the higher protein diets. The possible significance of a lack of individual amino acids in increasing the vitamin E requirement of rats is discussed.

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Induced Ovipositions in Relation to Age of Oviducal Egg in the Domestic Hen.

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The removal of the ruptured follicle from the ovary of the hen results in a delay in the time of lay of the egg which arose from that follicle.[1] The experiments reported here are an approach to the problem of how the ruptured follicle functions to determine the time of lay. Specifically, they were designed to determine whether the sensitivity of the chickens' uterus to an oxytocic agent increases as the time of normally expected lay approaches.

Methods and Materials. Five groups of laying hens were injected intravenously with the same dose of an oxytocic agent at various hours after the ovulation of an egg. The results were expressed as the percentage of birds showing premature ovipositions.

Birds selected for injection carried in their oviducts the second egg of a 2-egg clutch, the first egg of which was laid at the recorded hour of either 10:00 a. m. or 11:00 a. m. The time of lay of all eggs in this laboratory is recorded on the hour every hour between 8:00 a. m. and 4:00 p. m., so that there was a maximum spread of no more than 2 hours between the time of lay of the earliest and latest first egg of a clutch in any group. Of all the birds used, those selected for 10:00 a. m. lay of the first egg of a clutch comprised between 55 and 60% of the total group in 4 groups and 74% of the total in the fifth group.

The presence of the oviducal egg was determined by digital palpation through the rectum. The age of the oviducal egg at the time of injection was estimated from the relationships between time of lay and ovulation of intraclutch eggs,[2,3] and the time required for the egg to pass through the various portions of the oviduct.[2,3] For 2-egg clutches, the interval between lay of the first egg and ovulation of the second is about 45 minutes,[2,3] and the time required for the egg to reach the uterus, about 4 hours from the time it enters the oviduct.[2]

The injections were made during the interval between ovulation and the time of expected lay (a period of about 27 hours) at the following hours: 4:00 p. m., 8:00 p. m., midnight, 6:00 a. m. and 10:00 a. m. Usually, no more than one minute elapsed between the injection of one bird and the injection of the next in each group; the maximum difference between the given times of injection and the time of the earliest or latest injection in the group was 34 minutes. White Leghorn and Rhode Island Red hens were used: the number of one breed did not exceed the number of the other by more than one bird in each group, except in that injected at 6:00 a. m. In this group there were 33 White Leghorns and 12 Rhode Island Reds. The results for the groups as a whole were not appreciably different from the results obtained in each breed.

The agent used to induce premature oviposition was Parke, Davis & Co. "Pitressin." Although the main constituent of this preparation is the pressor principle of the posterior

1 Rothchild, I., and Fraps, R. M., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 79.

2 Phillips, R. E., and Warren, D. C., *J. Exp. Zool.*, 1937, **76**, 117.

3 Unpublished data from this laboratory.

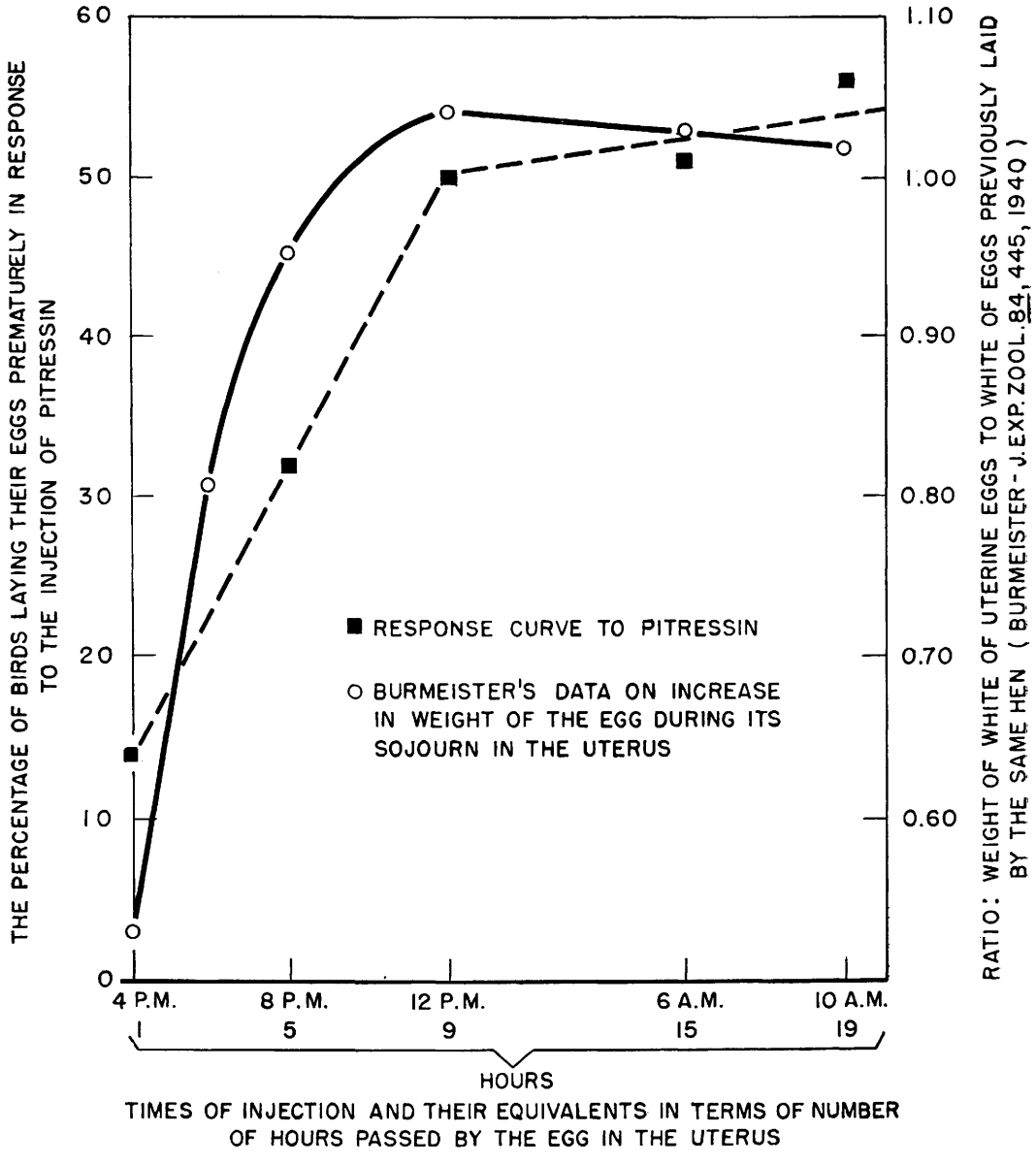

FIG. 1.

Comparison of the curve showing percentage response to Pitressin in relation to the age of the oviducal egg with the curve relating increase in size of the egg to the length of time spent by the egg in the uterus.

pituitary, it has been shown by Burrows and Fraps[4] to have a strong oxytocic action in the chicken. The dose used was 0.30 unit of labeled potency; this was prepared by diluting 1.0 cc, taken from a separate 1.0 cc vial, and containing 20.0 pressor units, to 10.0 cc with 0.9% NaCl. The amount injected was 0.15 cc. A diluted preparation was never used for more than 2 days in succession. The same 0.5 cc hypodermic syringe was used for all injections.

A premature oviposition in response to Pitressin was taken as lay of the oviducal

[4] Burrows, W. H., and Fraps, R. M., *Endocrinology*, 1942, **30**, 702.

TABLE I.
Relation Between the Percentage of Birds Reacting by Premature Oviposition to a Constant Dose of Pitressin (0.30 Unit) and the Hours After Ovulation That the Injection Was Made.

Injections		Birds injected		
			Positive reactions*	
Time	Approx. No. hr after ovulation	No.	No.	%
4 p.m.	5	22	3	14
8 "	9	31	10	32
12 "	13	44	22	50
6 a.m.	19	45	23	51
10 "	23	32	18	56

* A positive reaction is the expulsion of the oviducal egg in response to the injection of Pitressin within 30 minutes after injection.

egg within a period of 30 minutes from injection. Practically all premature ovipositions, however, occurred well within 15 minutes from the time of injection.

Results are shown in Table I. Between 4:00 p. m. and midnight following the ovulation of these particular eggs, the percentage of premature ovipositions in response to 0.30 unit of Pitressin increased substantially. From midnight to 10:00 a. m. the next day, however, there was only a slight, if at all significant further increase in the percentage of premature ovipositions. Since 10:00 a. m. was only 2 to 6 hours from the time of normally expected lay for these particular eggs, it can probably safely be assumed that there was very little change in the response to a given dose of Pitressin from midnight until the actual time of lay.

Removal of the ruptured follicle apparently did not affect the percentage of birds responding by premature oviposition to this dose of Pitressin. Of 17 birds so treated, 9, or 53%, laid prematurely. This compares very well with the group of intact birds injected at the same hour. This fact, together with the evidence of a plateau from midnight on, indicated that the response curve might be a manifestation of the mechanical stretching of the uterine walls as the egg increases in size (plumping) with the uptake of water.[5] To check this, Burmeister's data[5] on the increase in weight of the egg in relation to time spent in the uterus was compared with the response curve to Pitressin. The 2 curves are shown in Fig. 1. For comparison, the hour of injection was made equivalent to time spent in the uterus by means of the following rough calculations. The hour of lay of the first egg of the clutch for each group as a whole was taken as 10:00 a. m. (This probably errs somewhat in the direction of a later-than-actual average, but not enough to affect appreciably the overall picture). Ovulation of the test egg would therefore have occurred about 10:45 a. m., and the egg would have entered the uterus somewhere around 2:45 p. m.-3:00 p. m. (see above). The 2 curves were plotted, taking 3:00 p. m. as the 0 point for time spent in the uterus. The response curve shows the beginning of a plateau very close to the time that the egg becomes fully plumped, *i.e.*, reaches its almost maximum size. The only further increase in size that takes place from this point on is that due to shell formation (which is not shown by Burmeister's[5] figures), and it may well be that the slight increase in the percentage response to Pitressin during the period of shell formation is a reflection of this phenomenon.

Discussion. The increasing percentage of birds responding to a given dose of Pitressin as their eggs increase in size, and the lack of any substantial further increase in response after the egg reaches its almost maximum size, may be taken to indicate that the changes in reactivity of the uterus during the sojourn in it of the egg are not directly responsible for the determination of the time of normal oviposition. With the reservation that a response to Pitressin is only a probable indication of the sensitivity of the uterus to the stimulus which under normal conditions leads to oviposition, it may be assumed for the present, therefore, that the probable mode of action of the ruptured follicle is one that primarily involves a change in some system external to the uterus.

Summary. Hens carrying oviducal eggs were injected with 0.30 unit of Pitressin at times equivalent to 1, 5, 9, 15, and 19 hours of time passed by the egg in the uterus. The percentages of birds laying prematurely were respectively 14, 32, 50, 51, and 56.

[5] Burmeister, B. R., *J. Exp. Zool.*, 1940, **84**, 445.

Removal of the ruptured follicle shortly after ovulation did not alter the response at the 19th hour in the uterus stage. The beginning of the plateau of the response curve approximately coincided with the time at which the oviducal egg became fully plumped, *i.e.*, reached its almost maximum size. The response curve was interpreted as the expression of a change in the mechanical relations between the uterus and the contained egg, rather than a change in intrinsic sensitivity due to the action of the ruptured follicle.

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Physiological Disposition of Penicillin G and K in Dogs.

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The unexpected low activity of penicillin K in experimental syphilis first observed by Chesney[1] has been confirmed.[2] In addition it has been demonstrated that penicillin K is less effective than G in the treatment of mice infected with pneumococcus Type I,[3] *Streptococcus pyogenes*[3] and *Borrelia novyi*.[4,5]

This low activity of penicillin K has been correlated with an apparently low plasma concentration and it has been suggested that this is a result of more rapid destruction in the animal body.[3,6] A more detailed study of the disposition of penicillin G and K in dogs has led us to a somewhat different conclusion, and has cast considerable doubt on the supposition that inactivation plays a major part in the immediate fate of injected penicillin K.

Recovery of Added Penicillin G and K from Body Fluids and Tissues. The recovery of added penicillin from various biological materials was studied by the customary cup test procedure of assay for penicillin concentration. Known amounts of each penicillin* were added to body fluids and to tissue mashes, and the actual assay figures in each case were related to the assay figures of the same concentration of penicillin added to saline.

Plasma was obtained by centrifuging oxalated or heparinized dog blood. Tissue mashes were prepared with a homogenizer, using 2 parts of saline for one of muscle, and equal parts of saline and tissue in all other cases. The grinder was immersed in ice water during the preparation of each tissue. One-tenth of a volume of an appropriate saline solution of penicillin was added to a series of samples of tissue mashes, plasma, and saline. Final concentrations of penicillin ranged from 0.3 μg per ml to 24 μg per

1 Unpublished observations quoted by Eagle and Musselman, *Science*, 1946, **103**, 618.

2 Rake, G., Dunham, W., and Donovick, R., to be published.

3 Eagle, H., and Musselman, A., *Science*, 1946, **103**, 618.

4 Burk, M., Farr, A. C., and Schnitzer, R. J., *Science*, 1946, **104**, 370.

5 Richardson, A. P., Loeb, P., and Walker, H. A., unpublished observations.

6 Coghill, R. D., Osterberg, A. E., and Hazel, G. R., *Science*, 1946, **103**, 709.

* We are indebted to Drs. Wintersteiner and Adler of the Division of Organic Chemistry, and Mr. Lott and Mr. Dolliver of the Division of Medicinal Chemistry for these preparations. Elementary analysis and bioassay values for both penicillins agreed well with theoretical values. Analysis by Craig distribution methods showed the G preparation to be at least 92% homogeneous, the remainder consisting of inactive components. The K preparations were at least 88% of "K type" penicillins, not more than 10% of an active fraction which was probably an "F type" penicillin, and the remainder was an inactive component.