NORMAL PROGRESSIVE CHANGES IN THE OVARY OF THE STARLING (STURNUS VULGARIS) FROM DECEMBER TO APRIL[1].

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Plates VI–VII.

INTRODUCTION.

THE normal progressive part of the seasonal sexual cycle of the male Starling was described and discussed in relation to environmental factors by Bissonnette and Chapnick (1930); the regressive part of the cycle by Bissonnette (1930b); and modifications of the cycles in males by experimental manipulation of the seasonal light cycle to which the birds are exposed in a series of papers from this laboratory (Bissonnette, 1930a, c, 1931a, b, 1932a, b, d, e, f, 1933a, b; Bissonnette and Wadlund, 1931, 1932, 1933).

On the female side no such study appears to have been made. So this study of the normal progressive part of the seasonal cycle of the ovary with respect to (1) Changes in size of ovarian follicles, (2) Rate of growth of the largest follicle in each ovary, and (3) Yolk formation, was undertaken, to provide a necessary standard for comparison in future studies of the changes in ovaries induced by artificial modification of light cycles. These light manipulations have already been found to modify and even reverse the normal cycles of the testes in males of this species.

(1) Follicle size changes.

Like the testes, ovaries increase in size slowly through February and March, and rapidly in April. Earlier workers have expressed the sizes of ovarian follicles in terms of their diameters. With small follicles, this is not difficult because the usual techniques do not cause distortion by shrinkage, and diameters are easily measured by taking diameters at right angles from camera lucida outlines of the circular or oval outlines of the largest section of any given follicle. With large ripening follicles, however, the usual techniques cause shrinkage and collapse of the follicles, which give sections of irregular outlines when cut. In such cases the usual methods of measuring diameters become unreliable. A new method has been devised to determine the diameters of the larger follicles and, from them, their size, for correlation with intra-follicular changes.

Measurements of the 14 largest follicles in each ovary have served for the most part in this study. But in some cases smaller ones were also measured.

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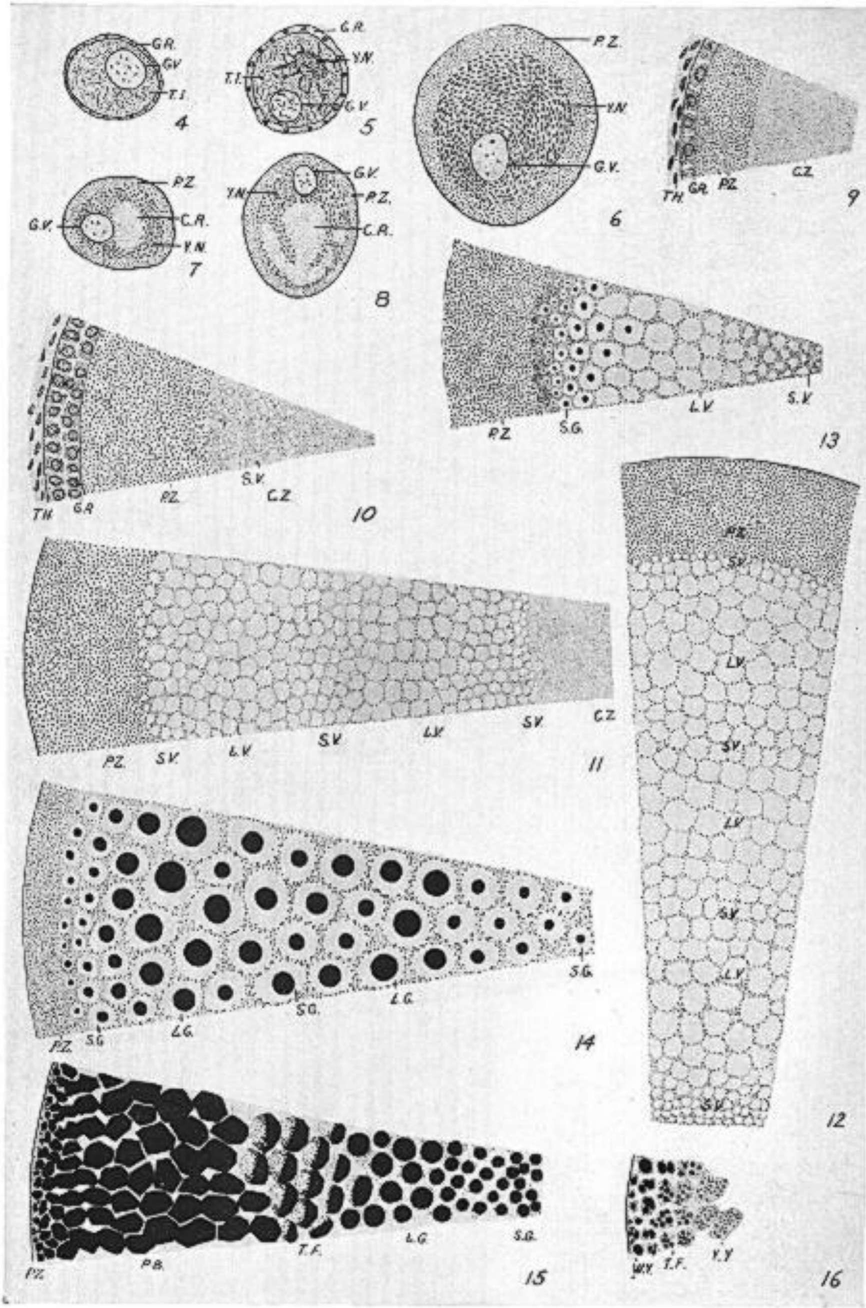


OVARIAN FOLLICULES IN THE STARLING.
STURNUS VULGARIS.

In the December ovary the 14 largest follicles are almost uniform in size. In February and March some of them increase in size, but at different rates, so that uniformity of size is lost and differences in size become more pronounced. From late March till April 20, these size differences in the 14 largest follicles increase rapidly as described by many earlier investigators for other animals.

(2) Growth Rate of Largest Follicles.

Riddle (1911) found that, in the Pigeon, 5–8 days before ovulation, when ova are at a diameter of about 6 mm., growth rate rises to about 8–20 times that formerly prevailing. Our measurements on starling material show that the average rate of growth of the largest follicle of each ovary is slow during December, January, and February; increases moderately through March; and becomes very rapid in April. During this April period, the rate of growth of several of the largest follicles increases appreciably from day to day.

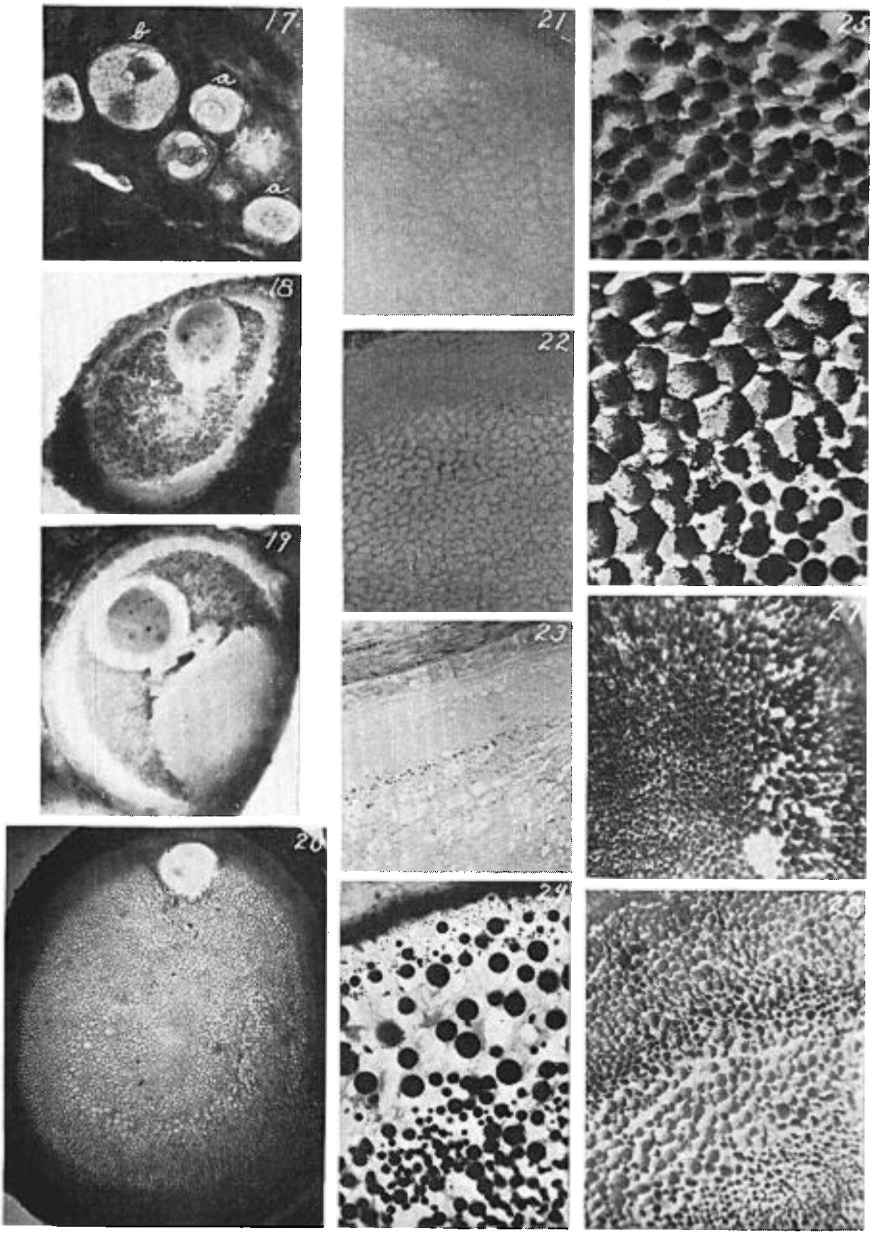
(3) Yolk formation.

The origin of yolk has been long discussed, but without general agreement. It is generally agreed, however, that yolk formation involves the transport of fat from the blood stream through the follicle wall, and its deposition in the maturing oöcyte or ovum, within the follicle. This study attempted to follow the intrafollicular changes from primordial to ripe follicles, covering modes of formation of vacuoles, of white and yellow yolk materials, thickness of peripheral zones of follicles, diameters of white yolk granules and of yellow yolk bodies, as deposited, in relation to follicle size.

Material and Methods.

The procedure followed in securing and killing birds was that used by Bissonnette (1930b) and Bissonnette and Chapnick (1930). At killing, oviducts were removed and fixed in extended condition to prevent curling and facilitate measurement. The left ovaries, syringes, and thyroid glands were fixed in the same reagent, Bouin's fluid. Pituitaries were fixed in Zenker's fluid with formic acid replacing acetic. Dehydration and paraffin infiltration were carried out by our own modification of a butyl alcohol technique suggested by Dr. Conway Zirkle, as follows:—

A stock mixture was prepared consisting of 50 units of 95% ethyl alcohol + 50 units of absolute butyl alcohol. From this, the following series of solutions was prepared. (1) 50 units of stock + 45 units water = 50% solution. (2) 70 units stock + 25 units water = 70% soln. (3) 80 units stock + 15 units water = 80% soln. (4) Stock alone = 95% soln. (5) Absolute butyl alcohol.



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Tissues for study were carried through these solutions in order, remaining about 2 hours in each, to a cold mixture of paraffin and butyl alcohol, left over night, transferred to warm paraffin in the bath, and embedded in the usual way, after 3 changes of paraffin at ½ hour intervals. All but the ovaries were filed away in paraffin blocks for future study.

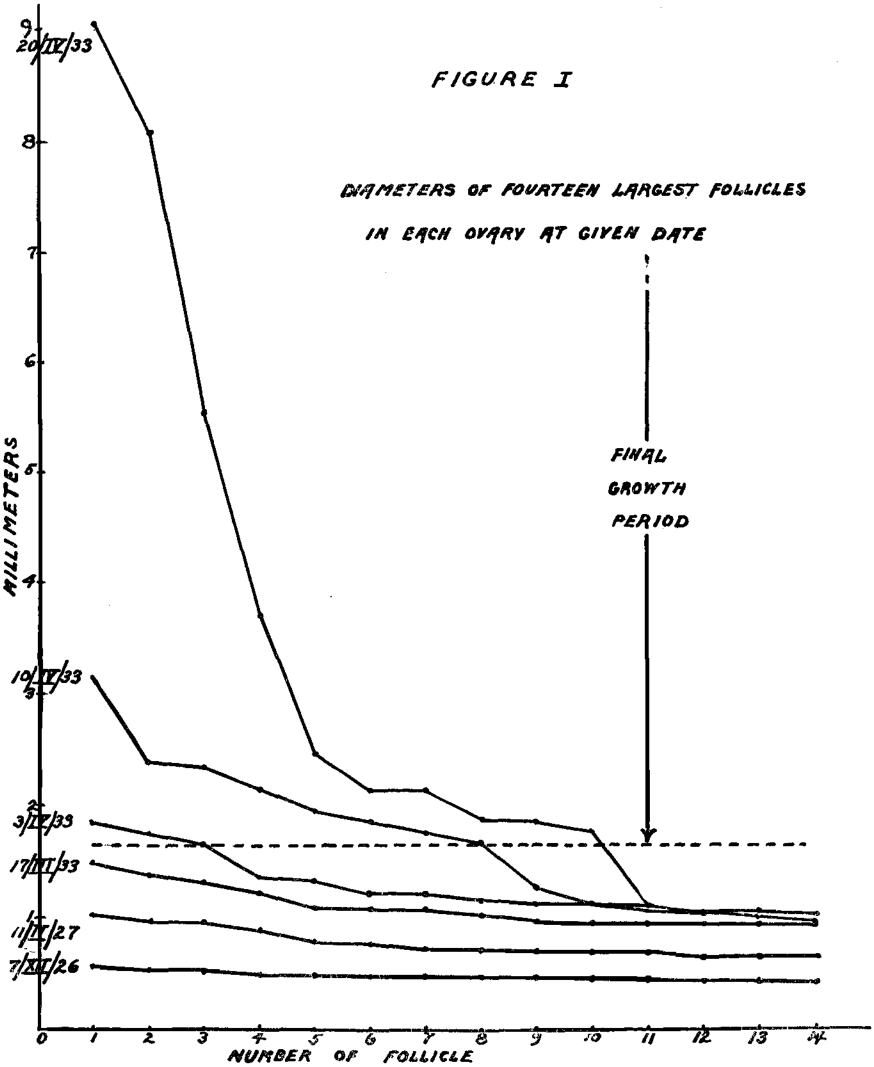
Ovaries were sectioned serially at 10–12.5 μ and mounted as usual. Stains used were Heidenhain's iron-hematoxylin with or without eosin, Mallory's triple connective tissue stain, and a combination of the two methods using hematoxylin first. All were cleared in Xylol and mounted in balsam.

In December, January, and February, the ovaries are rather compact organs and the follicles retain their circular outlines on the slide. But, as the season progresses, follicles become larger and give oval sections on the slide, though macroscopic observation of the freshly removed ovary shows that they are spherical. In large follicles shrinkage occurs during technical procedure, as a result of the dissolving out of certain lipoid substances, and sections of the follicles become irregular in outline upon the slide. Macroscopically, the irregularities of the surfaces of follicles become noticeable during infiltration with warm paraffin, indicating that removal of butyl alcohol and its dissolved substances is more rapid than penetration of warm paraffin. However, this is not peculiar to the butyl alcohol technique, but occurs also with ethyl alcohol and xylol.

Camera lucida drawings of all follicles above a certain size were made at about 34.4 diameters from about every 6th or 8th section, depending upon follicle size. In these drawings, the larger follicles of each ovary were numbered and followed through succeeding sections. As new follicles above the certain size appeared in the series of sections, they were given new numbers, and when the already numbered follicles disappeared from the sections their numbers were discontinued and not repeated. From the set of drawings covering an entire series of sections from an ovary, the largest follicles were again drawn at 140 diameters and measured along the base line of the granulosa layer so that both granulosa and oöcyte or "egg" are included. A map measurer was used in this last measurement.

On the assumption that the follicles were spherical in the untreated ovary, the diameters of the follicles were calculated from these circumferences of the largest cross-section of each follicle, using the formula, $D = \frac{C}{\pi}$; where C is the circumference, D, the diameter, and π has the value 3.1416.

The relative magnitudes of the calculated diameters are shown graphically (fig. I) for the 14 largest follicles in each of a number of typical ovaries at given dates. In the figure, the largest follicle is designated no. 1; the next in order of size, no. 2; and so on down to the 14th follicle in point of size. On the abscissa follicles are designated by number and the diameters of the follicles in millimeters on the ordinate axis. The curve, so formed,



shows the relative diameters of the maturing follicles at the dates given at the left of each curve.

It must be assumed that the largest follicle in the ovary on a given date must have grown fastest or started earliest or both. The average rate of growth of the largest follicles in each ovary was calculated and appears in Table I.

The time between December 7 and April 20, covered by this study, was divided for comparison into 5 unequal periods, based upon representative ovaries taken on the dates shown in Table II, which shows the number of days in each period and the increase in diameter for the period, as calculated.

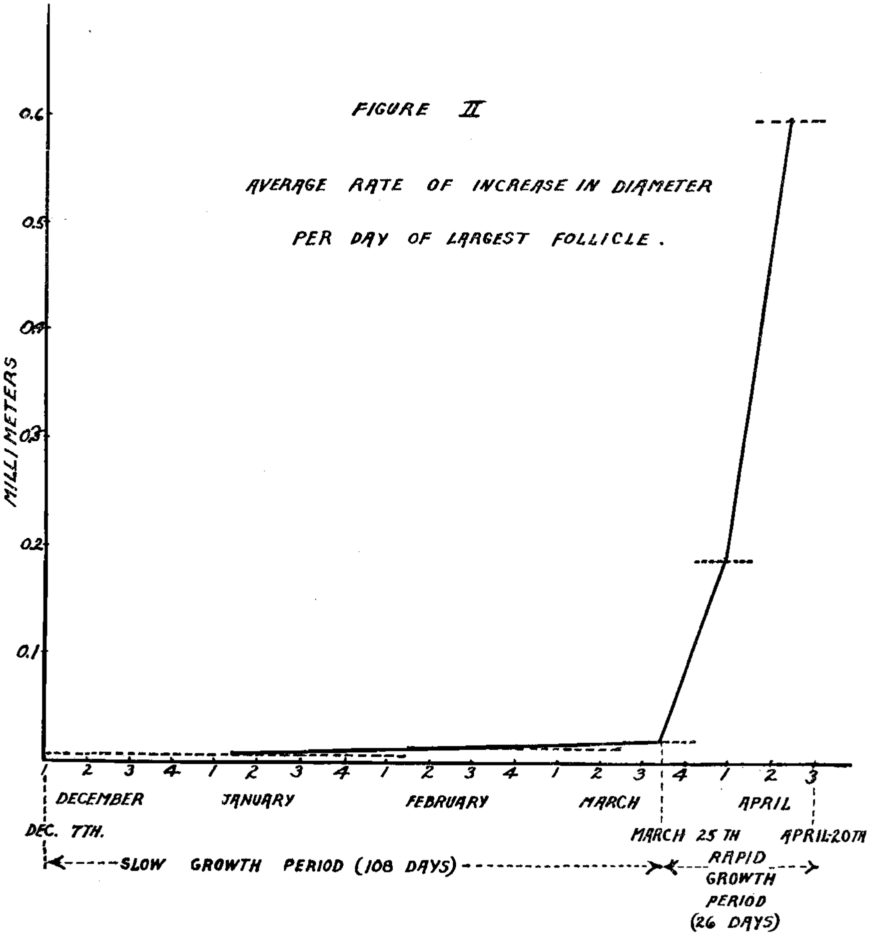


FIGURE II

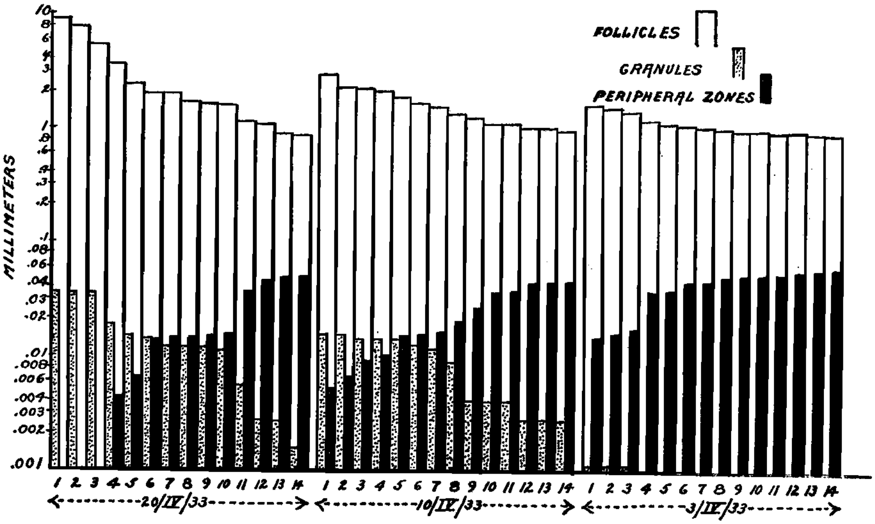
AVERAGE RATE OF INCREASE IN DIAMETER PER DAY OF LARGEST FOLLICLE.

During the measurement and study of the follicles, it became evident that the arrangement of the protoplasm of the oöcyte changed with enlargement of the follicle. So a thorough study of the protoplasm in each follicle measured was made. Sections of all follicles previously drawn at 34.4 and

140 diameters were studied again under a magnification of about 700 diameters and the diameters of white yolk granules and of yellow yolk bodies in them, and the thicknesses of their peripheral zones, were measured and calculated, except the peripheral zones of very early stages. The exceptions were made because the line of demarkation between peripheral zone and central region was not sharp or clear enough for accurate measurements.

FIGURE III

RELATION OF SIZE OF FOLLICLES, GRANULES AND PERIPHERAL ZONES IN FOURTEEN LARGEST FOLLICLES IN OVARY OF GIVEN DATE.



The relation between size of follicle, thickness of its peripheral zone, and diameters of yolk granules and polyhedral bodies is shown by a semi-logarithmic chart (fig. III).

Yellow yolk is said to be aggregated into spheres, which, after the usual techniques, appear on the slide as irregular polyhedral bodies. For lack of a better method of determining the size of these bodies, their perimeters in section were measured under camera lucida, and, on the assumption that their perimeters equal their circumferences before shrinkage, their proper diameters were calculated from the formula $D = \frac{C}{\pi}$, as was done for follicle diameters.

Size of Follicles.

Most observers have pointed out that one or more sets of follicles undergo a more rapid rate of growth than the others; but none has attempted to classify these sets of follicles as they occur in monoestrous or dioestrous birds; i. e. those with but one or two complete sexual cycles per year and a long period of anoestrum, or sexual quiescence, during autumn and winter.

By their macroscopic appearance, His (1868) distinguished four classes of follicles in the hen's ovary. In three ovaries, studied intensely, he found 5, 3, and 5 eggs, respectively, which were more than 9.0 mm. in diameter. Sonnenbrodt (1908) described the appearance of the ovarian egg of the hen and found that small follicles were grayish; larger ones, up to about 5 mm. in diameter, were whitish; while those over 5 mm. in diameter were quite yellow. He found that usually there were 3 or 4 follicles over 10 mm. in diameter. Patterson (1910) noted that, in hens, the follicles were arranged in sets, and that eggs were laid more or less rhythmically (Lillie, 1919).

We have classified the sets of follicles in the ovaries of the starling according to size from December to April 20.

The starling's ovary on December 7 is a small compact organ with its grayish follicles closely knit together by the connective tissue stroma. Three classes may be distinguished;—(1) primordial follicles, up to 0.04 mm. in diameter; (2) intermediate follicles, from 0.04 to 0.4 mm.; and (3) 14 large follicles ranging from 0.4 to 0.54 mm. in diameter, with an average of 0.463 mm. These 14 follicles are nearly uniform in size as shown by the small value (0.037 mm.) of the mean deviation about the mean diameter.

On February 11, the ovary is slightly larger, with a more or less uniform increase in the diameters of the 14 largest follicles over those of the December ovary. In each of the February ovaries, there are 4 classes of follicles in different stages of development. The diameters of the 14 largest range from 0.62 to 0.99 mm. Variations in size have become greater than those among the 14 largest follicles of the December ovary, as shown by the increase in the value of the mean deviation about the mean diameter.

In the March 17 ovaries, the diameter of the 14 largest follicles range from 0.90 to 1.48 mm. Eight of them have diameters over 1.0 mm. If these 8 are placed in a separate class, the ovary of this date will be said to have 5 classes of follicles.

On April 3, the 14 largest follicles range in diameter from 0.99 to 1.83 mm. The variation in size among them is still greater than in ovaries of the above mentioned dates. Mean deviation about the mean is 0.224 mm. The 3 largest follicles have just entered the final growth period and there are 5 distinct sets of follicles (fig. 1). Because the follicles in the final growth period vary so much in size, no definite classification as to size for the period was possible.

In addition to the above mentioned 5 sets of follicles, the ovary of April 10 has one follicle approaching the later stages of the final growth period, and 7 which have entered the final growth period.

The April 20 ovary contains the previously mentioned 5 sets of follicles, 6 follicles in the early stages, and 4 in the late stages of the final growth period.

The above classification is based upon the measurements of follicles as shown in Table I and Figure 1. Observations indicate that the number of small sized follicles in the early sets is great. In sets of larger follicles, the number in a set is smaller. In the final growth period, the variation in diameter among follicles in the late stages is greater than among those in the early stages, In other words, the number of follicles falling in a set decreases as the size of follicle increases. The follicles in ovaries of April 28 are smaller than those of April 20, indicating that the climax of activity is past and that ovulation of the largest, most advanced, follicles has occurred. So the study of these ovaries is not included here.

Rate of Growth of the Largest Follicle.

That the rate of growth of follicles in the ovaries of various animals is not uniform has been shown by Riddle (1911, 1916), Bartelmez (1912), D'Hollander (1904), Sonnenbrodt (1908), Hoffman (1892), Agassiz and Clark (1857) and others cited by them. Agassiz and Clark (1857) state that, in 7-year old turtles, there begins in the eggs a final rapid-growth period lasting for 4 years, in each successive set of eggs. Hoffman (1892) showed that, in Lacerta, an increased rate of growth of certain groups of ovarian follicles occurs in spring only. In pigeons, Bartelmez (1912) found a slow growth of the oöcyte to about 0.09 mm. in diameter. The smallest follicles then stop growth, and the others, increase to a diameter of 0.4 mm., apparently at the same rate of growth, and later, to 5.0 mm. From 5.0 mm. to ovulation size, growth is very rapid. Riddle (1911) found that 5–8 days before ovulation, when ova have reached a diameter of about 6 mm., the rate of growth suddenly increases about 8–20 times and Bartelmez agrees.

We have tried to determine the average rate of follicular growth for the various periods in the starling and the point at which this accelerated growth, characteristic of the final growth period, begins.

The diameter of the largest follicle in each of the representative ovaries is included in Table I, as stated. The number of days in each of the 5 periods between December 7 and April 20 and the increase in diameter of the tubules in the period are shown in Table II. The first 66 days from December 7 to February 11 constitute the first period, during which the diameter of the largest follicle increased from 0.54 to 0.99 mm. So the average rate of increase in diameter per day is 0.007 mm.

During the second period (Febr. 11–March 17), the average rate of increase in diameter rises to 0.014 mm. per day, double that of the first period (Table II).

In the third period (March 17–April 3) the average rate of increase in diameter advances to 0.02 mm. per day, about 3 times that in the first period.

With the fourth period (April 3–10), this average rate is 0.187 mm. per day, about 27 times that in the first period and 9 times that in the immediately preceding period.

In the fifth period (April 10–20), that preceding ovulation, this rate is 0.594 mm. per day, about 85 times that of the first, and 3 times that of the preceding period.

As may be seen from Figure II, growth of follicle falls into two divisions; one of slow growth at about 0.009 mm. per day on the average, for 108 days to March 25; and a period of 26 days to April 20, just before ovulation, with average growth rate for largest follicle of about 0.285 mm. per day; about 31.6 times that in the first division. This short period of rapid growth is called the "final growth" period and corresponds roughly with the one called by that name in the literature. In the pigeon, according to Riddle (1911), this "final growth" period (with growth rate 8–20 times that preceding it) begins 5–8 days before ovulation, when the ovum is 6 mm. in diameter. In the starling with its smaller egg, this period begins on or about March 25, when the ovum is about 1.65 mm. in diameter, and the average rate of growth is about 31.6 times that in the long period of slow growth preceding it.

TABLE I.

DIAMETERS IN MILLIMETERS OF 14 LARGEST FOLLICLES IN OVARIES AT GIVEN DATES.

	7/XII/26;	11/II/27;	17/III/33;	3/IV/33;	10/IV/33;	20/IV/33.	
1.	0.54	0.99	1.48	1.83	3.14	9.08	Diameter of the largest follicle
2.	0.52	0.94	1.39	1.76	2.40	8.06	
3.	0.52	0.93	1.29	1.66	2.32	5.55	
4.	0.49	0.89	1.22	1.32	2.11	3.71	
5.	0.48	0.77	1.08	1.31	1.94	2.42	
6.	0.48	0.77	1.07	1.20	1.85	2.14	
7.	0.47	0.70	1.04	1.19	1.79	2.13	
8.	0.46	0.70	1.00	1.15	1.65	1.85	
9.	0.45	0.68	0.93	1.10	1.21	1.83	
10.	0.44	0.68	0.92	1.10	1.11	1.75	
11.	0.42	0.67	0.91	1.05	1.11	1.07	
12.	0.41	0.62	0.91	1.01	1.05	1.03	
13.	0.40	0.62	0.90	1.00	1.05	0.97	
14.	0.40	0.62	0.90	0.99	1.01	0.94	
	0.463	0.756	1.074	1.262	1.695	3.038	Average diameter
	0.037	0.108	0.155	0.224	0.526	2.035	Mean deviation about the mean

TABLE II.

RATE OF GROWTH OF LARGEST FOLLICLE.

Period	*Increase in diameter*	*Average rate of increase in diameter per day*	*Approximate relative rates*
7/XII–11/II 66 days	0.45 mm.	0.007 mm.	1.
11/II–17/III 34 days	0.49	0.014	2.
17/III–3/IV 17 days	0.35	0.02	3.
3/IV–10/IV 7 days	1.31	0.187	27.
10/IV–20/IV	5.94	0.594	85.

YOLK FORMATION.

Most of those who have studied ovarian development divide it into more or less clearly defined periods. On the basis of nuclear phenomena, the analyses of D'Hollander (1904) for the embryonic stages, and Sonnenbrodt (1908) for the subsequent growth periods, are among the best. Bartelmez (1912) has given one of the best treatments of the subject so far as the pigeon is concerned. He describes four periods in development of the ovarian egg on the basis of changes within the follicles, as follows:—

1st; (Oöcytes up to 0.09 mm.). The cytoplasm in the follicles contains 3 kinds of granules suspended in a homogeneous ground substance; (1) large deutoplasmic spherules, absent in the peripheral zone, but present in the central zone of the oöcyte; (2) the yolk nucelus, a granular region, at the center of the spherule cap, close to the germinal vesicle; and (3) mitochondria, which alone stain with Janus green.

2d; (Oöcytes 0.09 to 0.4 mm.). More spherules are laid down outside those already present, so that the peripheral zone becomes narrower. Spherules are present throughout the oöplasm, except in the peripheral zone and in the region of the yolk nucleus, which has increased in size and begun to spread in irregular blocks and bands. The basophile granules of the yolk nucleus eventually become distributed throughout the oöplasm, except in the peripheral zone, on the inner side of which, spherules are still being formed. From studies of freehand sections of follicles in this period, stained with Sudan III, he concludes that spherules are confined for the most part to a zone just inside the peripheral zone, leaving a clearing in the central region of the oöcyte.

3d; (Oöcytes 0.4–5.0 mm.). For a short time at the beginning of this period, no deposition of yolk spherules occurs, and the peripheral zone becomes thicker. Then another zone of spherules is laid down. This is the first evidence of the periodicity in yolk formation which characterizes the final growth period (Riddle, 1911). While the germinal vesicle is still near the center of the oöcyte, the first definitive yolk granules (white yolk) appear in it. The germinal vesicle then migrates along the polar axis to the periphery at the animal pole. Shortly after the beginning of this migration, very fine yolk spherules appear in the central protoplasm, but not in the peripheral region. In oöcytes of 2–5.5 mm. diameter, growth is due chiefly to an increase in their fluid content, and a periodic deposition of yolk granules in the central protoplasm, while the peripheral protoplasmic zone becomes thinner. The path of the germinal vesicle to the periphery is marked by a trail of fine reticulum and the region that surrounded the germinal vesicle, during its stay near the center of the oöcyte, is clearly defined by its smaller yolk granules. This central region, together with the trail of fine reticulum to the periphery, constitute the latebra.

The great mass of yolk is laid down during the final growth period in such a way that the eccentricity of the latebra is preserved.

Riddle (1911), by feeding Sudan III to pigeons, found that the final period of rapid yolk secretion was characterized by an alternation of "white" and "yellow" yolk deposition in layers.

The ripening ovary of the pigeon is difficult to work with, because of its large size and the consequent difficulty of handling such large amounts of yolk in sections. For a study involving a great many sections of all types of follicles, the smaller ovary of the starling is much better.

VACUOLE FORMATION.

Under high powers of the microscope, after iron-hematoxylin-eosin, primordial follicles (about 0.037 mm. in diameter) in the adult ovary appear to consist of a germinal vesicle, or oöcyte nucleus proper, in cytoplasm which also contains thread-like inclusions (figs. 4, 17). These inclusions then appear to thicken and become more abundant throughout the egg cytoplasm. Near the center of the follicle, they seem to collect to form the yolk nucleus, a denser protoplasmic mass, with rays extending into the cytoplasm toward the periphery (figs. 5, 17). As the follicle develops, the yolk nucleus grows larger and spreads throughout the protoplasm. As to the extent of the spreading of yolk-nucleus material and the time and mode of origin of the peripheral zone, there appear to be at least two possibilities. One is that this yolk-nucleus material spreads throughout the cytoplasm of the follicle, till the whole follicle, with the exception of a thin zone next the granulosa layer, the peripheral zone, becomes completely invaded by this material of the yolk nucleus (figs. 6, 18). Another possibility is that the yolk-nucleus material spreads throughout the entire cytoplasm of the follicle at first, leaving no peripheral uninvaded zone, which appears later. The first would account for the origin and method of formation of the peripheral zone very simply. The second requires the assumption that the peripheral zone occurs either (1) from the transformation of the material of the yolk nucleus at the periphery into a more soluble material which disappears into solution, (2) from its later withdrawal from that region, or (3) from secretions from the granulosa or surrounding elements.

In follicles of about 0.2 mm. diameter (figs. 7, 19), a peripheral zone is present and a clearing appears in the middle region of the oöcyte. This clearing is the beginning of what will be called the central zone. Clearings may occur first in small areas scattered throughout the material of the yolk nucleus and may expand till the material of the yolk nucleus, as such, has completely disappeared or cannot be distinguished from the central zone (fig. 8). At this stage, the protoplasm of the follicle is differentiated into two regions; the peripheral zone and the central zone (fig. 9). The latter shows as a lighter pink than the peripheral zone after iron-hematoxylin-eosin, and appears to be coarse in texture, as compared with the more homogeneous peripheral zone.

When the follicle has reached a diameter of about 0.5 mm., small vacuoles appear at the periphery of the central zone (fig. 10), probably containing lipoids dissolved out by the fixing agent or other reagents used, and apparently the forerunners of white yolk. This may be called "clear yolk." As the follicle develops, the vacuolation of the central zone proceeds from the periphery inward. On the inner side of the vacuoles already formed, toward the center of the follicle, new vacuoles appear in the central zone and begin to enlarge (fig. 11). Later, more vacuoles arise on the inner side of those already formed. When the central zone first becomes completely vacuolated, there is a gradation in size of vacuoles from the periphery toward the center of the follicle. The material studied suggests that this zone of vacuoles enlarges, and there appear in it concentric thin layers of vacuoles which increase in size at a slower rate than the vacuoles which comprised the original thick zone, now becoming separated into three or more thinner zones of large vacuoles, with zones of smaller ones between them (figs. 12, 20). This is the first stratification in the development of the follicle. In sections of some follicles, this stratification of vacuoles into zones is not very evident (fig. 22).

In late stages of vacuole formation and growth, there is apparently a tendency for the vacuoles at the periphery and those at the center of the follicle to become nearly equal in size, by differential growth. The peripheral zone gradually becomes thinner, either by the formation of small vacuoles on its inner side or by stretching of the zone as the follicle enlarges, without corresponding increase in amount of the homogeneous peripheral protoplasm of the zone.

WHITE YOLK

Foster and Balfour (1883) described white yolk as being composed of vesicles, 4 μ to 75 μ in diameter, with a highly refractive body or spherule, often as small as 1 μ in diameter, in the interior of each; and of large spheres, each containing a number of spherules, similar to the smaller refractive bodies. Blount (1909), as already mentioned, described white yolk as consisting of round granules. The vacuoles were described above as containing a fluid substance which we called "clear yolk." With the technique used, the contents of the vacuoles was probably dissolved out giving the follicle section a vacuolated appearance on the slide. The presence of granules within these vacuoles suggests that precipitation of the clear yolk substance takes place. Following Blount's terminology, round granules in these vacuoles will be referred to as "white yolk" (figs. 13, 23).

In a 1.66 mm. follicle from an ovary of April 3, minute granules, staining red with acid fuchsin and black with iron-hematoxylin, appear within these vacuoles near the peripheral zone. In an ovary of April 10, these granules are already present in follicles 1.01 mm. in diameter (figs. 13, 23). In one of April 20, they appear in a 0.9 mm. follicle. This indicates that the deposition of granules is not correlated with size of follicle; but with degree of development of the ovary as a whole.

The granules first appear at the outer part of the vacuolated central region, next the peripheral zone, and subsequently enlarge. In the meantime, on the inner side of the zone of granules first laid down, appears another zone of granules which also enlarge. This centripetal granule formation and enlargement is repeated many times. As the follicle develops and granules appear in all the vacuoles, those last formed, at the center of the follicle, are smallest. The large granules of the thick zones are separated by thin zones of small granules. At this stage a section of a follicle (figs. 14, 24) shows a thin layer of small granules next the remaining unvacuolated peripheral zone, and layers of large granules alternating with layers of slightly smaller ones, with the smallest granules of all at the center of the follicle. A 1.85 mm. follicle of an April 10 ovary is completely filled with white yolk and illustrates this tendency to layer formation among the white yolk granules very well. The peripheral zone thins out while small granules make their appearance in the small vacuoles of the central region next its inner border.

This account of the formation of these zones agrees with Hoffman's findings that the zones of the Lacerta egg are developed centripetally; while Riddle (pigeon) and many others are of the opinion that they develop centrifugally. In the starling ovary of April 3, the peripheral zone of the 14th follicle, in order of size, measures about 0.64 mm. in thickness. As the follicle enlarges, this peripheral zone decreases to about 0.016 mm. in thickness in the largest follicle of this ovary. In the April 10 ovary, the thickness of the peripheral zone of the 14th follicle is about 0.48 mm. and this decreases, with the increase of follicle size, to about 0.006 mm. in the largest follicle of this ovary.

In many sections of follicles, a region of vacuoles appears in the outer border of the peripheral zone next the granulosa layer (fig. 23); but it is not possible to correlate this definitely with size of follicle or granule deposition.

In the three largest follicles of the April 3 ovary, white yolk granules appear as minute specks. In the April 10 ovary, the largest white yolk granules, in the follicle 14th in order from the largest one, are about 0.003 mm. in diameter. As larger follicles in this ovary are studied, these granules are found to be larger, until, in the largest follicle, the largest granules are about 0.016 mm. in diameter.

YELLOW YOLK

Balfour and Foster (1883) described yellow yolk as consisting of spheres, 25–100 μ in diameter, filled with numerous highly refractive granules. These spheres, when boiled or otherwise hardened in situ, assumed a polyhedral form. Blount (1909), in the pigeon's egg, distinguished two types of yolk; white yolk, consisting of spherical granules; and yellow yolk, of irregular polyhedral bodies. In keeping with their terminology, similar polyhedral bodies encountered in this study are referred to as "yellow yolk."

In sections of large follicles of the starling ovary, large white yolk granules are found in the vacuoles. When white yolk granules have reached a diameter of about 0.016 mm., the material in the vacuoles surrounding the granules apparently becomes sufficiently concentrated to withstand the dissolving action of the reagents used, and stains gray with iron-hematoxylin or blue with Mallory's triple stain (fig. 25). The outlines of the vacuoles, formerly somewhat rounded, then become irregular. The white granules break up into many smaller granules which form a mass, irregular in shape and eccentric in position in the vacuole (fig. 26). This eccentric irregular mass of small granules, so formed, stains red, while the rest of the vacuole becomes light blue with Mallory's stain. Vacuoles in this condition will be referred to as "transition forms." The large granule of white yolk may break up into two or more smaller granules, which do not form an irregular mass, eccentric in position in the vacuole, but become evenly distributed throughout the vacuole (fig. 16). These will also be referred to as "transition forms."

These smaller granules then break up into minute particles which become evenly distributed throughout the vacuole, which then stains black with iron-hematoxylin (fig. 15). After the complete dispersal of the particles, the transition forms become the polyhedral bodies of yellow yolk. In sections lightly stained in Mallory's stain, the polyhedral bodies contain yellow particles distributed in a blue homogeneous substance (fig. 16). Riddle (1911) concluded that, in development, yellow yolk spherules may arise from spherules of white yolk and, in normal digestion and utilization of the yellow yolk spherule, it may be reconverted to white yolk.

In a follicle of 3.71 mm. diameter, from an April 20 ovary, next the greatly reduced peripheral zone, are a thin zone of white yolk granules and one of transition forms. Next to these is a thick zone of polyhedral bodies and then a thinner zone of transition forms. The central region of the follicle contains white yolk granules only (figs. 15, 27).

In a 5.55 mm. follicle from an ovary of the same date, from the periphery inward, there appear the following 8 regions (fig. 28); (1) a thin peripheral zone, (2) a thin zone of white yolk granules, (3) a thin zone of transition forms, (4) a thick one of polyhedral bodies, (5) a thick one of transition forms, (6) a thin one of white yolk granules, (7) a thick one of transition forms, and (8) a central region with white yolk granules.

In the two largest follicles of the April 20 ovary, there are three zones of yellow yolk polyhedral bodies, between which are zones of white yolk granules. The peripheral zone has diasppeared everywhere except in the blastodisc region. The arrangement of the zones in these follicles, from the periphery inward, is;—(1) peripheral zone, only in blastodisc region, (2) thin zone of white yolk granules, (3) thick one of polyhedral bodies, (4) thin one of white yolk granules, (5) thick one of polyhedral bodies, (6) thin one of white yolk granules, (7) thick one of polyhedral bodies, (8) central region filled with white yolk granules.

In these two follicles, evidently breakdown of white yolk granules has occurred, thinning the white yolk zones considerably; and transition forms have largely or wholly turned into yellow yolk polyhedral bodies, causing the almost complete disappearance of the zones of transition forms found in the 5.55 mm. follicle. These 8 zones are complete and concentric except in the region of the latebra, which consists of a thin region of white yolk granules extending from the center of the follicle to the blastodisc region. In early stages, the peripheral zone is of uniform thickness over all parts of the follicle, but, when white yolk is being formed, that part of the peripheral zone, which is to become the blastodisc region, becomes slower in its rate of thinning and persists in this region after it has disappeared in other regions.

In the latebra, the granules are fairly large centrally, but become smaller toward the periphery of the follicle in the blastodisc region, where the peripheral zone persists, with its inner part containing many small vacuoles with minute granules.

At this stage, the granulosa layer has disappeared or, at least, become considerably reduced, so that its cells are imperceptible at the magnification used.

Parts of the above data are summarized in Table II and figure III.

Summary and Conclusions.

1. The normal spring changes in size and condition of ovarian follicles in (*Sturnus vulgaris*) the European Starling from December 7 to April 20 (just before ovulation) were studied.

2. A method was developed and described for the more accurate measurement of ovarian follicles rendered irregular in outline by the usual paraffin microtechnique.

3. December, February, and March ovaries have, respectively, three, four, and five different sets of follicles. April ovaries have follicles also in various stages of a sixth or final growth period.

4. The number of follicles falling in a set or class decreases as the size of follicle in the class increases, and the larger the follicles in a set, the greater are the variations in size among them.

5. From December 7 to March 25, the average rate of growth of the largest follicle is about 0.009 mm. increase in diameter per day. From March 25 to ovulation, the final growth period, after the follicle has reached a diameter of 1.65 mm., the average rate of growth of the largest follicle is about 0.285 mm. in diameter per day, about 31.6 times that of the preceding period.

6. (Stage 1) Primordial follicles (about 0.037 mm. diameter) contain the germinal vesicle and thin strands throughout the oögonial protoplasm (figs. 4, 17a). The strands then thicken and multiply throughout the protoplasm, and collect near the center of the follicle to form the yolk nucleus, a denser mass with rays extending into the surrounding protoplasm (figs. 5, 17b). In follicles from about 0.04 to 0.2 mm. in diameter, the yolk-nucleus material spreads throughout the protoplasm of the maturing germ-cell (figs. 6, 18).

7. (Stage 2) In follicles 0.2–0.5 mm. in diameter (figs. 7, 19), a clearing develops either in the central region or in small regions scattered throughout the material of the yolk nucleus (fig. 8) and expands until all of the material characteristic of the yolk nucleus has disappeared, leaving a central zone of clear substance surrounded by a homogeneous peripheral zone (fig. 9).

8. (Stage 3) In follicles from 0.5 to 1.20 mm. in diameter, from the April 3 ovaries, vacuolation begins at the periphery of the central zone and proceeds centripetally (figs. 10, 11).

9. (Stage 4) When the central zone has become completely vacuolated, alternate concentric zones of small and of larger vacuoles are apparent in sections. These are the result of unequal rates of growth of the vacuoles in

these zones, the smaller ones having grown less rapidly than the larger (figs. 12, 20, 21, 22). Finally, however, the zonation tends to disappear as the smaller vacuoles in one type of zone approach those of the other in size.

10. (Stage 5) The appearance of round granules within the vacuoles next the peripheral zone suggests that precipitation of the original substance or "clear yolk" takes place (figs. 13, 23). These precipitation granules are referred to as "white yolk." The fact that they appear in smaller follicles in more mature ovaries indicates that deposition of white yolk is not correlated with size of follicle directly, but with degree of development or maturity of the ovary as a whole.

11. (Stage 6) White yolk is laid down from the periphery inward, and, as in the cases of vacuoles, thick zones of large granules are separated by thin zones of smaller ones, with the smallest granules at the center of the follicle (figs. 14, 24). This indicates that the deposition of white yolk in a vacuole is related to the condition of the substance in it rather than to its size merely.

12. When white yolk granules have reached a diameter of about 0.016 mm., the material in the vacuole surrounding the granules becomes concentrated enough to stain (fig. 25). The granule loses its spherical form and either breaks up into many small granules, which mass together irregularly in an eccentric position in the vacuole (fig. 26), or breaks up into small granules evenly dispersed throughout the vacuole (fig. 16). These vacuoles are then referred to as "transition forms" between white and yellow yolk. Their small granules then break up into minute particles evenly distributed through the vacuoles which then become the polyhedral bodies of yellow yolk (fig. 16).

13. (Stage 7) Near the periphery and separated from it by a thin zone of white yolk granules and transition forms, appears a zone of polyhedral bodies of yellow yolk with a zone of transition forms on its inner side next the white yolk which fills the center of the follicle (figs. 15, 27).

14. (Stage 8) Then another thick zone of transition forms develops from white yolk farther toward the center of the follicle, separated from the polyhedral-body and transition-form zones, previously formed, by a thin zone of white yolk granules.

15. (Stage 9) In the two largest follicles of the April 20 ovary, are three concentric zones of yellow yolk polyhedral bodies separated by zones of white yolk granules. This results from conversion of transition forms into polyhedral bodies of yellow yolk.

16. As the follicle increases in size, its peripheral zone decreases and eventually disappears, except in the blastodisc region.

17. In follicles nearing ovulation, the granulosa layer either disappears

or becomes considerably reduced, so that it is not visible in sections after the technique used.

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PLATE VI.

Figures 4–16 are camera lucida drawings, others, photo-micrographs.

Fig. 4. Follicle showing germinal vesicle and thin strands in egg protoplasm in adult ovary. × 350.

Fig. 5. Follicle showing germinal vesicle, yolk nucleus, and thin strands in egg protoplasm, in adult ovary. × 350.

Fig. 6. Follicle showing material of yolk nucleus diffused throughout the egg protoplasm except in peripheral zone. × 350.

Fig. 7. Follicle showing clearing in center of material of yolk nucleus. × 70.

Fig. 8. Follicle showing the spread of the clear region throughout the material of the yolk nucleus. × 70.

Fig. 9. Sector of follicle showing peripheral and central zones. × 350.

Fig. 10. Sector of follicle showing vacuoles at periphery of central zone. × 350.

Fig. 11. Sector of follicle showing further vacuolation of central zone. × 350.

Fig. 12. Sector of follicle showing complete vacuolation of central zone and zones of large and smaller vacuoles. × 350.

Fig. 13. Sector of follicle to show white yolk granules in vacuoles at periphery of "egg." × 350.

Fig. 14. Sector of follicle showing tendency to formation of zones of larger and smaller granules. × 350.

Fig. 15. Sector of follicle showing zone of polyhedral bodies, zone of transition forms and white yolk granules. × 350.

Fig. 16. Part of section of follicle showing histological structure of one type of transition forms and polyhedral bodies of yellow yolk. × 350.

PLATE VII.

Fig. 17. Follicles showing (a) germinal vesicle and thin strands of egg protoplasm, (b) germinal vesicle, yolk nucleus, and strands in egg protoplasm. × 335.

Fig. 18. Material of yolk nucleus diffused throughout protoplasm of follicle except in peripheral zone; clearing beginning to appear in center of yolk-nucleus material. × 335.

Fig. 19. Clear region spreading throughout yolk-nucleus material in follicle. × 335.

Fig. 20. Zones of large and small vacuoles in vacuolated central zone of follicle. × 75.8.

Fig. 21. Zones of large and small vacuoles in part of follicle section. × 335.

Fig. 22. Section of follicle in which zones of large and small vacuoles are not evident. × 335.

Fig. 23. Part of section of follicle showing white yolk granules in vacuoles at periphery. × 335.

Fig. 24. Part of section of follicle showing tendency to form zones of large and small granules. × 335.

Fig. 25. Part of section of follicle showing deeply stained white yolk granules and remainder of vacuole less deeply stained. × 335.

Fig. 26. Transition forms in which minute particles collect into an irregular mass eccentric in position in the vacuole. × 335.

Fig. 27. Part of follicle showing zone of polyhedral bodies, zone of transition forms and white yolk granules. × 75.8.

Fig. 28. Part of follicle with zone of polyhedral bodies, zone of transition forms,

zone of white yolk granules, thick zone of transition forms and white yolk granules at center of follicle. × 75.8.

Key to Figures.

C.Z.	Central Zone.	S.V.	Small Vacuoles.
GR	Granulosa	Th	Theca.
G.V.	Germinal Vesicle.	T.F.	Transition Forms.
L.G.	Large Granules.	T.I.	Threadlike Inclusions.
L.V.	Large Vacuoles.	W.Y.	White Yolk.
P.Z.	Peripheral Zone.	Y.N.	Yolk Nucleus.
S.G.	Small Granules.	Y.Y.	Yellow Yolk.

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