tracks provided no anatomical basis for distinguishing between positive and negative responses. Unfortunately, brains from only 20 stimulated hens ovulating normally were saved for histological study and inaccurate bilateral placement of electrodes in some birds made comparisons difficult.

In view of the fact that the hypothalamus is a site of integration of a variety of metabolic and other somatic processes, as well as of external stimuli, it seems possible that the stimulus applied at 4:00 P.M. may have altered the course of some diurnal physiological process which must precede the onset of the open period for OIH release. It would follow that stimulation failed to affect ovulation in hens in which the postulated physiological state had already been attained. This hypothesis could easily be checked by stimulation at earlier or later times.

If the third of the foregoing assumptions is correct, electrical excitation of a hypothalamic release mechanism, as was our original objective, may not be possible within the period of natural inhibition. However, OIH release might be provoked during the open period following blockade of spontaneous ovulation by pharmacological means. Such a procedure has been eminently successful in the rat(2). We have not yet used it in the hen because the action of known blocking agents is not completely predictable in this species.

Summary. Ovulation was delayed in 20 of 63 hens following stimulation of a ventromedially limited region of the preoptic brain. The effect was produced following electrical stimulation with stainless steel or platinum electrodes, or by placement of electrodes without passage of current. In accord with our previous findings in the anterior median eminence, the delays in ovulation are believed to originate in the delay in or blockade of the release of ovulation-inducing hormone.

1. Opel, H., Fraps, R. M., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 291.

2. Everett, J. W., Radford, H. M., *ibid.*, 1961, v108, 604.

3. Ralph, C. L., *Anat. Rec.* 1959, v134, 411.

4. Ralph, C. L., Fraps, R. M., *Endocrinology,* 1959, v65, 819.

5. ———, *ibid.*, 1960, v66, 269.

6. Fraps, R. M., in *Control of Ovulation,* C. A. Villee, Ed., Pergamon Press, Oxford, 1961, p133.

7. van Tienhoven, A., Juhasz, L. P., *J. Comp. Neurol.,* 1962, v118, 185.

8. McCann, S. M., Taleisnik, S., Friedman, H., *Fed. Proc.,* 1960, v19, 292.

9. Bastian, J. W., Zarrow, M. X., *Poultry Sci.,* 1955, v34, 776.

10. Nalbandov, A. V., in *Comparative Endocrinology,* A. Gorbman, Ed., John Wiley, New York, 1959, p161.

11. Kurotsu, T., Kurachi, K., Ban, T., *Med. J. Osaka Univ.*, 1950, v2, 1.

Received April 17, 1963. P.S.E.B.M., 1963, v113.