between the two samples. It may be anticipated, furthermore, that asphyxia or hyperventilation (as by forced breathing) will lead to significant fluctuations in the prothrombin activity of the blood. Loss of CO_2 may also account in part for the diminution in prothrombin activity of plasma after being heated to 56°C for 15 minutes, or after standing at 5°C for several days.

Summary. Exposure of citrated or oxalated plasma to an air current is followed by a rapid diminution in its prothrombin activity; this is corrected by the addition of CO_2 and not affected by oxygen.

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Hormone-Induced Ovulation in Domestic Fowl.

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Multiple ovulations in hens have been effected in the laboratories of the Bureau of Animal Industry by intravenous administration of a luteinizing preparation after pretreatment with pregnant mare's serum. So far as the authors are aware, this is the first demonstration of direct experimentally induced ovulation in birds.

Ovulation has been induced in a number of mammalian species by administration of anterior pituitary hormones or anterior pituitary-like hormones.[1] Kirschbaum, *et al.*,[2] and Witschi[3] have described full ovarian function in small caged passerine birds following prolonged stimulation with pregnant mare's serum. Increase in rate of ovulation is implied in the results of Clark,[4] Gutowska[5] and Dubowik,[6] but Bates, Lahr and Riddle[7] clearly demonstrated that doses of follicle-stimulating hormone or pregnant mare's serum sufficient to cause marked follicular growth in the laying hen and pigeon also caused cessation of ovulation, usually by the fourth day. These results of Bates, *et al.*,[7] have been confirmed repeatedly in this labora-

1 Hartman, Carl G., Chapter IX, pp. 630-719, in *Sex and Internal Secretions*, Second Edition, Williams & Wilkins, Baltimore, 1939.

2 Kirschbaum, A., Pfeiffer, C. A., Van Heuverswyn, J., and Gardiner, W. U., *Anat. Rec.*, 1939, **75**, 249.

3 Witschi, Emil, *Wilson Bulletin*, 1935, **47**, 177.

4 Clark, L. N., *J. Biol. Chem.*, 1915, **22**, 485.

5 Gutowska, M. S., *Quart. J. Exp. Physiol.*, 1931, **21**, 197.

6 Dubowik, I. A., *Arch. f. Exp. Path. u. Pharmakol.*, 1930, **158**, 154.

7 Bates, R. W., Lahr, E. L., and Riddle, O., *Am. J. Physiol.*, 1935, **111**, 361.

tory.[8] Leonard[9] suggested that luteinizing hormone, the presence of which he demonstrated in chicken pituitaries, possibly played a rôle in ovulation in birds.

Materials and Methods. White Leghorn hens in laying condition and housed in individual laying cages served as experimental subjects. Hourly laying records were maintained from 8:00 a.m. to 4:00 p.m. during the experimental period. Daily palpations through the cloaca were made to determine the presence or absence of eggs *in utero*.

Each hen was injected daily subcutaneously for 6 to 11 days with pregnant mare's serum* (PMS) in doses of 100 or 200 rat units according to labelled potency. If the daily doses of PMS are too low the desired interruption of ovulation does not occur, and if markedly stimulating doses are continued beyond 10 or 12 days the larger follicles undergo regression.[8] These observations provided the basis for the dosages and number of days pretreatment with PMS.

The luteinizing preparation (LH) was a fractionated extract from horse pituitaries.† The LH potency of the extract was determined by increase in weights of the seminal vesicles of male rats according to the method described by Fevold.[10] Full maturity of the immature mouse ovary following appropriate injection of the preparation indicated some contamination with follicle-stimulating hormone.

The intravenously administered dosages of LH effective in causing ovulation, 10 to 20 Fevold units, were determined in preliminary experiments. No attempt was made to determine the limits of LH effectiveness, and the dosages used are not necessarily optimal.

In the first experiments PMS injections were begun in 20 hens in one day, in 14 on the following day. LH injections were made subsequently at the same time into hens of both groups. Since no significant differences were observed in the reactions of hens receiving 6 or 7 injections of PMS before the first LH administration, treatment of and results in the two groups are presented together.

In the second experiment, 32 hens were given 20 units of LH intravenously after 8 or 10 days of PMS injections. Four similarly PMS-injected hens served as controls.

Ovulation was checked first by palpation through the cloaca to determine the presence or absence of an egg *in utero* and finally by

8 Unpublished data.

9 Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 566.

* "Gonadin Serum Veterinary" of Cutter Laboratories, Chicago.

† The extract was especially prepared by Chappel Laboratories, Rockford, Ill.

10 Fevold, H. L., *Endocrinology*, 1939, **24**, 435.

postmortem inspection of the abdominal cavity for presence of yolks and of the ovary for presence of recently ruptured follicles. Diagnosis of ovulation following the first 2 LH injections in the first experiments depended wholly on palpation while all other diagnoses rested on use of all 3 technics.

Results. (1) *Repeated LH injections into PMS pretreated hens.* In these experiments each of 34 hens was injected daily with 200 units PMS. The first injection of LH was administered to 26 of these hens after 6 or 7 days PMS treatment, 13 hens receiving 10 units and 13 receiving 20 units. Ovulation occurred in 24 of the 26 LH-injected hens, as was shown by presence of single-yolked eggs *in utero* 22 hours later. Eight of the original 34 hens served as controls and continued to receive PMS but were not injected with LH; none of these hens had eggs in oviducts when the LH-injected birds were examined.

The second LH injections were made 22 hours after the first into the same 26 hens and at the same LH levels in individual hens. Seven of the LH-injected hens carried eggs *in utero* 24 hours later, while no eggs were found in oviducts of the 8 hens receiving PMS only. Each of the birds with eggs *in utero* following the second LH injection had ovulated following the first LH injection; the two hens failing to respond following either injection were killed at this time. One hen was found to have a highly stimulated ovary but with no follicles even approximately the size at which ovulation normally occurs. The other hen had ovulated twice, as was shown by the presence of two very recently ruptured follicles, but the yolk from neither ovulation had been picked up by the bird's oviduct.

The third LH injection was made into 17 of the 26 twice-injected hens 3 days after the second LH injection, all birds receiving 20 units of LH. Seven hens twice injected with LH served as controls against possible delayed ovulations consequent upon previous injections, and the original 8 hens receiving PMS daily were again carried without LH injection. Eight of the 17 LH-injected hens carried eggs *in utero* 24 hours later, while none of the hens in either group of controls had eggs in oviducts.

All birds were killed for direct examination of ovaries on the day following the third LH injection to the above 17 hens. The total count of recently ruptured follicles in ovaries of the 8 hens carrying eggs as a result of the third LH injection was 11. There was no evidence of recent ovulations in either group of controls.

The relatively small number of ovulating hens following second and third LH injections was probably due to ovulation of most of

the reactive follicles in many hens following the first LH injections, a conclusion borne out by the results of single LH injections described below.

While these experiments, resulting in 42 positively identified ovulations, demonstrated that ovulations were effected following the injection of the luteinizing preparation into PMS pretreated hens, the exact or total effect of any single LH injection was obscured by the repeated treatments. Furthermore, the presence or absence of an egg in the oviduct could alone be used as a criterion of ovulation following the first and second LH injections, and the possibility of multiple ovulations following single injections could not be determined. The second group of experiments, in which PMS pretreated hens were killed the day following LH injection for direct examination of ovaries, was designed to clarify this possibility.

(2) *Ovulations following a single LH injection into PMS pretreated hens.* The conditions and the results of tests for multiple ovulations are given in Table I. As is shown in the table, all hens on the lower level of PMS, 100 units daily, were caused to ovulate by the LH injection with an average of 3.5 ovulations per hen. Thirteen of the 16 hens on the higher PMS level ovulated. Follicular regression had occurred in the ovaries of 3 non-ovulating hens, suggesting that the level of PMS pretreatment was too high.

The total number of proved ovulations following single injections of LH into the 32 PMS pretreated hens was 99 (Table I), or an average of 3.41 ovulations in the 29 ovulating hens. No ovulations occurred in the birds which received PMS alone.

Discussion. The data presented in this paper furnish a striking demonstration that ovulation of ovarian follicles in hens can be directly effected by injection of anterior pituitary preparations rich in the luteinizing hormone. Multiple ovulations thus induced are

TABLE I.
Ovulations Following Single Intravenous Injections of LH into PMS Pretreated Hens and Absence of Ovulations in Hens Receiving PMS Only.

	PMS pretreatment			Ovulating hens			Ovulations	
				24 hr before LH	Following LH			
No. of hens	Daily dosage, rat units	Days	LH inj., units*	No.	No.	%	Total	Per ovulating hen
16	100	8-10	20	0	16	100	56	3.50
16	200	10	20	0	13	81	43	3.31
4	100-200	11	0	0	0	—	0	—

*Determined by increase in weights of seminal vesicles of rats according to the method described by Fevold.[10]

in accord with observations of hormone-induced ovulation in mammals. By counts of ruptured follicles, 7 ovulations were effected in one hen, 6 in another, 5 in each of 6 hens, and 4 in each of 5. In a number of birds the full complement of ovulated yolks was recovered from the oviduct and body cavity. Recovered yolks ranged from 12.4 to 23.1 g in weight, a rather large range for these hens. Only a single egg was found in the oviduct of any hen following a given injection and only a single yolk was recovered from any egg.

The PMS pretreatment induced great follicular growth in most birds and mechanical factors may account partly for failure of the oviducts to include more yolks. However, it is unlikely that mechanical difficulties accounted entirely for the uniformly observed singly occurring one-yolked eggs in the oviducts of the LH-treated hens.

Summary. Ovulations were induced in hens by the intravenous injection of a luteinizing preparation into hens pretreated with pregnant mare's serum for 6 to 11 days. Ovulation was induced in some hens following each of 3 successive LH injections. As many as 7 multiple ovulations were effected in individual PMS pretreated hens following a single LH injection.

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Effect of Thyroidectomy, Castration, and Replacement Therapy on Thymus, Lymph Nodes, Spleen in Male Rats.*

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A quantitative relationship between the thymus gland and other lymphoid tissues has been shown to exist for the effects of castration[1] and adrenalectomy[2, 3] in the rat. Clinically, castration and hyperthyroidism are conditions in which the thymus and lymphoid tissues

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1 Chiodi, H., *Rev. de la Soc. Arg. de Biol.*, 1938, **14**, 74.

2 Reinhardt, W. O., and Holmes, R. O., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 267.

3 Houssay, B. A., del Castillo, E. B., and Pinto, A., *Rev. de la Soc. Arg. de Biol.*, 1941, **17**, 26.