STUDIES ON THE EARLY DEVELOPMENT OF THE HEN'S EGG.

I. History of the Early Cleavage and of the Accessory Cleavage.[1]

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With 32 Figures.

I. Introduction.

The period extending from ovulation to the laying of the egg is a most obvious gap in our knowledge of the development of the hen's egg. It has been the writer's desire to fill in this break, and he is indebted to the trustees of the "Elizabeth Thompson Science Fund" for a grant which made it possible to undertake the work. If the problem contained no possibilities other than that of merely filling in a gap, it is doubtful whether the work would have been undertaken, since the results could not have been commensurate with the labor involved. But it was felt that certain points, brought out in a study of the pigeon's egg by several students at the University of Chicago (Harper, '04; Blount, '09; Patterson, '09), deserved further investigation. Among these were fertilization, accessory cleavage, and gastrulation.

On account of the importance centering in gastrulation and the accessory cleavage, their discovery in the hen's egg would be of the greatest interest; for a true gastrulation has never been found in this egg, and the accessory cleavage has been neither figured nor described. We have not even known whether fertilization in the hen's egg is monospermic or polyspermic.

[1]Contribution from the Zoölogical Laboratory of the University of Texas, No. 103.

It is not necessary to enter into an extensive discussion of the literature on the subject of the early development of the hen's egg, for the several papers touching on this subject are well known. The studies of Duval, '84, have, perhaps, received more attention than those of any other investigator, and yet it has been demonstrated that his fundamental conclusions are incorrect, and that he was probably misled in his interpretations through the use of pathological material (Kionka, '94; Barfurth, '95; Schauinsland, '99; Patterson, '09). Kionka, '94, although figuring stages throughout the greater part of the period to be considered in these studies, does not give us a good idea of the character of the very early cleavages. Neither of these workers, nor any one of the others who have investigated these stages, has had anything like a complete series from which to draw his conclusions; consequently it is not surprising to find that the majority of the interpretations do not accord with the principles of vertebrate development, and that the more fundamental points are obscure.

The recent discovery by Guyer, '09, of an "accessory chromosome" in the male germ cells of the chicken lends unusual interest to the study of fertilization in the hen's egg, for it ought to be possible to demonstrate from the study of the mitoses of the supernumerary sperm nuclei whether or not such nuclei are dimorphic.

It was the writer's intention at first to publish the entire history, from ovulation to laying, in a single paper, but the slow rate at which material naturally accumulates makes it desirable to publish the part already completed; the remaining parts, one on maturation and fertilization, and the other on late cleavage and gastrulation, will appear later.

II. Methods.

Since the methods employed are essentially the same as those used in handling the pigeon egg, they need be mentioned but briefly here. The picro-sulphuric-acetic mixtures, which were found to be so excellent for fixing the pigeon egg, do not work well, for they render the yolk too hard. The picro-acetic fluid, however, although not entirely satisfactory, gives fairly good results. For preparing

surface views, a weak solution of Flemming's chromo-osmic acid is excellent and has but one disadvantage, viz., that after its use the egg usually can not be sectioned.

III. Some Notes on the Laying Habits of the Hen.

The behavior of the hen during the breeding season would make an interesting topic for the student of animal behavior; for while one sees many evidences suggesting that domestication has wonderfully influenced the behavior of the hen, yet there are continually cropping out certain habits that evidently have been derived from her wild ancestors, and which even centuries of domestication have not completely eradicated. One of the most noticeable of these is seen in connection with the nest building. The hen never carries building material to the nest, but she often stands in the vicinity of the proposed site and makes a futile effort to get straws and feathers into the nest by tossing them over her back. In several of the other Gallinæ this same habit is observed. Many species of this group of birds are accustomed to building their nests in tufts of grass, where an abundance of material is ready at hand, and its building is a comparatively simple matter, consisting in the arrangement of the grass. Occasionally, however, other birds (*e. g.*, the quail) will engage in exactly the same futile effort as that cited above for the hen, only in a more pronounced manner.

The writer's study of the habits of the hen was not carried on with any intention of writing a paper on its behavior, but rather in order to find out if there is any regularity in its laying habits. If one is to collect eggs for the purpose of obtaining a close series of stages, it is of the greatest importance to be able to tell just when to kill the hen in order to secure a desired stage. It is only in this way that one can hope to obtain sufficient data for a correct interpretation of the history of development.

It is commonly supposed that the hen lays very irregularly, and while the writer finds this to be true for some few hens, yet in most cases he was soon able to predict the time of laying to within a few minutes. This is especially true of that class of hens laying daily. Such hens are found to lay slightly later each day, and

the difference between any two succeeding days is sometimes exactly one hour (hen 1).

HEN 1.

Laid	April	1, 8:30	A.M.
"	"	2, 9:30	"
"	"	3, 10:30	"
"	"	4, 11:30	"
"	"	5, 12:30	"

When a hen is not laying daily the matter of determining the time is not so simple, and yet even here one can approximately predict the exact time of the laying, as can be demonstrated in the following case:

HEN 2.

Laid	June	12, 2:00	P.M.
"	"	14, 10:00	A.M.
"	"	15, 2:00	P.M.
"	"	17, 10:00	A.M.

It is evident from these data that the hen was laying at 10:00 A.M. and 2:00 P.M. on succeeding days and then was missing a day. It was, therefore, predicted that she would lay early in the afternoon of the 18th, and since an early cleavage stage was desired, the hen was killed at 4:00 P.M. on the 17th. The stage secured is shown in Fig. 15.

There are some hens that apparently do not lay at any regular intervals, and in such it is quite impossible to predict the time of laying. As an example, I may cite the following case:

HEN 3.

Laid	July	25, 11:00	A.M.
"	"	28, 11:00	"
"	"	30, 11:45	"
"	Aug.	1, 11:00	"
"	"	6, 9:30	"
"	"	7, 3:00	P.M.
"	"	9, 1:00	"
"	"	11, 12:30	"
"	"	13, 2:00	"
"	"	16, 1:30	"
"	"	18, 1:00	"

Killed August 19, 5:00 P.M. (secured an early cleavage stage).

There are but few hens that ever lay before 8:00 A.M. or after 4 P.M. It is evident, therefore, that a hen in laying daily will eventually come to the 4 o'clock period, and will then miss a day (sometimes more) before beginning a new set. Indeed, this is true for all hens whether laying regularly or irregularly, for they lay in a sort of rhythm. In the case of irregular laying, cited above (hen 3), the eggs laid from the 25th of July (when the observations were begun) to the 1st of August, are the last of a set in which the hen was laying approximately every other day; while those laid from August 6th to 18th constitute another set. Evidence that the eggs will be laid in sets can be obtained by an examination of the ovary, which shows several graduate series of ovarian eggs.

It will be evident from the above considerations and data that if one is to secure a close series, it is necessary to study each hen individually and while this involves a great amount of labor, yet it is the only way in which one is able to meet with any success.

The collecting of the above data has another advantage besides that of aiding in securing a close series, for it makes possible the determination of the rate of development of the different stages. The time occupied by the egg in passing down the oviduct has been variously estimated at from eighteen to twenty-four hours, and even as high as thirty-six hours. This seems like a wide variation, and in taking up this work, the writer was prepared to find the normal time more constant than is indicated in such estimates.

The writer finds that in a hen kept under normal conditions, the egg traverses the entire length of the oviduct in about twenty-two hours. The time occupied in the different portions of the oviduct is as follows: Glandular portion, three hours; isthmus, two to three hours; uterus and laying, sixteen to seventeen hours.

As just stated, these estimates were made on hens kept under normal conditions; that is, hens that were given the freedom of the barnyard. It is possible to lengthen the time beyond the twenty-two hours by disturbing the hen when she is about to lay, and on one occasion the writer was able to delay the laying of the egg for twenty hours. When it was finally deposited an examination revealed the fact that it was in a stage of development equal to about twenty hours of incubation. This would account for the high esti-

mates of other writers, and perhaps also for those cases reported in the literature, where a freshly laid egg is said to contain an embryo with a well-developed vascular system. The writer is convinced that any appreciable extension beyond twenty-two hours is not due to an increase in the length of time that it takes the egg to traverse the reproductive passage, but rather to a rentention of the egg in the lower part of the oviduct, on account of some influence inhibitory to laying. The writer has found, however, slight variations in the length of time, but these are probably to be correlated with the variations in length of the oviduct in different hens.

When once it has been established that the time occupied by the egg in its passage through the different parts of the oviduct is practically constant, we are then in a position to determine the rate of development; because we need only to note the stage of development in eggs taken from the different parts, and from the data thus collected determine the time elapsing between any two stages. The following table will give the reader an idea of these estimates, which were determined from a study of hens laying daily (about one hour later each day). The table also gives the exact time of each of the stages described in the rest of the paper.

TABLE I.

Hen.	Last egg laid.	Succeeding taken from the oviduct.	Age.	Position in oviduct	Stage	Fig.
No. 1	1:30 A. M. May 28	4:00 P.M.. May 28	2½hrs.	11 inches from the infundibulum	pre-cleavage	5
No. 2	8:30 A.M., Aug. 10	2;30 P.M., Aug. 10	3 "	just entering the isthmus	two-celled	1
No. 3	8:30 A.M., Apr. 13	2:30 P. M., Apr. 13	3¼ "	in the isthmus	four-celled	12
No. 4	10:00 A.M. Sept. 1	5:00 P.M. Sept. 1	4 "	" " "	eight-celled	13
No. 5	11:00 A.M., Aug. 4	6:30 P.M., Aug. 4	4½ "	" " "	fifteen-celled	21
No. 6	12:30 P.M., July 31	8:15 P.M., July 31	4½ "	" " "	thirty-two celled	
No. 7	8:45 A.M., July 30	4:45 P.M., July 30	5½ "	in shell gland	64 cells in surface view.	25
No. 8	9:30 A.M., Aug. 7	7:30 P.M., Aug. 7	7 "	" " "	154 cells in surface view.	30
No.9	8:00 A.M., Aug. 27	7:00 P. M., Aug. 27	8 "	" " "	346 cells in surface view.	32

IV. Functions of the Oviduct.

The whole reproductive apparatus is a most delicately adjusted mechanism. The primary function of its oviducal portion is to transmit the egg from the body cavity to the exterior, but in the course of its evolution it has taken up several other functions, such as transmitting and storing sperms and the secreting of the accessory layers around the egg. The co-ordination between the infundibulum and the ovary is often very exact and delicate, but it is in the birds that we see this co-ordination reaching its highest degree of perfection. Coste describes the infundibulum as actually embracing the ovum in its follicle at the time of ovulation, and the writer has been able to confirm his statement by several observations. Coste believed that the infundibulum exerted some pressure on the follicle, and it may be that this is the direct cause of ovulation. Indeed, it is highly probable; for while ovulation may take place without the direct assistance of the oviduct, as in the lower vertebrates, yet the weight of evidence supports the opposite view. We have been able to show (in a paper not yet published) that the follicular orientation is preserved in the oviduct, and furthermore that this preservation probably occurs only when ovulation is directly caused by the activities of the infundibulum.

This explanation of ovulation also gives us the key to the solution of another problem, viz., why it is that normally but a single egg is found in the oviduct at a time. If we examine the oviduct of a hen that is laying daily, some time before the deposition of an egg, it will be found to be inactive; but an examination shortly after laying, reveals the fact that the oviduct is in a state of high excitability, with the infundibulum usually clasping an ovum in the follicle. In one case it was embracing a follicle containing a half-developed ovum, and with such tenacity that a considerable pull was necessary to disengage it. It seems certain, therefore, that the stimulus which sets off the mechanism for ovulation is not received until the time of laying, or shortly thereafter. So long as there is an egg in the lower part of the reproductive passage the infundibulum apparently does not clasp the ovum, and a second egg is thus prevented from entering the oviduct.

V. Fertilization.

Since it is intended to describe in detail the process of fertilization, the writer wishes here to make only a brief statement concerning the time of its occurrence. Harper, '04, has shown that fertilization in the pigeon's egg takes place immediately after ovulation, when the egg is in the region of the infundibulum. The writer finds the same to be true of the hen's egg also. Eggs taken from the upper part of the oviduct at distances varying from one to twelve inches from the infundibulum, give all the stages of development from maturation to the formation of the first cleavage spindle.

We have shown above that the egg is about twenty-two hours in passing down the oviduct, and since fertilization takes place immediately after ovulation, it is obvious that it occurs approximately twenty-two hours before the time of laying. Throughout this paper we shall, therefore, determine the "age" of any particular stage from this estimated time of fertilization.

VI. The Two-celled Stage.—Three Hours.

The first cleavage furrow makes its appearance just as the egg is entering the isthmus, about three hours after the estimated time of fertilization, and by the time the inner-shell membrane can be recognized as an extremely thin sheet covering the albumin, the furrow is well developed and covers a distance equal to about one-third the diameter of the area of primary cleavage. The furrow is usually situated in the central part of the disc (Fig. 1).

Any mention of the first cleavage furrow calls to mind the question of the relation of the plane of this furrow to the longitudinal axis of the later embryo. Of the five cases so far obtained, in which it was possible to determine absolutely the plane of the first furrow, only one showed it coinciding with the long axis of the future embryo. This would seem to indicate clearly that the plane of the first furrow does not necessarily parallel the median axis of the embryo. It may be, however, that in the case of each of the eggs mentioned, we were dealing with one in which the axis of the later embryo would be abnormal; that is, it would not meet the chalazal axis at right angles. Duval, '84, has pointed out that a

certain number of hen's eggs show abnormal relations existing between the two axes, and the writer has found a similar condition in the pigeon egg. In each of these species the percentage of abnormal axes was found to be small. It seems highly improbable, therefore, that four out of five eggs taken at random, and in the two-celled stage, would later show abnormal axes. The final answer to the question, however, could only be obtained by studying a two-celled stage and noting the plane of the furrow, and then after incubating the egg until the axis of the embryo became visible, it would be possible to determine the point in question. This would necessitate a much more extensive study than the object of this paper calls for. The problem, furthermore, has lost much of its earlier significance, inasmuch as it has not proved to be a fundamental law of development.

In sections taken transverse to the first cleavage furrow (Fig. 2) the membrane is seen to cut almost through the fine granular portion of the disc, and is peculiar in that it arises from a *membrane plate,* which, at both sides, is continuous with the perivitelline space. In section the membrane does not extend down from the membrane plate as a straight line, but is wavy (Fig. 3); and while this condition may be the result of unequal contraction of the materials, caused by the fixing and hardening fluids, yet it obtains for all of the earlier membranes.

Another point of interest brought out by the section (Fig. 2) is the depression in the surface of the disc just above the cleavage membrane. This is, of course, the cleavage furrow, which in this egg stood out with remarkable clearness in the living condition, but in most eggs it is practically wanting (Fig. 11). The lack of a furrow is the cause of the indistinctness of the early cells. In this respect, the early cleavages of the hen's egg differ greatly from those of the pigeon's egg, for in the latter their clearness is such as to permit photographing the living cells, while in the former, photographs are impossible, except in a few cases.

After the division of the first cleavage nucleus the daughter nuclei migrate peripherally in a line lying at right angles to the cleavage membrane, and are always elongated in the direction of motion. In

this egg they showed signs of preparation for the next division when they had reached a distance from the membrane equal to 0.175 mm.

Polyspermy. A close study of surface views of two-celled stages has failed to reveal any trace of the "accessory cleavage," which is such a characteristic morphological feature of the early pigeon blastoderm. It would be a great mistake, however, to conclude from this that fertilization in the hen's egg was monospermic, for a study of the sections brings to light the fact that ordinarily five or six supernumerary sperm nuclei are in the egg, and, as we shall see later, some of these may migrate to the periphery of the area of primary cleavage and there give rise to a *rudimentary accessory cleavage.*

In one egg (Fig. 5), which is in a precleavage stage of development, twenty-four extra sperm nuclei are found. This high number is very unusual, and led the writer at first to assume that the egg was abnormal. All the evidence, however, is against this assumption. In the first place, it can not be said that the physiological condition of the egg was such as to favor the multiplication of the sperm nuclei soon after their entrance into the egg; for if this were the case there ought to be evidences of nuclear multiplication, but not a single sperm nucleus gave any sign of undergoing division. In the second place, the egg in all probability was normal in so far as undergoing normal development is concerned, for the cleavage nucleus was in the act of producing a spindle at the time when the egg was fixed, and other eggs from this hen underwent normal development when incubated.

In the light of these facts it seems evident that in some few eggs a comparatively large number of sperms may enter. In such eggs this may be due to a greater attraction between the protoplasm of the disc and that of the sperms than ordinarily exists; or to a failure of the inhibitory agencies to become operative quickly enough after ovulation to prevent their entrance.

In the egg from which Fig. 1 was made only five supernumerary sperm nuclei are present, and none of these had reached the margin of the disc at the time when the egg was fixed, but all are located centrally. Two of the nuclei are situated quite superficially in the disc, while three have passed down deep and are resting on the coarse granular yolk (Fig. 9). One of the nuclei has undergone division

twice, and produced a "nest" of four nuclei (Fig. 6, *sn*). It is difficult to determine whether or not such nuclei later migrate from the nests to the margin of the primary area and there participate in the production of the accessory cleavage. The writer believes not, because nests containing many small fragments of nuclei are found in slightly later stages, and such nests are located in the same position as the earlier ones. This would seem to indicate that the nuclei, after sinking down into the coarse granules, continue to divide and fragment, finally disappearing altogether. If this be true, then we see the beginning of the degenerative agency which will cause all of the supernumerary nuclei to disappear.

An egg showing a case of fragmenting nuclei is outlined in Fig. 10. It is a three-celled stage, and the degenerating nucleus is close to the margin of the primary area. All of the sperm nuclei, excepting one, are located much more peripherally than in the preceding egg (cf., Figs. 9 and 10). The difference in position of the two sets indicates the distance traversed by the nuclei during the time intervening between the two stages.

A section of the egg figured above gives one a good idea of the character of the blastodisc in the early stage (Fig. 11). It also demonstrates the point made above, that very often the first cleavage membranes are not accompanied by a cleavage furrow.

This egg furnishes still other points of interest, for in fixing it, not all of the albumin was removed, and the thin chalazipherous layer adheres to the vitelline membrane. Embedded in this layer and next to the membrane are about ninety sperm heads, none of which is located peripherally to the terminal ends of the cleavage membranes (Fig. 8).

The presence of the sperm heads in such large numbers, together with their position at the central part of the disc, is important. It can not be said that the scarcity of sperm nuclei in the disc (as compared with the number found in the pigeon egg) is to be accounted for by the lack of sperms in the oviduct. Their location in the central part of the disc only, shows that they must be attracted there by a force which is strongest at the central point, and which gradually diminishes toward the periphery. The attraction between the protoplasm of the disc and that of the sperms is evidently neu-

tralized suddenly, because sperms are found lodged in the vitelline membrane, as though they had been stopped in the very act of entering the egg (Fig. 7).

VII. The Four-celled Stage.—Three and One-fourth Hours.

The four-celled stage is produced by a vertical division in each of the blastomeres of the two-celled stage, and the two furrows meet the first one approximately at right angles (Fig. 12). While the division in one blastomere may slightly precede that of the other, usually they occur simultaneously. It has been stated that the point where the second furrows meet the first is situated eccentrically, the displacement being toward the posterior border of the blastoderm. It is not uncommon to find eggs with the center of the cleavage eccentric, but the displacement may be in any direction from the center. The writer does not believe, therefore, that any importance can be attached to the eccentricity of cleavage.

The rudimentary accessory cleavage makes its appearance in the four-celled stage, and in the egg shown in Fig. 12 there are three such furrows present. These cut across the margin of the area of primary cleavage, and their planes are approximately radial. In most eggs the furrows lie entirely without the margin. This blastodisc shows, in addition to the three accessory furrows, two other small ones lying well within the margin, but their position in the anterior blastomeres makes it clear that they are the approaching divisions of these two cells.

VIII. The Eight-celled Stage.—Four Hours.

In the formation of the eight-celled stage, the third division furrows, at least in some cases (Fig. 16), tend to remain regular; that is, each of the third furrows is vertical and meets the second furrow at right angles. There is thus produced two parallel rows of four cells each. In the majority of eggs, however, the form of the cleavage in this stage apparently is not regular, though probably if it were possible to follow the divisions in the living cells it would be found that there was considerable regularity. The cells do not always divide simultaneously, and since there is a tendency for the

early blastomeres to flow together, it may be that the original relationship between the cleavage planes is modified. If this is not the case, then the variation in the form of cleavage which characterizes the later stages is anticipated in the eight-celled stage.

As in the two- and four-, the cells of the eight-celled stage are not true cells, in the sense that they are not completely delimited by cell walls, but are open to the periblast both below and peripherally. Occasionally, however, one of the blastomeres may be surrounded (in surface view) by cell walls (Fig. 13). We have, therefore, two regions of cleavage, in which the cells are usually designated as central and marginal.

The connections between both the central and marginal cells with the periblast are very clearly brought out in the section (Fig. 18), which also gives one a clear idea of the beginning of the horizontal cleavage planes. These planes not only separate the blastomeres from the underlying or central periblast, but also mark the position of the future segmentation cavity. Such an interpretation for the origin of the cleavage cavity is not in accord with the account of Duval and others. Duval contends that a very narrow space situated between a single superficial layer of cells and the deeper cells represents the segmentation cavity, and, furthermore, that the deeper cells are derivatives of the deeper parts of the disc, and have arisen by additions upward to the parts already segmented. Duval's view has been shown to be untenable for the pigeon's egg, and I find it is also incorrect for the hen's egg; but exactly the same thing occurs in the latter egg as described for the pigeon's egg by Miss Blount. The increase in the number of cell layers in the disc is not brought about by the addition upward of cells from the underlying material, but by the appearance of horizontal cleavages, which occur between the segmentation cavity and the surface of the blastodisc and thus the large central cells are cut up into a number of cell layers.

The accessory cleavage remains distinct up to the eight-celled stage (Figs. 14, 16, 13), and in the reconstruction of a series from the seven-celled stage there are shown ten supernumerary sperm nuclei (Fig. 14). Seven of the nuclei can be arranged into three groups, indicating that originally but five sperms entered the egg and not ten. Four of the nuclei are in the last stages of degenera-

tion, while two are associated with a rudimentary accessory cleavage furrow. The furrow was clearly visible in the living egg, where it appeared as a shallow groove. In section it is characterized by having a broad shallow depression, at the bottom of which is found a distinct membrane plate of exactly the same appearance as that of an early primary cleavage furrow, and differs from the latter only in the absence of a membrane (Fig. 19, a. f.). It is, therefore, rudimentary, and the presence of two supernumerary sperm nuclei in its immediate vicinity leaves no doubt as to the interpretation that it is an accessory furrow. One of the nuclei (Fig. 19, s. p., nucleus on right) has passed down into the coarser granular yolk and undergone almost complete fragmentation, and this may be the reason the furrow never proceeds to the point of forming a membrane.

In addition to the rudimentary cleavage this egg also presents an interesting case of *horizontal accessory cleavage* (Fig. 4, *c.*). In reality this is not a cell division, because there is associated with the cleft but a single nucleus, which lies just below the cleft. Such cases would seem to be attempts at cell formation without the accompanying nuclear division. They are not of any great importance, as but two examples have been found.

So far the writer has not observed the accessory cleavage after the ten-celled stage, and in all probability it completely disappears shortly after this period. It seems highly probable that many of the sperm nuclei degenerate soon after their entrance into the egg. The degeneration occurs when they pass down into the coarse granular yolk; but so long as they remain superficially situated they seem to possess the power of migration. Undoubtedly, there are not a few eggs in which all of these nuclei degenerate before reaching the margin, and hence such eggs would at no time show an accessory cleavage. The writer does not believe, however, that this would account for the failure of previous investigators to discover the accessory cleavage of the hen's egg. Only a few of them have described the four-celled stage, and, so far as we are aware, none have figured the eight. The brief period during which they would most likely observe this peculiar form of cleavage is, therefore, the one to which they have given least attention. Furthermore, the acces-

sory cleavage, except in a few cases, is extremely difficult to detect in the living egg; and it is only after the use of Flemming's fluid that it becomes clearly demonstrable. Its difficulty of demonstration is further increased by the fact that the furrows most often occur in radial planes, thus leading the observer to believe that they are only the terminal ends of the primary cleavage furrows. We have been unable to find a satisfactory explanation as to why the clefts take this radial direction, unless it be that they follow the course of least resistance. Not all of the accessory cleavages, however, have their furrows lying in radial planes, for the writer has found several cases in which they were lying in various planes; and, furthermore, he has observed two very clear cases of completely formed accessory cleavage cells (Figs. 13 and 17).

The contention of Blount that the accessory cleavages in the pigeon's egg completely disappear and, therefore, these cells take no part in the formation of the embryo, receives a full confirmation from these studies. The demonstration of that conclusion is even clearer in the hen's egg than in the pigeon's egg. In the hen's egg the marginal cells never become closed, thus indicating that their closing in the pigeon's egg must be in response to a stimulus received from the numerous accessory cleavage cells. Probably the closed margin cuts off influences which emanate from the accessory cells, and which might interfere with the normal development of the blastoderm.

At best the accessory cleavage in the hen's egg is but a weak attempt at cell formation, which has become inhibited, shortly after its initiation, by the degenerative tendency of the accompanying nuclei.

IX. Fifteen to Seventeen Cells.—Four and One-half Hours.

In the stage consisting of approximately sixteen cells there are four or five central and eleven or twelve marginal ones (Figs. 17 and 20). The central cells increase by the cutting off of the inner ends of the marginal cells, while the latter multiply by the formation of radial furrows. In some cases one can still see a tendency in the form of the cleavage to remain regular. In Fig. 20 the anterior

half shows exact regularity, there being just eight cells, but the posterior half is slightly irregular, with nine cells.

There are no accessory cleavages in this stage, but a large number of short radial furrows lie just inside the margin of the primary area (Fig. 20). These furrows can be seen in the living egg, but are brought out more clearly in osmic acid preparations. Under the higher power of the microscope they are seen to be due not so much to the formation of furrows as to the arrangement of the granules. In the median lines they are composed of fine granules, while to either side are several rows of larger granules. Occasionally one can detect a membrane, which appears as a delicate thread running through the median streak of fine granules.

At first sight it might seem that these furrows represent an abundant accessory cleavage, but such is not the case. In the first place, the furrows are situated entirely within the primary area, while the accessory furrows lie without the area. In the second place, there are no nuclei directly associated with these short furrows. They are simply the beginnings of the peripheral extensions of the primary cleavage furrows; for it will be noted that for the most part they lie either in the same radial planes with the primary furrows (marginal), or in positions where the next divisions of the marginal cells will soon occur. This interpretation is fully substantiated when slightly later stages were studied, when it was found that not only are the short furrows greatly diminished in numbers, but that the primary furrows now reach the margin of the primary area (Fig. 15), and at the same time the marginal cells are practically double in numbers.

These short furrows do not occur in all blastoderms, but when they do appear it is at the sixteen-celled stage, though they may last until comparatively late cleavage stages (Fig. 30). In significance these furrows indicate that the cytoplasmic division of the marginal cells is felt at the margin of the primary area earlier than in regions lying somewhat more centrally; and the attempted division at the margin probably immediately follows that of the marginal cell nucleus, while the intervening space awaits the approach of the central portion of the primary cleavage membranes.

In median sections of the fifteen-celled stage the horizontal cleavages have progressed to the point of effecting a complete separation of the central cells from the central periblast (Fig. 22), and these cells, are therefore, completely delimited by cell walls. The segmentation cavity is expanding laterally beneath the marginal cells by the extension of the horizontal clefts (Fig. 22, on left), and will eventually increase in depth by the accumulation of fluid within it.

X. Thirty-two to Thirty-five Cells.—Four and Three-fourths Hours.

In this stage (Fig. 23) the central cells have begun to multiply more rapidly than those of the margin, and the two kinds are now of equal numbers. The form of the cleavage is irregular, and, therefore, very variable in different eggs. At the posterior border of the blastoderm are seen two short radial furrows (Fig. 23, *m*), and while it is possible that there were more at an earlier period, yet probably this is an egg in which there were never many; because if numerous at an earlier period of development, the primary cleavage furrows should give some evidence of having extended to the margin of the primary area, and it will be noted that they fall quite short of reaching the margin.

The median section of a blastoderm, which showed thirty-two cells (sixteen marginal and sixteen central) in the living egg, is seen in Fig. 24, A. The cleavage cavity is especially well developed, and the one-layered condition of the blastoderm is unusually clear. The vertical cleavage separating the two cells which lie slightly to the left (anterior) of the center has not completely cut through to the cavity. Such a connection between adjacent cells is very slender, and, in this particular case, is absent in a few sections lying to either side.

While the planes of the early cleavages are, for the most part, vertical (that is, meeting the surface of the blastoderm at right angles), yet some of the planes take an oblique course. Under such conditions it is not difficult to find many places where the blastoderm appears to be two cells thick; especially is this true of sections that are taken some little distance to either side of the median line (Fig.

24, B). Here, the lower portions of the two cells (on right) appear to be "buds" or outgrowths from the floor of the cavity; but if the succeeding sections on each side are carefully examined, it will be found that such "buds" are nothing more nor less than the lower ends of cells whose upper portions reach the surface of the blastoderm in another section—and vice versâ, the cells that appear to form a free upper layer are found to have portions extending obliquely downward to, and connecting with, the floor.

This point is of considerable interest, because a failure to appreciate its import probably has been the source of error on the part of some embryologists (*e. g.,* Duval), who have stated that cells are cut off from the floor and are added upward to the blastoderm, thus contributing to its increase in thickness. The deception arising from the appearance of these buds is all the more striking in the cases where the nucleus of the cell concerned lies deep enough to be included in the "bud" (*e. g.,* Fig. 24 B, *n*). In some sections both the upper and lower portions of the cell are included (Fig. 24 B, *c*), and in such the connection of the cell with the floor is clearly shown. In later stages the connection will be severed by the extension of the horizontal cleavage planes.

The accessory cleavage has entirely disappeared by the thirty-two celled stage, but occasionally a supernumerary sperm nucleus will be found. The sections of the egg considered just above were subjected to a very careful examination for the purpose of determining how many such nuclei were present. This study gave the following results: In the sixteen central cells eighteen nuclei were found, but three of the cells had two nuclei each; that is, the cytoplasmic division of these three cells had not yet taken place. In the sixteen marginal cells, together with the surrounding periblast, nineteen nuclei were found, and two of the marginal cells had two nuclei each. The extra nucleus was found far out in the periblast—so far out, indeed, that there is no possibility of its being considered as a derivative of a marginal cell nucleus. It must be, therefore, a sperm nucleus. This could have been determined without counting the other nuclei, for the one in question is in an advanced stage of degeneration, and rapidly disappearing.

It is certain, therefore, that not only does the accessory cleavage

disappear at an early cleavage stage, but the extra sperm nuclei also disappear early. The writer has never found sperm nuclei after the thirty-two-celled stage.

XI. Sixty-four Cells in Surface View.—Five and One-half Hours.

This stage brings out more clearly than any we have so far considered the method by which the region of central cells increases. At the inner ends of many of the marginal cells are large central ones, which have been just recently cut off; and located centrally to these are smaller cells (Fig. 25). The number of central cells increases, therefore, in two ways: First, the central region grows at the expense of the marginal cells, and in this manner the central region gradually extends peripherally; second, the central cells thus formed multiply *inter se*. If we compare this stage with the preceding one (Fig. 23), in which there were seventeen cells in each region, it will be seen at once that while the number of marginal cells has increased but six, the central ones have increased twenty-four (in surface view). This comparison becomes all the more striking when it is stated that the central region averages two cells in depth, due to the formation of horizontal clefts, so that there are probably a total of some eighty central cells, or an increase of sixty-three. The central cells multiply, therefore, more than ten times as rapidly as the marginal ones.

The manner in which the central region becomes more than a single layer deep is made clear in a study of a section of a blastoderm in this same stage of development (Fig. 26). The segmentation cavity is very distinct and above it the original one-layered disc (see Fig. 22) is now two cells deep; and this condition has been brought about not by the addition of cells from that portion of the disc lying beneath the cleavage cavity, as supposed by Duval, but entirely by the formation of horizontal clefts in the cells lying above the cavity. As we might have expected, the method of increase in cell layers is identical with that of the same process in the pigeon blastoderm. At this stage there is no possibility of cells being added to the disc from the central periblast, because that region is entirely void of nuclei.

On account of the obliquity of the so-called horizontal clefts, the central cells are characterized by great irregularity both in shape and size. Many of them are shaped like squamous epithelium, and often give the appearance of stratified epithelium in section.

A detailed drawing of the anterior end of a section will show the manner in which the marginal cells add their products to the central area (Fig. 27). The cleavage membranes cut down deep into the disc, and at their terminal points the horizontal clefts begin to spread beneath these large cells, thus separating them from the underlying portion; when this is accomplished, the large cells are split up into smaller ones by vertical and horizontal divisions (on right of Fig. 27).

XII. One Hundred and Fifty-four Cells in Surface View.—Seven Hours.

During the next hour and a half the marginal cells undergo but few divisions, but the central ones multiply very rapidly, and since they receive but few additions from the marginal cells, their increase must be due to their own activity in division. This results in producing a large number of small cells in the central area, and the smallest cells lie at the very center of the blastoderm (Fig. 30). It would seem, therefore, that the early cleavage of the hen's egg follows the rule which states that the time occupied between any two successive cleavages grows shorter and shorter as the volumes of the cells decrease.

The average depth of the central part of the blastoderm at this period is about three cells (Fig. 31). The cleavage cavity, although not so distinct as in the preceding stage, is, nevertheless, clearly recognizable and there are no connections between its floor and the lower cells of the blastoderm. The anterior and posterior ends of the section show different conditions in the character of the cells. At the posterior end there are three large cells, in addition to the marginal one, which have not been broken up by horizontal clefts. At the anterior end, on the other hand, all of the cells, except the marginal, have undergone division. This difference is probably only a local condition, and, therefore, is not fundamental.

The periblast still remains free of nuclei, but the marginal cell nuclei are beginning to show a tendency to migrate farther peripherally than usual (Figs. 28 and 29).

XIII. Three Hundred and Forty-six Cells in Surface View.—Eight Hours.

The final stage that we shall consider in this paper is shown in Fig. 32. During the hour intervening between this and the previous stage the marginal cells have added many more cells to the central area than at any former period of like duration, and consequently the (radial) length of the marginal cells has greatly decreased, and their furrows now are beginning to cut out into the periblast.

This stage is one of the most important of all the early cleavages, because it represents the transitional period between the "unorganized" and "organized" periblast; but we shall not consider the sections at this time.

XIV. General Summary.

The absence in this paper of comparisons between the development of the hen's egg and that of other vertebrate eggs is not due to a lack of appreciation of the importance of such comparisons, but rather to the fact that in the main these have been pointed out for the corresponding stages of the pigeon's egg. In this connection the writer wishes, therefore, to confine himself to emphasizing the close similarities between the development of the hen's egg and that of the pigeon, although to those who have followed closely the work on the latter egg this may seem unnecessary.

Exact agreement in all details of development, even in the eggs of two species as closely connected as those of the hen and the pigeon, is not to be expected, but the fundamental processes should certainly agree. And such has proved to be the case. The minor differences in development of these two forms have to do primarily with time relations; for although the eggs of these two species are in about the same stage of development at the time of laying, yet the pigeon's is forty-one hours old and the other's twenty-two. The holomogous processes, therefore, necessarily do not occur at exactly the same time after fertilization.

The more important comparisons are as follows:

1. The process of fertilization (that is, the entrance of the sperm) in each egg occurs immediately after ovulation, when the egg is in the region of the infundibulum.

2. At the time of fertilization in the pigeon's egg, from twelve to twenty-five supernumerary sperm nuclei enter the egg. In the hen's egg only five or six such nuclei are found (except in one case where twenty-four were present).

3. Upon their entrance into the egg these sperm nuclei, in each egg, migrate toward the periphery of the disc. In the pigeon's egg the nuclei, on reaching the margin, become active, divide and give rise to an accessory cleavage, which disappears between ten and twelve hours after fertilization. In the hen's egg some of the supernumerary nuclei pass down into the deeper portions of the disc and there undergo complete fragmentation; others may succeed in reaching the margin, and there give rise to a rudimentary accessory cleavage, which disappears shortly after the eight-celled stage, or between four and five hours after fertilization.

4. In the pigeon's egg the marginal cells become closed and remain so throughout the period occupied by the accessory cleavage. In the hen's egg the marginal cells always remain open to the periblast both below and peripherally. This would seem to indicate that the condition of a closed marginal cell in the pigeon's egg is to be correlated with the presence of a large number of accessory cleavages. Perhaps it is for the purpose of cutting off some influence emanating from the accessory sperm nuclei.

5. In neither egg does the direction of the first cleavage plane, or the eccentricity of cleavage, if present, seem to bear any constant relation to the axis of the future embryo.

6. Immediately after the disappearance of the accessory cleavages and their accompanying nuclei in the pigeon's egg the marginal cells open to the periblast, and their nuclei divide and some of the daughter nuclei migrate into the periblast and "organize" it. In the hen's egg there is a period of from two to three hours after the disappearance of the accessory sperm nuclei during which the periblast is void of nuclei of any kind.

7. In each egg the first horizontal cleavage plane marks the position of the segmentation cavity.

AUSTIN, TEXAS, November 8, 1909.

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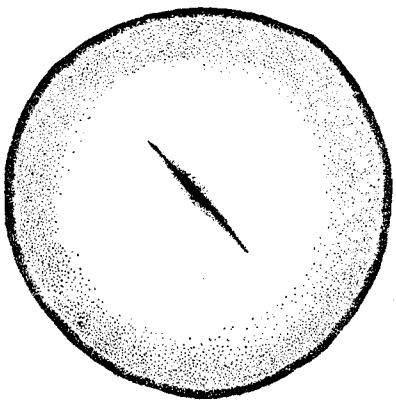

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FIG. 1.—A two-celled stage, which was drawn from a free-hand sketch and measurements of the living egg.[2] For the history of this egg see Table 1, hen No. 2. × 18.

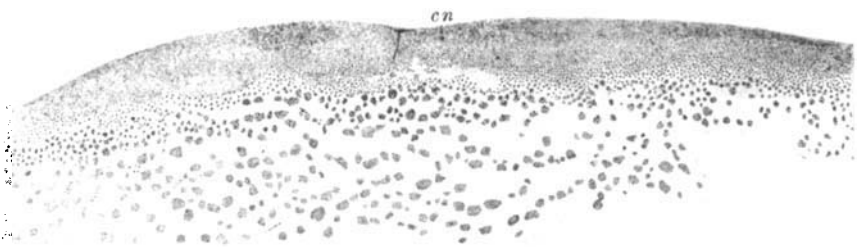

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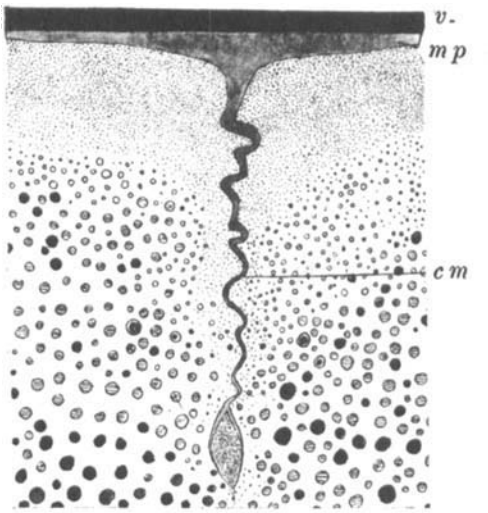
FIG. 2.—A median section taken transverse to the furrow of the egg illustrated in the preceding figure. The nucleus of Pander is very poorly developed in this egg. *c.n.*, cleavage nucleus. × 39.

[2] In this, as in the succeeding figures of surface views, the anterior margin of the blastodisc is toward the top of the page, and hence the median axis of the later embryo will parallel the sides of the page. In sections the anterior end is always toward the left. In the surface views the area occupied by the primary cleavage, together with the first "ring" of periblast, are shown.



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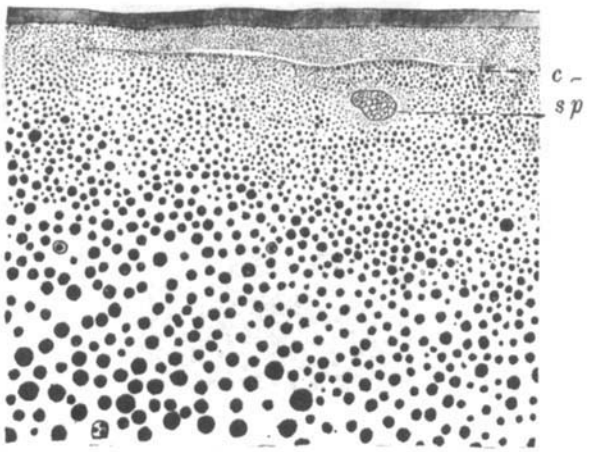

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FIG. 3.—Enlarged drawing of the cleavage membrane of the preceding figure. *c.m.*, cleavage membrane; *m.p.*, membrane plate; *v.*, vitelline membrane. × 525.

FIG. 4.—Enlarged drawing of the extreme left end of the section shown in Fig. 18. This shows a supernumerary sperm nucleus, *s.p.*, about which cell formation is attempted, as evidenced by the horizontal cleft situated just above the nucleus. × 525.

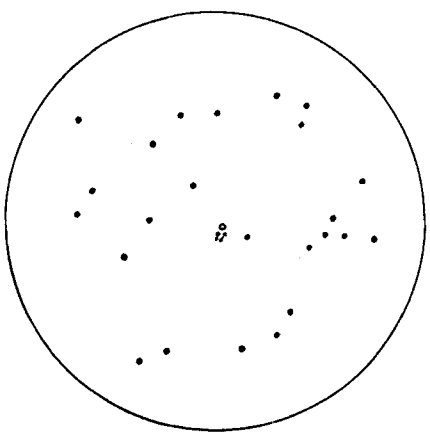

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FIG. 5.—Diagram of an unsegmented blastodisc showing the distribution of twenty-four sperm nuclei. This egg was removed from the oviduct about two and a half hours after the estimated time of fertilization, when it was eleven inches from the infundibulum. See Table 1, hen No. 1. × 18.

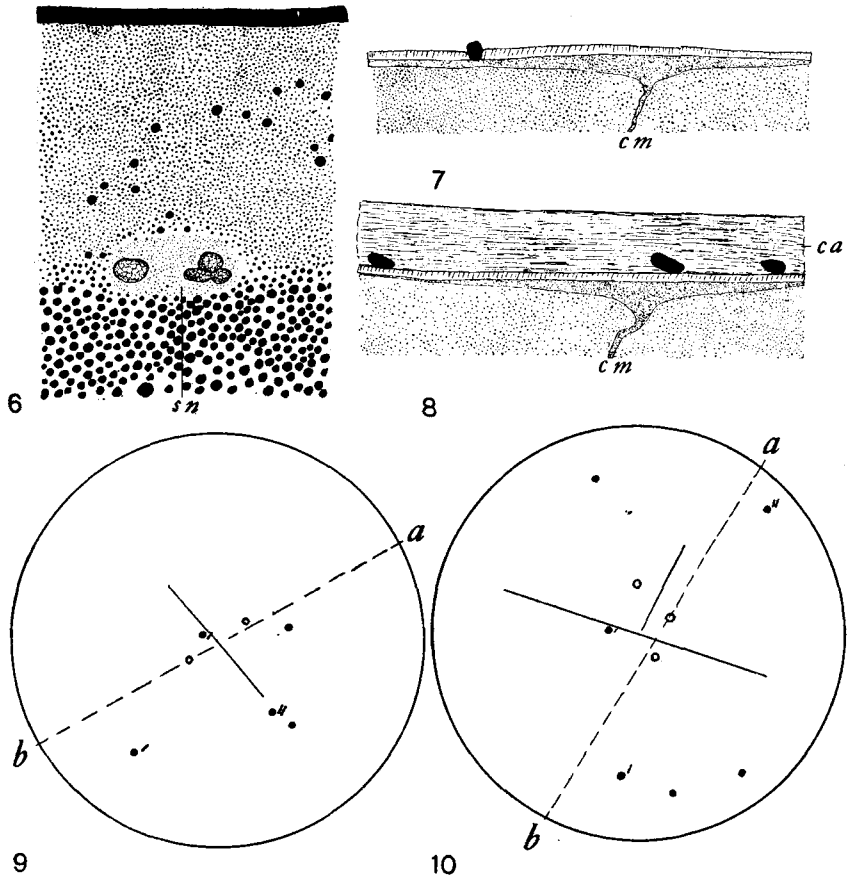

FIG. 6.—A nest of four supernumerary sperm nuclei. × about 600.

FIG. 7.—A sperm head lodged in the vitelline membrane. The sperm had evidently been stopped in the act of entering the egg. × about 600.

FIG. 8.—Three sperm heads embedded in the chalazipherous layer of albumin and located next to the vitelline membrane. Both of these figures (7 and 8) are taken from the central region of the egg shown in Fig. 10. × about 600.

FIG. 9.—A diagram of the blastodisc shown in Fig. 1, showing the distribution of the supernumerary sperm nuclei. The black dot indicates that the nucleus is located more or less superficially in the disc; while a dot marked prime one shows the location of a nucleus that is situated deep in the disc, and one marked prime two, a nucleus that is undergoing fragmentation. The broken line, A—B, is the plane of the section shown in Fig. 2. × 18.

FIG. 10.—Diagram of a blastodisc of an egg taken from the oviduct three hours after fertilization, shortly after it had entered the isthmus. This shows six supernumerary sperm nuclei. Line A—B is the plane of the section shown in Fig. 11. × 18.

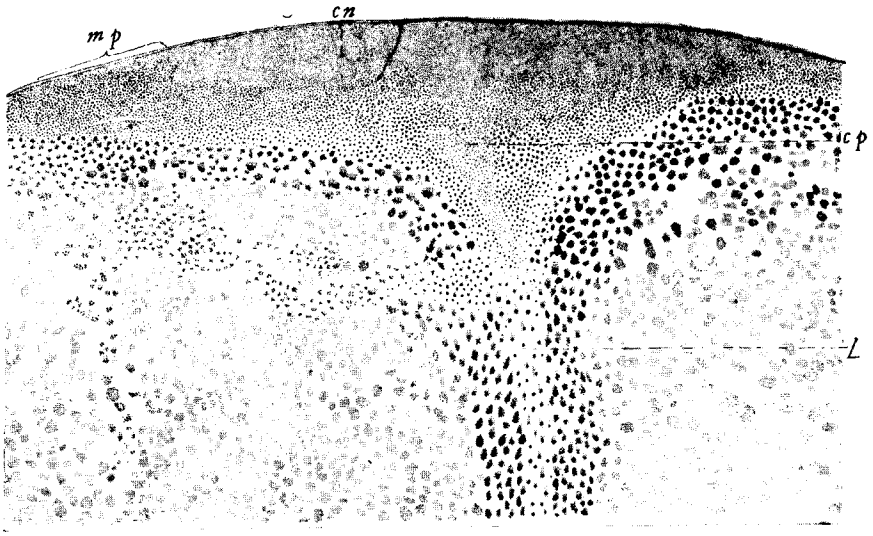

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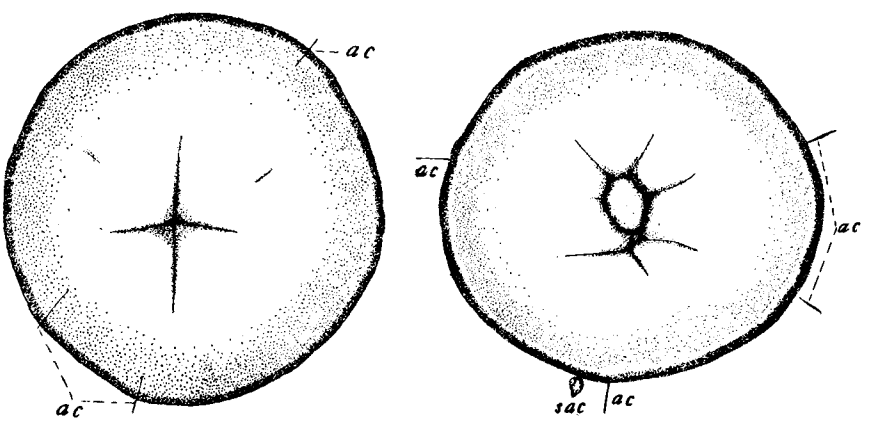
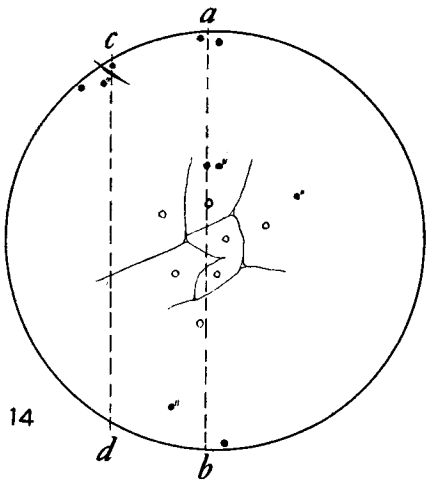
FIG. 11.—A section through line A—B of Fig. 10. *m.p.*, marginal periblast; *c.n.*, cleavage nucleus; *l*, neck of the latebra. × 52.



12 13

FIG. 12.—The four-celled stage, drawn from a whole mount preparation. The egg as taken from the isthmus (see Table 1, hen No. 3). *a.c.*, accessory cleavage furrows. × 18.

FIG. 13.—An eight-celled stage, drawn from a whole mount preparation. The history of this egg is given in Table 1, hen No. 4. It shows one central and seven marginal cells. *a.c.*, accessory cleavage furrows; *s.a.c.*, small accessory cleavage cells. × 18.



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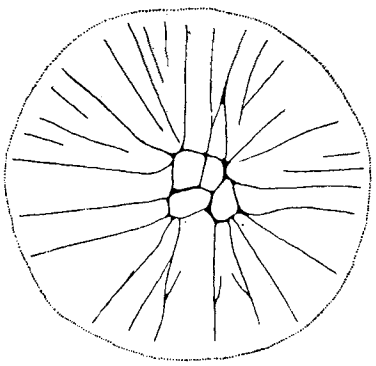

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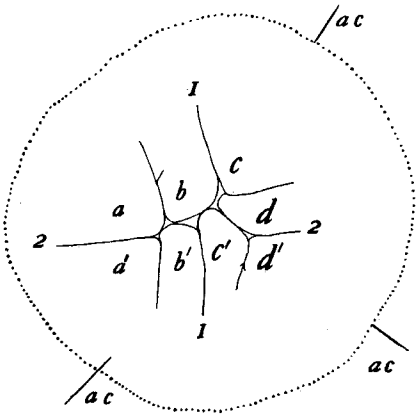

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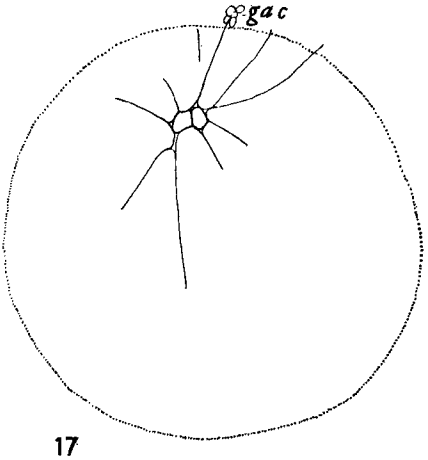

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FIG. 14.—A diagram of a seven-celled stage, showing the distribution of the cleavage and supernumerary sperm nuclei. The egg was taken from the isthmus about three and three-fourths hours after the estimated time of fertilization. A—B, plane of the section shown in Fig. 18, and C—D that of Fig. 19. × 18.

FIG. 15.—An interesting blastodisc showing a large number of marginal cells in the process of formation. The egg was taken from the isthmus. × 18.

FIG. 16.—A blastodics showing a comparatively regular form of cleavage in the eight-celled stage. The egg was taken from the isthmus about four hours after fertilization. The letters indicate the cells that are probably homologous; and the numbers, the first and second cleavage planes. Three accessory cleavage furrows are shown. × 18.

FIG. 17.—An early stage showing an eccentric cleavage, with the displacement toward the anterior. *g.a.c.*, a group of five small accessory cleavage cells. The egg was taken just as it was passing into the shell-gland.

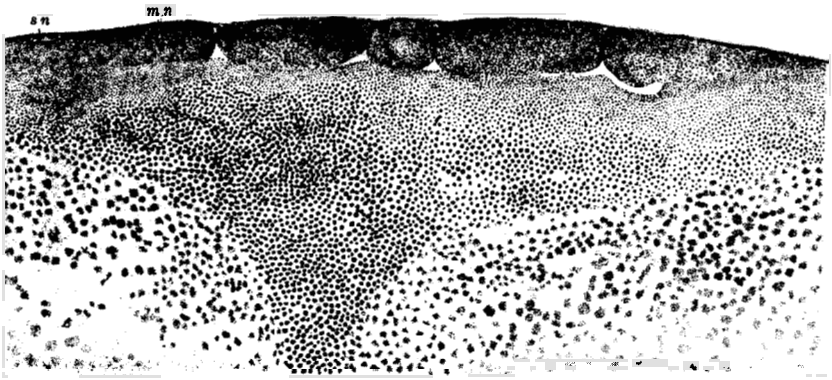

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FIG. 18.—Section through plane *a*—*b*, Fig. 14. *m.n.*, marginal cell nucleus; *s.n.*, supernumerary sperm nucleus with a cleft lying just above it. × 73.

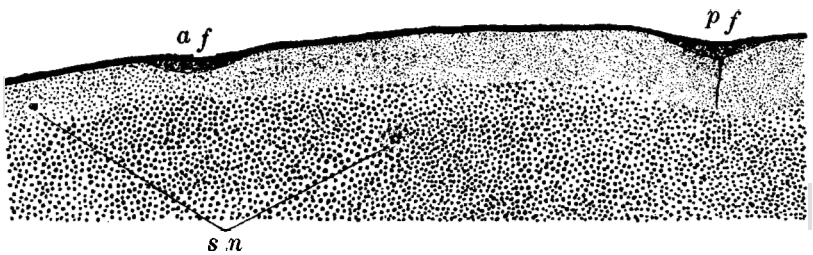

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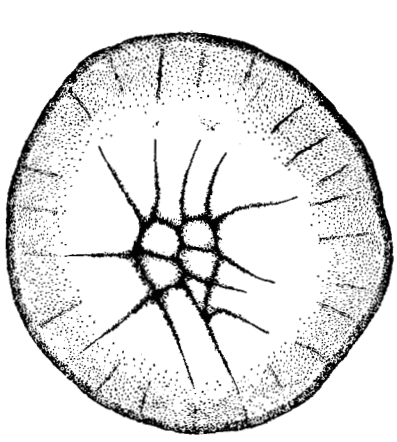
FIG. 19.—Anterior portion of a section through *c*—*d*, Fig. 14. *s.n.*, sperm nucleus; *a.f.*, accessory cleavage furrow; *p.f.*, terminal portion of a primary cleavage furrow with membrane. × 66.



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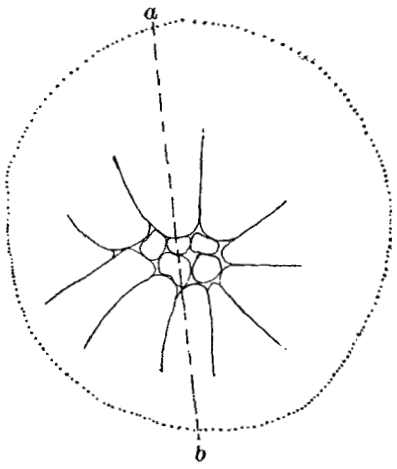

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FIG. 20.—A seventeen-celled stage, drawn from a whole mount preparation. The egg was taken from the isthmus between four and five hours after fertilization. The blastoderm is remarkable in that it has many short radial furrows situated near the margin of the primary area (see text for a description of these furrows). × 18.

FIG. 21.—A fifteen-celled stage, drawn from the living egg (see Table 1, hen No. 5). *a*—*b*, plane of section shown in Fig. 22. × 18.

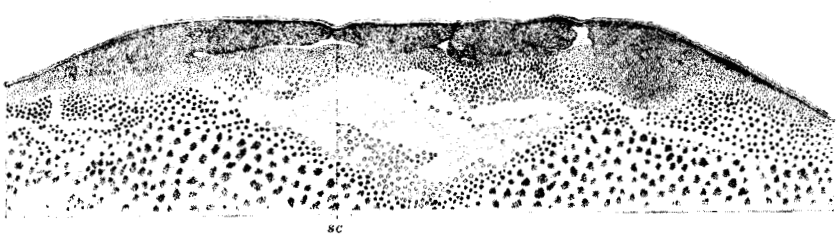

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FIG. 22.—Section through plane *a*—*b*, Fig. 21. *s.c.*, segmentation cavity. × 55.5.

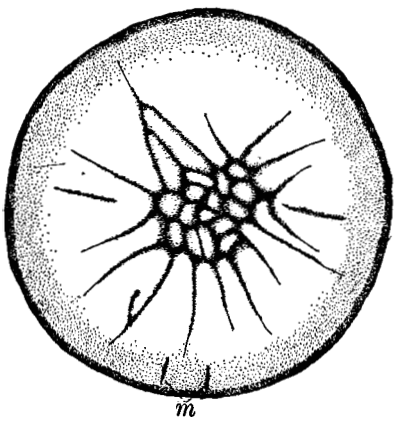

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FIG. 23.—A thirty-four-celled stage, drawn from a whole mount preparation. **The egg was taken from the shell-gland.** There are seventeen marginal and **seventeen central cells. At the posterior** margin are shown two of the short radial furrows which were noted in Fig. 20. × 18.

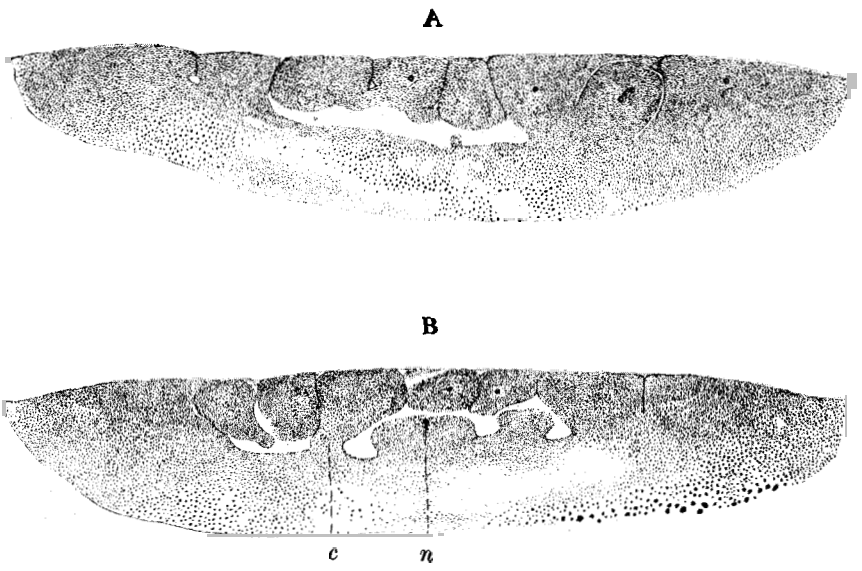

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FIG. 24.—A. Median section of a blastoderm showing thirty-two cells. B. A section taken seven sections to the left of the preceding. *n*, nucleus; *c*, cell showing a connection with the floor of the segmentation cavity (see text for a description of these figures). **Both × 59.**

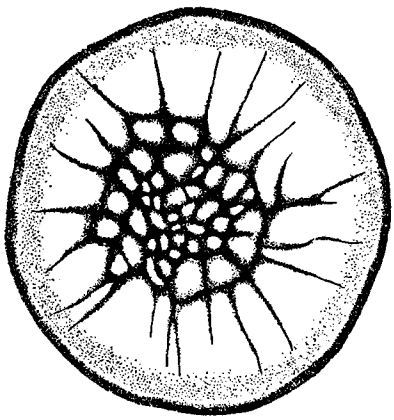

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FIG. 25.—Blastodisc showing sixty-four cells in surface view—forty-one central and twenty-three marginal (see Table 1, hen No. 7). × 18.

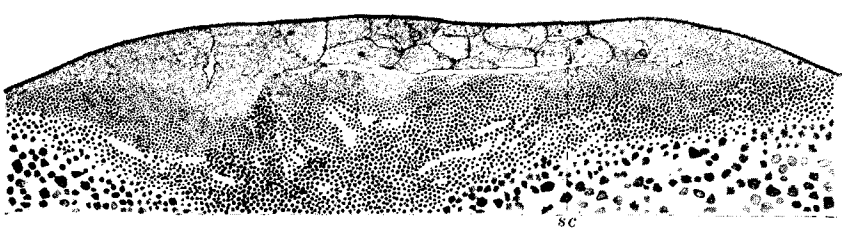

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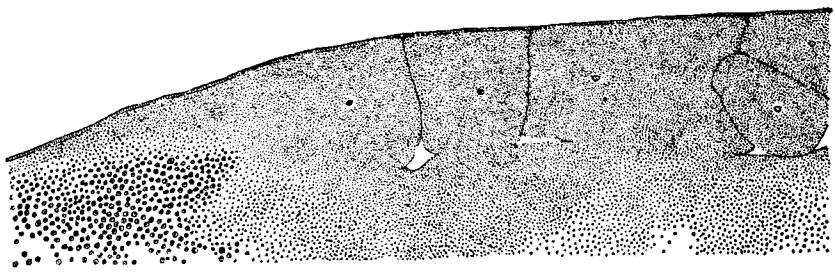
FIG. 26.—Median longitudinal section of a blastoderm in a stage of development corresponding to that of the preceding figure. *s.c.*, segmentation cavity. × 55.



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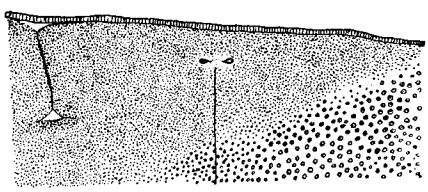

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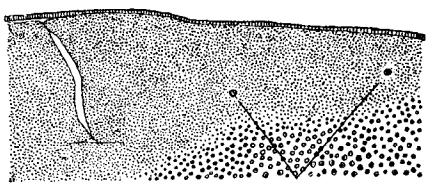

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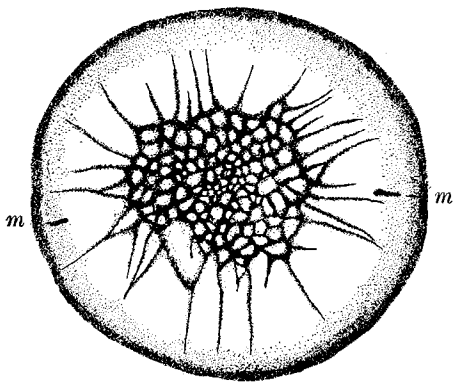
FIG. 27.—The anterior end of a section located to the right of the one shown in the preceding figure. This gives the details of structure of the margin of the disc. Some of the nuclei are taken from adjacent sections. × 139.

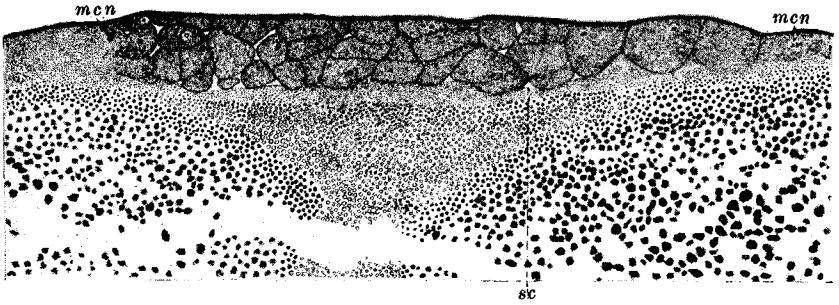
FIG. 28.—Section of a marginal cell from the same series as the section shown in Fig. 31. *m.c.n.*, marginal cell nucleus, undergoing division. × 139.

FIG. 29.—Another marginal cell from the same series, showing how the sister nuclei have migrated apart. *m.c.n.*, sister nuclei of the marginal cell. × 139.



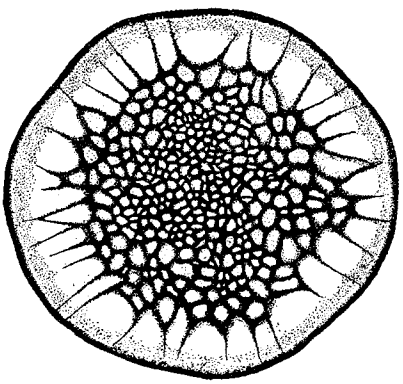
30

FIG. 30.—Surface view of a blastoderm of an egg taken from the shell-gland seven hours after fertilization (see Table 1, hen No. 8). There are 31 marginal and 123 central cells, or a total of 154 in the surface view. *m.*, short radial furrows at the margin. × 18.



31

FIG. 31.—Median longitudinal section of a blastoderm in a stage of development corresponding to that shown in Fig. 30. *m.c.n.*, marginal cell nucleus; *s.c.*, segmentation cavity. × 65.



32

FIG. 32.—A blastoderm showing 346 cells in the surface view, 34 marginal and 312 central (see Table 1, hen No. 9). Note that the marginal cell furrows are beginning to cut out into the periblast. × 18.