quite as rapidly as cholesterol, such that in severe dystrophy the proportion of cholesterol in the total lipids was slightly but perhaps not significantly increased. In brain tissue of dystrophic animals cholesterol, both free and total, markedly increased, especially the free—such that the proportion of cholesterol esters was insignificant. Total lipids increased in moderate dystrophy; they were subnormal in amount in severe dystrophy and the significance of the high proportion of cholesterol in total lipids is not understood. These results are in general similar to those obtained in rabbits,[1] with 2 or 3 exceptions. One of these is the marked decline in the total lipids in the brain of severely dystrophic rats just mentioned. The course of the disease in rats and in rabbits is dissimilar and the chronic condition in which rats, for months, miserably drag themselves around to secure food and drink, is very different from the complete prostration of rabbits. The brain tissue of normal and control rabbits contains practically no esterified cholesterol, whereas in normal and control rats, about a third of the total cholesterol was esterified. Finally, the differences in muscle cholesterol as between normal and control animals may be related to the high fat diet of the latter. Furthermore, some dog biscuits are not a rich source of vitamin E[6] and the control animals were more generously supplied with it than the normal animals. The significance of these lipid changes will not be understood until the role of vitamin E in oxidative processes[7] is clarified.

Summary. In rats on a vitamin E-deficient diet the cholesterol content of muscle was significantly increased, the total lipid less markedly. In brain tissue the cholesterol content was markedly increased, especially that of the free cholesterol, such that cholesterol esters represented about 5% of the total; in control animals this figure was 35%. These changes generally parallel those reported for rabbit tissue but are not as striking, perhaps because in grown rats dystrophy develops slowly. An understanding of these changes must await further knowledge of the functional activity of vitamin E.

6 Mason, K. E., *J. Nutrition*, 1942, **23**, 71.

7 Houchin, O. B., *J. Biol. Chem.*, 1942, **146**, 313; Kaunitz, H., and Pappenheimer, A. W., *Am. J. Physiol.*, 1943, **138**, 328.

14151

Occurrence of Premature Ovulation in the Domestic Fowl Following Administration of Progesterone.*

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During the course of tests undertaken to determine the possible value of steroids in controlling the release of pituitary gonadotrophins in fowl, it was unexpectedly found that progesterone, injected under appropriate conditions, regularly caused the premature ovulation of ovarian follicles in laying hens.

Progesterone is known to induce ovulation in amphibia,[1,2,3] but we are not aware that a similar result has been obtained previously either in birds or in normal mammals. Pearl and Surface[4] concluded that the administra-

* This investigation was carried out under grant of funds provided by the Bankhead-Jones Act.

1 Shapiro, H. A., *Chem. Indus.*, 1936, **55**, 1031.

2 Zwarenstein, H., *Nature*, 1937, **139**, 112.

3 Langan, W. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 59.

4 Pearl, R., and Surface, F. M., *J. Biol. Chem.*, 1914, **19**, 263.

tion of a fat-free lutein preparation inhibited ovulation in the laying hen, and Dunham and Riddle[5] have reported that crystalline progesterone tends to inhibit ovulation of the second of the 2 follicles constituting the usual clutch sequence of ring doves and pigeons. Previous findings in birds thus appear to be in agreement with the generally accepted conclusion that the administration of progesterone inhibits ovulation in mammals.[6,7]

Differential follicular reactivity in the hen. In the steadily laying hen each of a series of follicles is ovulated at intervals of 25 to 28 hours over a number of successive days. Ovulation of the follicle initiating a cycle occurs generally between 4:00 and 7:00 o'clock (E.W.T.) of the morning following a day of missed ovulation. This first follicle of the cycle, or clutch sequence, is more sensitive to progesterone and other ovulation-inducing substances administered under stated conditions than are subsequent follicles of the sequence.[8] We shall therefore make the following distinctions:

C_1 *follicle,* the first or primary follicle of a clutch sequence, normally ovulated 14 or more hours following lay of last egg of the preceding cycle, or at least 38 hours following a prior ovulation.

C_s *follicle,* any follicle except the C_1 follicle of a clutch sequence, typically ovulated within an hour following lay of the preceding egg, or from 25 to 28 hours following a preceding ovulation. It is not necessary, at this time, to distinguish between constituent C_s follicles.

Materials and Methods. Experimental and control material consisted of laying hens of several standard breeds, including Rhode Island and New Hampshire Reds, White Leghorns, Columbians and some crossbreds. Most of the hens were in first to third year of production. No tests were limited to hens of a single breed or age. All birds were maintained in individual cages in laying batteries, were subjected to a constant 14-hour light day and had access, *ad libitum,* to a standard laying mash. Before any bird was injected for test, the hour of lay was recorded from 8:00 a.m. through 4:00 p.m. over at least the number of days required to establish the characteristics of the individual hen's laying cycle.

All tests were based on a single injection of progesterone for effect on the single normally maturing follicle next due to ovulate at a known time. Hour of normally expected ovulation of C_1 follicles was determined by autopsy of some 75 uninjected control hens during the course of reported tests. Estimation of time of next expected normal ovulation of C_s follicles was based on a previously described procedure,[9] but control hens were autopsied regularly in confirmation of the relations involved.

Diagnosis of forced ovulation of C_1 follicles was made in most hens by palpation for presence or absence of egg *in utero,* identification of such eggs preceding by at least 6 hours the similar identification of eggs dependent on ovulation at the normal hour. Other hens were sacrificed for estimation of minimal prematurity of forced ovulation.

Practically all hens injected for effect on C_s follicles were autopsied for identification of forced ovulation in accord with tested criteria.[9] The few exceptions consisted of hens which laid prematurely the egg in the oviduct at the time of injection, and which carried *in utero,* at or before the hour of normally expected ovulation, the egg consequent upon forced ovulation.

Crystalline progesterone was administered in all but a very few tests in which a corpus luteum preparation† was injected subcutaneously with good result. Progesterone was dissolved in propylene glycol for intravenous injection (wing veins), and usually an equal volume of distilled water was added to the solution prior to injection of low dosages. Not more than 1.0 ml of propylene glycol per kilo body weight was injected into any bird, a quantity shown by control tests to have no

[5] Dunham, H. H., and Riddle, O., *Physiol. Zool.,* 1942, **15**, 383.

[6] Makepeace, A. W., Weinstein, G. L., and Friedman, M. H., *Am. J. Physiol.,* 1937, **119**, 512.

[7] Hartman, C. G., *Sex and Internal Secretions,* 1939, Baltimore.

[8] Fraps, R. M., and Dury, A., *Anat. Rec.,* 1942, **84** (No. 4), 3.

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

† "Lipo-Lutin," Parke, Davis and Co.

TABLE I.

Effect of Progesterone, 4:00 p.m. Injections, in Forcing Ovulation of Ovarian Follicles of the Hen at Least 6 Hours Prematurely.

Injection		Hens with C_1 follicles			Hens with C_8 follicles		
			Ovulating			Ovulating	
Route	Level, mg	Injected, No.	No.	%	Injected, No.	No.	%
Intravenous	.01	—	—	—	10	0	0
"	.02	9	1	11.1	20	0	0
"	.05	13	4	30.8	18	0	0
"	.20	27	19	70.4	11	0	0
"	.50	9	8	88.9	6	0	0
"	1.00	10	9	90.0	7	0	0
"	1.5-5.0	—	—	—	8	0	0
"	10.0	17	10	58.8	7	1	14.3
"	15.0-40.0	—	—	—	16	1	6.3
Subcutaneous	.05	20	0	0	—	—	—
"	.125	21	4	19.0	9	0	0
"	.25	29	20	69.0	8	0	0
"	.50	25	19	76.0	15	11	73.3
"	1.0	19	18	94.7	14	10	71.4
"	2.0	11	10	90.9	7	5	71.4
"	3.0	—	—	—	13	10	76.9
"	5.0	7	7	100.0	11	8	72.7
"	10.0	10	9	90.0	10	5	50.0

effect on ovulation in the hen. Progesterone was dissolved in sesame or in corn oil for subcutaneous injection (dorsal neck region).

Results and Discussion. The main body of data, based on injection of 417 hens, is summarized in Table I. The C_1 follicle is shown to be highly reactive to progesterone administered either intravenously or subcutaneously, while the reaction of the C_8 follicle differs to an extreme degree with route of injection. It is notable that a fairly constant percentage, 71 to 77, of C_8 follicles ovulated prematurely following subcutaneous injection of progesterone at all levels between 0.5 and 5.0 mg.

The striking contrast in reaction of the C_8 follicle with route of progesterone administration suggests that the hormone must act on the follicle over a sufficiently long time as well as at a minimally effective level in order to force ovulation prematurely, conditions satisfied by subcutaneous but not by intravenous injection. This interpretation of action on C_8 follicles implies a relatively rapid elimination of progesterone from the blood stream, and supposes further that the more sensitive C_1 follicle reacts to progesterone within a relatively limited time.

Direct comparison of effects on C_1 and C_8 follicles is not permissible in Table I, since time from injection to expected normal ovulation of C_1 follicles is approximately 3 hours less than is the same interval for C_8 follicles. Progesterone was therefore injected at 11:00 a.m. for effect on C_1 follicles, thus substantially increasing the interval from injection to normal ovulation of the C_1 follicle over that for C_8 follicles following the 4:00 p.m. injections of Table I. Intravenously injected, progesterone at the 0.20 mg level forced ovulation in 53% of 17 treated hens. Progesterone subcutaneously injected at the 0.25 mg level effected premature ovulation in 54% of 24 treated hens. These yields are sufficiently high, at the submaximal doses injected, to indicate that differences appearing in Table I with respect to C_1 and C_8 follicles can be attributed only in small part to differences in intervals from injection to expected normal ovulation.

It is of interest to note that the time from administration of progesterone (intravenously, 0.2 to 0.6 mg per hen or subcutaneously, 0.5 to 1.5 mg per hen) to subsequently forced ovulation of C_1 follicles is about the same, 7 to 9 hours, required for effect on C_8 follicles by

anterior pituitary and equine chorionic gonadotrophins.[10] Under comparable conditions the interval from progesterone injection to forced ovulation of C_8 follicles falls between 8 and 11 hours. The details of these and other tests now in progress will be reported elsewhere.

Summary. The administration of crystalline progesterone to the common domestic hen is highly effective, under stated conditions, in causing premature ovulation of normally developing ovarian follicles. Results differ with route of injection, time from injection to expected normal ovulation, injection level, and place of the follicle in the clutch sequence. First follicles of clutch sequences are ovulable at least 6 hours prematurely in 90 to 95% of hens receiving 0.5 and 1.0 mg progesterone intravenously, or 1.0 to 10.0 mg subcutaneously. Follicles other than the first of clutch sequences are ovulable by at least 7 hours prematurity in 70 to 75% of subcutaneously injected hens at dosage levels of 0.5 to 5.0 mg progesterone. Under identical conditions the intravenous administration of progesterone is relatively ineffective. Observed intervals from injection to ovulation varied from 7 to 11 hours.

10 Fraps, R. M., Riley, G. M., and Olsen, M. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 313.

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Effects of Adrenal and Thyroid Hormones on Water Exchange in Hypophysectomized Rats.*†

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After hypophysectomy in the rat there is a transient high diabetes insipidus (d. i.) which subsides fairly rapidly and is followed by a mild chronic diuresis. If only the neurohypophysis is removed or inactivated a permanent d. i. results.[1,2,3]

It has been found that under certain circumstances anterior pituitary extract could reestablish a latent d. i. in hypophysectomized rats,[1,4‡] as it does in other species, whereas neither pituitary preparations containing primarily adrenotropic hormone, nor whole adrenal cortical extract, would do so.[4] In view of reports[5,6] that desoxycorticosterone acetate (DCA) can reinduce a polyuria in hypophysectomized rats, further observations on adrenal cortical materials are reported here,

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2 Fisher, C., Ingram, W. R., and Ranson, S. W., *Diabetes Insipidus and the Neuro-hormonal Control of Water Balance*, Edwards Bros., Ann Arbor, 1938.

3 Gersh, I., *Res. Publ. Assn. Nerv. Ment. Dis.*, 1939, **18**, 436.

4 Schweizer, M., Gaunt, R., Zinken, N., and Nelson, W. O., *Am. J. Physiol.*, 1941, **132**, 141.

‡ It has been more difficult to maintain d. i. with anterior pituitary preparations in hypophysectomized rats than in other species. We have found that different lots of crude beef anterior pituitary extract, apparently prepared in a similar manner, and equally effective as stimulants of growth and appetite, do not all have the marked diuretic effect previously reported in hypophysectomized rats.[4] This is perhaps due to variation in the amounts of antidiuretic substances contained in the extracts, although quantitative studies have not been made.

5 Selye, H., and Bassett, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 272.

6 Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1941, **133**, 511.