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On the function of the Ruptured Ovarian Follicle of the Domestic Fowl*

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The relatively few accounts dealing directly with the ruptured avian follicle are limited largely to descriptions of histological changes incident to its regression and to discussions, notably lacking in experimental support, of presumed homology or lack of homology with the corpus luteum of the mammal.¹⁻⁴ In any

case, previous investigators and writers have been unable to assign any demonstrable physiological function to the ruptured follicle of birds.

In this report we wish to present evidence for the conclusion that the ruptured follicle of the hen participates decisively in control of time of lay of the egg normally arising from ovulation of its previously contained ovum or yolk. Some evidence suggests that the ruptured follicle also participates in regulation of the interval between successive ovulations. At this time, however, we shall consider only those relations demonstrating the dependence of time of lay of the oviducal egg on its cor-

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¹ Pearl, R., and Boring, A., *Am. J. Anat.*, 1918, **23**, 1.

² Hett, J., *Verh. Anat. Ges. (Jena)*, 1922, **31**, 153.

³ Novak, J., and Duschak, F., *Zeit. Ges. Anat. (Munchen)*, Abteil I, 1923, **69**, 483.

⁴ Fell, H. B., *Quart. J. Micr. Sci.*, 1925, **69**, 591.

responding ruptured follicle, leaving aside also all questions of the mechanism responsible for this specific function.

Material and Methods. All hens used in this study were selected from an extensive stock kept in individual laying cages. Most of the birds were from standard breeds, but some cross-bred hens were included. The hens were subjected to a constant 14-hour light-day and were given unlimited access to feed and water. Lay of eggs was recorded hourly from 8:00 a.m. through 4:00 p.m. and again at 8:00 p.m. for a sufficient time before operation to establish the individual hen's schedule of lay. Lay of eggs following operative procedures was recorded similarly as well as at hourly intervals after 4:00 p.m. whenever called for by possibly modified schedules of lay.

The experiments reported here consisted in removing maturing or ruptured follicles (or both simultaneously) and determining the effect on time of lay of eggs in oviducts at the time of operation. It was therefore essential to know accurately the time relations underlying ovulation and lay in individual hens. All hens operated on were known, on the basis of records of lay, to be highly regular in laying schedule and to miss lay on but a single day between clutches (*i.e.*, eggs laid on successive days). Moreover, the oviducal egg was always accounted for at the time of operation, and by reference to hour of lay of the preceding egg, all possibility of this egg having been ovulated at an unusual hour was eliminated.

The ovulated ovum of the hen remains in the oviduct from 24 to 30 hours, the exact time varying with the number of eggs in the clutch and the place of a given egg in the series. Ovulation occurs about 0.5 hour following a previous lay except following lay of the last egg of the clutch, which takes place during afternoon hours generally.⁵ The next ovulation, initiating a new clutch sequence in such hens as were used in our tests, occurs during the early morning hours (4:00-8:00 a.m.) of the day following clutch termination, *i.e.*, some 16 to 20 hours following lay of the

last egg of the preceding clutch. On the basis of these known relations and the individual hen's record of lay it is possible to ascertain, with a relatively high degree of accuracy, the day and hour of origin of any ruptured follicle borne by the ovary, age of an oviducal egg, and hour of at least the next-due ovulation, which of course is the same as the hour of expected origin of the next-due ruptured follicle.

Like the maturing follicles, the ruptured follicles are pendant to the ovary, and excision of either type is relatively simple. Immediately following ovulation the ruptured follicle weighs about 0.4 to 0.5 g. It loses weight rapidly and decreases correspondingly in size, making identification of any one of at least the 3 largest ruptured follicles practically certain on direct inspection. As a final check, however, each excised follicle was weighed on removal for comparison with expected weight based on recentness of its origin.

Identification of maturing follicles was carried out similarly. Only the largest follicle in the oncoming series was removed in tests reported here. Upon removal, this follicle was weighed and its weight referred to expected weight at maturity, due account being taken of time at which the follicle was removed. The results of Table I do not include any follicle, maturing or ruptured, of questionable place in its appropriate series.

All surgical operations were carried out under Nembutal anesthesia. Operative details were essentially similar to those previously described by Phillips and Warren.⁵ The pedicle of the maturing or ruptured follicle was clamped with a hemostat before the desired structure was removed. Whenever possible, the severed stalk was ligated, but bleeding in any case was never excessive and was easily controlled. Twenty to 30 minutes were usually required for the complete operation. Moderately aseptic conditions were maintained, and in most cases sulfathiazole was used sparingly in local wound areas. With very few exceptions the birds operated on exhibited practically no subsequent effects except those immediately involving the ovary or the oviducal egg.

Results. The operated birds of the first

⁵ Phillips, R. E., and Warren, D. C., *J. Exp. Zool.*, 1937, **76**, 117.

TABLE I.

The Effect of Removing Ruptured and Maturing Follicles on Retention of the Egg in the Oviduct at Time of Operation.

Group	Follicle removed	Age of oviducal egg, hr	Birds		Duration of delay in laying, range
			Operated on, No.	Retaining eggs, No.	
I	R ₁	0.5-26	22	17	9 hr to 3 days
II	M ₁	0.5-24	30	2	3 and 5 hr
III	R ₁ and M ₁	2.5-25	15	14	1 to 7 days
IV	R ₂	1.0-21	18	4	2 to 5 hr

R₁—The most recently ruptured follicle.

R₂—The second ruptured follicle in order of recency of origin.

M₁—The oldest maturing follicle, next due to ovulate.

experiments constituted Group I and II of Table I. The most recently ruptured follicle was removed from hens in Group I, the oldest maturing follicle from hens of Group II. Of the 17 hens holding their eggs following removal of the most recently ruptured follicle, only 1 held for as little as 9 hours; 8 hens held their eggs for 1 day, 4 for 2 days, and 4 for 3 days. In sharp contrast with these results, all except 2 hens of Group II laid at the expected hour. The 2 hens holding their eggs for 3 and 5 hours appear to belong to a definite category which will be discussed later.

Both the most recently ruptured follicle and the most mature of the maturing follicles were removed from the hens of Group III. Of the 15 operated hens, 14 held their eggs over the following intervals: 5 for 1 day, 3 for 2 days, 2 for 3 and 2 for 5 days, and 1 each for 6 and 7 days.

As a final check on the foregoing results the second most recently ovulated follicle was removed from 18 birds, the most recently ruptured follicle being left intact (Group IV of Table I). Fourteen hens laid at the expected hour, and 4 held their eggs for periods not exceeding 5 hours. These are discussed in connection with the 2 similarly holding hens of Group II.

Not shown in Table I is an additional group of 17 hens on which sham operations were performed, the follicles being handled but not removed, or the birds being carried through other phases of complete operation. Of these, 16 laid at the expected hour and one hen held its egg for a period of one day, possibly as the result of follicular injury although this cannot be said with certainty.

Discussion. Follicular regression was

brought about in many of the operated birds, with resultant interruption of ovulation for 1 or more days. In view of the normally close relation between ovulation and lay (excepting always the terminal egg of the clutch), it might therefore be argued that we have prevented lay not by removing the organ directly controlling this process, but rather by causing follicular regression and thus preventing ovulation. In the hens of Group II, however, all possibility of ovulation near the hour of expected lay was eliminated by excision of the ovulable follicle with minor effect in only two of 30 operated birds. Furthermore, only similar minor delays occurred in any of the hens of Group IV, although 17 of the 18 hens failed to ovulate in conjunction with lay. It may be remarked finally that the terminal egg of the clutch of the normal intact hen is laid 16 to 20 hours before the next ensuing ovulation, and is not, therefore, immediately dependent upon ovulation for lay. In view of the evidence from these several lines, we conclude that normally timed lay depends mainly upon some property of the ruptured follicle from which the oviducal egg has taken its origin.

Although the last-ruptured follicle appears to be the dominant factor in control of lay of the oviducal egg, the maturing follicle is not without secondary or substitutional function, within limits. Of the 5 hens in Group I which laid normally, 3 did so in conjunction with normally due ovulation. The higher percentage of birds holding their eggs following excision of both the maturing and ruptured follicles (Group III) is obtained in association with elimination of all possibility of next due ovulation. These results suggest, when

taken together, that the follicle ovulating about the time of lay exhibits some of the properties of the ruptured follicle.

It has been shown that the terminal egg of a clutch remains in the oviduct for an appreciably longer time than do mid-clutch eggs.⁶ The 2 birds of Group II and the 4 of Group IV, in which lay was delayed only a few hours, were entirely dependent upon the most recently ruptured follicle for lay, next-due ovulation having been precluded by operation or regression. The postponement in time of lay by only a few hours is thus approximately comparable with the behavior of the terminal egg of a clutch, which egg also is laid in the absence of closely ensuing ovulation.

It would be premature to speculate at this time upon the mechanism underlying the action of the ruptured follicle in determining the hour of lay of its ovulated yolk. We believe, however, that the results presented here provide adequate proof that the avian ruptured follicle is not an organ without func-

tion, but that this structure, like its mammalian homologue, exerts an influence on the oviducal history of the ovum it once contained. Since the oviducal histories of ova of the two classes of vertebrates are so dissimilar, it will be interesting to observe what parallels and divergences exist in physiological behavior of the two organs.

Summary. Removal of the ruptured ovarian follicle or of this follicle together with the oldest maturing follicle of the hen at the time when the egg taking its origin from the ruptured follicle was in the oviduct almost invariably resulted in the holding of the egg beyond the expected time of lay. Most eggs were held from 1 to 7 days. Removal of other parts of the ovary at comparable times, without simultaneously removing the most recently ruptured follicle, practically never resulted in comparable holding of the oviducal egg. The ruptured follicle of the hen is believed therefore to be an important factor in determining the time of lay of the egg formed from its previously contained ovum.

⁶ Heywang, B. W., *Poultry Sci.*, 1938, **17**, 240.

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Treatment of Experimental Renal Obstruction from Sulfadiazine.

I. "Forcing of Fluids" and Alkalinization.*

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The value of massive alkalinization in the prevention of renal complications from intratubular precipitation of sulfadiazine and its acetylated product has been established clinically^{1,2,3,4} as well as in the animal experi-

ment.^{5,6} The application of this procedure in combination with the "forcing of fluids" has also been suggested in fully developed renal obstruction and anuria occurring during the course of sulfadiazine therapy,⁷ but its usefulness and advisability lacks experimental foundation.

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¹ Gilligan, D. R., Garb, S., and Plummer, N., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 248; Gilligan, D. R., Garb, S., Wheeler, C., and Plummer, N., *J. A. M. A.*, 1943, **122**, 1160.

² Barnes, R. W., and Kawaichi, G. K., *J. Urol.*, 1943, **49**, 324.

³ Jensen, O. J., Jr., and Fox, C. L., Jr., *J. Urol.*, 1943, **49**, 334.

⁴ Fox, C. L., Jr., Jensen, O. J., Jr., and Mudge, G. H., *J. A. M. A.*, 1943, **121**, 1147.

⁵ Lehr, D., *Bull. New York M. College, Flower and Fifth Ave. Hosps.*, 1943, **6**, 70.

⁶ Jensen, O. J., Jr., *Am. J. Med. Sci.*, 1943, **206**, 746.

⁷ Lowell, F. C., *M. Clin. North America*, Sept., 1943, p. 1247.