NEURAL BASIS OF DIURNAL PERIODICITY IN RELEASE OF OVULATION-INDUCING HORMONE IN FOWL

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Communicated by B. H. Willier, March 11, 1954

Everett¹ has recently reviewed the extensive evidence favoring the hypothesis that spontaneous and reflex-induced ovulation are both controlled by similar hypothalamico-hypophyseal mechanisms. This mechanism, in the rat, is believed to "be activated by a hypothalamic center in which resides the property of spontaneity and which undergoes a well-defined diurnal fluctuation of excitability." The potentiation of excitability in the rat by estrogen and progesterone is emphasized.

Like the rat, the hen ovulates "spontaneously," and the release of ovulation-inducing hormone (OIH $\equiv$ the luteinizing hormone, LH) appears to be controlled by a nervous mechanism. The neural apparatus differs however, in at least some respects, from that of the rat. Certain barbiturates which block ovulation in the rat² induce premature ovulation in the hen.³ Two of these, diallylbarbituric acid (Dial) and pentobarbital sodium (Nembutal), enhance in the hen the ovulation-inducing action of progesterone administered at near subovulatory levels. In contrast, phenobarbital sodium does not cause premature ovulation in the hen,³ but, like Nembutal in the rat,⁴ it blocks progesterone-induced ovulation when the steroid is injected at usually effective levels.^{3, 5} In this connection it is significant that progesterone is without direct effect on the ovarian follicle of the hen,⁶ and it does not induce ovulation more effectively when placed on the exposed pituitary than when applied at distant sites.⁷

The diversity of the hen's ovulatory responses to barbiturates in itself constitutes good evidence that the OIH release mechanism includes a neural component.⁸ In addition, Zarrow and Bastian⁹ have recently reported both adrenolytic and parasympatholytic drugs to block naturally occurring as well as progesterone-induced ovulation. These authors suggest that a neural link, possibly similar to that postulated for the rat and rabbit, must be present in the mechanism regulating ovulation in the hen. It may be noted also that Huston and Nalbandov¹⁰ and van Tienhoven¹¹ have reported experimentally induced irritation in certain regions of the hen's oviduct to suppress ovulation without follicular deterioration, an effect assumed to be mediated by neurogenic suppression of the episodic release of OIH (or LH) required for ovulation.¹⁰⁻¹²

While nervous control of OIH release in the hen thus seems fairly certain, little direct evidence has been adduced for the "well-defined diurnal fluctuation in excitability" so convincingly demonstrated by Everett and Sawyer in the rat.² In the ringdove, Dunham and Riddle¹³ found estradiol benzoate to cause a 24-hour delay in ovulation of the second egg in the typical 2-egg clutch of this species. Subsequently, it was reported from this laboratory that estradiol benzoate suppressed ovulation (or OIH release) in the hen under conditions believed to indicate diurnal periodicity in sensitivity of the postulated neural apparatus.⁸ The results of a more extended study of estradiol benzoate-suppressed ovulation in the hen,

presented here, suggest that estrogen decreases excitability of the neural component of the OIH release mechanism during the hours of normally high excitability. However, the action of estradiol on first ovulations of the cycle is different from its action on second (or subsequent) ovulations. These differences are explained in terms of a hypothesis formulated to account for expressions of diurnal periodicity seen in timing of the succession of ovulations constituting the hen's normal ovulation cycle. Because of the significance, experimentally and theoretically, of time relationships appearing in the ovulation cycle, these are described briefly at the outset.

THE OVULATION CYCLE

In the regularly laying hen the time of occurrence of any ovulation can be estimated fairly satisfactorily from records of lay.¹⁴ Under usual conditions of lighting and maintenance, ovulation takes place only within some 8 hours of the 24. In hens of interest here, ovulation typically occurs on each of two or more consecutive days, fails to occur on one day, and then takes place on the following day to initiate another series. The series of ovulations occurring on consecutive days is known as a sequence. The sequence, together with the single day of "missed" ovulation, constitutes the ovulation cycle. In the restricted sense in which the term is used throughout this paper, the cycle is understood to include events from the first, or C_1 , ovulation of one sequence to, but not including, the C_1 ovulation of the succeeding sequence. Sequence length is the number of ovulations occurring on successive days; cycle length refers to the number of days elapsing from the C_1 ovulation of one cycle to the C_1 ovulation of the succeeding cycle and is numerically the same as sequence length plus 1.

Except under limiting conditions in long cycles, each ovulation beyond the first of the cycle occurs later in the day than did its predecessor.¹⁴ This difference in *times of day* at which ovulations take place on successive days is denoted lag and corresponds to the "asynchronous rhythm" of Bastian and Zarrow.¹⁵ Lag decreases, in general, with increasing cycle length. The greatest value of lag is found in the 2-member sequence (3-day cycle), where it is commonly of the order of 4-5 hours. In long cycles, lag is typically greatest at the second ovulation (2.5 to 3.5 hours), decreasing thereafter to a minimal value which may approximate or equal zero hours, and then increasing with the last ovulation or two in the cycle.

Since successive ovulations within a cycle occur at later hours on consecutive days, the first, or C_1 , ovulation of the next cycle occurs at an *earlier* hour on the second day following the last, or C_i , ovulation of the preceding cycle. The interval between C_i and C_1 ovulations varies, therefore, from about 40 hours in long cycles to as much as 44 hours in a 3-day cycle. The terminal 16 hours of these intervals between C_i and C_1 ovulations are those of the 24 within which ovulation does not normally take place. The same hours recur each day of the cycle but are of special significance in the interval between C_i and C_1 ovulations. The follicle passing through the 16 terminal hours of the last day of the cycle is highly responsive, and the anterior pituitary body is capable of secreting OIH to effect its ovulation, at any time during these hours.^{6, 14} Failure of ovulation to occur during the terminal 16 hours of the last day of the cycle consequently rests in failure of the OIH "release-provoking system" during a similarly circumscribed part of the 24-hour day.⁶ The existence of diurnal periodicity in some component of the OIH

release mechanism is therefore clearly expressed in the timing of events in the ovulation cycle.

MATERIALS AND METHODS

The hens used included White Leghorns, Rhode Island Reds, and crosses between these breeds. The birds were individually caged in laying batteries, under electric lights from 6:00 A.M. through 8:00 P.M., and had access to feed and water at all times. The usual records of lay at hourly intervals from 8:00 A.M. through 4:00 P.M. served as the basis of selection of experimental hens. Most of the hens were laying in 2-egg sequences, a few in longer sequences, but all were selected on the expectation that only a single day would elapse between lay of the terminal egg of one sequence and the first egg of the following sequence. Neglecting the few hens laying in sequences of more than 2 eggs, the first, or C_1 , ovulation was estimated¹⁴ to occur at about 6:00 A.M., the second, or C_2 , ovulation at about 10:30 A.M. of the following day. The interval between C_1 and C_2 ovulations was thus approximately 28.5 hours. Following C_2 ovulation at 10:30 A.M., the C_1 ovulation of the next sequence takes place at about 6:00 A.M. of the second day thereafter, that is, after an interval of 43.5 hours. On the basis of these relationships, estradiol benzoate was injected 14–41 hours before expected C_1 ovulation, 11–28 hours before expected C_2 ovulation. The estrogen, dissolved in corn oil (Mazola) in concentrations of 5 mg. per milliliter, was found to be about equally effective following intramuscular injection at levels of 1–2.5 mg. per hen. The higher level was used in the great majority of experiments.

The occurrence of normal or suppressed ovulation was determined by palpation, according to well-established procedures,³ for presence or absence of the oviducal egg. When ovulation was suppressed, palpation was timed, through the second day particularly, to give approximate information on the hour of the delayed ovulation. The reliability of palpation in diagnosis of results was established by autopsy of a group of nine hens at 12–14 hours following the occurrence of C_2 ovulations delayed for one day, or at about 60 hours following the administration of estradiol benzoate. The ovaries of these hens were normal in all respects. The absence of atresia in any maturing follicle was conclusive evidence that the estrogen was not causing selective atresia (of the next ovulable follicle) when delay in ovulation was indicated by palpation.

One small but prevailing element of error in results arises out of the failure of an occasional hen, however regular her previous record, to ovulate as anticipated. Of any considerable number of hens selected for C_1 ovulation some 5–10 per cent may fail to ovulate as expected. Failure of C_2 ovulation to occur as anticipated is less frequent. Ordinarily such failures are of little consequence, but obviously they must be borne in mind in evaluating any very low incidence of presumed delays in ovulation.

RESULTS

Under "Ovulations Suppressed," in Table 1, are recorded the numbers and percentages of injected hens failing to ovulate on the day of expected normal ovulation, regardless of duration of delay. Numbers and percentages of delayed ovulations (1 day or more than 1 day) are also given in terms of all injected hens in respective

groups. Ovulations delayed by more than 1 day usually occurred on the second day following initial suppression, but a few failed to occur until the third day.

As is evident in Table 1, estradiol benzoate suppressed few expected C₁ ovulations; the incidence of actually suppressed ovulations is almost certainly even less than indicated, since, as has been noted, a small proportion of anticipated C₁ ovulations may fail to occur on the day following completion of a sequence. Taking the results of Table 1 at face value, however, the effect of estradiol either in suppressing C₁ ovulation or in delaying it by 1 day is fairly independent of the time elapsing from injection to normally expected ovulation. Of the total of 12 suppressed ovulations, 8, or 67 per cent, were delayed by only a single day. All delayed C₁ ovulations subsequently occurred at or close to the hour of normally expected C₁ ovulation, therefore at 24 hours, or some multiple of 24 hours, beyond the hour of normally expected ovulation.

In contrast with its extremely limited effect on C₁ ovulation, estradiol benzoate suppressed C₂ ovulation in 66, or 55 per cent, of the total of 119 treated hens (Table 1). Of the 66 hens in which C₂ ovulation was suppressed, 54, or 82 per cent,

TABLE 1

INCIDENCE OF SUPPRESSED AND DELAYED OVULATIONS FOLLOWING INJECTION OF ESTRADIOL BENZOATE AT INDICATED HOURS BEFORE EXPECTED NORMAL OVULATION

EXPECTED NORMAL OVULATION	INJECTION (HOURS BEFORE EXPECTED OVULATION)	NO. HENS INJECTED	OVULATIONS SUPPRESSED		OVULATIONS DELAYED		OVULATIONS DELAYED	
			No.	Per Cent	No.	Per Cent	No.	Per Cent
C ₁ at 6:00 A.M.	14	56	7	13	5	9	2	4
	24	16	3	19	2	13	1	6
	41	16	2	13	1	6	1	6
	14-41	88	12	14	8	9	4	5
C ₂ at 10:30 A.M.	11	13	9	69	6	46	3	23
	15	15	7	47	5	33	2	14
	19	60	31	52	28	47	3	5
	28	31	19	61	15	48	4	13
	11-28	119	66	55	54	45	12	10

ovulated on the day thereafter. The incidence either of suppressed C₂ ovulations, or of C₂ ovulations delayed by a single day, was not related to the increasing interval from injection to normally expected ovulation (Table 1); in other words, increasing the time over which estradiol benzoate might act to suppress the incidence of C₂ ovulations did not increase its effectiveness.

Delayed C₂ ovulations always occurred within the restricted hours of the 24 during which ovulation normally takes place, that is, from approximately 6:00 A.M. to 2:00 P.M. Most of the delayed ovulations occurred, however, at around 6:00 A.M., indicating that when ovulation of the C₂ follicle is delayed, this follicle subsequently behaves, as a rule, like a normal C₁ follicle. When C₂ ovulation is delayed by 1 day, the delay is therefore actually of the order of 19-20 hours (10:30 A.M. of one day to 6:00 A.M. of the next), not 24 hours, as is usually the case when the C₁ ovulation of the hen, or the second of the clutch in ringdoves, is suppressed.

Estradiol dipropionate and diethylstilbestrol have much the same suppressing action on ovulation as does estradiol benzoate. The differential effect on C₁ and C₂ ovulations is of approximately the same order as that found with estradiol ben-

zoate following injection of 2–5 mg. per hen some 14 hours before expected C_1 ovulation, or 19 hours before expected C_2 ovulation. It is of some interest that injection of as much as 5 mg. (per hen) of estradiol benzoate or dipropionate, or of diethylstilbestrol, did not appreciably increase the incidence of suppressed C_1 or C_2 ovulations.

DIURNAL PERIODICITY IN THE EXCITATION CYCLE

Since the incidence of suppressed ovulations (C_1 or C_2) was found to be independent of the time over which estradiol might act, it is not likely that estrogen interfered to any great extent either with processes of follicular maturation or with the capacity of the anterior pituitary body to produce and to secrete gonadotrophin for ovulation. On these grounds alone, it seems probable that the suppression of ovulation observed in the hen might be attributed to decreased excitability of the postulated neural component of the OIH release mechanism during the relatively limited hours of normally high excitability. The inference of diurnal periodicity in the neural apparatus is corroborated in the observation that ovulation, following suppression, always occurred during those hours of the 24 within which the event normally takes place. However, the full import of the ovulatory responses to estrogen can be evaluated critically only on the basis of some consideration of the possible role of diurnal periodicity in the normal cycle.

1. *The Normal Excitation Cycle.*—Excitation refers here to onset of the specific neural activity which results, over whatsoever pathways, in the episodic release of OIH from the anterior pituitary body, and consequently in ovulation. In cycles of given length, each excitation is assumed to precede the corresponding ovulation by a constant interval. The excitation-ovulation interval may be taken as of the order of 8 hours, a figure based on time elapsing from injection of progesterone to subsequent ovulation of the C_1 follicle.¹⁶ If the excitation-ovulation interval is constant (regardless of its absolute value), successive excitations in excitation cycles must stand in the same relationship to each other as do successive ovulations in ovulation cycles.

The neural apparatus is assumed to respond to definite levels of hormones from the ovary, and, more specifically, from the maturing follicle; these are denoted excitation hormones. Some assumption must obviously be made as to the course by which blood levels of excitation hormones increase with time. Perhaps the simplest view is to suppose excitation hormone levels associated with follicles maturing on successive days to be substantially the same at equal times following the preceding excitation (or OIH release or ovulation). This means that each curve of increasing hormone concentrations, beyond the first of the follicular sequence, is displaced, *in time of day*, by the extent of lag at the preceding excitation.

The relationships implicit in the foregoing premises are represented formally for a 7-day cycle in Figure 1. Shaded abscissae indicate the hours of darkness, 8:00 P.M.–6:00 A.M. Hours of the cycle day are 0–24. The curve passing through E_1 , E_2 , . . . , E_6 and E_1' describes the postulated diurnal rhythm in neural thresholds; here thresholds (of response) represent the inverse of excitability or sensitivity in the neural apparatus. This curve is understood to be repeated each day of the cycle. Ordinates to the curve measure levels of excitation hormones required for neurogenic response. Curves C_1 , C_2 , . . . , C_6 and C_1' represent terminal concentra-

tions of excitation hormones associated with follicles of the same designations. Curves $C_2, C_3, \dots, C_6$ reach values required for excitations $E_2, E_3, \dots, E_6$ at later hours on successive days. Curve C_1' , however, though displaced only by the extent of lag at E_6 , fails to attain to threshold values on day 7 of the cycle but does so at zero hour of the following day to initiate a new cycle. The first excitation, E_1 , of the complete cycle shown in Figure 1 is assumed to have occurred under the same conditions as did E_1' .

The characteristics of timing in ovulation cycles follow clearly from the relationships shown in Figure 1. The restriction of ovulation to within about 8 hours of the 24-hour day—the most obvious aspect of diurnal periodicity—depends upon the appearance of relatively low thresholds in the neural apparatus (i.e., high sensitivity or excitability) during a similarly restricted part of the 24 hours. The characteristic pattern of lag is seen to arise out of an orderly change in relationship between successively displaced excitation curves and variation in neural thresholds

FIG. 1.—Relationships between diurnal rhythm in thresholds of response in the neural apparatus and excitation hormone concentrations in a 6-member sequence followed by 1 day with no excitation, thus constituting a 7-day cycle.

with time of day. Failure of the C_1 follicle to ovulate on the last day of the cycle results from the relatively high thresholds exhibited by the neural apparatus during some 16 hours of every day, but evident only when the excitation hormone curve associated with a given follicle is so displaced that excitation cannot occur during these hours. But if excitation hormone concentrations associated with such a follicle, the C_1' of Figure 1, continue to increase during these terminal 16 hours of the last day of the cycle, excitation of the neural apparatus should occur finally at higher thresholds than will be possible elsewhere in the cycle. In the hen, this means at an earlier time of day.

2. *The Estrogen-modified Cycle.*—Neglecting small differences associated with cycle length, the first excitation of the 3-day cycle may be supposed to occur at zero hour in Figure 1, the second (and last) at about hour 4.5, thus at about the time of E_3 in the figure. Excitation hormone curves C_1, C_3 , and C_1' of the figure may likewise be supposed to represent curves C_1, C_2 , and C_1' in the 3-day cycle.

It is clear that even a moderate increase in neural thresholds during hours when such thresholds are normally low would result in failure of excitation to occur between, for example, hours 4 and 8 of the cycle day. Suppression of the expected C_2 ovulation would, of course, follow. A considerably greater increase in thresholds (that is, decrease in excitability) would be required to prevent excitation E_1 , normally occurring at zero hour of the cycle day. The results given in Table 1, showing the much lower incidence of suppressed C_1 than of C_2 ovulations, are thus in good accord with theoretical expectations.

It has already been pointed out that a large proportion of suppressed C_2 ovulations (82 per cent) occurred following delay of a single day. Estradiol benzoate is known to be effective for several days, and any increase in neural thresholds caused by this estrogen should be as evident on the day following suppression of ovulation as on the previous day. If, however, the C_2 follicle continued to produce excitation hormones at about the usual rate following failure of excitation to occur at the normal time, concentrations of these hormones on the day thereafter should be of the same order as are concentrations normally associated with the C_1 follicle (C_1' of Fig. 1). Thus the incidence of C_2 ovulations delayed for more than 1 day should be comparable with the incidence of C_1 ovulations delayed by a single day. An examination of the results recorded in Table 1 shows that, of 66 suppressed C_2 ovulations, only 12 or 18 per cent, failed to ovulate on the day thereafter. This figure, 18 per cent, is to be compared with the *initial* suppression of 14 per cent of 88 possible C_1 ovulations. The 19–20-hour delay (rather than 24 hours) typical of C_2 ovulations occurring on the day following their suppression is also to be expected in view of the foregoing considerations.

DISCUSSION

A most interesting outcome of this study of estrogen-suppressed ovulation lies in the consonance of results with the hypothesis proposed to account for timing in the normal ovulation cycle of the hen. Nevertheless, it is to be recognized that "the excitation cycle" represents a highly formal and no doubt oversimplified statement of relationships much in need of further analysis. The conveniently designated excitation hormones, for example, may include effects of estrogen, probably of progesterone or of a physiologically equivalent steroid,¹⁷ and possibly of androgen.¹⁸ The diurnal rhythm in neural thresholds may reside in a well-defined hypothalamic center, but thresholds in this postulated center would almost certainly be subject to neural stimuli arising elsewhere,¹⁹ and these, in turn, to possible modification by one or more of the several "excitation" hormones. The significance of the "neural connection" between oviduct and pituitary gland¹⁰ and that of the theory of the ovulation cycle developed by Nalbandov¹² on the basis of this relationship also calls for attention. Despite the claims of these and of other questions of specific mechanisms involved, the proposed hypothesis of the excitation cycle may serve to emphasize the fundamental importance of diurnal periodicity in the interpretation both of normal cycle relationships and of a considerable body of experimental results.

In contrast with the suppression of ovulation by estrogen in the hen, appropriately timed injection of estrogen advances ovulation in the 5-day cycling rat by about 24 hours.²⁰ Advancement of ovulation in this species is attributed to *in-*

creased excitability of the presumably hypothalamic center in consequence of increased estrogen levels;^{20, 21} suppression of ovulation in the hen has been attributed to *decreased* excitability of a comparable apparatus. We are confronted here with another of those dissimilar responses in the rat and in the hen for which no satisfactory explanation can be offered at present. A clue to these differences may possibly lie in Harris' observation that the hypothalamus may be "regarded as a region where the effect of neural stimuli, arising in the external environment and in the higher centers of the brain, are integrated with humoral factors."¹⁹ Conceivably, these "stimuli" from higher centers may include inhibiting as well as stimulating influences. Quantitative differentials in the effects of certain agents (e.g., estradiol benzoate) on neural centers outside the hypothalamic center immediately concerned in activation of the hypophysis might then lead to completely contrary responses such as advanced or delayed ovulation, in the rat and the hen. The differing and sometimes opposed effects of different barbiturates on ovulation in the hen may have a similar basis in differential neural responses antecedent to the final reaction of the presumed hypothalamic center to excitation hormones.

A direct comparison of the effect of estradiol in the hen and the ringdove is not possible, since Dunham and Riddle were able to observe delayed ovulation only of the second egg of the ringdove's clutch.¹³ Moreover, ovulation of the first egg of the ringdove's clutch takes place around midnight, while the second ovulation occurs 4-6 hours *earlier* on the second day thereafter. Timing of first and second ovulations of the dove's clutch is thus not comparable with timing of C_1 and C_i ovulations of the hen's sequence, but rather with the C_i ovulation of one sequence and the C_i ovulation of the succeeding sequence. In both species, it is the ovulation normally occurring earliest, *in time of day*, that is delayed by 24 hours or some multiple thereof. The existence of diurnal periodicity in some element of the ovulation-inducing mechanism of the dove seems implicit in the 24-hour delay, as was recognized by Dunham and Riddle. It seems likely that a neural apparatus similar to that believed to exist in the hen is involved also in the periodicity observed in the ringdove. If so, the "lateness" of the first ovulation in the dove may result from partial suppression of neural thresholds in consequence of higher estrogen levels presumably associated with ovulation of the first follicle of the pair.¹³

SUMMARY

Estradiol benzoate injected intramuscularly into regularly ovulating hens at levels of 1.0-2.5 mg. per hen suppressed 14 per cent of expected first (C_1) ovulations of the cycle, in contrast with 55 per cent of expected second (C_2) ovulations in similar cycles. Sixty-seven per cent of suppressed C_1 ovulations and 82 per cent of suppressed C_2 ovulations occurred within the hours of normally occurring ovulation on the day following suppression. The incidence of suppressed ovulations (C_1 or C_2) and the proportion of ovulations delayed by 1 day were independent, over wide ranges, of the time from injection to normally expected ovulation. These observations constitute strong evidence that the neural mechanism believed to control the release of ovulation-inducing hormone from the anterior pituitary body exhibits diurnal periodicity in its thresholds of response to excitation hormones. This concept is shown to provide a basis for explanation of many of the characteristics of timing of the hen's ovulation cycle, as well as of effects following the administration of estradiol benzoate.

The author wishes to express his appreciation to Dr. M. H. Conner for carrying out many of the experiments. Estradiol benzoate and other estrogens were supplied most generously by the Research Department of Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

- ¹ J. W. Everett, *Ciba Found. Coll. Endocrinol.*, **4**, 167-178 (1952).
- ² J. W. Everett and C. H. Sawyer, *Endocrinology*, **47**, 198-218 (1950).
- ³ R. M. Fraps and J. F. Case, *Proc. Soc. Exp. Biol. Med.*, **82**, 167-171 (1953).
- ⁴ J. W. Everett, *Anat. Rec.*, **109**, 291 (1951).
- ⁵ R. M. Fraps, *Poultry Sci.*, **32**, 899 (1953).
- ⁶ I. Rothchild and R. M. Fraps, *Endocrinology*, **44**, 141-149 (1949).
- ⁷ I. Rothchild, *Federation Proc.*, **8**, 135 (1949).
- ⁸ *Report of Chief of Bureau of Animal Industry* (United States Department of Agriculture, 1953), p. 41.
- ⁹ M. X. Zarrow and J. W. Bastian, *Proc. Soc. Exp. Biol. Med.*, **84**, 457-459 (1953).
- ¹⁰ T. M. Huston and A. V. Nalbandov, *Endocrinology*, **52**, 149-156 (1953).
- ¹¹ A. van Tienhoven, *Anat. Rec.*, **115**, 374 (1953).
- ¹² A. V. Nalbandov, *Poultry Sci.*, **32**, 88-103 (1953).
- ¹³ H. H. Dunham and O. Riddle, *Physiol. Zool.*, **15**, 383-394 (1942).
- ¹⁴ I. Rothchild and R. M. Fraps, *Endocrinology*, **44**, 134-140 (1949).
- ¹⁵ J. W. Bastian and M. X. Zarrow, *Proc. Soc. Exp. Biol. Med.*, **79**, 249-252 (1952).
- ¹⁶ R. M. Fraps and A. Dury, *Proc. Soc. Exp. Biol. Med.*, **52**, 346-349 (1943).
- ¹⁷ R. M. Fraps, C. W. Hooker, and T. R. Forbes, *Science*, **108**, 86-87 (1948).
- ¹⁸ A. V. Nalbandov, (unpublished; cited by Huston and Nalbandov, *op. cit.*).
- ¹⁹ G. W. Harris, *Ciba Found. Coll. Endocrinol.*, **4**, 106-114 (1952).
- ²⁰ J. W. Everett, *Endocrinology*, **43**, 389-405 (1948).
- ²¹ C. H. Sawyer, J. W. Everett, and J. E. Markee, *Endocrinology*, **44**, 218-233 (1949).

MENDELIAN AND NON-MENDELIAN INHERITANCE OF STREPTOMYCIN RESISTANCE IN *CHLAMYDOMONAS REINHARDI*

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Communicated by M. Demerec, March 4, 1954

Studies of inheritance in the green alga *Chlamydomonas reinhardi* were initiated in the search for material in which heritable alterations affecting the chloroplast and other cytoplasmic bodies could be analyzed. It has been shown^{1, 2} that this organism possesses a readily controlled, complete sexual cycle and that a number of factors affecting mating type,³ pigment formation,⁴ and streptomycin resistance⁴ exhibit the expected 2:2 segregation and recombination. In addition, one instance of non-Mendelian segregation has been observed; the character concerned here, too, is streptomycin resistance. It is with the analysis of the nonchromosomal nature of this inheritance that the present report is concerned.

Inheritance of resistance to streptomycin and other growth inhibitors has previously been studied primarily in bacterial systems⁵ where conventional methods of genetic analysis were not available.

Materials and Methods.—Nutritional studies with *C. reinhardi*, control of the mating reaction, and methods of genetic analysis are described elsewhere;^{2, 4, 6} relevant