

OBSERVATIONS CONCERNING THE MECHANICS OF OVULATION
IN THE FOWL

by

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INTRODUCTION

The physiological causation of ovulation in the vertebrates is a phenomenon which is not well understood. Many theories have been proposed as to the cause of the follicular rupture, but until recently few direct observations have been made in support of the theories advanced.

The fowl is an excellent subject for the study of the phenomenon of ovulation since the ova carry a large amount of deutoplasm and when necessary, they can be adjusted into full view, by virtue of the fact that the follicles are suspended from the ovary by means of a follicular stalk. This eliminates many of the handicaps encountered in the study of the microscopic mammalian ova which are embedded in an ovarian stroma.

The purpose of this study is to investigate by observation and varied experimental technique, the mechanics of ovulation in the fowl. The bearing of the observations on theories proposed by workers on other vertebrates, was kept in mind during the course of the study.

REVIEW OF THE LITERATURE

Bartelmez (1912) observed the act of ovulation in the pigeon, and by measurements of the follicle before ovulation and of the ovum after it was liberated, concluded there

was a great pressure inside of the follicle resulting from yolk secretion which caused the follicular rupture. Although egg laying, and thus ovulation, does not normally occur in the pigeon until mating has taken place, Craig (1913) caused laying to start in the pigeon, without previous mating, by merely stroking the head of the bird. This psychological effect as a cause of ovulation, has later been reported in two mammals (rabbit - cat) which normally do not ovulate until after copulation has occurred.

Patterson (1910) reported that ovulation in the fowl resulted from the muscular exertion of the infundibulum which grasped either mature or immature Graafian follicles and caused them to rupture. However, Pearl and Curtis (1914) showed ovulation could occur when the oviduct was entirely removed.

The most detailed study of the process of ovulation in the fowl is the work of Warren and Scott (1935). This work, however, was concerned primarily with the process of egg formation in relation to the different divisions of the oviduct rather than the direct causes of the liberation of the ovum from the follicle. The findings of these workers also substantiate the view of Pearl and Curtis concerning the role of the funnel in the follicular rupture.

A review of the different ovulation theories by Hartman (1932) is one of the most comprehensive recent sources

of the literature in the field.

MATERIAL AND TECHNIQUE

The 77 birds used in this study were White Leghorn females in their first or second year of production. The birds were trapnested hourly so that the characteristics of each bird's clutch could be studied before it was selected for the observation. In the operative work only those birds were used, the laying record of which gave fairly good assurance that the next ovum would be ovulated without any delay such as occurs between clutches. The hens available for the celiotomy had a wide variation in intensity of laying so a study of the relationship between the time elapsing between oviposition and ovulation and length of interval between eggs was possible.

Normally, all of the birds were allowed to lay in their usual quarters while under close observation. Immediately after laying they were taken to the operating room and anaesthetized with liquid nembutal (pentobarbital sodium 1 gr. per cc.). All injections were made intra-venously utilizing the vena humeri profunda of the wing. For the initial injection three-fourths of a cubic centimeter of nembutal was usually sufficient to completely anaesthetize the bird. Succeeding injections varied from one-fourth to one-half cubic centimeter depending somewhat upon the size

of the bird. Respiratory shock resulted in the death of 12 hens when the dosage was in excess, or when the injection of nembatal was made too rapidly.

As soon as the bird was under the influence of the anaesthetic, the feathers were removed from the left lateral surface of the abdomen. The incision (approximately 5 inches in length) was made parallel to the back slightly above the mid-line between the vertebral column and the sternum. By making the incision at this locus, only two blood vessels needed to be clamped off. One was a small blood vessel located on the underneath surface of the skin, and the other a large artery ramifying the surface of the abdominal muscles. Serious hemorrhage resulted if this larger blood vessel was not clamped off near the vertebral column before it was cut. Since the fowl is only slightly susceptible to infection and few were desired after the operation, no special antiseptic technique was used for the operations. Approximately 10 to 15 minutes were required to anaesthetize and open the body cavity of a bird after oviposition had occurred.

With a little experience one can readily recognize the next ovum to be liberated provided this is to take place within an hour. Not always was the mature follicle visible from the incision on the left side. If an immature follicle was uppermost, the mature follicle was located and then

gradually adjusted into full view for observation from the left side. The presence of a follicular stalk and the lack of ovarian stroma permit considerable adjustment of the position of the follicle without much difficulty and the change in position seemed to have no effect on ovulation. Only when the mature follicle was in the extreme cephalic end of the coelom near the heart did the adjustment tend to subject the bird to a shock. Sometimes to facilitate the observation of follicles in the anterior part of the body cavity the usual incision was lengthened by disarticulating the diarthrodial joint between the dorsal and ventral parts of the last rib. Because of the location of the lung, the sixth rib marked the most anterior point of the incision.

The only deviation from this operative routine was that nine hens were removed from the nest, anaesthetized, and opened before laying occurred. This was done so that more time would be available for studying the visible changes in the mature follicle and the peristaltic movement of the oviduct before and after laying. Provided the incision through the lateral muscles did not extend too far posteriorly the anaesthetized hen had little difficulty in the expulsion of the egg at the expected time. It was of interest to learn that the passage of the fully formed egg through the vagina required only about one minute.

Since blood pressure has been considered as one of the

factors influencing ovulation, care was taken when making the abdominal incision to ligate the two large blood vessels in the abdominal wall before cutting across them. By employing this technique, only a very small amount of blood was lost, so ovulation could not have been influenced by this factor.

When the incision was to be kept open for a considerable period, the opening into the body cavity was covered with cellophane and sealed with vaseline to conserve the humidity and body heat. Usually, however, after the mature follicle was located and adjusted into view if necessary, the coelomic incision was only opened intermittently to observe the follicle until the rupture appeared ready to occur.

Exposure of the reproductive organs to low temperatures was believed to have an effect in delaying or inhibiting the follicular rupture, so all of the operations were performed in a warm room. Usually the birds were killed after the observations were made, but in seven individuals, the incision was sutured and they were returned to the flock. Of these, six lived for several months and two (1919 and 1967, table 1) were operated upon the second time.

OBSERVATIONS AND EXPERIMENTAL RESULTS

Several different methods were used in this study to investigate the cause of the follicular rupture. The following list gives a brief summary of the number of cases that were used in the various techniques for testing the different theories offered as an explanation of the follicular rupture:

| | |
|--|----|
| Ovulations recorded | 40 |
| Birds anaesthetized before laying | 9 |
| Excised follicles | 4 |
| Follicles clamped off but attached to the ovary | 4 |
| Follicles ruptured by applying pressure (squeezing). | 4 |
| Pituitrin injections | 2 |
| Electrical stimulation of follicle | 2 |
| Removal of a portion of the yolk | 2 |
| Intra-follicular injections of water | 2 |

Changes in the Appearance of the Follicular
Blood Vessels

Immediately after gaining entrance into the coelom, the slightly vascular serous ovarian pocket was torn in order that the ovary could be seen more easily. The oviduct usually exhibited slight peristaltic movement when first exposed (about 15 minutes after laying), but as the time of

ovulation approached, greater motility was observed, especially in the infundibular region.

The stigma has been referred to as the non-vascular area of the follicle, but there is a great variation in the width of this non-vascular area due to the changes which occur in the blood vessels of the follicle within an hour previous to ovulation. Many small finger-like blood vessels (branches from two large blood vessels that parallel the stigma on either side) extending out to this non-vascular area, gradually became obliterated before the follicular rupture occurred. The blurring out of these blood vessels caused an apparent widening of the stigma. This was the characteristic by which identification was made of the next follicle to be ruptured. The wide non-vascular area is referred to as the stigma in this study.

Two indications of the impending ovulation were the changes in the appearance of the blood vessels of the follicle and the slight to prominent bulged area or areas which appeared on the stigma. Practically all of the blood vessels of the mature follicle were very red and conspicuous when the ovary was first exposed, provided ovulation was not to occur immediately. Later the small blood vessels throughout the follicle became blurred or obliterated. Figures 1 and 2 show the difference in the appearance between a follicle that would have ruptured within a few minutes and a

Figures 1 and 2.

Fig. 1. Appearance of a follicle immediately before rupturing. Note the wide stigma and the blurred blood vessels.

Fig. 2. A follicle approximately 26 hours before the follicular rupture is to occur. The stigma is narrow and the blood vessels are distinct and prominent.

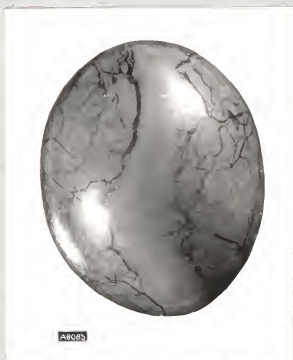


Figure 1.

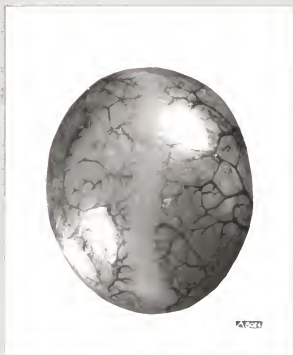


Figure 2.

follicle that would not have ruptured before approximately 26 hours.

When the tension in the follicular wall was released, due to the expulsion of the ovum, all of the blood vessels in the follicle again became prominent and had a dark red color. This would indicate that there had not been a collapse of the smaller blood vessels, but that on the contrary they had been compressed to the extent that they temporarily had become invisible. Since there was no apparent change in the appearance of the blood vessels of the remaining follicles before or after ovulation it was believed that the tension on the follicular membranes was responsible for the reduction in the blood supply to these tissues rather than some external factor. Tension artificially applied caused the blood vessels to disappear in the same manner that they did immediately before a normal ovulation.

Muscle Fibers in the Follicular Wall

Histological study of the follicular wall showed that muscle fibers were present in this tissue (Figs. 3, 4, and 5). The long, slender fibers were very compact in the region of the stigma, slightly more so than in the region of the follicular stalk.

There seemed to be a slight periodic variation in the turgidity of the follicle previous to the time of ovulation,

but since the change seemed slight and this impression was gained by palpation of the follicle, there was the possibility that the observation was incorrect.

The contraction of the muscle fibers was apparently the cause of the follicular turgidity which resulted in the blurring or obliteration of the smaller blood vessels. Evidence supporting this view was found in observations made on three hens that died just before ovulation was about to occur. With the loss of muscle tonus just before death, the follicles became flaccid and the small blood vessels again became dark red and prominent. Thus the accumulation of additional yolk material inside the follicle was not responsible for the visible changes that occur in the appearance of the follicular wall, but instead served only as a body on which the muscle fibers can contract and increase the tension. In two cases ovulation was prevented by removing, by means of a syringe, approximately three-fourths of a cubic centimeter of deutoplasm from the follicle. This caused the follicle to become flaccid and as a result the tension in the follicular wall was lowered. Apparently this tension is essential before ovulation can occur since the birds that died just previous to ovulation were observed three hours afterward and the follicular rupture had not occurred.

Expulsion of the Ovum from the Follicle

Histological evidence that there is an inhibition in the growth of the follicular tissue at the non-vascular stigma region is shown in figures 3, 4, and 5. In figure 3 is shown the stigma region of a follicle that was expected to rupture within a few minutes. Figure 4 shows the same region of a follicle approximately 26 hours before ovulation was expected to occur and figure 5 shows the appearance of a follicle which was estimated would not have ruptured before 10 days.

When the mature follicle was about to rupture, the stigma area was very thin and the muscle fibers were under great tension. It was thought that the decrease in the thickness of the follicle had not resulted from a proteolytic digestion, because the corona radiata was intact in all places. If enzymatic action had taken place it was expected that this layer of cells would have been destroyed.

The follicular rupture practically always began as a tiny point at one of the stigma ends. In only one of the cases observed did the point of rupture occur about midway between the ends of the stigma.

Exposure of the ovary to the air resulted in a drying out of the follicular tissue, and because of this there was a variation in the time required for the actual follicular

Figures 3 and 4.

Fig. 3. A transverse section of the stigma region of a follicle immediately before rupturing. The inside of the follicle is uppermost. The corona radiata is intact and the muscle fibers are stretched. Magnification 240x.

Fig. 4. A transverse section of the stigma region of a follicle about 26 hours before ovulation. Particles of deutoplasm can be seen next to the corona radiata. The muscle fibers are not fully stretched and the follicular wall is thicker than that of the mature follicle. Magnification 240x.



Figure 3.

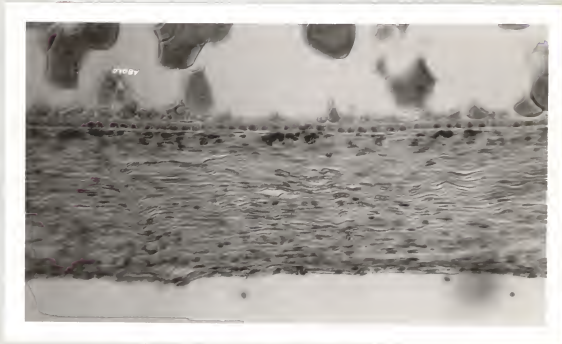


Figure 4.

Figure 5. [Faint, illegible text]

Figure 5.

Fig. 5. A tranverse section of a portion of a follicle which was estimated would not have ruptured before 10 days. The vitelline membrane can be seen next to the poorly defined corona radiata. Magnification 240x.

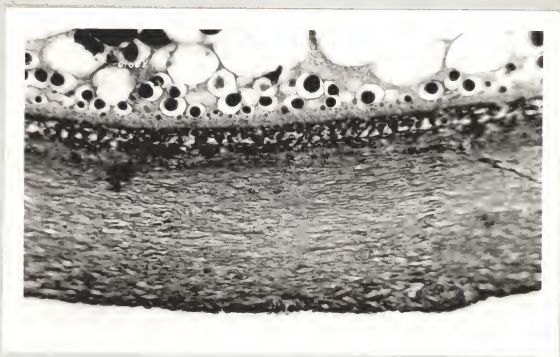


Figure 5.

split. If lymph was removed from the coelom by means of a pipette and dropped on the follicle when there was evidence of the tissue drying, the rupture of the well-moistened follicle was practically instantaneous, and very little could be observed concerning the nature of the rupture. However, during this study, motion pictures were taken of ovulation and under these conditions, with a marked drying out of the follicle as a result of the heat given off from the arc lights, the follicular rupture was considerably slower and a more critical observation could be made.

The inside layers of the follicular membrane began to break first and as a consequence a bulged area appeared at the point of rupture. If the rupture was exceptionally slow it was not uncommon for the entire stigma region to be bulged. The germinal epithelium was firmly attached to the theca cells at the stigma, but at other places on the follicle the attachment between the two cell layers was very loose. Thus, the germinal epithelium at the stigma region was actually torn when the theca cells below it had broken apart.

As soon as the tiny rupture point was visible, the ovum was rapidly expelled from the follicle as a result of the slit-like tearing of the follicular wall through the center of the stigma.

Effects of Different Techniques on Ovulation

When it was found that ovulation was not prevented (excepting one case that will be discussed later) in three birds by clamping off the follicle at the stalk, it was apparent there was not a last minute deposition of yolk which was responsible for ovulation. In all cases immediately after the follicular stalk was clamped, the bird was not disturbed until the ovum was observed in the body cavity. Because of this, the actual rupture of the follicle was not observed, but the time between laying and ovulation was recorded for one of these birds in Table 1. The follicle of this hen (2053) was clamped off 11 minutes before the actual rupture occurred.

In all cases after ovulation had taken place, the clamp was not unclamped until after the bird had been killed and the sternum removed to make certain that the entire follicular stalk had been between the jaws of the clamp. To make certain that the clamp was definitely inhibiting the blood supply and nerve impulses, a follicle which was expected to rupture within a few minutes was clamped off and then completely excised from the ovary. The normal turgidity of the follicle was maintained as nearly as possible by the application of a second clamp just inside of the first one. Then the follicle was returned to the warm,

moist body cavity of the hen from which it had been taken, but had otherwise been handled in the usual manner. Four of these excised follicles ruptured normally at the stigma. No accurate record was made of the time elapsing between oviposition and these follicular ruptures because the follicles did not always retract enough to see the ovum just as it was released and sometimes the follicles were placed under loops of the intestine so that they would be warm and moist. The time of observation of the recently released ova, however, closely approximated the time estimated for the follicular rupture had the follicle not been molested. Two of these mature follicles were excised from the ovaries before the hens had laid so the minimum time, before ovulation was expected to have occurred normally, was estimated at one hour or longer. From this evidence it would seem that all of the deutoplasm must have been deposited in the ova at least an hour before ovulation.

Applying this same technique to follicles which were not expected to rupture normally before 26 hours did not result in a follicular rupture. The lack of a prolonged tension in the follicular wall might have been one cause that the follicles did not rupture, because enough pressure could not be applied to obliterate the smaller blood vessels, and the pressure was not applied for a long time.

The muscle fibers of the follicle could not be made to

contract sufficiently to cause ovulation by applying a tetanic current to the sides of the follicle. Two cases were used to study this technique. By palpation there was no indication of an increase in the turgidity of the follicle at the time of application of the current although many of the other muscles of the body reacted violently. These results would seem to cast doubt on the view that ovulation is due to any sudden nerve impulse reacting on the muscle fibers of the follicular wall. Since a point electrode was used, it is possible that not enough of these fibers were simultaneously stimulated to give a noticeable contraction.

The mature follicles of four hens that died just before ovulating were caused to rupture at the stigma by squeezing them with the fingers. The pressure was applied at the region of the follicular stalk. However, not all of the mature follicles ruptured normally when this technique was used. This would indicate that some change in the follicular wall might have taken place just previous to ovulation although other observations did not support this view. It was thought that perhaps the pressure was increased too rapidly for the membranes to tear normally at the stigma region. Application of the same methods to follicles in which it was estimated that rupture would not take place before 26 hours usually caused them to rupture at points other

than the stigma.

Intra-follicular injections of water into the mature follicles of two hens did not result in ovulation. The rapid injection of 3 to 4 cc. of the fluid would cause the obliteration of all the blood vessels in the follicular wall and yet ovulation would not occur even though the turgidity of the follicle was far greater than that observed in any normal ovulation. From other evidence it seems probable that the follicular rupture would have occurred under these conditions if the internal pressure could have been prolonged, but it was impossible to maintain this pressure because of the expulsion of the deutoplasm and water.

Two birds were each injected with five international units of obstetrical pituitrin. It was thought that this might contract the muscle fibers in the follicular wall enough to cause the liberation of two or more ova at one time, but multiple ovulations did not result. It was apparent that this dosage had a tremendous effect upon the smooth muscles of the uterus, because one of the hens was given the intravenous injection before laying had occurred, and within two minutes after the injection was completed the fully-formed egg was expelled from the uterus with great force. There was indication in one bird that the injections diminished the normal length of the clutch to a slight degree.

Relation of Ovulation Time to Interval

In Table 1 are presented the data for the 40 ovulations recorded in this study. The recorded time for the 7 hens that had ovulated before the ovary could be exposed was determined from the time of laying until the recently liberated ovum was observed in the coelom. The birds were arranged according to the length of interval between eggs. Where possible 10 eggs were considered in obtaining the mean for this interval, and in all cases these data were obtained from successive eggs in the clutch. The mean of the time elapsing between the laying of the previous egg and ovulation was 32.2 minutes. This closely approximates the time recorded by Warren and Scott (1935) and McNally and Byerly (1935). The mean length of interval was 26.3 hours. The 20 birds that ovulated the most rapidly (mean 25.7 minutes) after oviposition had a mean interval length of 25.8 hours. Fourteen birds that were celiotomized between November 1 and March 1 ovulated 33.1 minutes after laying the previous egg and the mean length of interval was 27.1 hours. The mean time recorded on the 26 birds operated on during the period of March 1 to June 1 was 27.4 minutes for the time elapsing between oviposition and ovulation, with a mean length of interval of 25.9 hours, so there was some indication that length of interval as well as

Table 1. Data on Ovulations Recorded

| Hen number | : Minutes elapsing : : between oviposition : : and ovulation : | : Length of : : interval : : in hours : | : Hen : : number : | : Minutes elapsing : : between ovipositions : : and ovulation : | : Length of : : interval : : in hours : |
|------------|--|---|-----------------------|---|---|
| * 942 | : 10 | : 24.3 | : ***2048 | : 41 | : 26.4 |
| * 959 | : 20 | : 24.5 | : 1918 | : 60 | : 26.5 |
| 3294 | : 35 | : 24.6 | : 2078 | : 43 | : 26.5 |
| *1919 | : 15 | : 24.7 | : 1990 | : 17 | : 26.5 |
| *3374 | : 25 | : 24.8 | : 1923 | : 43 | : 26.6 |
| 1966 | : 34 | : 24.8 | : 1992 | : 32 | : 26.7 |
| 3024 | : 17 | : 25.1 | : 1967 | : 7 | : 26.7 |
| 1980 | : 22 | : 25.1 | : *3296 | : 15 | : 26.8 |
| 1961 | : 25 | : 25.2 | : *** 961 | : 44 | : 26.8 |
| 1920 | : 36 | : 25.3 | : 3147 | : 60 | : 27.0 |
| 3307 | : 26 | : 25.4 | : 1939 | : 19 | : 27.1 |
| 1965 | : 25 | : 25.5 | : 1519 | : 35 | : 27.2 |
| **2053 | : 31 | : 25.5 | : 2051 | : 28 | : 27.7 |
| *1984 | : 19 | : 25.6 | : 1020 | : 33 | : 27.8 |
| 3312 | : 11 | : 25.7 | : 1993 | : 74 | : 27.9 |
| ***3172 | : 45 | : 25.8 | : 1967 | : 25 | : 27.9 |
| 1945 | : 29 | : 25.9 | : 1939 | : 60 | : 28.0 |
| 859 | : 19 | : 26.0 | : 2081 | : 34 | : 28.0 |
| *1998 | : 15 | : 26.1 | : 1988 | : 50 | : 28.1 |
| 1940 | : 55 | : 26.2 | : 994 | : 52 | : 28.7 |

* Birds had ovulated before the ovary was exposed.

** Bird ovulated with the follicle clamped off at the follicular stalk.

*** Severe adjustment of the follicle had to be made.

the time intervening between the expulsion of the previous egg and ovulation are less during the months of March to June.

BEARING OF THE RESULTS ON PROPOSED THEORIES

There is a great difference in the structure of the ovary of the fowl and that of the mammals on which most of the previous studies of ovulation have been made. Each follicle is attached to the ovary by means of a follicular stalk and the ova are not embedded in ovarian stroma. The ova of the mammals are microscopic in size while those of the fowl are very large on account of the large amount of deutoplasm the ova contain. Because of this, the fowl is an excellent subject to use for ovulation studies. It is possible that the steps by which the release of the ovum is accomplished may vary in the different vertebrates, the major underlying principles must be the same. It is, therefore, of interest to summarize the bearing of the observations made in this study on the theories more commonly offered to explain the phenomenon of ovulation.

Internal Pressure Theory of Ovulation

Liquor folliculi. A review of the literature concerning ovulation in the vertebrates seems to disclose the fact that the internal pressure theory resulting from the forma-

tion of the liquor folliculi is the most frequent explanation of ovulation. Walton and Hammond (1928) made direct observation studies of the ovulation process in the rabbit and concluded that the liquor folliculi was responsible for the follicular rupture. The fluid was described as being gradually expelled from the point of rupture. However, recent motion pictures of this phenomenon, prepared by the U. S. Department of Agriculture, showed the mature follicle gradually increasing in size until it became a prominent excrescence on the ovary, but the act of ovulation appeared to be a miniature explosion. The thin liquor folliculi was rapidly expelled first, followed by the expulsion of the more viscous fluid which probably contained the ovum.

This theory seems not to be applicable to the bird, however, because of the 33 ovulations observed, only a very minute amount, if any, liquor folliculi was present inside the mature follicle. The evidence proving this resulted from the observation of those follicles which had become slightly dried before the follicular rupture occurred. In the more extreme cases it was about four seconds before the follicular rupture was completed, but in no case was so much as a drop of fluid observed. Apparently, however, there was some lubricating consistency inside the follicle, because in one case while the adjustment of the follicle was being made, the germ spot was observed to rotate inside

the follicle and come to the top of the ovum.

Blood pressure. Heape (1905) working with the rabbit, was of the opinion that ovulation resulted from the blood vessels bursting and pouring their contents into the follicle, thus resulting in a sufficient pressure to cause the follicular rupture. This belief could not be supported in the fowl as no follicular cavity surrounds the ovum into which the blood could be liberated. Also if such were the nature of ovulation in the fowl, eggs containing blood spots should be of a more frequent occurrence. However, in all of the operations performed during this study, no blood vessels were seen to break in the follicular membrane. After observing the apparent widening and changes in the blood supply at the stigma prior to ovulation, it seems rather probable that blood spots in eggs result from a rupture in the vascular system of the oviduct, rather than in the follicular membrane as the slit-like tearing of the follicle wall always occurred in the non-vascular middle portion of the stigma.

Heape (1905) working with the rabbit and Rugh (1935) with the frog were able to stop ovulation by limiting the blood supply to the ovary, but such was not the result in the fowl, because as previously stated, a mature follicle will break when excised from the ovary even an hour before a normal rupture might have been expected, provided the

muscle fibers in the stigma area were held under the approximate normal tension.

The one case where ovulation did not occur when the follicle was slamped off, but not excised from the ovary, might be explained by the muscle tonus of the follicle being relaxed when the clamp was applied, and some controlling force not being able to stimulate the muscle fibers to contract sufficiently to cause the follicle to tear at the stigma.

The visible changes in the appearance of the mature follicle of the fowl, resulting from the blurring or obliteration of the smaller blood vessels just previous to ovulation, was caused by the muscle tension in the follicular wall rather than a change in blood supply, so apparently this was hardly the responsible factor for the follicle rupture.

Deposition of yolk. Pearl and Curtis (1914) support the belief of Bartelmez (1912) that internal pressure due to continued yolk formation was probably the most important factor causing ovulation. The results of this study would not support this view, because although the deutoplasm serves as a body over which the muscle fibers in the follicle wall can contract and increase the tension, the amount of yolk material would not cause ovulation in those follicles where the muscle tonus was decreased due to the

death of the bird. Also the follicles clamped off an hour before the ovulation was expected to occur ruptured in a normal manner.

Smooth Muscle Theory of Ovulation

Muscular force of the infundibulum. Pearl and Curtis (1914) completely removed the entire oviduct from several hens and ovulation continued to occur so this was definite evidence that the force exerted by the funnel was not responsible for the follicular rupture. However, in this study the infundibulum was usually observed exhibiting great motility in the ovarian pocket adjacent to the mature follicle before ovulation.

Muscular force of the ovarian stroma and follicular wall. Some investigators have thought that the muscle fibers in the ovarian stroma are responsible for ovulation, but Rugh (1935) reported that ovarian contractions occur in both mature and immature frogs and does not believe this to be a causative factor in ovulation, except by improving the circulation to that region. In the case of the fowl, no ovarian contractions were noted at any time, and since the attachment of the ovary is so restricted, the musculature of this cannot be considered a factor contributing to the follicular rupture.

The compactness and abundance of the muscle fibers in

the follicular wall (Figs. 3, 4, and 5) would indicate that working as a unit these fibers would have a marked effect on the control of the turgidity of the follicle. The obliteration of the finger-like blood vessels along the non-vascular area of the follicle was partially if not entirely due to these muscle cells. Figures 3 and 4 show the difference in tension of these fibers prior to the time of ovulation. Since these muscle fibers have the organization which makes possible a three-way tension at the ends of the stigma, and only a two-way pull in the center, it seems reasonable to assume this is the point of greatest contraction and as a result, this was thought to be responsible for the first point of the follicular rupture to occur at one of the stigma ends.

Chemical Change Theory of Ovulation

Enzyme action. Schochet (1916) reported the liquid from the mature ovarian follicles of a hog was capable of digesting the ovarian tissue, and as a result he concluded that the digestion of the follicle wall was partially responsible for the follicular rupture. He further stated that another factor causing ovulation was the compression of the blood vessels, thus cutting off the nutrient supply to the stigma, causing it to atrophy and break.

There was no indication of any digestive action in any

of the follicles of the fowl irrespective of size. Figure 3 shows the corona radiata is intact in a follicle immediately before rupturing so this would indicate that there is no digestive action inside the follicle since it would have acted first on this layer of cells. However, the more mature the ovum the wider the stigma and the thinner the follicular membranes at this region. The narrowness of the follicular membrane at the stigma seems to result from an inhibition of the growth of the tissue as a consequence of the non-vascular condition in this region rather than from any proteolytic digestion or atrophy.

Anterior pituitary. Many workers have investigated the relationship between the pituitary gland and ovulation. The rabbit seems to be the most favorable subject for this study, since ovulation usually does not occur until approximately 10 hours after copulation. Bellerby (1929) was able to induce ovulation in the rabbit without copulation by subcutaneous injections of an extract from the anterior lobe of the pituitary. The rabbit was kept under the influence of an anaesthetic to avoid any "mental effects" and after giving the extract, ovulation occurred in about 10 hours. Thus, Bellerby concluded that copulation stimulates the pituitary to secrete substances into the blood stream which induce ovulation. Fee and Parkes (1929) found that the removal of the pituitary within one hour after copulation

inhibited ovulation, but when the hypophysectomy was performed later, ovulation was not impeded. Smith and White (1931) reported that ovulation would occur in the rabbit as long as the pituitary was not removed from the body until 1 1/4 hours after mating. Rugh (1935) reported that substances from the pituitary gland instigated the follicular rupture in the frog.

No attempt was made to remove the pituitary gland from the hens used in this study, but in two cases pituitrin injections were made without performing the celiotomy. One bird stopped laying for three days and then returned to the normal intensity or rate of laying. The second case indicated that the injections had diminished the number of eggs in the clutch to a slight degree, although the bird laid four consecutive days after the injection. This latter bird was injected 10 minutes after oviposition had occurred. The first bird was injected before oviposition.

DISCUSSION

In some mammals, ovulation seems to be associated with copulation in one way or another, but not always does ovulation occur after coitus. However, it is well known that in the fowl, ovulation is in no way dependent upon copulation.

Since ovulation did not occur in the three cases where

the birds died after bulged areas had appeared on the stigma for a considerable period, it is doubtful that any digestive action is responsible for the release of the ovum. Such digestive action should have continued sufficiently long after death to rupture the follicle. Also digestive action would not be expected to be so specific as to act upon the tissues at each end of the stigma and not effect the other portions of the same region. The histological evidence presented indicates that the portion of the follicle next to the vitelline membrane is intact, and this would not be expected if digestive action had taken place. Also there is no indication of pressure necrosis or vacuolization of the cells in the follicular membrane, so it is doubtful that any of these theories aid in explaining the ovulation phenomenon in the fowl.

Since many of the follicular ruptures observed in this study took place very slowly as a result of the drying of the follicular tissue, very accurate observations could be made concerning the liquor folliculi in the follicle, and in the 33 cases observed, no fluid was seen even when the initial point of the rupture was very small. Also no blood vessels were observed to break in the follicle and no blood was seen to be expelled when the ovum was liberated, so it is improbable that these are causative factors of ovulation in the fowl.

One of the causes of ovulation in the fowl is apparently the prolonged tension of the muscle fibers in the follicular membrane, as the muscle fibers are very abundant in the inside layers of the follicular membranes where the rupture is initiated. The initial point of the rupture is at the ends of the stigma where the fibers have the greatest power of contraction. The factor or factors responsible for the contractions of these muscle fibers was not determined.

Apparently the yolk merely serves as a body upon which the muscle fibers can contract, because ovulation occurred when the mature follicle was excised from the ovary, provided the original turgidity was maintained with clamps. The addition of deutoplasm is not responsible for the increased tension of the follicle as it becomes very flaccid when the muscle tonus is decreased as a result of death or excision.

SUMMARY

1. Thirty-three normally occurring ovulations were observed in this study.
2. The mean period of time elapsing between oviposition and ovulation was 32.2 minutes, with a range of 7 to 74 minutes.
3. The mean length of interval between successive eggs

of the hens operated upon was 26.3 hours, with a range of 24.3 to 28.7 hours. No association was found between the length of interval and the time intervening between oviposition and ovulation.

4. A last minute deposition of yolk was not responsible for the follicular rupture.

5. Observations of follicles that failed to rupture and the study of histological preparations indicates that neither enzymatic action nor vacuolization of cells in the follicular membrane is responsible for ovulation in the fowl.

6. Mature follicles ruptured normally when excised from the ovary so blood pressure was apparently not responsible for ovulation.

7. No liquor folliculi was observed in any of the follicles.

8. Pressure resulting from the prolonged tension of the muscle fibers of the follicular membrane seems to be one of the factors responsible for ovulation in the fowl, but the factor or factors stimulating these fibers to contract was not determined. No critical studies were made of the influence of hormones on ovulation.

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