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Time Required for Induction of Ovulation Following Intravenous Injection of Hormone Preparations in Fowl.

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In both the normal (non-pretreated)¹ and the pretreated² hen, ovulation is induced by the intravenous injection of appropriate hormone preparations, and it has become a matter of interest to determine the interval between injection of the ovulatory preparation and rupture of the ovarian follicle. In most hens under the conditions of experiment thus far employed the interval is between 6.5 and 8.5 hours, indicating a remarkably direct effect of the intravenous injection.

Materials and Methods. Only White Leghorn hens approximately one year of age were used in the tests of pretreated hens. White Leghorn and Single Comb Rhode Island Red hens of differing ages were injected in establishing the ovulatory interval for normally laying (non-pretreated) hens. Most of the hens in this latter group served simultaneously in supplying data on rupture of the premature normal follicle.¹ Additional hens were used in the present connection, particularly in checking the minimal time of response to the ovulatory injection.

All hens were kept in laying batteries under a constant 14-hour light day, and hourly laying records were maintained for two weeks prior to and during the experimental period.

The pretreated hens were all intravenously injected with the luteinizing preparation from horse anterior pituitaries previously used in the induction of multiple ovulation.² In the normally laying (non-pretreated) hens the same preparation was used in addition to the predominantly follicle-stimulating preparation, Prephysin, and two preparations from the serum of pregnant mares, Gonadin serum and Anteron.* Each preparation was administered over a considerable range of dosages.

The procedure used in pretreatment was essentially that described

¹ Fraps, R. M., Olsen, M. W., and Neher, B. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **50**, 308.

² Fraps, R. M., and Riley, G. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **49**, 253.

* These products are briefly described in citation 1, above.

earlier,² 100 rat units of pregnant mares' serum being administered subcutaneously, daily, through 8 days. After pretreatment the ovulatory injection (the luteinizing preparation only) was made at the uniform level of 20 rat units (Fevold seminal vesicle test) per hen. The injected hens were sacrificed at known times and upon autopsy the number of ovulated follicles was determined. If an ovulated yolk had entered the oviduct, the oviducal level at which it was found was ascertained as carefully as possible. The time required by the yolk, following ovulation, to reach definitive regions of the oviduct was then estimated on the basis of the time relations described by Warren and Scott.³ The time of ovulation was thus arrived at, and the difference between known time of injection and estimated time of ovulation was taken as the interval required for ovulatory effect. This estimate of the ovulatory interval applies of course only to the yolk picked up by the oviduct. In some hens ovulated yolks were found only in the body cavities at autopsy; it was assumed that in these hens ovulation had occurred not more than 15 minutes prior to the time of autopsy.⁸ The progress of ovulated yolks may not be entirely normal in pretreated hens, and the data reported for such hens are accordingly limited to groups in which yolks were in the anteriormost levels of the oviduct or free in the body cavities of autopsied hens.

Estimation of time of ovulation of yolks in normally laying hens (non-pretreated) was identical with procedures just described except that estimates were made also for yolks in more advanced oviducal regions in a number of instances, but in no case for yolks beyond the isthmus.

Results. (1) *Pretreated Hens.* A considerable number of pretreated hens were autopsied at various intervals following injection of the ovulatory preparation, intervals which proved much too long to permit of accurate estimates of times of ovulation and hence of the ovulatory interval. Results on these birds and others which served to determine the approximate ovulatory interval need not be discussed here.

Injection and autopsy data on 4 groups of hens sacrificed at around 7 hours following the ovulatory injection are given in Table I. Fourteen of the 15 injected hens had ovulated at the time of autopsy, the single failure occurring in a hen of the group injected at 8:30 a.m. The number of ovulations per ovulating hen also was lowest in the 8:30 a.m. group, the average being 1.5. While the differences between the 8:30 a.m. and the other groups may be

³ Warren, D. C., and Scott, H. M., *Poultry Sci.*, 1935, **14**, 195.

TABLE I.
Time of Ovulation Following Intravenous Injection of a Luteinizing Preparation,
20 Units per Hen, into White Leghorn Hens Pretreated with Pregnant Mares'
Serum, 100 Units Daily, through 8 Days.

No. of hens	Time of ovulatory injection	Injection to autopsy, hr	Ovulations per ovulating hen, No.	Injection to ovulation	
				Avg, hr	Range, hr
5	8:30 a.m.	7.2	1.5	7.0	6.6-7.2
4	1:00 p.m.	7.1	2.3	6.7	6.3-7.1
3	6:00	7.1	3.3	6.9	6.6-7.1
3	9:15	7.1	2.7	6.6	6.1-7.0

of some significance in other connections, there appears to be no consistent trend in either average time from injection to ovulation or in range of the ovulatory interval in hens injected at different times of the day.

The time from injection to ovulation, averaged by groups, varied by only 0.4 hour, and the range in ovulatory interval for all ovulating hens was from 6.1 to 7.2 hours. Since the average time from injection to autopsy was only 7.1 hours, the average time from injection to ovulation, 6.8 hours for the 14 ovulating hens, cannot possibly be more than 0.3 hour in error on the maximal side. The time of first ovulation following injection is not so positively determined, since this involves estimation of age of yolks in oviducts and must assume that the first yolk to be ovulated is also the first to be picked up by the oviduct. The minimal figures for the ovulatory interval (Table I, "range") are accordingly to be considered high rather than low.

(2) *Normally Laying (Non-pretreated) Hens.* The interval between injection of the ovulatory preparation and ovulation was estimated for each of 69[†] hens. The percentages of hens ovulating during one-half hour periods from 6.5 to 10.0 hours following the ovulatory injection, are shown in Fig. 1. Ninety percent of all ovulations occurred between 6.5 and 8.5 hours following the ovulatory injection, the largest percentage, 31.9, falling between 7.0 and 7.5 hours, and almost equal percentages, 21.7 and 23.3, occurring in the 6.5-7.0 and 7.5-8.0 hour periods respectively.

Seven yolks were found in body cavities of hens autopsied between 8.1 and 9.6 hours following ovulatory injection, and it is perhaps a question whether the oviduct failed to pick up these or whether the hens were killed very soon after ovulation had occurred. The latter

[†] The same hens were used in the induction of ovulation prematurely (citation 1, above); of the 70 prematurely ovulating hens one was omitted in the present connection because of inadequate autopsy record.

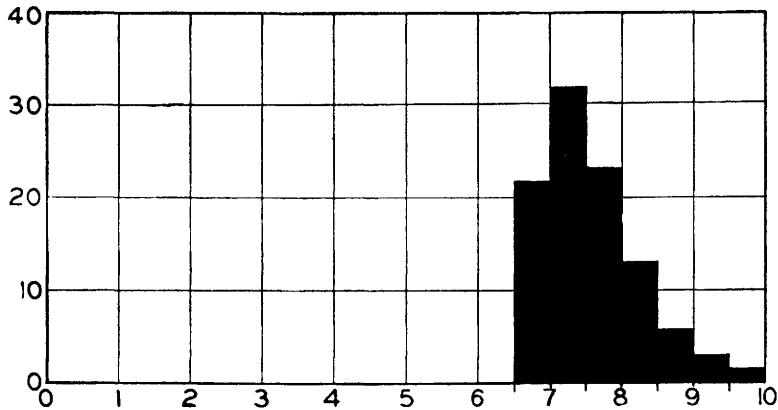


FIG. 1.

Distribution of induced ovulations, non-pretreated hens, in time from injection of ovulatory preparation. Abscissas, time in hours from injection (0 hour); ordinates, percentage of ovulations occurring within indicated 0.5-hour intervals.

possibility seems more probable, in some instances, at least, since yolks were found in infundibula of 2 hens, one killed during the 8.0-8.5 and the other during the 9.0-9.5 hour periods following injection. Furthermore, the mean estimated ovulatory interval decreases as yolks are found in increasingly caudal oviducal regions. The average interval for yolks found in the infundibulum is 8.8 hours, in the magnum, 7.6 hours, and in or very close to the isthmus, 6.9 hours. Such a relation is of course expected, since in general these yolks first to be ovulated would also be the first to enter the oviduct.

In order to check directly the minimal ovulatory interval 12 hens were divided into 3 groups of 4 each and sacrificed at precisely 6, 6.5, and 7.0 hours following injection of 1200 units Anteron per hen. No ovulations occurred in the hens killed at 6 hours, one occurred while the ovary of a hen in the 6.5 hour group was being removed, and 2 of the 4 hens killed at 7 hours had ovulated. This result is in good agreement with the relations shown in Fig. 1.

Comment. The time from injection of the ovulatory preparation to ovulation was, on the average, significantly shorter and the interval showed considerably less variation in pretreated hens than in non-pretreated hens. It should be emphasized, however, that in the pretreated hens—all of the same breed and age—a uniform, relatively high dosage of a potent ovulatory preparation was administered, whereas the non-pretreated hens were of differing breeds and ages into which injections of four different preparations were made at varying dosage levels to induce ovulation of follicles in varying

degrees of maturity. Furthermore, it may be assumed that most of the ovulating follicles of the pretreated ovary are, in consequence of pretreatment, wholly mature at the time of injection of the ovulatory preparation and therefore respond more uniformly to the ovulatory stimulus.

The minimal interval between injection and ovulation in either pretreated or non-pretreated hens appears to be appreciably less than are the intervals for even the most reactive of mammalian ovaries. However, the differences are not of large order, and the approximate similarity of interval rather than differences should perhaps be remarked upon. In any event, the response of the avian follicle within 6 to 9 hours is the more striking in view of the relatively enormous size of the ovarian follicle, the structural and physiological peculiarities associated with the deposition of deutoplasm, and the marked differences in ovulatory sequences of mammals in comparison with birds generally and with the domestic hen specifically.

Summary. The time at which ovulation occurs following intravenous injection of appropriate hormone preparations into pretreated and normally laying (non-pretreated) hens has been determined. The interval in pretreated hens averaging more than 2 ovulations per hen at time of autopsy was 6.8 hours, range 6.1-7.2 hours. Ninety percent of all normally laying hens (69) ovulated from 6.5 to 8.5 hours following ovulatory injection, the remaining 10% of injected hens requiring up to 9.6 hours for ovulation. The non-pretreated hens were injected with 4 different hormone preparations administered at differing dosage levels and at times calculated to effect ovulation of follicles at varying degrees of prematurity. The minimal ovulatory interval recorded for pretreated hens was 6.1 hours, for non-pretreated hens, 6.5 hours.

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Effect of Air Currents on Plasma Prothrombin.*

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Tocantins¹ recently reported that plasma exposed to air currents

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¹ Tocantins, L. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 251.