regard to tumor potential) is formed in the absence of the MTV than in its presence.

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Blockade of Gonadotrophin Release for Ovulation in the Hen following Stimulation with Stainless Steel Electrodes. (26918)

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The release of pituitary gonadotrophin for ovulation following electrical stimulation of certain regions of the brain has been demonstrated in several mammalian species(1). Similar responses appear not to have been described in birds. The experiments reported here were undertaken to ascertain the possible effectiveness of stimulation in the induction of ovulation prematurely in the hen. Our procedures proved of no value in this respect, but unexpectedly resulted in apparent postponement of ovulation for some hours beyond the time of its normal occurrence in a substantial proportion of treated birds. These experiments, some results of which have been reported in abstract(2) are described here. Evidence is presented also for the conclusion that the observed delays in ovulation are dependent upon delay in, or blockade of, release of the ovulation-inducing hormone (OIH) in quantities adequate to assure ovulation.

Procedures. All experiments were carried out with White Leghorn hens in first or second years of production, individually caged in batteries under electric lights from 6:00 a.m. to 8:00 p.m., and with free access to feed and water. Hourly records of lay, routinely maintained from 8:00 a. m. through 4:00 p. m., served to identify hens regularly ovulating sequences of two or three members and failing to ovulate on but a single day between repeated sequences(3). Under conditions prevailing in this Laboratory, ovulation of the first or C_1 follicle in such sequences takes place at about 6:00 a.m. The administration of exogenous luteinizing preparations or of progesterone at around 4:00 p. m. of the day preceding ovulation of the C_1 follicle (the day of "missed" ovulation between sequences) forces its ovulation some 7 to 8 hours thereafter, or 6 to 7 hours prematurely. On the basis of these well-documented normal and experimental relationships(3), electrical stimulation was applied between 3:00 and 5:00 p. m. for effect on C_1 follicles normally destined to ovulate at about 6:00 a.m. of the following day.

The hour of ovulation was estimated by routine digital palpation of the oviducal egg (4), supplemented by observations at autopsy. Two orders of delayed ovulations were recognized; (i) presumptively delayed ovulations, completed before autopsy; and (ii) definitively delayed ovulations, based on presence of the intact C_1 follicle and complete absence of follicular atresia at time of autopsy.

Using the stereotactic procedures described by Ralph(5), bipolar stainless steel electrodes were placed bilaterally in the diencephalon, 1.0 mm on either side of the midline. The electrodes consisted of 2 parallel lengths of 29 gauge wire, each completely insulated except for a 0.5 mm ring at the tip, with the exposed ends separated by 0.5 mm.

The standard stimulus consisted of biphasic 1 msc pulse pairs, 100 cps, applied during

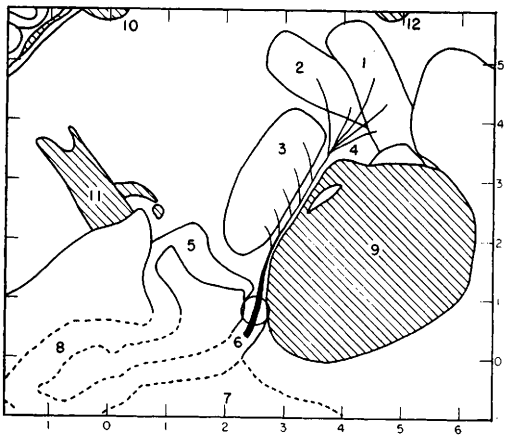

FIG. 1. Diagram of a parasagittal section of chicken diencephalon showing 1-mm stereotaxic coordinates. Dotted lines indicate approximate position of the hypophysis. 1, preoptic paraventricular nucleus; 2, magnocellular paraventricular nucleus; 3, lateral hypothalamic nucleus; 4, anterior hypophyseal tract; 5, tubero-mammillary nucleus; 6, median eminence; 7, anterior pituitary; 8, posterior pituitary; 9, optic chiasma; 10, posterior commissure; 11, oculomotor nerve; 12, tractus septomesencephalicus. (Based on C. L. Ralph, *Am. J. Physiol.*, 1959, v197, 1279.)

alternate 15 second intervals for a period of 10 minutes. The current was delivered from a Grass model S-4 stimulator and was passed through a model UTC - M 1019 pulse transformer. Current was continuously monitored oscilloscopically and was maintained at approximately 200 μA.

In an exploratory study including 67 birds, stimulation was applied routinely for 10 minutes with stainless steel electrodes in the preoptic hypothalamus, along the dorso-caudal surface of the optic chiasma in the anterior hypophyseal tract, or in the region of the median eminence. Stimulation in regions rostral to the median eminence had no effect on subsequent ovulation. Results of stimulation in a region of the median eminence at the level of the anterior hypophyseal tract (Fig. 1) were unexpected and of particular interest.

Control experiments included stimulation with platinum electrodes and placement of stainless steel and platinum electrodes for 10 minutes without passage of current. The platinum electrodes were of the same construction as the stainless steel electrodes, and were used under identical conditions in the stimulation experiments.

To verify the placement of electrodes at or close to the selected coordinate intercepts (Fig. 1), thionin stained frozen sections were made of the brains, selected at random, of approximately half the treated birds. Sagittal reconstructions were made for purposes of localization.

The luteinizing hormone (LH) preparation* used to test ovulability of the C_1 follicle following stimulation was dissolved in distilled water, 5 mg/ml and injected intravenously. In similar tests, progesterone was administered intravenously or intramuscularly. The steroid was dissolved in propylene glycol, 5 mg/ml, for intravenous use. Progesterone in corn oil, 5 mg/ml, served for intramuscular injections.

Results. The 18 hens of Group A of Table I were sacrificed 24 hours following stimulation, or 10 hours later than the hour of normally expected ovulation. Ovulation occurred at the normal hour in 9 birds. In 6, ovulation was delayed, but had taken place before the hour of autopsy. As judged by palpation records and stage of egg formation at autopsy, ovulation was delayed by some 3 to 6 or 7 hours in 5 of these hens. The ovulated yolk was found in the body cavity of the sixth hen, so located as to indicate its ovulation some hours before autopsy. The C_1 follicles of the remaining 3 hens were intact.

The finding of a yolk free in the body cavity of one hen of Group A led to the suspicion that ovulation might have occurred at the normal hour in some of the 6 hens ovulating before autopsy, the apparent delays in ovulation signifying impaired oviducal function, *e.g.*, failure to engulf the yolks within the usual brief intervals following ovulation. If this were so, an increasing proportion of yolks should be expected in body cavities as the interval from the hour of normal ovulation to autopsy was decreased. Hens were accordingly sacrificed 7 and 4 hours following the hour of normally expected ovulation (Groups B and C respectively, Table I). While 2 hens of Group C did carry yolks in body cavities, none were found in the hens of Group B.

* "PLH" of Armour Pharmaceutical Co., Kankakee, Ill.

TABLE I. Follicular Responses Following Stimulation 14 Hours before Expected Normal Ovulation.

Group	Stim'l'n to autopsy, hr	Normal ovulation time to autopsy, hr	No. hens	Ovulations*				Yolk in body cavity
				No. normal	No. delayed			
					P†	D†	Total	No. hens
A	24	10	18	9	6	3	9	1
B	21	7	16	7	3	6	9	0
C	18	4	18	7	3	7	10	2
	Totals		52	23	12	16	28	3

Follicular atresia was not observed in any hen.

* Ovulation was induced prematurely in one of the 18 hens of group C.

† P, presumptively delayed ovulations; D, definitively delayed ovulations.

The "average" incidence of yolks in body cavities—one per group—is clearly of little significance beyond suggesting that the oviduct of an occasional hen may fail to engulf the ovulated yolk.

Other results recorded in Table I bear more directly on the fact of actual delay in presumptively delayed ovulations. The incidence of normal ovulations is about the same in all 3 groups, as is the incidence (total) of delayed ovulations. In Groups B and C, however, the proportion of presumptively to definitively delayed ovulations is nearly the reverse of that seen in Group A. The inference to be drawn from these relationships is that some follicles not ovulated at 4 hours (Group C) or even at 7 hours (Group B) later than the hour of normally expected ovulation would have ovulated between 4 and 10 or 7 and 10 hours thereafter (cf. Group A). There could be no question concerning actual delay in the hour of ovulation of these follicles. If this be so, it seems probable that all ovulations of the "presumptively delayed" category, except the 3 resulting in yolks in body cavities, were in fact delayed to the extent indicated by palpation records and autopsy findings.

If any doubt remains as to delay of ovulation in hens ovulating before autopsy, there need be none concerning those hens in which ovulation had not occurred at autopsy, the definitively delayed ovulations of Table I. The absence of follicular atresia, even at 24 hours after stimulation, is especially worthy of note in this connection.

It was recognized that some proportion of delayed ovulations of Table I might result from palpation itself, or from an uncommon spread in the hour of normal ovulation. Fifty-six control hens ovulating in cycles comparable with those of the stimulated birds accordingly were palpated in the same routine. Ovulation occurred within normally expected limits in all hens.

Stimulation with platinum electrodes, or placement of either stainless steel or platinum electrodes without passage of current, all failed to delay ovulation (Table II). Placement of stainless steel electrodes without passage of current resulted, however, in the appearance of follicular atresia in those hens failing to ovulate at the normally expected hour, a condition not encountered in hens of the other groups nor in any hen subjected to stimulation with steel electrodes (Table I).

Follicular responses to LH and to progesterone administered at differing intervals following stimulation are recorded in Table III. Ovulation occurred in all of 17 hens injected with LH 6 hours following stimulation, *i.e.*, at about the hour of normal OIH release. Ovulation would have occurred in about half of these hens in response to normal release of OIH (Table I); the injected LH thus induced ovulation in the remaining half or

TABLE II. Effects of Stimulation with Platinum Electrodes and of Electrode Placement on Hour of Ovulation of C_1 Follicle.

Electrodes	Current passed	No. hens	No. ovulations		Atresia, No. hens
			Normal	Delayed	
Platinum	Yes	15	15	0	0
Steel	No	27	21	0	6
Platinum	"	13	13	0	0

TABLE III. Follicular Responses to LH and to Progesterone (Pgst) Administered Systemically at Stated Intervals Following Stimulation with Stainless Steel Electrodes.

Substance	Route*	Quantity, mg	Stim'l'n to inj., hr	No. hens	No. ovulations		Atresia, No. hens
					Total	Forced	
LH	Intrav.	2.5	6	17	17	†	0
"	"	2.5	18	7	5	2/4	5
Pgst	Intrav.	10	6	10	5	None	5
"	Intramusc.	1	6	11	11	†	0
"	"	1	0	14	14	12	2

* Intrav., intravenous; Intramusc., intramuscular. † About half of total; see text.

thereabouts in which delay was expected. No follicular atresia was seen in the ovaries of these birds at autopsy, 24 hours following stimulation.

The 7 hens of a second group were injected with LH 18 hours following stimulation. Ovulation was induced in only 2 of 4 hens in which it had not occurred before injection. Curiously, the C_1 follicles of the 2 hens failing to respond to LH showed no signs of atresia at autopsy 42 hours following stimulation, although most follicles of intermediate size were atretic.

Progesterone, administered intravenously 6 hours after stimulation, apparently failed to induce ovulation in any of the hens in which delay might have been expected (Table III). In contrast, ovulation occurred in all hens injected intramuscularly 6 hours after stimulation, and in about half of these progesterone must have induced the ovulation. Progesterone was similarly administered to hens of the last group of Table III immediately following stimulation, thus about 6 hours before normal release of OIH. Ovulation was induced in 12 of the 14 hens. Yolks were found in the body cavities of the 2 remaining hens upon autopsy 24 hours after stimulation, but times of ovulation could not be known. Atresia was seen in the follicles of only 2 hens; ovulation had been forced in both birds.

A final experiment was carried out to ascertain the fate of C_1 follicles which appeared ovulable 18 to 24 hours following stimulation, as were those of the 16 hens of Table I in which ovulation had not occurred at the hour of autopsy. Twenty-one hens were subjected to stimulation in the usual manner. Nine of the 21, a rather high proportion, were found not to carry a palpable egg 24 hours later. These 9 birds were palpated routinely the next day, and were then sacrificed at 4:00 p.m., 48 hours following stimulation. In 4 hens, palpation records and post-mortem findings established the occurrence of ovulation, at about 6:00 a.m., of the C_1 follicles whose ovulation had been delayed by stimulation. Follicular atresia had supervened in the remaining 5 hens.

Discussion. The observation that delays in ovulation in hens of the first group of Table I were of the order of 3 to 6 hours, or greater than even 10 hours, but not between some 6 and 10 or more hours, was indicative clearly of delay in or blockade of OIH release. The results recorded in Table III confirm this. Of particular interest is the response to progesterone, for this steroid acts through central nervous channels rather than on either the anterior pituitary gland or the follicle(3,6). Perhaps the most convincing evidence of blockade of OIH release by stimulation is afforded, however, by those 4 hens in which ovulation occurred without further intervention 24 hours later than would have been expected in absence of stimulation.

The timing relationships between the hour of stimulation and normal or delayed OIH release are represented in Fig. 2, which includes the final hours of the terminal day of one cycle and day 1 of a succeeding cycle. The open period embraces those 6 to 8 hours of the 24 within which OIH release may occur; the closed period includes the 16 to 18 hours of the 24 within which OIH release does not normally occur. The onset of OIH release, R_1, for ovulation of the C_1 follicle takes place about 10:00 p. m., some 2 hours following the onset of darkness. If an ap-

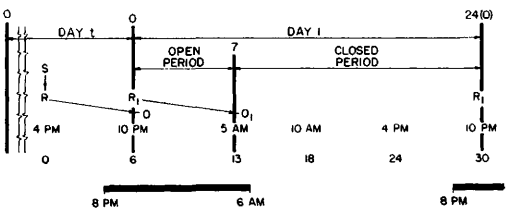

FIG. 2. Schematic representation of timing relationships involved in normal, blocked or advanced release of OIH for ovulation of the C_1 follicle. Open period: hours of the 24 within which OIH may be released. Closed period: hours of the 24 during which OIH is not normally released. R_1 at hour 0 of cycle day 1, normal OIH release for ovulation, O_1, of the C_1 follicle; R_1 at hour 24 of day 1, OIH release for ovulation of the C_1 follicle following delay of 24 hr (blockade through one open period). R, induced release of OIH in response to stimulus, S, at 4:00 P.M., or 6 hr before onset of cycle day 1. The horizontal bars at bottom of the figure represent the hours of darkness.

propriate stimulus, such as progesterone, evokes OIH release at 4:00 p. m., ovulation is correspondingly advanced.

It is clear that if electrical stimulation at 4:00 p. m. is to delay OIH release at all, it must act, directly or indirectly, at least some 6 hours later (Fig. 2). OIH releases delayed by 3 to 6 hours thus call for effective action 9 to 12 hours following stimulation. But it is clear also that duration of effect of stimulation need not exceed some 12 or 14 hours to account for the continuing delay in OIH release, since by these hours suppressive or inhibitory factors associated with the closed period come into play. With the termination of this period and onset of the succeeding open period, the blockade of OIH release is or may be ended, as was attested by OIH release in the 4 hens spoken of earlier.

Everett and Radford(7,8) have demonstrated very recently that the release of gonadotrophin for ovulation induced in the rat by brief stimulation with stainless steel electrodes is in fact mediated by electrolytically deposited iron. The delay in or blockade of OIH release in the hen following stimulation with stainless steel electrodes appears to be mediated similarly, for the effect cannot be produced by comparable stimulation with platinum electrodes nor by simple placement of electrodes. The physical basis for prolonged effect following stimulation for a brief interval thus seems to be established.

The physiological processes through which "irritative deposits" of iron may delay or block OIH release remain a matter of conjecture. A simple mechanical obstruction to transmission of nervous impulses or to passage of neurohumoral substances over fibers of the anterior hypophyseal tract seems improbable in view of the absence of atresia in the follicles of any of the 52 hens subjected only to stimulation (Table I). The fact seems the more notable in view of the advanced follicular atresia seen in some hens following the secondary treatments recorded in Table III, or placement of electrodes without passage of current (Table II). It is true that atresia was observed in 5 of the 9 hens autopsied 48 hours following stimulation alone, but this might well have been the result of OIH release at subovulatory levels, some 30 hours following stimulation (Fig. 2). In any event, follicular integrity was maintained in the remaining 4 hens in which OIH release was blocked over the initial open period and through the succeeding closed period of the cycle.

Stimulation in the tuber cinereum, bilaterally just above the median eminence, induces release of gonadotrophin for ovulation in the rat(9). Under the conditions of our experiments, stimulation in a comparable region of the hen's brain evokes the contrary response. But as in the rat, we might reasonably expect the differing response in the hen to be imposed by some positive (rather than impaired) function of the anterior pituitary. One possibility is that stimulation causes the secretion of gonadotrophin(s) at levels conducive to maintenance of estrogen concentrations capable of suppressing OIH release. Estradiol benzoate may delay ovulation of the second follicle of the ring dove's sequence by about 24 hours(10), and delays ovulation of the second follicle of the hen's 2 or 3 member sequence(11). It must be noted, however, that delay in the hour of ovulation of C_1 follicles was seldom achieved. Intramuscular injection at appropriate times of anterior pituitary preparations, or of LH or of LH + FSH preparations of chicken origin, effectively blocks the release of OIH for effect on the second follicle of the hen's 2 member se-

quence(12). It was suggested that the blockade of OIH release by the same gonadotrophic preparation (LH) so effective in forcing ovulation under differing conditions might be the outcome of increased blood estrogen levels, but we have no direct evidence that this is so. It would be of much interest to know estrogen levels not only before and after gonadotrophin administration, but also during the course of the specific activity consequent upon electrical stimulation in the hen.

Our results do not exclude the possibility that stimulation at other regions of the hen's brain, or with current of differing parameters, may induce OIH release in the hen as it does in some mammalian species. The single forced ovulation recorded in Table I may be of some import in this connection, since LH release at 4:00 p. m. of the terminal day of the cycle is not encountered normally. The differing response of the hen to stimulation may nevertheless betoken differences in organization and function of nervous or endocrine factors in birds and mammals. It is of interest in this connection that some barbiturates regularly blocking OIH release in the rat may induce OIH release prematurely in the hen(13).

Summary. Bilateral stimulation with bipolar stainless steel electrodes was applied for 10 minutes in a selected region of the median eminence of the hen, 14 hours before expected normal ovulation of the C_1 follicle. In 28 of 52 hens, ovulation was delayed by 3 to 10 hours or more. The delays in ovulation were shown to arise out of delays in or blockade of the release of ovulation-inducing hormone (OIH). The effects of stimulation on delay of OIH release may extend over a period of 12 to 14 hours. Stimulation with platinum electrodes or placement of steel or platinum electrodes without passage of current failed to delay OIH release. The prolonged effects of stimulation with stainless steel electrodes are consistent with the conclusion of Everett and Radford that electrolytically deposited iron may act irritatively long after application of the stimulatory current.

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Discrimination in Intestinal Absorption of Strontium and Calcium.* (26919)

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The preliminary results here reported represent an effort to study the effect of calcium on absorption of trace levels of radiostrontium from the intestinal tract of rats using a method which focuses attention on the absorption process. To eliminate the influence of excretory and bone deposition processes, many recent studies of intestinal absorption have been conducted *in vitro,* using the everted intestinal-sac technic described by

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