MATURATION, FERTILIZATION, AND EARLY CLEAVAGE IN THE EGG OF THE DOMESTIC FOWL

By

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INTRODUCTION

The egg of the domestic fowl has been a classical subject for the study of vertebrate embryology during the past two centuries. The popularity of the hen's egg for this purpose has been and still is largely due to two facts: (1) Chickens are raised almost universally and consequently eggs are available throughout the year; (2) This type of egg, unlike those of the mammals, can be incubated artificially and the embryo studied at any desired stage of development.

Since the egg of the domestic hen has been used so commonly, one would naturally expect that all stages in its developmental history would have been studied in great detail. It is surprising to find, therefore, that very little published material has been presented on the early stages of embryonic development. This is especially true of such processes as occur during maturation, fertilization and early cleavage.

Van Durme (62) in discussing the suitability of different birds' eggs for early embryological studies gave three reasons why she preferred to use the eggs of smaller birds such as the pigeon and sparrow. In her opinion smaller birds are more easily handled, require less space, and represent less investment than the domestic hen. The fact that many zoological laboratories are not equipped for the housing and care of such large birds as the domestic fowl doubtless has been a contributing factor in causing many investigators to

choose the eggs of smaller birds for experimental material. To obtain eggs in the maturation and fertilization stages of development, the birds must be sacrificed since these stages normally occur either before or soon after ovulation. Since it is impossible to predict the exact time of either ovulation or fertilization, the chances of obtaining an egg at a desired stage of development are relatively slight. This means that in a study of this nature many birds must be sacrificed in order to secure a complete series of the various stages of development. This necessarily entails not only a great amount of time and labor but considerable expense as well.

Inasmuch as the processes of maturation and fertilization in the hen's egg have not been adequately described, it has been assumed by some authors that maturation and fertilization are essentially the same for all birds. This assumption is probably justified for general purposes of presenting an unbroken account of the developmental processes. Nevertheless it is important to have a complete description of all phases of development in the hen's egg since it is so commonly used in descriptive and experimental embryology of vertebrates. It would also be of interest to learn definitely if the processes of maturation and fertilization are the same in two groups of birds such as the domestic fowl and pigeon, which differ so greatly in their breeding habits, time of ovulation, rate of production, and size of egg.

The purposes of the author in undertaking the present

study were twofold - (1) To study the processes during maturation, fertilization and early cleavage in the hen's egg in order to complete the knowledge of the early stages of development of the chick, and (2) If possible, to develop a technique whereby infertile hen eggs could be artificially impregnated and induced to develop under controlled laboratory condicitions outside of the hen's body. Such a technique obviously would be of much value to students of embryology since early stages of development could be obtained and studied at will.

REVIEW OF LITERATURE

Before entering into a detailed discussion of the work connected with the present study it may be helpful to review briefly some of the physiological processes which occur during the formation of the hen's egg.

The origin of the primordial germ cells in the case of the chick has been studied by Swift (61), Firket (20), Woodger (70), Goldsmith (23), and Willier (68). According to Swift (61) and Woodger (70), the primordial germ cells arise in the anterior and lateral portions of the blastoderm outside the embryo. The germ cells arise during the primitive streak stage up until the time the embryo has developed three somites. The germ cells remain in this position, according to Swift (61), until the mesoderm extends to the anterior and lateral portions of the blastoderm (about 10 somite stage). The germ cells are then carried by the blood to the area of gonad formation. Here

the germ cells undergo further development beginning, according to Marza and Marza (35), about the tenth day of incubation. At this stage the germ cells are known as primary oogonia. Each primary oogonium multiplies rapidly, giving rise to eight daughter cells, one of which, according to Munsen (40), is destined to become the egg. The other seven cells, according to Munsen (40), produce the follicular epithelium which surrounds the egg.

The eight-fold multiplication of the original oogonia takes place principally at the periphery of the ovary, the daughter cells now being known as secondary oogonia. These secondary oogonia cease dividing about the twenty-first day of incubation. A detailed study of the growth and nuclear changes of the primary and secondary oogonia of the fowl was made by d'Hollander (16).

Following the period of multiplication the secondary oogonia become what is termed first order oocytes. In the ovary of the young hatched chicks these first order oocytes undergo development and continue to do so until time of ovulation. This lengthy development of the oocytes involves many nuclear and cytoplasmic changes, such as variation in size, position and staining qualities of the nucleus and variations in composition, structure, and amount of cytoplasmic materials. These changes have been studied in detail by several investigators including Holl (28), Harper (25), Loyez (34), Sonnenbrodt (59), Bartelmez (3), Van Durme (62), and Brambell (8). During this last stage of growth which is

termed the intrafollicular period by Marza and Marza (35), the young oocyte increases more than 2000 times in diameter, i. e., from about fifteen to twenty microns to approximately 3.5 centimeters.

Soon after formation, the young oocytes become surrounded by a layer of flat cells which is known as the follicular epithelium. The follicle wall which acts as a protective covering of the ovum while it is in the ovary, is composed of three layers. Beginning with the outermost layer, they are known as (1) the external theca, (2) the internal theca, and (3) the follicular epithelium. The follicular epithelium in larger follicles lies next to the vitelline membrane and separates the ovum proper from the rest of the ovarian tissue. All the material which goes to form the yolk material of the ovum must pass through this membrane. According to Marza and Marza (35), this membrane exhibits great selective permeability to material present in the blood.

The various stages of yolk formation have been studied in birds' eggs by several investigators, including Van Durme (62) and Marza and Marza (35). According to the latter authors, yolk formation can be divided into three phases. The first is characterized by the appearance of fat globules in the ooplasm of the oocyte; the second by the appearance of transparent vacuoles and the formation of yolk material within these vacuoles; the third by the appearance of the yellow yolk.

The ovary of the pullet prior to time of sexual maturity appears as a mass of immature follicles which, according to

Conrad and Scott (11), range from microscopic size to about six mm. in diameter. The individual oocytes or follicles are now in what is termed the second phase of growth. At this period of development the oocytes contain no yellow yolk material such as is found in older ones during the third and last stage of growth. Shortly before the pullet begins production, certain of these oocytes enter into the third phase of yolk formation. As one of these oocytes matures and is liberated from the ovary another young ovum enters the third phase of growth so that in the ovary of a laying bird several follicles can be found which differ greatly in size. According to Conrad and Scott (11) there are eight to ten follicles in the third phase of growth at any given time. During this third period of yolk formation these few follicles greatly increase in rate of growth which has been estimated by Riddle (55) to be twenty-five times faster than that of the previous level. The yellow yolk material is laid down in the form of alternate rings of lighter yellow-colored and darker yellow-colored yolk material. Riddle (54), Gage and Gage (21), Rodgers (57) and others have demonstrated that the concentric rings of lighter and darker yellow yolk material could be differentially colored by feeding a red aniline dye, Sudan III, to laying hens. Riddle (54) and (55) by this method was able to calculate the amount of yolk deposited in a given time. He found that approximately two grams of yolk material were deposited daily in the ova during the third or last stage of yolk formation. Warren and Conrad (63)

used a modification of that method in their study on the growth of the hen's ovum. These latter authord injected the dye directly into the blood stream of the hen.

The nucleus, which formerly occupied an approximately central position in the ovum, moves toward the periphery as more and more yolk material is deposited in the ovum. As the nucleus migrates toward the periphery of the ovum it leaves behind a flask-shaped mass of light-colored yolk material, the spherical portion of which is in the center of the ovum. This flask-shaped structure is known as the latebra.

As the oocyte approaches maturity, i. e., during the last forty-eight hours of its sojourn in the ovary, there are marked changes which occur in the large nucleus or germinal vesicle. The nuclear changes which occur during this brief interval of time have not been adequately described for the hen's egg. These nuclear changes,which will be discussed in detail in this paper, are concerned with the maturation process, whereby the number of chromosomes in the egg is reduced from a diploid to a haploid number. During the last forty-eight hours of the growth of the ovum the nuclear wall of the germinal vesicle begins to disintegrate allowing the fluid contents of the germinal vesicle to escape into the surrounding protoplasm.

At least one of the two polar bodies is extruded from the egg prior to the time of ovulation. Harper (25) studied and described these structures in the egg of the pigeon and later Van Durme (62) published a detailed account of the same structures in the eggs of the pigeon, swallow, sparrow, and

green finch. Thus far no description has been made of polar body formation in the hen's egg.

When the ovum of a hen reaches a size of approximately thirty-seven mm. in diameter or eighteen to twenty-two grams in weight, the walls of the follicle rupture along a preformed, non-vascular line called the stigma. This process, called ovulation, allows the ovum to leave the follicle and enter either the oviduct or the body cavity. Coste (12) was among the first investigators to observe and report the processes attending ovulation. It was his opinion that the rupture of the mature ovarian follicle was brought about by the pressure exerted by the walls of the infundibulum portion of the oviduct as it enclosed the follicle just prior to ovulation. In this manner the egg was thought to be directed into the oviduct. Bartelmez (3) also reported that in the case of the pigeon the infundibulum enclosed the mature follicle just prior to ovulation. Curtis (13), on the other hand, believed that the rupture of the follicle was chiefly due to the increased internal pressure within the ovum.

Phillips and Warren (51) working on the mechanics of ovulation in the fowl concluded that the contraction of muscle fibers which they found present in the follicular wall was largely responsible for ovulation. Warren and Scott (65) using an operative technique on twelve anesthetized hens were able to directly observe the processes attending ovulation. These observations led them to conclude that (1) the infundibulum of the oviduct seldom encloses a follicle at the time of ovulation and therefore is not the causative factor of this

process, and (2) the ovum does not always enter the oviduct immediately after ovulation, since in the case of ten of the twelve birds under observation the ova were seen to fall into the body cavity. The ova which entered the body cavity were later picked up by the infundibulum. It should be pointed out, however, that the birds used by Warren and Scott (65) were under the effect of anesthesia and were lying in an abnormal position while the observations were taken. These observations, therefore, do not necessarily mean that under normal conditions the same percentage of eggs as they reported fall into the body cavity before entering the oviduct. The orientation of the embryo with respect to the long axis of the egg is of interest in this connection.

When the blunt end of a fertile egg of a bird which has been incubated for a few days is held to the left and the upper portion of the shell removed, the embryo usually will be found lying on its left side with the head turned to the right and directed away from the observer. This orientation has been noted in various birds' eggs by Pander (45), Dalton (15), Haswell (26), Fere (19), Peebles (50), Blount (5), Patterson (47), and Bartelmez (3). It is logical to assume that to attain this orientation the ovum must enter the oviduct in a predetermined manner. Bartelmez (3) reported that in the case of the pigeon's egg a definite polarity could be found even while the ovum was still in the ovary and Munsen (40) found the same to be true in the case of the tortoise egg. The latter author observed that the eccentric position of the nucleus and the central position of what he terms the

"cytocenter" were the early landmarks of polarity. If ova entered the body cavity with the frequency reported by Warren and Scott (65) and later were picked up at random by the oviduct, one would expect to find a greater incidence of embryos in inverted positions with respect to the long axis of the shell. However, in the case of hen eggs, more than eighty-eight percent of the forty-eight-hour embryos are found in a position on the yolk which is considered as normal with respect to the long axis of the shell.

The time of ovulation with respect to the time of laying has been studied by several investigators, including Warren and Scott (66) and (67) and McNally and Byerly (37). The former investigators found that ovulation takes place on the average 30 minutes after time of laying except in cases where the egg is laid in the late afternoon. These investigators found that there was a range of fourteen to seventy-five minutes in the time of ovulation following laying in the group of birds under observation. McNally and Byerly (37) found that in the case of thirty-two hens killed one-half hour after laying twenty, or 62.4 percent of the birds, had already ovulated.

The histological structure of the oviduct of birds, including the oviduct of the domestic hen, was studied by Giacomini (22) and Cushing (14). Detailed and exhaustive studies of the same structure in the domestic hen were later made by Pearl and Curtis (49), Surface (60), and Richardson (53). Pearl and Curtis (49) recognized five different regions

of the oviduct, namely (1) the infundibulum, (2) the albumen-secreting portion, (3) the isthmus, (4) the uterus, and (5) the vagina.

The time that an egg remains in the different regions of the oviduct has been studied by a number of workers including Patterson (47), Pearl (48), Pearl and Curtis (49), Asmundson (1), and Warren and Scott (65). Patterson (47) reported that the ovum spent three hours in the glandular portion, two to three hours in the isthmus, and sixteen to seventeen hours in the uterus. Pearl (48) and Pearl and Curtis (49) estimated that an egg remains in the albumen portion for three hours, less than one hour in the isthmus, and twelve to sixteen hours in the uterus. Warren and Scott (65) and (67) found that on an average an egg required eighteen minutes to pass through the infundibulum, two and nine-tenths hours to traverse the albumen-secreting portion, and one and two-tenths hours to pass through the isthmus.

The egg is fertilized while in the infundibulum, the spermatozoa penetrating the relatively thin vitelline membrane soon after ovulation. A description of the different stages of fertilization in the pigeon egg was given by Harper (25), Blount (5), and Van Durme (62). The last author also has studied the same process in the eggs of the swallow, sparrow, and green finch. No comparable study has been made of fertilization in the hen's egg.

As the egg passes through the albumen portion forty to fifty percent by weight of its total albumen is acquired,

according to Pearl and Curtis (49). During this time, the non-cellular vitelline membrane also increases in thickness due to a deposit of thick albumen on the surface of this structure, Lecaillon (31) and McNally (38). This increase in thickness may be as much as one hundred percent, according to McNally (38).

In the isthmus the egg receives its two shell membranes, which, according to Jull (29) are composed of matted organic fibers of protein. The formation of the first two cleavage furrows in the hen's egg also occurs while the egg is in this portion of the oviduct. Coste (12), V. Kolliker (30), Duval (17)(18), and Patterson (47) have studied the formation of the first cleavage furrow in the hen's egg, especially as to its direction with respect to the long axis of the future embryo. Cleavage in the pigeon egg was studied by Harper (25), Blount (5), and Van Durme (62). The last author also used the eggs of the swallow, sparrow and green finch in her studies.

While in the uterus the volume of white in the egg increases by nearly one hundred percent, Pearl and Curtis (49), The uterine fluid which is added enters the egg by osmosis and, according to Beadle, Conrad and Scott (4), is essentially a solution of inorganic salts. The egg slowly rotates in the uterus, according to Hansen (24) and Conrad and Phillips (10), which results in the twisting of strands of mucin fibers at the two ends of the yolk. These two twisted strands of fibers are known as the chalazae. It is also in this portion of the oviduct that the egg receives its calcareous shell and the

so-called bloom which is deposited on the outer surface of the shell. During the sojourn of the egg in the uterus the developing blastodisc passes through all the cleavage stages from the eight-cell stage to that found at the time of gastralation.

The egg as it passes through the oviduct is formed usually with the small end caudal but may in some instances be formed with the blunt end directed toward the cloaca, as was shown by Olsen and Byerly (43). The same authors in a later publication (44) presented data showing that the embryos in naturally laid eggs are inverted on the yolk in 11.8 percent of the cases with respect to the orientation considered as normal. These data indicated that most of the eggs containing such inverted embryos were formed in the oviduct with the large end of the shell directed toward the cloaca.

MATERIALS AND METHODS

Origin of eggs used. Most of the fertile eggs used in the study of maturation and fertilization were collected from the ovaries and oviduct of a group of naturally fertilized White Leghorn and Rhode Island Red hens which were being slaughtered for other purposes. The material was fixed in Bouin's as it was collected. A record was kept of the date and time of collections, the position of the egg in the oviduct and the previous laying schedule of the bird.

The uterine eggs used for obtaining the series of normal cleavage stages shown in Plates 5 and 16 were secured from a

mass-mated flock of White Leghorn and Rhode Island Red hens. Some of these eggs were obtained by palpation, using the technique described by Olsen and Byerly (43). The majority of these eggs, however, were obtained by injecting three rat units of posterior pituitary extract into the leg muscle of each bird. By this procedure, described by Riddle (50) and by Burrows and Byerly (9), it was possible to obtain premature eggs five hours after the estimated time of ovulation. After obtaining these premature uterine eggs, the procedure followed was to mark the anterior end of the blastodisc by inserting a coarse hair into the yolk near the future head region. The shell membranes and albumen were next removed and the yolk placed in Bouin's fixing fluid.

The infertile uterine eggs used for developing the technique of artificial impregnation as well as those used to study fragmentation were secured from birds which were kept in individual cages of laying batteries. These birds had not been with males for more than three months prior to the time their eggs were obtained for this study. As an added precaution, eggs laid by these birds were tested for fertility prior to the time the birds were used. The fertility test, which consisted of incubating the eggs for forty-eight hours and then examining the germinal discs for signs of development, showed that all eggs laid by the group of birds were infertile. These particular birds were observed each hour in order to know the approximate time when the previous egg was laid. From these data it was possible to estimate the time of ovulation

of the next ovum. In this regard it will be recalled that both Warren and Scott (66) and McNally and Byerly (37) reported that ovulation occurs in the majority of birds within one-half hour after laying. Therefore, by knowing the time of ovulation and the time when the premature soft-shelled egg was obtained from the uterus, it was possible to estimate the age of any given egg. These premature infertile eggs were obtained between the hours of 2 and 4 p. m. on the afternoon preceding the day when they normally would have been laid. In other words, the infertile eggs used in this phase of the study were approximately five to seven hours old.

Artificial impregnation. Several procedures were tried in developing a satisfactory method of artificially impregnating infertile eggs. One procedure was to remove the shell membranes and albumen. The yolk was then placed in a small porcelain cup, and a drop of undiluted semen deposited on the germinal disc. The porcelain cup containing the yolk was then placed in a covered dish within an incubator for fifteen minutes and kept at 105° F. After this interval the albumen was again replaced and the egg allowed to incubate for the length of time desired. Other methods which were slight modifications of that just described were also tried. These modifications consisted of such procedures as washing the vitelline membrane with either normal saline or Ringer's solution following removal of the albumen and before impregnation and in using different types of containers, including portions of egg shell for incubating the yolk.

The most successful method found to date consisted of simply injecting one drop of a one to fifty dilution of fresh semen beneath the vitelline membrane near one edge of the blastodisc. An ordinary hypodermic syringe fitted with a No. 27 gauge needle proved satisfactory for this purpose. A modified Ringer's solution of the composition described by Bonnier and Trulsson (6) was used as a diluent for the semen. This solution was of the following composition:

In 10,000 cc. of water were dissolved:

NaCl	68.00 grams
KCl	17.33 grams
$CaCl_2$	6.42 grams
$MgSO_4$	2.50 grams
$NaHCO_3$	24.50 grams

Using this method of artificial impregnation, the premature egg was kept practically intact since it was only necessary to cut a small circular hole about one-half inch in diameter in the shell membrane providing it was made directly over the germinal disc. After impregnation the egg, with its membranes intact except for the small aperture, was placed in a small porcelain cup which acted as a holder. The cup containing the egg was then placed within a covered dish in which a high relative humidity was maintained. The egg was then incubated for the desired length of time at a temperature of 105° F. The shell membranes and albumen were then removed and the yolk fixed in Bouin's.

After fixation, the entire yolk with the exception of those blastodiscs which were to be photographed, were removed from the fixative solution and placed in sixty percent alcohol.

The germinal disc was then carefully removed by cutting around it with a small pair of scissors. The germinal disc was then placed in seventy percent alcohol followed later by the higher alcohols and xylol and finally embedded in paraffin.

Stains used. Delafield's hematoxylin, Heidenheim's iron hematoxylin, and Feulgen's stains were used in these studies. In all cases the tissues were stained after they were sectioned. Heidenheim's iron hematoxylin proved very helpful in studying structures of the germinal vesicle, the mitotic figures and the polar bodies. Delafield's hematoxylin was useful for studying the gross structures of sections of the germinal disc and the underlying yolk material. The yolk granules are not stained as darkly with this stain as when Heidenheim's iron hematoxylin is used. Feulgen's stain, because of its specificity for chromatin material, was used in determining the presence or absence of chromatin.

Tissue carrier. Difficulty was encountered in the earlier part of these studies due to breakage of some of the tissues as they are being transferred from one reagent to another. To overcome this difficulty, the device shown in fig. 55 was developed. This consisted of a flat, circular plate of non-corrosive metal which was fitted with a series of 35 small chambers (one-half inch in depth and one-half inch in diameter) made of the same material. The perforated disc was so designed as to fit in a small-sized petri dish. The tissues to be dehydrated were placed in the small perforated chambers with an accompanying marker. This tissue

carrier saved much time and effort in that a large number of tissues could be changed from one solution to another with one operation. The greatest advantage, however, was that once such fragile tissues were placed in their respective compartments they were not handled again until the time of embedding.

Photographing whole mounts. In the study of whole mounts, the yolk containing the blastodisc to be photographed was transferred from the fixing fluid to sixty percent alcohol. The vitelline membrane was then carefully removed from that area of the yolk immediately over the germinal disc. This operation left the germinal disc intact and in its normal position upon the yolk. The position of the marker was then determined and the anterior portion of the blastodisc marked with laboratory ink. The blastodisc could then be either photographed while on the yolk or removed and placed in a depression slide and photographed. In cases where the germinal disc was removed, it was found advisable to cover the tissue with fifty percent alcohol and a cover glass before photographing. Both procedures of handling this type of material were used but the latter was preferred due to ease in handling the tissue.

It was found very helpful in the case of cleavage stages to stain the blastodisc before photographing. Gentian violet stain proved useful for this purpose. The blastodisc to be photographed was first overstained, then destained with fifty percent alcohol until a point was reached where most of

the color was removed from the upper surfaces of the cells but some still remained in the cleavage furrows. This created a marked contrast between the cleavage furrows and the cells proper. The cells in some blastodiscs which were barely visible in an unstained condition could easily be seen by this method. All photomicrographs of whole mounts shown in plates 5 and 6 were taken at magnifications of twelve to twenty-one times.

RESULTS AND DISCUSSION

Nuclei of immature oocytes. The nucleus or germinal vesicle in the smallest follicles of the adult ovary is located near the center of the developing oocyte. At this stage of growth the nucleus is relatively large in comparison to the total volume of the follicle. The chromatin material in the nucleus stains deeply with such basic stains as Delfield's hematoxylin and Heidenheim's iron hematoxylin. In plate 1, fig. 1, is shown a very young oocyte with its relatively large nucleus. This oocyte was .09 mm. in diameter while its nucleus measured .036 mm. This means that the nucleus in follicles of this stage of growth represents approximately 6.4 percent of the total volume of the oocyte.

In fig. 2 is shown a cross section of a slightly older oocyte. The diameter of the follicle in this case was .41 mm. while that of its nucleus was .09 mm. The nucleus therefore at this stage of development represents approximately 1.6 percent of the total volume of the oocyte. This means that during these early stages of growth, the nucleus, although increasing

in size, does so at a slower rate than the cytoplasmic material.

Figs. 1 and 2 also show another marked change in the nuclei of follicles at these stages of growth. These two follicles were stained simultaneously in Heidenheim's iron hematoxylin. Nevertheless the chromatin material in the nucleus of the younger follicle stains more deeply than that of the older oocyte. This shift in staining reaction from a basophilic to a more acidophilic condition was considered by Brachet (7) to be due to the production of thymonucleic acid during the course of yolk formation.

In fig. 3 is shown a follicle at a still later stage of development. In this case the follicle has a diameter of 1.7 mm. while that of its nucleus measures .28 mm. The nucleus or germinal vesicle in an oocyte of this size is found in an eccentric position having migrated from the more central position shown in fig. 2 toward the periphery of the cell. The shift in the staining reactions of the nucleus of ova of this size is even more pronounced than was the case of the follicle shown in fig. 2. In follicles of this size both the chromatin material and the nuclearplasm appears to stain less intensely than in the stages of growth represented in figs. 1 and 2.

The germinal vesicle of mature follicles. In fig. 5 is shown a median cross sectional view of the blastodisc of an oocyte which was removed from the ovary forty-eight hours prior to the estimated time of ovulation. The germinal vesicle

as shown in this photomicrograph has migrated to one edge of the follicle and now lies, as noted by Lillie (33), in the center of a thickened mass of protoplasm known as the germinal disc. In follicles of this size (approximately thirty-three mm. in diameter) the germinal vesicle almost invariably assumed the shape shown in fig. 5. The outer surface of the germinal vesicle is flattened against the vitelline membrane while its inner surface remains convex in shape. The germinal vesicle as shown in fig. 5 is surrounded by a definite nuclear membrane. The interior structure of the germinal vesicle of nearly mature follicles appears to be composed of a fine network of delicate fibers. Within this network of fibers is a clear nuclear sap which at this stage of growth is quite resistant to nearly all nuclear stains. In sections stained in Delafield's hematoxylin, for example, the surrounding protoplasm stains deeply while the germinal vesicle retains its original grayish color.

Within the mesh work of the germinal vesicle at this stage of development are found scattered strands of chromatin which can be seen in certain well stained sections of this structure. In later stages, to be discussed later, the chromatin is less scattered and is found near the center of the germinal vesicle.

The germinal vesicle in mature follicles is relatively large in comparison to the blastodisc. In the case of twenty specimens the average size of the germinal vesicle, as seen in cross section, was found to be 350 microns in width and 105 microns in depth. Variations of 310 to 400 microns in width

and 90 to 110 microns in depth were found.

As viewed from the surface, the germinal vesicle appears to be nearly circular in shape. Its relative position and size in relation to the surrounding protoplasm can be judged from the photomicrograph shown in fig. 4. In this particular case the greatest diameter of the germinal vesicle was found to be 350 microns.

A cross sectional view of the blastodisc of a follicle taken from the ovary approximately twenty-four hours prior to the estimated time of ovulation is shown in fig. 6. The walls of the germinal vesicle as shown in this photomicrograph are beginning to disintegrate. This degeneration of the nuclear wall is indicated by the vacuoles which have formed on either side of the upper surface of the germinal vesicle and also by the fact that the contents of the germinal vesicle are beginning to spread laterally. It will be observed by comparing figures 4 and 6 that the inner surface of the germinal vesicle of the older egg has flattened.

In the photomicrograph shown in fig. 7 of plate 2, the degeneration of the germinal vesicle appears to be even in a more advanced state than that shown in fig. 6. The upper surface of the germinal vesicle has separated from the surrounding protoplasm leaving a vacuolated area which is partially filled with a black residue. This residue which is derived from the disintegrated germinal vesicle resembles chromatin as to color when stained with Heidenheim's iron hematoxylin. This material, however, probably is not chromatin since it is not colored when Feulgen's stain is used.

In fig. 8 is shown a cross sectional view of the blastodisc of a mature follicle. This follicle was removed from the ovary of a hen just prior to the time of ovulation. This photograph clearly shows the ultimate fate of the germinal vesicle. Its walls at this stage have completely broken down allowing the fluid contents to spread laterally in a thin sheet beneath the vitelline membrane. The material from the germinal vesicle in this instance was found in more than seventy-five sections cut ten microns in thickness.

By comparing figures 5, 6 and 8 it will be observed that the protoplasm of the germinal disc, as well as the contents of the germinal vesicle, has spread laterally. This thinning and spreading of the protoplasm and the contents of the germinal vesicle is undoubtedly associated with the increased internal pressure within the follicle and with the weakening of the follicle wall.

Cross sections of the germinal discs of follicles removed from the ovary a few hours prior to time of ovulation show small areas of chromatin material near the center of the germinal vesicle. The chromatin threads at this period of development are extremely variable in shape and size (figures 9, 10 and 11). In one instance the chromosomes appeared to be arranged in tetrads, a stage preparatory to the first maturation division. The tetrads were found in a small group near one edge of the germinal vesicle. It was possible to count eighteen of these structures, the most of which appear in the photomicrograph shown in fig. 12. Only slight variations in shape and size were noted among the individual tetrads.

If the chromosomes shown in fig. 12 are in a true tetrad condition, it would indicate that in the fowl there are at least eighteen pairs of chromosomes. Wilson (69), it will be recalled, has shown that the number of tetrads in a given species is always one-half the usual number of chromosomes for that species.

Formation of first polar body. A still later stage in the maturation process is shown in figures 13, 14 and 15. These photomicrographs taken of two succeeding sections through the germinal vesicle of a mature ovarian follicle show an early anaphase stage in the formation of the first polar body. The chromosomes appeared in two sections and in these photomicrographs are shown in the process of being drawn to opposite ends of the spindle. The spindle, although not visible in either of the three photographs, could be seen by careful focusing and was found to be lying with its long axis at right angles to the surface of the egg. The reconstructed drawing shown below was made of the two sections of tissue to show the relative size and position of the chromosomes.

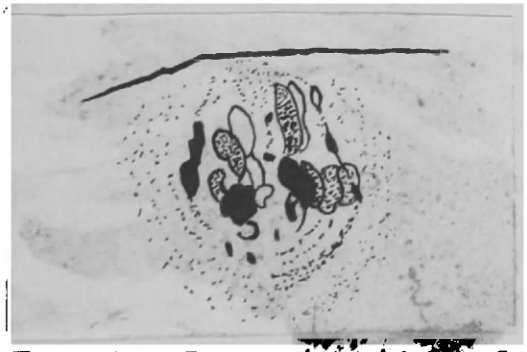


Fig. A. Reconstruction of the spindle of the first polar body shown in figs. 13, 14, and 15.

In plate 3, figure 16, is shown a cross sectional view of the germinal disc of a mature follicle which was removed from the ovary about one hour prior to the estimated time of ovulation. This photograph shows the first polar body which has been extruded from the egg lying in a small depression in the protoplasm immediately beneath the vitelline membrane. What appears to be the spindle formation of the second polar body is shown a short distance to the left of the polar body. The polar body in this case was found to be seven microns in length, three microns in depth, and ten microns or less in thickness. In this particular case the cytoplasm made up the largest proportion of the polar body.

In fig. 20 is shown a cross sectional view of the blastodisc of another mature ovarian follicle. In this tissue only small isolated portions of the germinal vesicle could be found. In the two small vacuoles shown in fig. 20 which were filled with material from the germinal vesicle, were found small portions of chromatin material. In the case of the smaller vacuole, the chromatin was present in three sections of tissue and might possibly be interpreted as an early stage in the formation of the first polar body. The smaller quantity of chromatin shown in the second vacuole in fig. 20 probably represents portions of this material from the disintegrated germinal vesicle which was not involved in the maturation process. Wilson (69) in discussing the fate of some of the chromatin material in the eggs of other animals states "It is a very interesting and important fact that during the growth and maturation

of the ovum a large part of the chromatin of the germinal vesicle may be lost either by passing out bodily into the cytoplasm, by conversion into supernumery or accessory nucleoli which finally degenerate, or by being cast out and degenerating at the time the polar bodies are formed."

In figures 17, 18 and 19 are shown cross sectional views of the germinal discs of three newly ovulated yolks. The eggs from which these tissues were obtained were found in the body cavities of three hens not as yet having been picked up by the infundibulum. In each of these photomicrographs a first polar body is shown lying in a small depression in the protoplasm near the center of the germinal disc and immediately beneath the vitelline membrane. The polar body shown in fig. 17 was found to be twenty microns in length, seven and six-tenths microns in depth, and more than ten microns in width. The one shown in fig. 18 measures twelve and eight-tenths microns in length, six microns in depth and ten microns in width. The polar body shown in fig. 19 is the largest of the three, measuring twenty-eight and two-tenths microns in length, eight and five-tenths microns in depth, and more than ten microns in width. The dimensions of the three polar bodies shown in figures 17, 18 and 19 were larger than the polar body found in the ovarian follicle and shown in fig.16. This would suggest that the polar bodies increase in size after being expelled from the egg. Van Durme (62) also noted the difference in size of the first polar bodies found in the ovary and those found in the infundibulum of the pigeon and the swallow. It is obvious from figures 17, 18 and 19 that

the polar bodies in different hens' eggs vary a great deal in size and shape. In general, however, it can be said that the polar bodies in hens' eggs are oval in form, elongated, and more or less pointed at either end. In the case of the pigeon and the swallow, the polar bodies are on the average about one-third the size of those found in the chicken egg. Van Durme (62) who studied the polar bodies in the pigeon and swallow reported that in the pigeon the greatest diameter of the polar body was seven microns. In the case of the swallow two first polar bodies were found. One polar body had a diameter of seven microns while the greatest diameter of the second was approximately fifteen microns.

The polar bodies of the hen's egg not only are different in shape and size but also vary greatly in the apparent amount of chromatin material which they contain. For example, the polar body shown in fig. 18, although the smallest of the three, contains nearly twice as much chromatin as either of the other two shown in figures 17 and 19. The shape and location of the chromatin within the different polar bodies are also of interest. In fig. 17 the chromatin is arranged in threads which are quite evenly distributed throughout the polar body. In the polar body shown in fig. 18 the chromatin material appears to be subdivided and is found in small more or less circular patches which are distributed evenly throughout the polar body. The chromatin shown in fig. 19, on the other hand, appears in the form of a narrow band about the periphery of the polar body with the greatest quantity being

concentrated near the upper surface of this structure. Only one case was encountered (fig. 18) where there was more chromatin than cytoplasmic material in the polar body. In some of the photographs reported by Van Durme (62) for polar bodies in the pigeon and swallow, almost the entire bulk of the polar body appears to be chromatin material. The polar bodies of the hen's egg shown in figures 16, 17 and 18 with respect to the amount of chromatin material more nearly resemble those shown by Harper (25) for the pigeon. In shape and size, however, the polar bodies more closely resemble those described by Van Durme for the pigeon and the swallow.

The second polar body and fertilization. The second polar body in the hen's egg is extruded a short time after ovulation and following the entrance of the spermatozoa. Since fertilization and the formation of the second polar body are intimately connected, these two phenomena will be discussed together.

It will be recalled that the spindle for the second maturation division is formed while the egg is still in the ovary and just prior to ovulation, fig. 15. The second polar body after reaching this stage of its formation passes so to speak into a dormant condition and only proceeds with its development following the entrance of the sperm. The sperm in all probability provides the stimulus which is necessary to induce further development.

In fig. 21 is shown an early stage in the process of fertilization. In this cross sectional view of the blastodisc the male and female pronuclei are in close proximity to each

other and to the chromatin material whose ultimate fate is to be extruded from the egg with the second polar body. The egg in which this stage was found was taken from the body cavity of a hen which was killed forty-five minutes after laying. This is the same egg as that represented in fig. 19. The first polar body was found eight sections or eighty microns from the section shown in fig. 21. If we consider that ovulation in this case took place thirty minutes after the laying of the hard-shelled egg it would mean that the egg had been in the body cavity of this hen approximately fifteen minutes. Nevertheless, in spite of this short time, the sperm had already penetrated the vitelline membrane and had reached an advanced stage in its characteristic change in preparation to uniting with the female pronucleus.

The nucleus shown on the right in this photomicrograph is considered to be the female component because of its position with respect to the polar body and due to its deeper location in the tissue. Both the male and female pronuclei shown in fig. 20 are of approximately the same size and shape. These pronuclei measure 2.2 microns in length and 1.6 microns in width and both are "egg shaped", i. e., one end is blunt while the opposite end is quite pointed. The chromatin material, as might be expected, makes up the greater proportion of the two pronuclei and at this stage of development stains intensely with iron hematoxylin. There is a characteristic haziness in the appearance of the tissue surrounding the two pronuclei and the polar body. This appears to be characteristic since the same condition was found in other

preparations where pronuclei were present.

Another male pronucleus is shown in fig. 22. This pronucleus was found in a cross section of the blastodisc of another egg removed from the body cavity. In general this pronucleus is of the same shape and size as those shown in fig. 21. The same hazy appearance of the tissue is also present.

During the brief interval between ovulation and fusion of the two pronuclei, the sperm goes through several characteristic changes. The head and middle portion of the sperm is transformed eventually into an oval nucleus, which is composed of approximately equal parts of chromatin and nucleoplasmic material and which has definite nuclear walls. The following diagram shows a few of the transitional stages of the sperm after it enters the egg and prior to its fusion with the female component.

Fig. B. Stages in the transformation of sperm heads.

Later stages in the developmental process were encountered in naturally fertilized eggs which were taken from the infundibulum and albumen portions of the oviduct. Polar bodies were

found in eggs from these regions but because of the similarity of the first and second polar bodies it was impossible to determine with any degree of certainty which was the first and which was the second. This same difficulty was encountered by Van Durme (62) in her studies of the two polar bodies in the eggs of the swallow and pigeon. She reported that the first and second polar bodies in these birds were almost identical in appearance and size and there was no morphological difference which could be used to differentiate the two.

According to Van Durme (62) the first polar body, which is extruded while the egg is still in the ovary, has completely disintegrated by the time segmentation begins, i.e., approximately three and one-half hours after the time of ovulation. This would mean that by the time an infertile egg reached the isthmus the first polar body would not be found in sections of the blastodisc. Therefore, if an infertile uterine egg could be induced to resume its development, any polar body found later would logically have to be considered the second polar body.

In this present study, a technique has been developed whereby infertile uterine eggs impregnated with sperm will resume their development. This technique, which will be discussed in greater detail later, has made it possible to study the structure of the second polar body. It has also made it possible to determine with certainty whether fertilization or at least the entrance of the sperm is a necessary step before the second polar body can be extruded.

In plate 6, fig. 39, is shown a photomicrograph which was taken of a cross section of the blastodisc of an infertile uterine egg after being impregnated with a one to eighty dilution of sperm and then incubated for twenty-five minutes at 105° F. The egg which supplied this tissue was removed from the uterus of an infertile hen five hours after the estimated time of ovulation. In this photograph, the second polar body can be seen in a small depression in the protoplasm and just beneath the vitelline membrane. Portions of the second polar body shown in fig. 39 were present in three sections making this structure thirty microns in width. The polar body as seen in cross section was twelve microns in length and six microns in depth. The chromatin material made up approximately one-half the total volume of this polar body. To the left of the polar body is the large egg nucleus which, judging from its size, is the fusion nucleus. The nucleus appears to be preparing for the first mitotic division.

In plate 6, fig. 41, is shown another second polar body which was found in sections of a blastodisc from an infertile uterine egg. This egg was impregnated with undiluted semen and then incubated for four hours. The polar body in this case is much larger than any of those previously discussed. In this instance, the polar body had not been completely extruded from the egg. The greatest portion of this polar body is composed of cytoplasmic material. The chromatin material which stained deeply with Delfield's hematoxylin is shown in a small compact mass near the center of the polar body.

These two photographs (figures 39 and 41) therefore bring out two important facts, namely:

(1) The second polar body in the case of birds' eggs is extruded only after the sperm has entered.

(2) The infertile egg can remain in a dormant state for several hours in the oviduct of the hen and still be capable of undergoing further development.

Harper (25) reported that in the case of the pigeon egg the second polar body was given off after the entrance of the sperm. Van Durme (62) on the other hand reported that the expulsion of the second polar body in the case of pigeon eggs was not dependent upon the penetration of the sperm. The findings reported in this paper therefore confirm Harper's observation but are not in agreement with those reported by Van Durme.

Segmentation of naturally fertilized eggs

The germinal disc of the hen's egg at the time it is about to undergo cleavage appears in a surface view as a whitish circular area which is about three mm. in diameter, Patten (46). The lighter-colored central portion of the disc is surrounded by a somewhat darker area known as the periblast. These divisions can be seen in the blastodisc shown in plate 8, fig. 51. In the case of the hen's egg segmentation begins in the lighter-colored central area of the blastodisc.

The two-cell stage. The first cleavage plane in naturally fertilized hens' eggs usually appears while the egg is in the isthmus, i. e., approximately three and one-half hours after the estimated time of ovulation. Patterson (47) who has studied the early cleavage stages in the hen's egg found that the first furrow appeared as the egg was entering the isthmus. Although the isthmus is usually the region of the oviduct where segmentation begins, cases were encountered where the first cleavage occurred in the albumen-secreting portion.

From surface view, the first cleavage plane appears as a narrow furrow which in some cases is practically straight while in other instances it is curved. The first cleavage furrow usually only extends part way across the central portion of the germinal disc. Some cases are encountered, however, as shown in plate 4, fig. 23, where the furrow extends almost all the way across the germinal disc.

In cross section, the cleavage furrow appears as a relatively deep V-shaped groove in the upper surface of the protoplasm. Cross sectional views of the first cleavage plane, as found in two different eggs are shown in plate 6, figures 35 and 36. One of these eggs was found in the albumen-secreting portion while the other was taken from the isthmus.

The question arises in a discussion of the first cleavage plane as to the relationship which exists between the direction of the first furrow and the future long axis of the embryo. Of the three cases encountered where it was possible

to determine the direction of the first cleavage plane, one coincided exactly with the embryonic axis. Another was encountered where the first furrow was at right angles to the long axis of embryo. In the other case the first cleavage furrow deviated from the long axis of the embryo by about forty-five degrees. These observations, however, do not necessarily mean that no relationship exists between the first cleavage furrow and the long axis of the embryo since it is known that the long axis of over forty percent of the embryos in hens' eggs deviate from the position considered as normal. Patterson (47) reported that in the cases which he observed only one case was encountered where the first cleavage furrow and the long axis of the embryo coincided.

Hitherto, only drawings of cleavage in the hen's egg have been made. These drawings, although carefully made, fail to show the variations which exist in the length, shape and location of the cleavage planes which are found in naturally fertilized eggs. This creates the impression that cleavage furrows always form in a definite manner and in a certain set relationship with respect to other cleavage planes. To show the variations in the shape and size of cells which actually exist, photographs were obtained of each of the early cleavage stages. In some cases several photographs are shown of the same cleavage stages. This was done to show the marked variations in cleavage patterns which are commonly found in different eggs from the same and different hens.

The four-cell stage. The four-cell stage is produced by a vertical division of the blastomeres of the two-cell stage.

The cleavage furrow which subdivides the first two cells crosses the first cleavage plane at approximately right angles. Usually the second cleavage plane meets the first at separate points along the first furrow. Such a condition is clearly shown in the two four-cell stages shown in plate 4, figures 24 and 25.

In the case of the four-cell stage shown in fig. 24, the cleavage is occurring near the center of the blastodisc. Unfortunately this specimen was broken when it was removed from the yolk for photographing. The relationship of the cleavage planes to the blastodisc, however, is still discernible. It will be observed in fig. 25 that cleavage in this particular egg is taking place near one edge of the blastodisc. Duval (17) observed similar cases in hens' eggs where the first two furrows formed in an eccentric position in the blastodisc. He was of the opinion that this condition was normal for the hen and that this point of active cell formation marked the posterior end of the future embryo. Only a few such cases as that shown in fig. 23 were encountered in naturally fertilized eggs.

The eight-cell stage. In fig. 26 is shown an early eight-cell stage. Two parallel cleavage furrows which are connected by a third but less distinct furrow can be seen in this photograph. In the case of this egg, the cell formation is occurring at a point near the center of the blastodisc. The relative length of the furrows and the size of the cells with respect to the entire blastodisc can be readily judged from this photograph.

The rudimentary accessory cleavage which is of common occurrence in the case of the pigeon egg was not observed in any of the cleavage stages occurring in naturally fertilized hens' eggs. Patterson (47), however, reported finding such a condition in hens' eggs at the four-cell stage of development. This accessory cleavage was encountered commonly in eggs which had been artificially impregnated with fresh, diluted semen. In this case, however, as will be discussed later, the condition was caused by injecting a large number of sperm into the egg.

The sixteen- to thirty-two-cell cleavage stages. In fig. 27 is shown a blastodisc with twenty-two cells visible from a surface view. In this photograph the furrows which form the boundaries of the large marginal cells are shown to have extended to such an extent that the entire blastodisc has become segmented. The smaller central cells in this case stand out prominently in marked contrast to the large marginal cells. In this particular specimen there are twelve central and ten marginal cells which are visible in the surface view.

In figures 28, 30 and 31 are shown photomicrographs of three different blastodiscs which have reached the thirty-two-cell stage of development. The great variations which are found in pattern of cell formation in the different eggs are shown in these three photomicrographs. In fig. 28 the cells are quite large and involve approximately one-third of the area of the blastodisc. In fig. 31, on the other hand, segmentation is limited to a relatively small central region with the marginal cells extending only a short distance into the

surrounding protoplasm. In fig. 30 cleavage is taking place near the center of the blastodisc. The central cells in this case are relatively large, some of which appear to surpass certain of the marginal cells in size.

The 64- to 240-cell cleavage stages. In figures 29 and 32 of plate 5 are shown two blastodiscs each of which contain approximately sixty-four cells. The variations which occur in cell shape and size in nature are again demonstrated in these photomicrographs. In the case of the tissue shown in fig. 32 the cleavage planes involve nearly the entire surface of the blastodisc, the marginal cells being relatively large in comparison to the central cells. In fig. 29, on the other hand, only about two-thirds of the blastodisc is segmented. In this case the marginal cells are relatively small in comparison to the largest central cells.

In figures 33 and 34 are shown still later stages in the development of the hen's egg. Fig. 33 shows a blastodisc in which about one hundred cells are visible in surface view. In this case there appears to be at least seventy-four central cells and twenty-four or more marginal cells. This photograph is interesting in that it shows the great variation which exists in the shape and size of the different cells in blastodisc at this stage of development. This same condition is shown in fig. 34 where 240 or even more cells are visible in surface view. The marginal cells shown in this photograph are still relatively large at this stage of development.

Artificial impregnation of infertile eggs

It was pointed out earlier in this paper that the first maturation division in the hen's egg occurs while the egg is still in the ovary. The sperm enters the egg within a few minutes after ovulation and while the egg is in the infundibulum. This is followed by the extrusion of the second polar body, the fusion of the male and female pronuclei and the appearance of the first cleavage furrow. Thus in the case of a naturally fertilized hen's egg there is a continuous chain of uninterrupted developmental processes from the time the egg enters the oviduct until it is laid. Such is not the case, however, with an infertile egg. Such an egg, after extruding the first polar body, enters what might be considered a dormant condition with no further visible changes occurring in the blastodisc until the egg has entered the uterus.

The length of time that an infertile hen's egg can remain in this dormant state and yet be capable of undergoing development when the proper stimulus is applied has not been determined previously. According to Morgan (39) the eggs of some of the lower forms of animal life can remain dormant for several days and yet be fertilized. No published reports could be found in the literature of any attempt to artificially impregnate infertile hens' eggs although this has been commonly practiced in the case of eggs of some lower forms.

One of the objects of the present study was to develop a technique whereby infertile hens' eggs could be artificially impregnated and caused to resume their development. Some

success has been attained.

An early stage of development in an artificially impregnated uterine egg is shown in plate 6, fig. 39. This egg after being impregnated was incubated at 105° F. for twenty minutes. In this case a 1-80 dilution of sperm was used and the impregnating fluid was placed beneath the vitelline membrane near one edge of the blastodisc. In this photomicrograph the second polar body is shown in a depression in the protoplasm just beneath the vitelline membrane. The large fusion nucleus appears also in this photograph a short distance below and to the left of the second polar body. The nucleus in this case appears to be preparing for the first segmentation division.

There is little question that the development shown in this photograph was initiated by the sperm which was injected. Infertile uterine eggs injected with Ringer's solution served as controls. These control eggs, after being incubated at 105° F. for three hours showed no signs of development.

In plate 4 fig. 23 is shown the first cleavage furrow of an artificially impregnated egg after twenty-five minutes of incubation at 105° F. The first furrow in this case extends nearly all the way across the center of the blastodisc. In plate 6 fig. 40, however, which shows another early cleavage stage, the furrow is much shorter and only involves a small portion of the blastodisc. In this respect the cleavage shown in fig. 40 resembles the two-cell cleavage stages found in naturally fertilized eggs.

Another point of interest in connection with the

artificially impregnated eggs is the accessory cleavage. The extra spermatozoa have established an area of accessory cleavage about the periphery of each of the two blastodiscs which can be seen in figures 23 and 40. This condition which is of common occurrence in the pigeon egg is rarely found in the case of naturally fertilized hens' eggs. In the case of the pigeon egg, Blount (5) attributes this condition to the twenty-five or more extra sperm which enter the egg prior to the fusion of the male and female pronuclei.

Accessory cleavage has also been reported as occurring in naturally fertilized hens' eggs. Patterson (47) who reported this condition in chicken eggs found that the accessory cleavage furrows appeared at the four-cell stage of development approximately four hours after the estimated time of fertilization. This condition may occur in some naturally fertilized hens' eggs, but was not observed during the course of this present study.

The fact that accessory cleavage is commonly found in artificially impregnated hens' eggs but rarely occurs in naturally fertilized eggs shows that this condition is brought about by the supernumerary sperm. Although only one drop of a 1-80 sperm dilution was injected into each egg, nevertheless this small quantity contained more than five hundred sperm. This observation therefore confirms the findings of Blount (5) who reported that accessory cleavage is caused by the extra sperm which enter the egg.

The fact that accessory cleavage is of rare occurrence in normally fertilized chicken eggs indicates that very few

sperm enter the hen's egg at the time of fertilization. The largest number of sperm nuclei found in each of ten different eggs from the infundibulum was three. This finding therefore is in accord with the observations made by Patterson (47) who reported that usually only four to five sperm enter the egg at the time of fertilization.

Another point of interest in connection with the study of artificially impregnated eggs is the rate at which development takes place. The first cleavage plane in naturally fertilized eggs appears while the egg is in the isthmus or in other words approximately three and one-half hours after the estimated time of fertilization. In artificially impregnated eggs, however, where the time factor can be determined accurately, the first cleavage furrow appears in twenty-five minutes. In other words, in artificially impregnated eggs the first cleavage furrow forms in one-eighth the time that is normally required for this degree of development. The difference in rate of development of artificially impregnated and naturally fertilized eggs is shown in the following table:

TABLE I

Time table of different developmental stages in naturally fertilized and artificially impregnated eggs.

Naturally fertilized eggs					Artificially impregnated eggs
No. of cases	Stage of development	Position of egg in oviduct	Average time after laying preceding egg Hours	Range in time Hours	Time needed to produce an equivalent stage Minutes
2	Fertilization	Infundibulum	0.3	0.1 - 0.3	0
2	2nd polar body	Albumen portion	3.5	--	20
3	2-cell stage	Isthmus	5.0	4.5 - 5.2	25
3	4-cell stage	"	5.5	5.0 - 6.3	27
3	8-cell stage	"	6.0	5.0 - 8.0	--
8	16-cell stage	Uterus	6.3	5.0 - 6.5	29
7	32-cell stage	"	6.8	5.8 - 7.8	
2	64-cell stage	"	7.0	6.5 - 7.5	
2	120-cell stage	"	7.5	7.0 - 8.0	

No definite explanation can be offered at the present time for this greatly accelerated rate of development in artificially impregnated eggs. The two following conditions, however, may be contributing factors and will be investigated later.

(1) The number of sperm. In the case of naturally fertilized hens' eggs only three or four sperm normally enter the egg. In the case of the artificially impregnated eggs even when a 1-80 dilution was used, several hundred sperm were injected in the egg next to the blastodisc. This difference suggests two possibilities, namely:

(a) The spermatozoa may contain either an emzyme, hormone, or some other chemical substance which acts as a catalyst, thereby speeding up development.

(b) The fusion of the male and female pronuclei may occur sooner than under natural conditions. The large number of sperm injected into the egg would greatly increase the chances of a sperm being in closer proximity to the female pronucleus than would be the case under natural conditions. In such a case it is logical to assume that the fusion of the two pronuclei might occur sooner than under natural conditions.

(2) The difference in activity of the sperm. Under normal conditions, only the head and middle portion of each spermatozoa enter the egg, the tail taking no part in the fertilization process, McEwen (36). Such tailless portions of sperms would naturally be much more sluggish in their movements than fresh sperm. In the case of the artificially impregnated eggs, fresh active spermatozoa were injected into the egg. The difference in the activity of the sperm in the naturally fertilized and artificially impregnated eggs may therefore be a contributing factor

in the increased rate of development. In this connection the observation reported by Warren and Kilpatrick (64) is of special interest. These authors found that if a Black Minorca hen which had been previously impregnated with the semen of a White Leghorn male was again impregnated using the semen of a Black Minorca male, the fresh sperm would immediately replace the older White Leghorn semen. Warren's observation shows therefore that sperm in the oviduct become less active with age. The possibility of a ripening process occurring in the nucleus of the egg due to aging should also be considered. If the nucleus did undergo a ripening process it might result in accelerated nuclear changes upon entrance of the sperm. However, as mentioned previously, all the possibilities listed are only speculative and experimental proof is lacking.

Plate 7, figures 38 and 39, shows the blastodiscs from two artificially impregnated eggs. Both of these eggs after being impregnated were incubated for five and one-half hours at 105° F. It is apparent from these photographs that many cells have been formed in this relatively short space of time, during which naturally fertilized eggs undergo only two or three cleavages. Cell formation therefore has taken place several times faster than is the case in naturally fertilized eggs.

The cells found in artificially impregnated eggs vary greatly in shape and size. These variations are greater than those found among the cells of naturally fertilized eggs. In fig. 43, for example, one especially large cell appears near the center of the blastoderm in marked contrast to the very

small neighboring cells. The same variation in cell size and shape can be seen in the blastodisc shown in fig. 38.

Figure 45 shows a cross sectional view of the blastodisc shown in fig. 43. The vitelline membrane was removed for the purpose of obtaining the photomicrograph shown in fig. 43 and therefore does not appear. This photograph shows an area of cell formation near the center of the blastodisc. The variations in cell size and shape which were pointed out in fig. 43 can be seen in this photograph. A segmentation cavity, although not as distinct as those which are found in naturally fertilized eggs, nevertheless can be seen in fig. 45. Two of the large marginal cells from which some of the smaller central cells are derived can also be seen in fig. 45.

In fig. 46 is shown a photomicrograph of the germinal disc of another artificially impregnated egg. In this case the egg was incubated for seven and one-fourth hours at 105°F. following impregnation. The development is much more advanced than was the case of the blastodisc shown in figures 43 and 44 and there appears to be less variation in size and shape of cells. On close examination of this photograph, however, it can be seen that there is still much variation in cell size and shape. Although some variation exists in cell size in late cleavage stages of naturally fertilized eggs, the variations which occur are not as marked as those shown in fig. 46. At this stage of development all evidence of accessory cleavage has completely disappeared.

In figures 47 and 48 are shown cross-sectional views of cell formation of two other artificially impregnated eggs.

The cells in this case were impregnated by placing one drop of undiluted semen on the vitelline membrane directly over the blastodisc. The eggs were then incubated for different lengths of time, the one represented in fig. 47 for five hours and forty-five minutes, the other for six hours. The cells found in each of these two eggs are more uniform in shape and size than those which were found in other artificially impregnated eggs but still are not as uniform as those which form under natural conditions. Nuclei were found in the cells of both these artificially impregnated eggs, three of which can be seen in the cells shown in fig. 48. It is apparent from fig. 47 that a segmentation cavity has been formed, which closely resembles those which are found in naturally fertilized eggs.

Fragmentation

If an egg upon entering the oviduct is not fertilized it enters into what might be considered a dormant state. Upon first entering the uterus, the blastodisc of such an egg looks like the blastodisc of either a fertile or infertile egg taken from the anterior region of the oviduct, i. e., it is unsegmented, circular in shape, and about three mm. in diameter. Four to five hours after entering the uterus, however, the blastodisc of the infertile egg begins to undergo fragmentation. During this period of fragmentation the protoplasmic blastodisc fragments into small pieces which in surface view closely resemble true cell formation. The protoplasm during fragmentation is progressively subdivided by fragmentation furrows which closely resemble true cleavage furrows. This fragmentation must be

considered as an abortive type of parthenogenesis since some of the cell-like bodies, as shown in fig. 54, possess nuclei. The larger fragments of protoplasm divide and this apparent multiplication is accompanied in some instances at least by corresponding nuclear divisions. This fragmentation or parthenogenetic development continues until the entire blastodisc is segmented. The exact age of the egg when fragmentation of the blastodisc is completed has not been definitely determined. However, it was found that the blastodisc of infertile uterine eggs which were obtained from hens at 11:30 p.m. were still undergoing fragmentation. This means that the final stages of fragmentation occur between midnight and the normal time of laying.

The cell-like structures which are formed as the result of fragmentation of the blastodisc of infertile hens' eggs resemble in some respects the cells which have already been described for artificially impregnated eggs. However, when a careful stody is made of the sections of the two types of blastodiscs the following differences can be observed:

(1) Fragmentation usually begins at the margin of the blastodisc where there is a considerably smaller amount of protoplasm per unit area than at the center. The final result is the formation of many small cell-like bodies at the periphery of the disc and fewer but larger cells at the center of the disc. In the case of artificially impregnated eggs, the first cleavage furrows usually form near the center of the blastodisc, figures 23 and 40. Consequently the cells in

this region are more numerous and smaller than at the margin of the blastodisc.

(2) Cell formation in fragmented infertile eggs does not take place as rapidly as in the case of artificially impregnated eggs. Most infertile eggs removed from the uteri of hens ten to eleven hours after the estimated time of ovulation contained relatively few cell-like bodies, figures 51 and 52. In contrast to this, unfragmented, infertile eggs removed from the uteri of hens five hours after ovulation, artificially impregnated and incubated for five to seven hours at 105° F., contained several hundred cells, figures 43, 44, and 46.

After the fragmentation of the blastodisc is completed the numerous cell-like bodies congregate at the center of the blastodisc, forming the small dense mass of protoplasm which is seen in freshly laid infertile eggs. The appearance of the blastodiscs of infertile and fertile eggs at the time of laying is shown in plate 8, figures 49 and 50.

Several attempts were made to impregnate freshly laid infertile eggs. Serial sections of these blastodiscs showed no indications of development even after the eggs had been incubated for as long as five hours. It is evident therefore that the blastodiscs of newly-laid infertile eggs have reached a stage of disintegration where they are incapable of resuming development even though the proper stimulus is applied.

Two different stages of fragmentation in premature infertile eggs are shown in figures 51 and 52. In fig. 51 a

fragmentation furrow which might easily be mistaken for a true cleavage furrow is shown at one edge of the blastodisc. This fragmentation furrow was found in an infertile egg which was obtained from the uterus of a hen at 8:00 p. m. the day before it would normally have been laid. This was eleven hours after the estimated time of ovulation and six hours after the egg had entered the uterus. This photograph also shows the beginning of a second fragmentation furrow in the upper portion of the blastodisc. A large number of faint radial furrows can be seen forming about the periphery of the blastodisc. In fig. 52 is shown a later stage of fragmentation in an infertile egg. This egg was obtained at 7:30 p. m. the day before it would have been laid or ten hours after the estimated time of ovulation and five hours after the egg had entered the uterus. The blastodisc in this case has been subdivided by numerous fragmentation furrows, the majority of which run in a radial direction. Three exceptionally wide furrows appear near the center of the blastodisc.

Several premature infertile eggs which were obtained between 3:00 and 4:00 p. m. the day before they were due to be laid were incubated for short periods of time in order to study the formation of the fragmentation furrows. These eggs were removed from the incubator at given intervals and sketches made of the blastodisc. Sketches made at ten-minute intervals showing the progressive development of the fragmentation furrows in two of these infertile eggs are shown in plates 9 and 10.

Several investigators have studied the blastodiscs of freshly laid infertile hens' eggs and have reported finding parthenogenesis. Oellacher (41) was the first to report what he thought was parthenogenesis in infertile hens' eggs. He reported finding nucleated cell-like bodies in the germinal area which he considered to be true cells because of their location and their ability to multiply by division. Duval (17) also observed these cell-like bodies in freshly laid infertile eggs and like Oellacher considered them to be true cells. Lacaillon (32) also reported finding indications of definite parthenogenetic development in freshly laid, infertile hens' eggs. The last named author made a detailed study of freshly laid, infertile eggs and presented fifty-six carefully made drawings of the cell-like bodies which he found. Some of these drawings showed the cell-like bodies in the process of undergoing mitotic division.

Cell-like bodies in freshly laid infertile hens' eggs were also observed by Barfurth (2). This author could find no nuclei in these structures and therefore concluded that they were not true cells but only the result of fragmentation. Lillie (33) also cincluded that what had been reported to be parthenogenesis in hens' eggs was only a type of fragmentation.

Hays and Nicolaides (27) examined sections of the blastodiscs of freshly laid infertile hens' eggs for evidence of parthenogenesis. These authors found no cell-like structures in their material and concluded that parthenogenesis does not occur in the case of chicken eggs.

Since all of these investigators used the blastodiscs of either freshly laid infertile eggs or infertile eggs which were several days old it is not surprising to find conflicting opinions as to whether or not parthenogenesis occurs in hens' eggs. It has been shown in this study that fragmentation of the blastodisc is completed and the cell-like bodies have congregated in a compact mass at the center of the blastodisc (fig. 49) previous to the time the egg is laid. It was further shown that the blastodisc of an infertile egg has reached a stage of disintegration by the time the egg is laid where it is incapable of undergoing further development. It is improbable therefore that infertile eggs would undergo development upon being incubated as has been reported by Oellacher (41), Duval (17), and Lacaillon (32). Therefore in a study of parthenogenesis or fragmentation premature uterine eggs should be used rather than newly laid or older infertile eggs.

In the course of this study numerous cell-like structures have been observed in cross sections of blastodiscs of infertile uterine eggs obtained at 11:00 p. m. from the uteri of hens. Some of these cell-like bodies contained nuclei in which chromatin material could be observed. This shows that some sort of transitional parthenogenetic development occurs in the case of infertile birds' eggs.

SUMMARY

The present study has dealt with the sequences of developmental changes which occur in the hen's egg from the time the egg forms in the ovary of the hen until it reaches the uterus. It has been shown that the nucleus of the egg which first occupies a central position in very young oocytes migrates towards the periphery of cell as the cell increases in size. Forty-eight hours prior to the time of ovulation, the nucleus or germinal vesicle is found in an extreme eccentric position near one edge of the ovum a few microns beneath the vitelline membrane. The germinal vesicle at this period is relatively large and is found in the middle of a peripheral layer of protoplasm called the blastodisc. Approximately twenty-four hours before the time of ovulation the walls of the germinal vesicle begin to disintegrate. As the internal pressure within the follicle increases the germinal vesicle spreads in a lateral direction and its inner surface becomes somewhat flattened. Shortly before ovulation the wall of the germinal vesicle completely breaks down allowing the fluid contents of this structure to spread laterally in a thin sheet beneath the vitelline membrane. Accompanying this breakdown of the germinal vesicle are other marked nuclear changes. The chromosomes which were formerly found in thin threads and scattered throughout the nucleus are now found congregated near the center of the germinal vesicle. The first maturation division then occurs, the first polar body being extruded while the egg is still in the ovary. The spindle for the second maturation division is then formed after which time

ovulation occurs. The egg is fertilized when it enters the infundibulum portion of the oviduct. The second polar body is extruded following the penetration of the sperm and before the fusion of the male and female pronuclei occurs. The first segmentation division occurs as the egg enters the isthmus portion of the oviduct. This is followed in approximately fifteen to thirty minutes by the formation of the second cleavage furrow. Upon entering the uterus portion of the oviduct the egg undergoes further development. In this region all the early cleavage stages from the sixteen-cell stage to the 240-cell stage occur in a space of four hours.

If an egg is not fertilized as it enters the oviduct it passes into what might be considered a dormant condition. No further visible changes occur in the blastodisc until the egg enters the uterus,

Fragmentation of the blastodisc begins approximately four to five hours after the infertile egg has entered the uterus. This fragmentation continues until the blastodisc has been subdivided into many small cell-like bodies some of which are nucleated closely resembling true cells in appearance. These small portions of protoplasm congregate near the center of the blastodisc as a dense white mass, when the fragmentation of the blastodisc has been completed. This fragmentation of the protoplasm and the congregation of the cell-like bodies at the center of the blastodisc takes place prior to time of laying. This study has shown that when infertile eggs have reached this stage of disintegration the protoplasm is incapable of

further development. Infertile eggs, however, taken from the uterus five to seven hours after the estimated time of ovulation can be artificially impregnated and induced to undergo further development.

CONCLUSIONS

The following conclusions are based upon the study of serial sections and whole mount preparation of more than two hundred blastodiscs of ovarian and oviducal eggs in various stages of development.

1. The nucleus or germinal vesicle of the egg of the domestic fowl begins disintegrating approximately twenty-four hours prior to the time of ovulation. As more yolk is added, there is an increase in the internal pressure within the follicle causing the fluid contents of the germinal vesicle to spread laterally in a thin sheet just beneath the vitelline membrane. At the time of ovulation only rudiments of the germinal vesicle can be found.

2. The first polar body is extruded and the spindle of the second polar body formed before ovulation occurs.

3. The first polar body increases in size after being extruded from the egg. The first polar bodies found in different freshly ovulated yolks vary greatly in size and shape. Those found in freshly ovulated yolks averaged 20.3 microns in length, 7.4 microns in depth, and 10 microns in width. This is approximately four times the size of the same structures found in pigeon eggs.

4. The egg is normally fertilized within fifteen minutes after ovulation and while the egg is in the infundibulum. These studies have shown that only three or four sperm enter the egg at time of fertilization which is in accord with the observations of Patterson (47).

5. The second polar body is extruded from the egg after the entrance of the sperm. This fact was demonstrated in the case of artificially impregnated uterine eggs as well as in cases of natural fertilization.

6. The first cleavage plane appears in surface view about the time the egg is entering the isthmus portion of the oviduct which is approximately three and one-half hours after the time of ovulation. The four- and eight-cell cleavage stages also occur while the egg is in the isthmus. No accessory cleavage was observed at two- and four-cell stages as was reported by Patterson (47).

7. Infertile uterine eggs begin fragmentation three to four hours after entering the uterus. The fragmentation furrows subdivide the protoplasm in much the same manner as true cleavage resulting in the formation of nucleated and non-nucleated cell-like bodies. These fragments eventually congregate at the center of the blastodisc forming a dense white mass of protoplasm. The blastodiscs of such eggs are incapable of resuming development even though the proper stimulus is applied.

8. Infertile, non-fragmented, uterine eggs can be artificially impregnated with semen and caused to resume their development.

LIST OF SYMBOLS USED

A. C. - Accessory cleavage

Chr. - Chromatin

F. E. - Follicular epithelium

F. N. - Female pronucleus

G. V. - Germinal vesicle

M. N. - Male pronucleus.

N. - Nucleus

Ov. - Ovum

P_1 - First polar body

P_2 - Second polar body

S. C. - Segmentation cavity

Tr. - Tetrads

V. - Vitelline membrane

PLATES

EXPLANATION OF PHOTOMICROGRAPHS OF PLATE 1

Fig. 1 - Cross sectional view of a very young ovarian follicle with its relatively large and centrally located nucleus. (Dia. of follicle .09 mm. Dia. of nucleus .036 mm.) 720 x

Fig. 2 - Cross sectional view of a young ovarian follicle. (Dia. of follicle .41 mm. Dia. of nucleus .09 mm.) 75 x

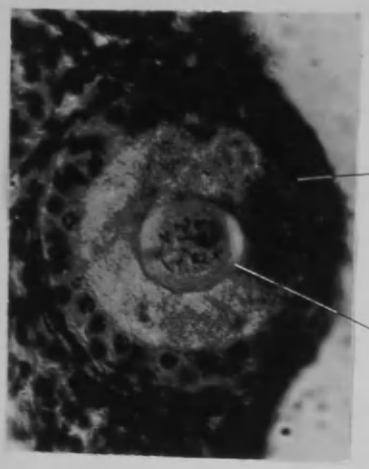
Fig. 3 - Cross sectional view of a slightly older ovarian follicle showing the nucleus in an eccentric position within the egg. (Dia. of follicle 1.7 mm. Dia. of nucleus .25 mm.) 60 x

Fig. 4 - Horizontal section of a blastodisc of a nearly mature, ovarian follicle (33 mm. dia.) showing the relative position and shape of the large germinal vesicle within the blastodisc (Germinal vesicle 370 microns in dia.) 75 x

Fig. 5 - Cross sectional view of a blastodisc of a nearly mature ovarian follicle showing the relatively large germinal vesicle at the periphery of the egg and in the center of the germinal disc. 75 x

Fig. 6 - Cross sectional view of germinal disc of an ovarian follicle 35 mm. in dia. showing the initial stages in the breakdown of the walls of the germinal vesicle. 75 x

PLATE 1



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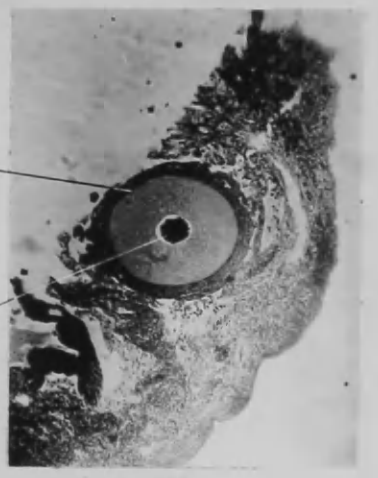

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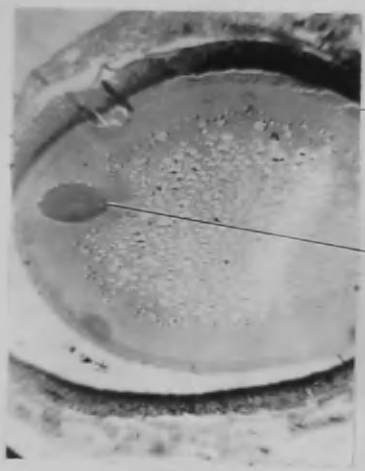

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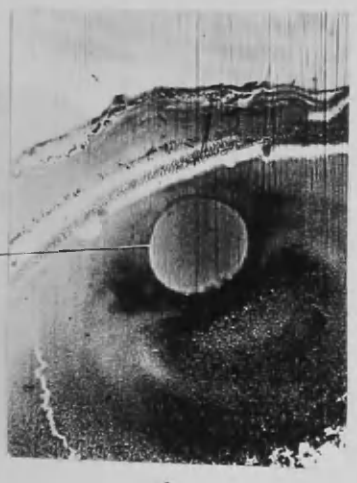

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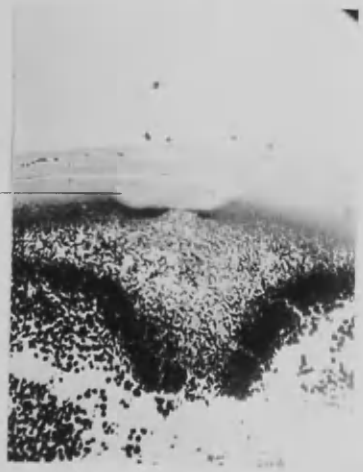

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EXPLANATION OF PHOTOMICROGRAPHS OF PLATE 2

Fig. 7 - Cross sectional view of germinal disc of a mature ovarian follicle (size 16 mm. dia.) showing the disintegration of walls of the germinal vesicle and the chromatin-like residue. 360 x

Fig. 8 - Cross sectional view of the germinal disc of a mature ovarian follicle (37 mm. in dia.) just prior to ovulation. This photograph shows the germinal vesicle spreading laterally in a thin sheet beneath the vitelline membrane. 75 x

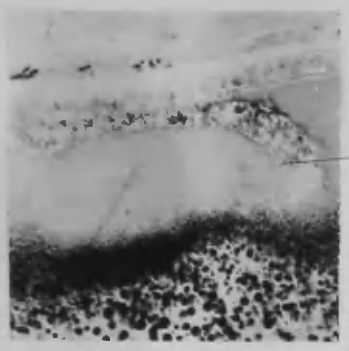
Fig. 9 - Cross sectional view of the germinal disc of a mature ovarian follicle (37 mm. dia.) which was removed from the ovary one hour prior to the estimated time of ovulation. This photomicrograph shows the chromatin material near the center of the disintegrating germinal vesicle. 360 x

Figs. 10 and 11 - Same as above. 360 x

Fig.12 - Photomicrograph of a horizontal section of the germinal vesicle of a mature ovarian follicle (36 mm. dia.) showing eighteen tetrads near one edge of this structure. 360 x

Figs.13 and 14 - Cross sectional views of two successive sections of the blastodisc of a mature follicle (37 mm. dia.) which was removed from the ovary just prior to time of ovulation. These photographs show the anaphase stage of the formation of the first polar body. The long axis of the spindle lies at right angles to the surface of the egg. 720 x

PLATE 2



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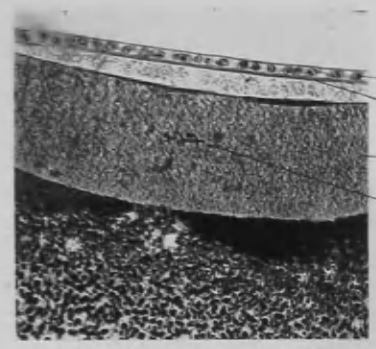

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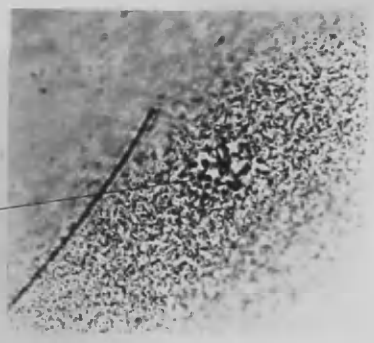

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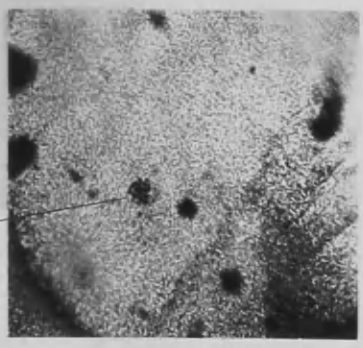

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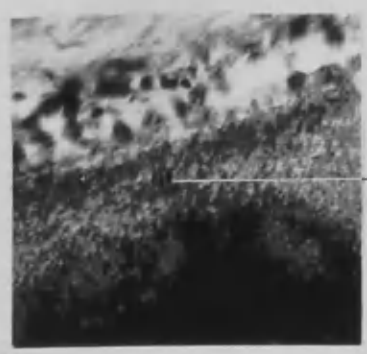

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EXPLANATION OF PHOTOMICROGRAPHS OF PLATE 3

Fig. 15 - Cross sectional view of blastodisc of same egg as represented in figures 13 and 14 showing another view of the formation of the first polar body. 720 x

Fig. 16 - Cross sectional view of germinal disc of a mature ovarian follicle (36 mm. in dia.) showing the first polar body. The polar body has been extruded from the egg and lies in a small depression just beneath the vitelline membrane. 360 x

Figs. 17, 18 and 19. Cross sectional views of the blastodiscs of three freshly ovulated yolks from the body cavity. These photographs show three first polar bodies lying in depressions in the protoplasm just beneath the vitelline membrane. 720 x.

Fig. 20 - Cross sectional view of the germinal disc of a mature ovarian follicle (37 mm. in dia.) showing chromatin material in each of the two small vacuoles beneath the vitelline membrane.

Fig. 21 - Cross sectional view of the blastodisc of a freshly ovulated yolk showing the male and female pronuclei near the second polar body (male and female pronuclei 2.5 x 1.8 microns). This photograph was taken of a section of the same blastodisc as represented in fig. 19. 360 x

Fig. 22 - Cross sectional view of a section of the blastodisc of a freshly ovulated yolk showing a male pronucleus. (The pronucleus measured 2 x 1.8 microns) 360 x

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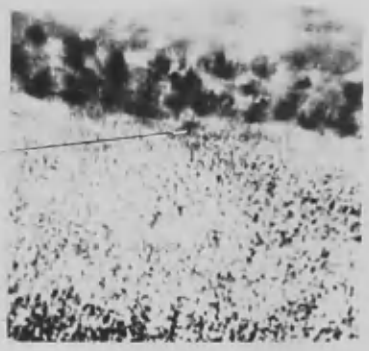

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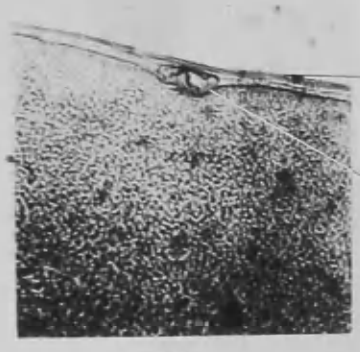

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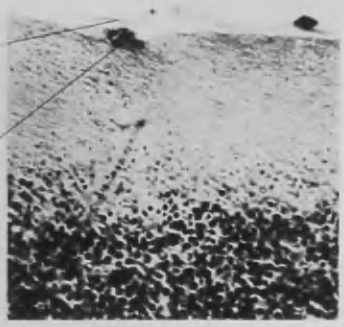

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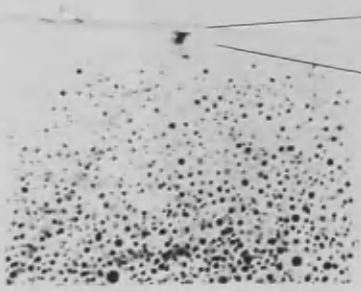

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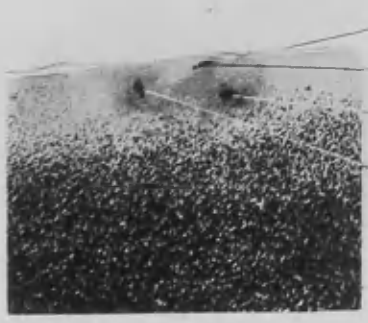

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EXPLANATION OF PHOTOMICROGRAPHS OF PLATE 4

Fig. 23 - Blastodisc of chicken egg showing first cleavage plane. 18 x

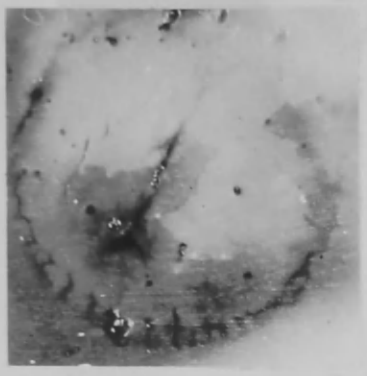
Figs. 24 and 25 - Blastodiscs of chicken eggs showing four-cell stages. Eggs were removed from isthmus portion of oviduct. 18 x

Fig. 26 - Blastodisc of chicken egg showing six cells. Egg removed from uterus at 4:30 p. m. or five hours after the estimated time of ovulation. 18 x

Fig. 27 - Blastodisc of chicken egg showing sixteen cells. This egg was obtained from the uterus of a hen at 1:30 p. m. or five hours after the estimated time of ovulation. 12 x

Fig. 28 - Blastodisc of chicken egg showing approximately thirty-two cells. This egg was obtained from the uterus of a hen approximately six and one-half hours after the estimated time of ovulation. 18 x

PLATE 4



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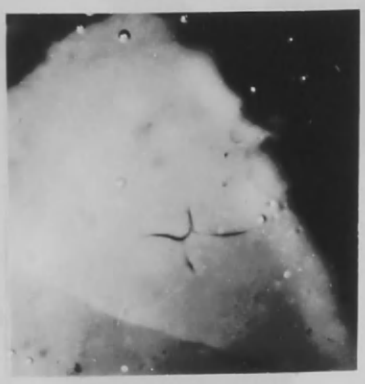

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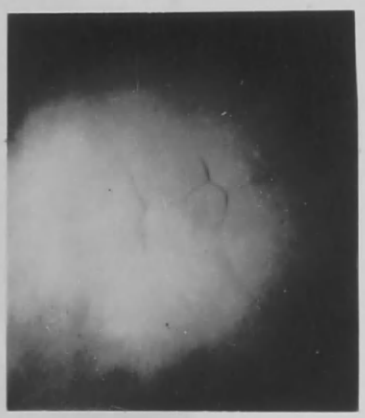

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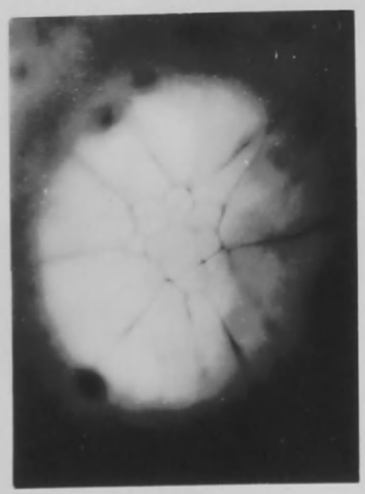

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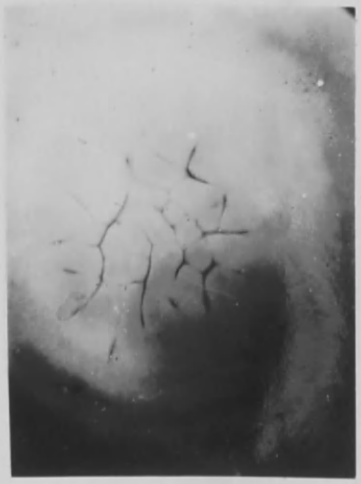

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EXPLANATION OF PHOTOMICROGRAPH OF PLATE 5

Figs. 29 and 30 - Segmented blastodisc of hens' eggs each approximately thirty-two cells. Both of these eggs were obtained at 3:00 p. m. or six and one-half hours after the estimated time of ovulation. 12 x

Figs. 31 and 32 - Blastodisc of hens' eggs showing approximately sixty-four cells. The blastodiscs were secured from eggs removed from the uterus six and one-half hours and seven and one-half hours after the estimated time of ovulation. 18 x

Fig. 33 - Blastodisc showing approximately one hundred cells in surface view (seventy-four central and twenty-four marginal cells). This blastodisc was from an egg which was obtained at 3:10 p. m. or six and two-thirds hours after the estimated time of ovulation. 18 x

Fig. 34 - Blastodisc showing a late cleavage stage (probably 360 cells). This egg was obtained at 4:00 p. m. or seven and one-half hours after the estimated time of ovulation. 12 x

PLATE 5

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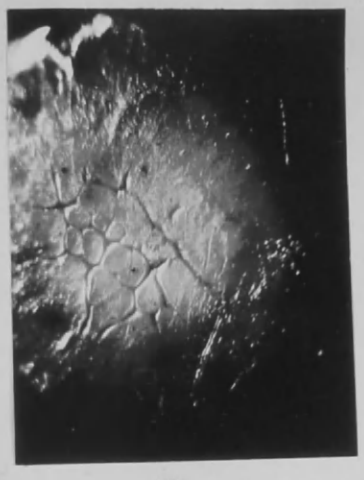

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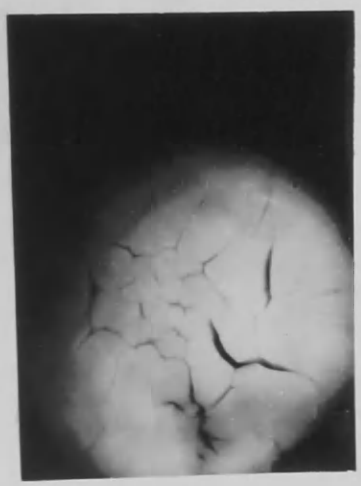

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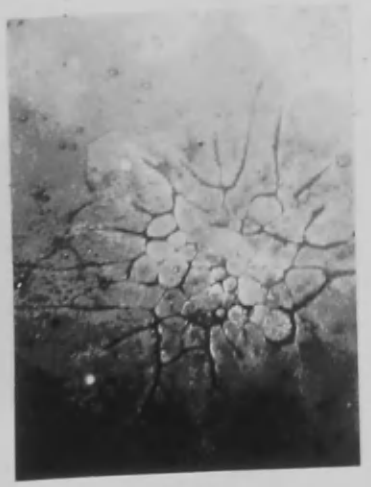

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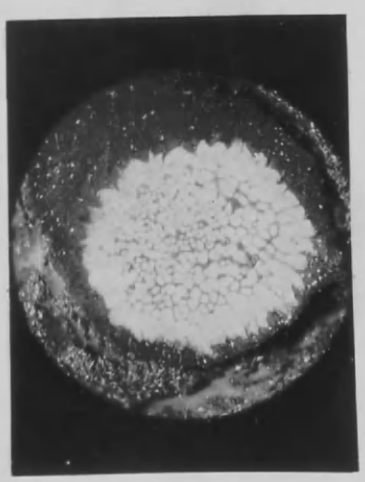

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EXPLANATION OF PHOTOMICROGRAPHS OF PLATE 6

Figs. 35 and 36 - Cross sectional views of the first cleavage plane of two different naturally fertilized eggs. One of these eggs was secured from the albumen-secreting portion and one from the isthmus, 320 x

Fig. 37 - Cross sectional view of a blastodisc which showed thirty-two cells in surface view. 75 x

Fig. 38 - Cross sectional view of a blastodisc of a naturally fertilized egg which was obtained from the uterus at 7:15 p. m. or eleven hours after the estimated time of ovulation. This photograph shows a very late cleavage stage. 75 x

Fig. 39 - Cross sectional view of a section of a blastodisc from an artificially impregnated egg showing the second polar body and the large fusion nucleus. This egg was incubated at 105° F. for twenty minutes. 720 x

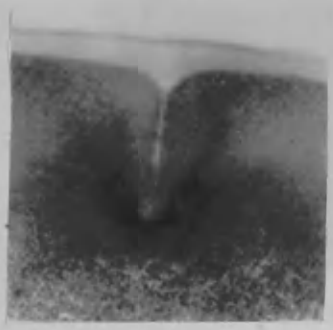
Fig. 40 - Blastodisc of an artificially impregnated egg after twenty-five minutes incubation showing first cleavage plane. This photograph also shows accessory cleavage about the periphery of the blastodisc. 12 x

Fig. 41 - Cross sectional view of the blastodisc of another artificially impregnated egg showing a large polar body. The round dark mass shown near the center of this polar body is chromatin material. 320 x

Fig. 42 - Blastodisc of an artificially impregnated egg showing approximately sixteen cells. This egg was incubated for thirty minutes at 105° F. 18 x

PLATE 6

35



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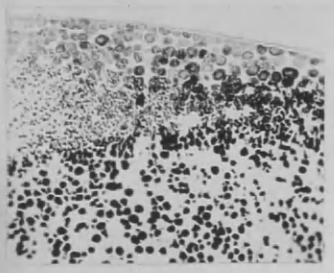

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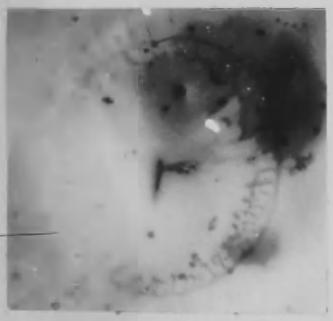

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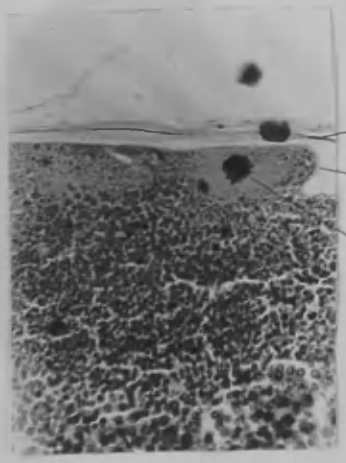

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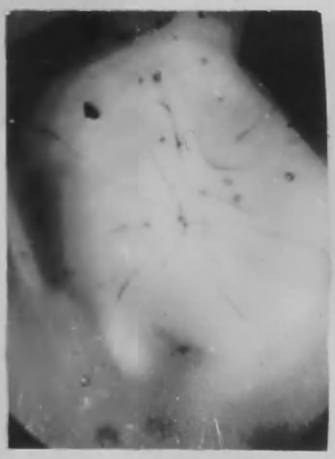


42

EXPLANATION OF PHOTOMICROGRAPHS OF PLATE 7

Fig. 43 - Blastodisc of an artificially impregnated egg showing late cleavage. This egg was incubated for five hours and thirty-four minutes at 105° F. following impregnation. 18 x

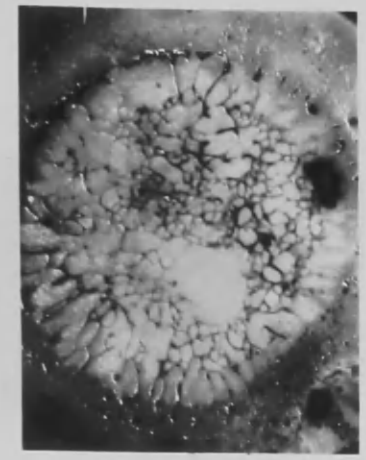
Fig. 44 - Blastodisc of an artificially impregnated egg showing late cleavage. This egg was incubated for five and one-half hours at 105° F. following impregnation. 18 x

Fig. 45 - Cross sectional view of the blastodisc showing cell formation. 75 x

Fig. 46 - Blastodisc of an artificially impregnated egg after being incubated for seven hours and eleven minutes at 105° F. 21 x

Figs. 47 and 48. Cross sectional views of two blastodiscs of artificially impregnated eggs showing cell formation. These uterine eggs were impregnated by placing the sperm upon the vitelline membrane directly over the germinal disc. The eggs were then incubated for five and one-half hours. 75 x

PLATE 7



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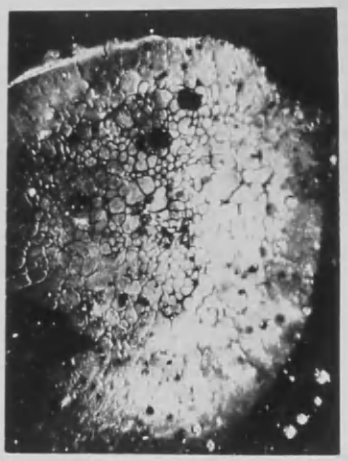

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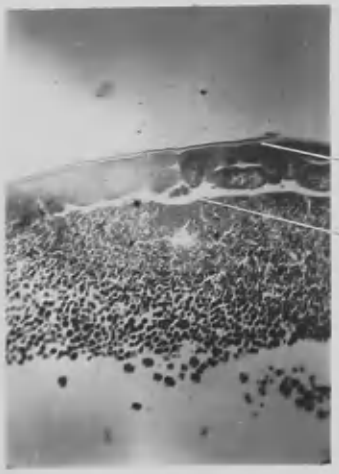
46



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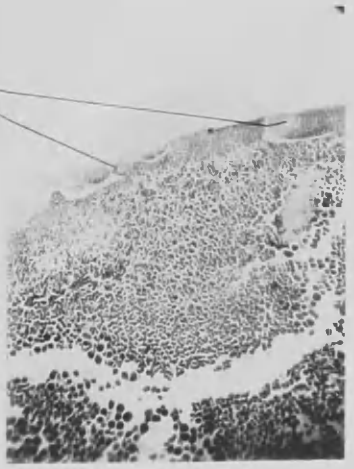


48

EXPLANATION OF PHOTOMICROGRAPH OF PLATE 8

Fig. 49 - Photograph of the yolk of a freshly laid infertile egg showing the dense, white mass of protoplasm in the center of the germinal disc. This condition which is a result of fragmentation is characteristic for nearly all newly laid infertile eggs. Natural size.

Fig. 50 - Photograph of the yolk of a freshly laid fertile egg showing the appearance of the blastodisc at the time of laying. Natural size.

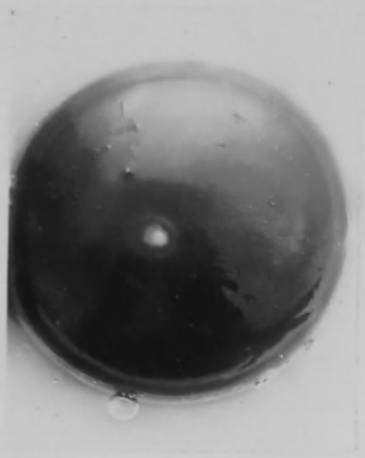
Fig. 51 - Blastodisc of an infertile uterine egg showing the beginning of fragmentation. This egg was obtained at 8:00 p. m. or ten hours after the estimated time of ovulation. 12 x

Fig. 52 - Blastodisc of another infertile uterine egg showing fragmentation. This egg was obtained at 8:30 p.m. or ten hours after the estimated time of ovulation. 18 x.

Fig. 53 - Cross sectional view of the fragmented blastodisc of an infertile egg showing the cell-like structures. This egg was obtained from the uterus at 11:30 p. m. or fifteen hours after the estimated time of ovulation. 75 x

Fig. 54 - Cross sectional view of the blastodisc of the same infertile uterine egg shown in 53. This photograph shows one of the many cell-like bodies of this blastodisc. The nucleus can be seen in this particular cell. 720 x

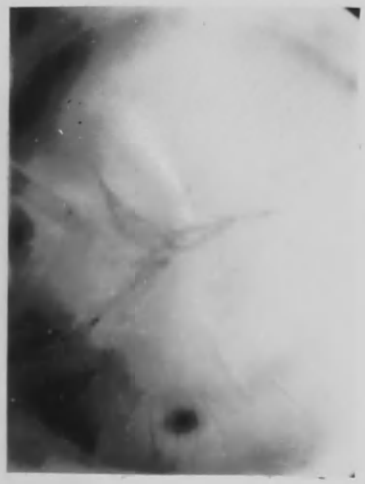
PLATE 8



49

50

51



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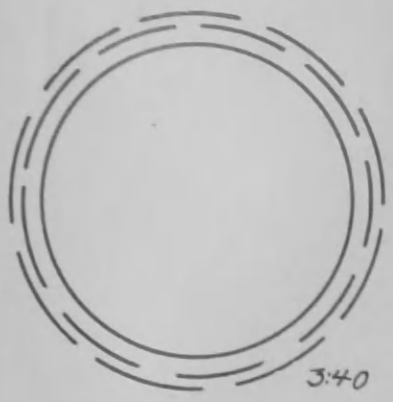
53

54

EXPLANATION OF PLATE 9

Drawings showing the progressive formation of fragmentation furrows in the blastodisc of an infertile egg.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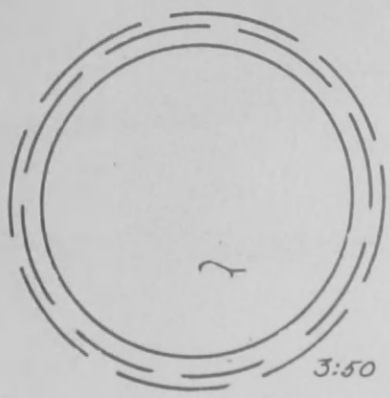
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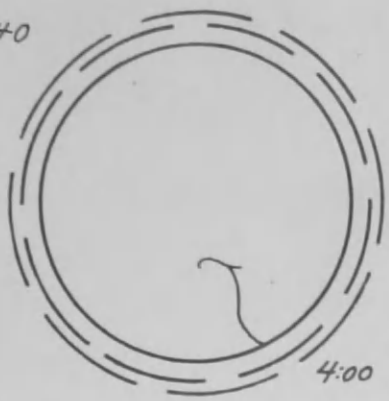
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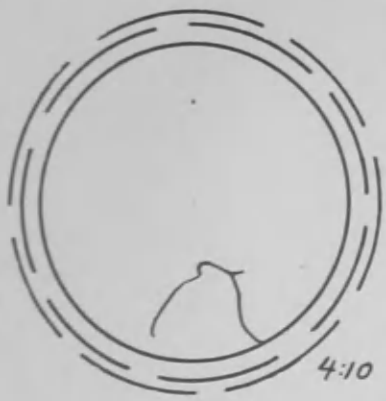
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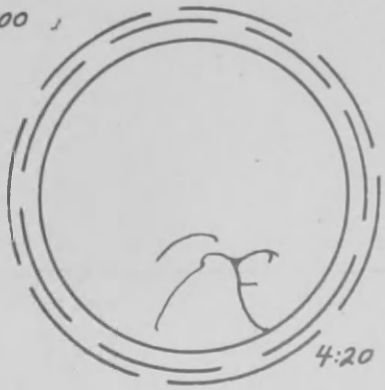
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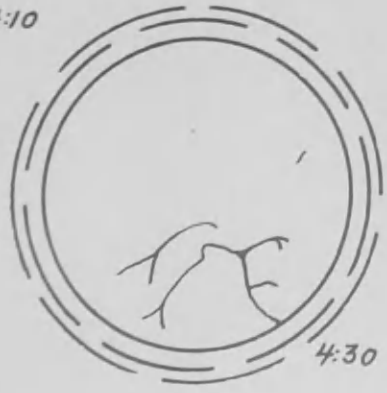
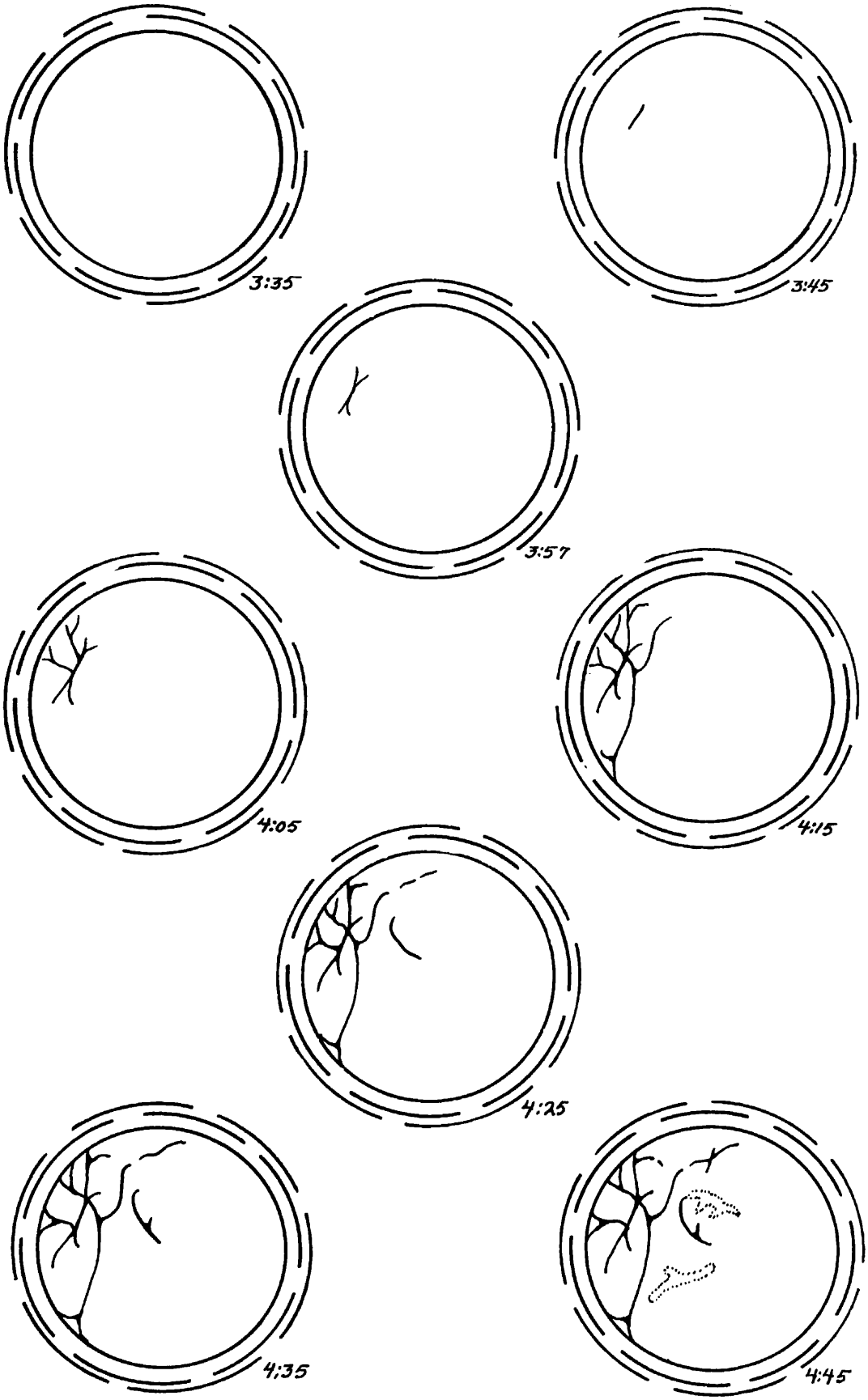

4:30


4:40


4:50

EXPLANATION OF PLATE 10

Drawings showing the progressive formation of fragmentation furrows in the blastodisc of a second infertile egg.


3:35
3:45
3:57
4:05
4:15
4:25
4:35
4:45

EXPLANATION OF PLATE 11

Carrier used to transfer tissues

PLATE 11

BIBLIOGRAPHY

(1) Asmundson, V. S., The formation of the hen's egg. Sci. Agric. 11:590-606. 1931.

(2) Barfurth, D., Versuche uber die Parthenogenetische Forschung des Huhneries. Archiv. Entwick. Mech. 2:303-351. 1896.

(3) Bartelmez, G. W. The bilaterality of the pigeon's egg. I. A study in egg organization from the first growth period of the oocyte to the beginning of cleavage. J. Morph. 23:269-330. 1912.

(4) Beadle, B. W., R. M. Conrad and H. M. Scott, Composition of the uterine secretion of the domestic fowl. Poul. Sci. 17:498-504. 1937.

(5) Blount, M. The early development of the pigeon's egg with especial reference to polyspermy and the origin of the periblast nuclei. Jour. Morph. 20:1-64. 1909.

(6) Bonnier, G., and S. Trulsson. Artificial insemination of chickens with semen diluted in Ringer solution. Proc. Seventh World's Poul. Congress:76-79. 1939.

(7) Brachet, J. Recherches sur le Comportement de l'acide Thymonucleinique au Cours de l'oogenese Chez diverse especes animales. Arch. de Biol. 39:676-697. 1929.

(8) Brambell, F.W. The oogenesis of the fowl. Phil. Trans. Royal Soc. B. 214:113-151. 1925.

(9) Burrows, W. H. and T. C. Byerly. Premature expulsion of eggs by hens injected with posterior pituitary substances. Poul. Sci. In press: 1941.

(10) Conrad, R. M., and R. E. Phillips. The formation of the chalazae and inner thin white in the hen's egg. Poul. Sci. 17:143-146. 1938.

(11) Conrad, R. M., and H. M. Scott. The formation of the egg of the domestic fowl. Physiol. Reviews 18:481-494. 1938.

(12) Coste, M. History general et particuliere du developpement des Corps organises. Paris. 1853.

(13) Curtis. Studies on the physiology of reproduction in the domestic fowl. VI. Double- and triple-yolked eggs. Biol. Bul. 26:55. 1914.

(14) Cushing, A. R. On the glands of the oviduct in the fowl. Proc. Am. Physiol. Soc. 14th Ann. Meeting. Am. J. Physiol. 6:XVIII-XIX. 1902.

(15) Dalton, J. C. The position of the embryo in the fowl's egg. Med. Record 20:589-590. 1881.

(16) d'Hollander, F. Recherches sur l'oogenese et sur la Structure et la Signification du Noyau Vitellin de Balbiani Chez les Oiseaux. Archives D'Anatomie Microscopique, 7:117-180. 1904.

(17) Duval, M. De la Formation du Blastoderme dans l'oeuf d'oisseau. Annales des Sci. Nat., 6 Series, 18:1-208. 1884.

(18) _____ Atlas d'embryologie. Paris. 1889.

(19) Fere, Chas. Note Sur l'orientation et sur l'allure du development de l'embryon de Canard. C. R. Soc. Biol. 10 Ser. T. 3:909-911. 1896.

(20) Firket, Jean. Recherches sur l'organogenese glandes sexuelles chez les oiseaux. Arch. de Biol. 29: 201-351. 1914.

(21) Gage, S. H., and S. P. Gage. Suden III deposited in the egg and transmitted to the chick. Sci. (N.S.) 28: 494-495. 1908.

(22) Giacomini, E. Sull' ovidutte dei Saurosidi. Monitore Zool. Ital. An. IV: 202-265. 1893.

(23) Goldsmith, J. B. The history of the germ cells in the domestic fowl. J. Morph. Physiol. 46:275-315. 1928.

(24) Hansen, C. H. On the formation of the hen's egg with especial reference to the white. Proc. 5th World's Poultry Congress 2:587-593. 1934.

(25) Harper, E. H. The fertilization and early development of the pigeon's egg. Am. J. Anat. 3:349-386. 1904.

(26) Haswell, W. A. Observations on the early stages in the development of the emu. Proc. Soc. of New S. Wales, Ser. B 2:577-599. 1887.

(27) Hays, F. A. and Costas Nicolaides. Variability in development of fresh-laid hen eggs. Poul. Sci. 13: 74-91. 1934.

(28) Holl, M. Ueber die Reifung der Eizelle des Huhn's. Sitzb. d. K. Akad. d.w.wien. Bd. 99 Abt.III:311-370.1890.

(29) Jull, M.A. Poultry Breeding - John Wiley and Sons, New York. 483 pp. 1940.

(30) Kolliker, A Grundriss der Entwickelungsgeschichte des Menschen und der hohern Thiere, Zweite Auff. Leipzig. 1880

(31) Lecaillon, A. Vitelline capsule, Compt.Rend. Acad.Sci. 150:240. 1910.

(32) _____ La Parthenogenes Chez les Oiseaux. Archiv. D'Anat. Micro. 12:511-638. 1910.

(33) Lillie, F. R. The development of the chick. 2nd Ed. Henry Holt and Co. New York. 472 pp. 1919.

(34) Loyez, M. Recherches sur de Developpement ovarien des Oeufs Meroblastiques. Archives d'Anatomie Microscopique, 8:69-397. 1905.

(35) Marza, V. D., and E. V. Marza. The formation of the hen's egg. Quat.J. Micros. Sci. 78:133-249. 1935.

(36) McEwen, R. S. A textbook of vertebrate embryology. Henry Holt and Co., New York. 544 pp.1924.

(37) McNally, E. H. and T. C. Byerly. Variation in the development of embryos of hens' eggs. Poul. Sci., 15:280-283. 1936.

(38) McNally, E. H. The physiology of yolk formation, especially the vitelline membrane and the mechanism of ovulation in the fowl. Doctorate thesis, University of Maryland, 41 pp. 1940.

(39) Morgan, T. H. Experimental embryology. Columbia University Press, New York. 776 pp. 1927.

(40) Munson, J. P. Researches on the oogenesis of the Tortoise, Clemmys marmorata. Amer. Jour. Anat. 3:311-347. 1904.

(41) Oellacher, J. Untersuchungen uber die Furchung and Blatterbildung in Huhnerie I. Studien aus dem Institute fur experimentelle pathologie in wien, T. I.:54. 1869.

(42) _____ Die Veranderungen des unbefruchteten Keimes des Huhnereies im Eileiter und bei Bebrutungsversuchen. Zeitsch. Wissenach. Zool. 22:181-234. 1872.

(43) Olsen, M. W. and T. C. Byerly. Orientation of the hen's egg in the uterus and during laying. Poul. Sci. 11: 266-271: 1932.

(44) _____ and _____. The orientation of the embryo in the egg of the domestic fowl. Poul. Sci.14: 46-53. 1935.

(45) Pander, Ch. Hysteria metamorphoseos quan ovum incubation prior quinque diebus subit. Wiresburgi Bertrage zur entwicklungsgeschichte des Huhnchens im ei. Wurzburg. 1817.

(46) Patten, B. M. The early embryology of the chick; 2nd ed. P. Blakiston's Son and Co., Philadelphia, 177 pp. 1925.

(47) Patterson, J. T. Studies on the early development of the hen's egg. I. History of the early cleavage of the accessory cleavage. Jour. Morph. 21: 101-134. 1910.

(48) Pearl, Raymond. The secretory activity of the oviduct of the domestic fowl. Proc. of the Soc. for the Promotion of Agric. Sci.:20-34. 1911.

(49) Pearl, Raymond, and M. R. Curtis. Studies on the physiology of reproduction in the domestic fowl. V. Data regarding the physiology of the oviduct. Exp. Zool. 12: 99-132. 1912.

(50) Peebles, Florence. The location of the chick embryo upon the blastoderm. Jour. Exp. Zool. 1:369-385. 1904.

(51) Phillips, R. E. and D. C. Warren. Observations concerning the mechanics of ovulation in the fowl. J. Exp. Zool. 76:117-136. 1937.

(52) Rabaud, E. La position et l'orientation de l'embryon de poule sur le jaune. Arch. Zool. Exp. Gen. (Notes et Revue) 4th Ser. T.9:1-7. 1908.

(53) Richardson, K.C. The secretory phenomena in the oviduct of the fowl including the process of shell formation examined by the microincineration technique. Philos. Trans. of the Roy. Soc. of London, 225:159-195. 1935.

(54) Riddle, O. The rate of growth of the egg yolk of the chick and the significance of white and yellow yolk in the ova of vertebrates. Sci. (N. S.)27:945. 1908.

(55) _____ The occurrence and measurement of a sudden change in the rate of growth of avian ova. Am. J. Physiol. 41:387-396. 1916.

(56) _____ A simple method of obtaining premature eggs from birds. Science 54:664-666. 1923.

(57) Rogers, C. A. Feeding color - an aid in studying physiological development. Proc. Inter. Asso. of Ins. and Investigators. 1:77-81. (1908-1910)

(58) Ruckert, J. Zur Entwickelungsgeschichte des Ovarialeies bei Selachiern. Anat. Anz. Bd. VII:107-158. 1892.

(59) Sonnenbrodt. Die Wachstumsperiode der Oocyte des Huhnes. Archiv. f. Mikr. Anat. und Entwicklungsgeschichte-Bd. 72:415-480. 1908.

(60) Surface, F. M. The histology of the oviduct of the domestic hen. Maine Agric. Exp. Sta. Bul. 206: 395-430. 1912.

(61) Swift, Charles H. Origin and early history of the primordial germ cells in the chick. Am. Jour. Anat. 15:483-516. 1914.

(62) Van Durme, M. Nouvelles recherches sur la Vitellogenese des oeufs d'oiseaux aux stades d'accroissement de Maturation, de Fecondation et du debut de la segmentation. Arch. de Biol. 29:71-200. 1914.

(63) Warren, D. C. and R. M. Conrad. Growth of the hen's ovum. Jour. Agr. Res. 58:875-893. 1939.

(64) Warren, D. C. and L. Kilpatrick. Fertilization in the domestic fowl. Poul. Sci. 8:237-256. 1929.

(65) Warren, D. C. and H. M. Scott. The time factor in egg formation. Poul. Sci. 14:195-207. 1935.

(66) _____ and _____ Ovulation in domestic hen. Science 80:461-462. 1934.

(67) _____ and _____ Physiological factors influencing the rate of egg formation in the domestic hen. J. Agr. Res. 51:565-572. 1935.

(68) Willier, B. H. On the origin and differentiation of the sexual gland. Amer. Naturalist 67:1-8. 1933.

(69) Wilson, E. B. The cell in development and inheritance. 2nd Ed. The Macmillan Co., London. 1911. 483 pp.

(70) Woodger, J. H. Observations on the origin of the germ cells of the fowl (Gallus domesticus) studied by means of their golgi bodies. Q. J. M. Sci. 69:445-462. 1925.