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GASTRULATION IN THE PIGEON'S EGG— A MORPHOLOGICAL AND EXPERIMENTAL STUDY

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GASTRULATION IN THE PIGEON'S EGG—A MORPHOLOGICAL AND EXPERIMENTAL STUDY.[1]

BY

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I. INTRODUCTION.

In view of the fact that the bird has long been the classic type in the field of embryological research, it is surprising that the question of the origin of the entoderm[2] in this form should have

[1]From the Department of Zoölogy, University of Chicago.

[2]The terms entoderm, gut-entoderm, and invaginated-entoderm will be used synonymously throughout this paper.

remained unsatisfactorily answered. Nearly all recent writers acknowledge that the problem is far from being solved. Thus Nowack, writing in 1902, admits that he has failed to make clear the exact manner in which the entoderm takes its origin. He says, "Ich bin leider nicht in der Lage, auf Grund meiner Präparate eine absolut sichere Erklärung über die Entstehung des inneren Keimblattes zu geben. Das aber kann ich mit aller Bestimmtheit behaupten, dass das Entoderm nicht als eine Einstülpung am Rande des Blastoderms entsteht, wie es nach Duval der Fall sein soll."[3] The study of comparative embryology, nevertheless, would lead us to expect to find this germ layer arising by a process of gastrulation. Aside from a few descriptions of isolated stages, however, the theory of gastrulation is supported, by actual observations, only in the work of Duval ('84); but Duval's interpretation has been disputed on the ground that he was probably misled through the use of pathological material (Kionka, '94, Barfurth, '95, Schauinsland '99); and, as I have previously pointed out ('07, *b*), this author's work tends to support the idea of *delamination*. In this connection the statement of Hertwig ('03) is of special interest, in that he has often quoted Duval in support of gastrulation, but now says, "Der Darstellung Duval's war ich in meinem Lehrbuch längere Zeit gefolgt, halte sie aber jetzt nicht mehr für richtig und glaube, dass die in Fig. 482[4] am hinteren Rand der Keimhaut abgebildete Spalte zwischen Embryonalzellen und peripherem Dottersyncytium durch die Hartung oder beim Schneider künstlich erzeugt ist und mit einer Gastrulation nichts zu thun hat."[5]

Before a complete history of the early development of the bird can be written, therefore, it is necessary to give a detailed account, not only of gastrulation itself, but also of the stages preceding and immediately following it. Such an account is rendered possible by the fact that the writer has been able to secure a close series of stages covering this period of development.

The results recorded here are the outcome of a line of investiga-

[3] *Loc. cit.*, p. 27.
[4] Hertwig here refers to Fig. 8, Plate I, of Duval ('84).
[5] *Loc. cit.*, p. 861.

tion suggested to me by Professor Whitman, to whom I am indebted, not only for scholarly criticism, but also for his inspiring ideals of research. This paper is one of the series designed by Professor Whitman for the purpose of giving an account of the Natural History of Pigeons.[6] I also wish to express my gratitude to Professor F. R. Lillie for his assistance, and to the other members of the department for their interest in the work.

II. Material and Methods.

For the purpose of these studies the egg of the common pigeon offers several advantages over that of any other bird. (1) Its small size makes it especially easy to handle in preparing sections. (2) The fact that this bird breeds readily in confinement renders it possible to secure absolutely fresh material. (3) Undoubtedly the greatest advantage, however, is that of being able to secure all the early stages of development in definite sequence. This is made possible by the regularity of the laying habits of the pigeon, which ordinarily lays two eggs for each sitting. The first is laid late in the afternoon, usually between four and six p. m., and the second between one and two p. m. on the second day following. Harper ('04) has shown that fertilization in the latter egg occurs shortly before it enters the oviduct, about four hours after the first egg is laid, that is, at about eight p. m. The second egg is, therefore, forty-one hours in passing down the oviduct. Hence, by killing birds at various hours in the interval between the two eggs a close series of developmental stages can be secured. Such a series is indispensable to the discovery of demonstrative evidence of gastrulation and to a correct interpretation of the attendant phenomena.

In fixing the egg I have followed the method employed by Harper, in that the whole yolk is fixed and hardened before any attempt is made to cut out an oriented block of yolk containing the blastoderm. For fixing, various reagents have been employed, but the picro-acetic mixtures have proved superior to all others and during

[6] The series so far embraces the following: Guyer ('00), Harper ('04), Blount ('07), Patterson ('07 b), Riddle ('08).

the past year have been used almost exclusively. It was found advisable to vary the percentage of acetic acid with the age of the blastoderm.

For the most part, Delafield's hæmatoxylin has been used for staining, although iron hæmatoxylin and carmine have been employed. In connection with the cytological work I have used the anilin dyes to good advantage.

In stages prior to the appearance of the primitive streak it is necessary to determine the orientation of the blastoderm before using the fixing fluid. Fig. I, the scheme for orienting, shows that the axis of the embryo meets the chalazal axis at an angle of 45° instead of at right angles, as is the case in the chick. For experi-

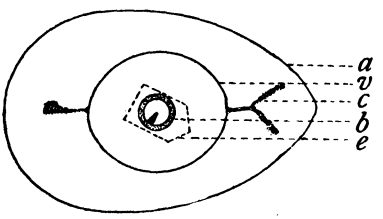

FIG. I. Scheme for orienting the blastoderm of the pigeon's egg in cutting sections. *a*, shell; *b*, blastoderm at the first appearance of the primitive streak; *c*, chalaza, which is sometimes double at the pointed end of the egg; *v*, vitelline membrane; *e*, wedge-shaped block of yolk containing the blastoderm which is cut out and embedded for sections.

mental work it is very important to know whether or not this angle is constant, particularly in experiments designed to demonstrate the movement of materials in the blastoderm. In order to determine this point, the record of about 200 eggs was kept, from which it was found that eight per cent show abnormal chalazæ. Of those with normal chalazæ, ninety per cent show the angle to be 45°, while in the remaining ten per cent it varies 1-5° from this angle. In the case of abnormalities the defect is usually found at the broad end of the egg, where the chalaza is either rudimentary or entirely wanting, or else its place of attachment to the vitelline membrane varies. In any of these cases the angle may vary greatly, even as much as 180°.

In most eggs the attachment of the chalaza to the membrane at the pointed end of the egg is much more intimate than at the opposite end. This, together with the fact that the position of the embryo upon the blastoderm is constant, has led the writer to believe that the chalazæ play an important rôle in maintaining the orientation of the egg in the oviduct. It seems very probable that the place for the attachment of the chalazæ to the vitelline membrane, at least at the small end of the egg, is determined in the ovary.[7]

From what has just been said it is obvious that eggs with abnormal chalazæ can be used neither for experimental work nor for sections, because the plane of section can not be determined. Consequently the utmost care has been taken in this work to detect and discard such eggs.

Special attention has been given to methods and means of experimentation, for it became increasingly apparent as the work progressed that there was need of a much more refined technique than that used by previous workers in this field. I have, in sterilization and in opening and closing the window in the shell, employed, in the main, the methods described in a previous paper (Patterson, '07, *a*), and they therefore need little explanation. A one tenth per cent solution of bichloride of mercury is used for sterilizing all instruments, except the operating needle, which is sterilized in alcohol. The window in the shell is made by the aid of a fine pair of forceps, and after the operation is performed, this opening is sealed with a piece of shell from a fresh egg, and a piece of sterilized cotton is placed over the closed window. The egg is then revolved until it is completely inverted, and thus, as the yolk turns, the blastoderm is brought uppermost into a normal environment. "Control" eggs show that by this method, not only is infection reduced to the minimum but also that the retardation in development which ordinarily accompanies this kind of work, is greatly reduced. After the operated egg has been developed for the desired time, it is taken from the incubator and the upper half of the shell removed. This allows one to determine the relation existing between the axis

[7]The writer has made observations and experiments to determine this point, but as yet they are incomplete.

of the embryo and that of the chalazæ, and consequently enables one to decide whether or not it is necessary to discard the egg.

For making the injury a No. 16 "bead" needle is employed. Although the diameter of this needle is small, yet it is entirely too large for very fine work, and so it was ground down to the desired diameter on an emery stone and then polished on a fine water-stone. By this means I have been able to secure a needle-point with a fineness of about 0.04 mm.

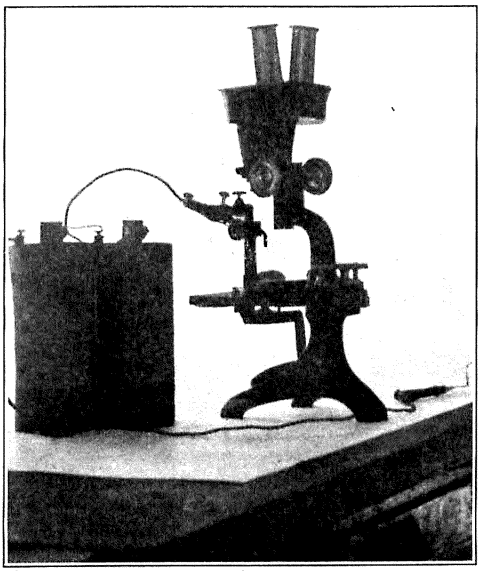

FIG. II. Apparatus used in operating.

By the aid of a special piece of apparatus, which is, in part, a modification of the one described by McClendon ('06), the needle is inserted in the blastoderm at the desired point. This apparatus is attached to a binocular and consists of an upright post fastened to the left end of the sliding bar of a Spencer mechanical-stage. Within the post the vertical end of an elbow is moved up and down by means of a rack and pinion. On the free end of the horizontal part of the elbow is a clamp which works on a universal joint. In operating, the needle-holder, which is connected with two dry battery

cells (see Fig. II), is held in the clamp, and by means of the universal joint the point of the needle is brought to bear directly over the blastoderm. To make the injury, the operator observes the magnified blastoderm (magnified 12.6 diameters) through the binocular, and with the right hand moves the needle horizontally by the mechanical-stage until the needle-point is directly above the place to be injured. The point is then inserted by adjusting the rack and pinion with the left hand, and the circuit is completed immediately by touching the second needle to the albumin. The extent of the injury can be regulated either by the number of battery cells included in the circuit, or by the length of time the current is allowed to run.

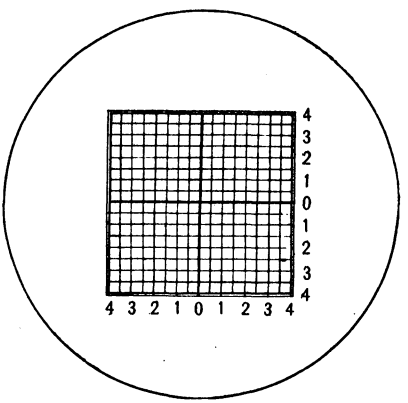

FIG. III. Eye-piece micrometer which is placed in one of the oculars of the binocular, and thus the blastoderm appears to the observer as plotted into small squares.

In order not to expose the blastoderm unduly while operating on early stages, it is highly desirable to have some quick and easy method for locating the place to be injured. This is done by using a net-micrometer, which is placed in one of the oculars, and thus the blastoderm is plotted into small squares. Two grades of micrometers are used, one ruled into 0.1 mm. and the other 0.5 mm. squares. A drawing of the latter kind is shown in Fig. III. In practice, the egg is placed in a depression at the top of a large cork, which, with the egg, can then be moved about on the stage of the binocular. In this way

the center of the blastoderm can be made to coincide with that of the micrometer. The numbers at the sides of the ruled area allow one to determine quickly the dimensions of the blastoderm, and at the same time the record of an injury in any quadrant is easily read in the terms of its co-ordinates.

In operating with this instrument there is a three-fold advantage

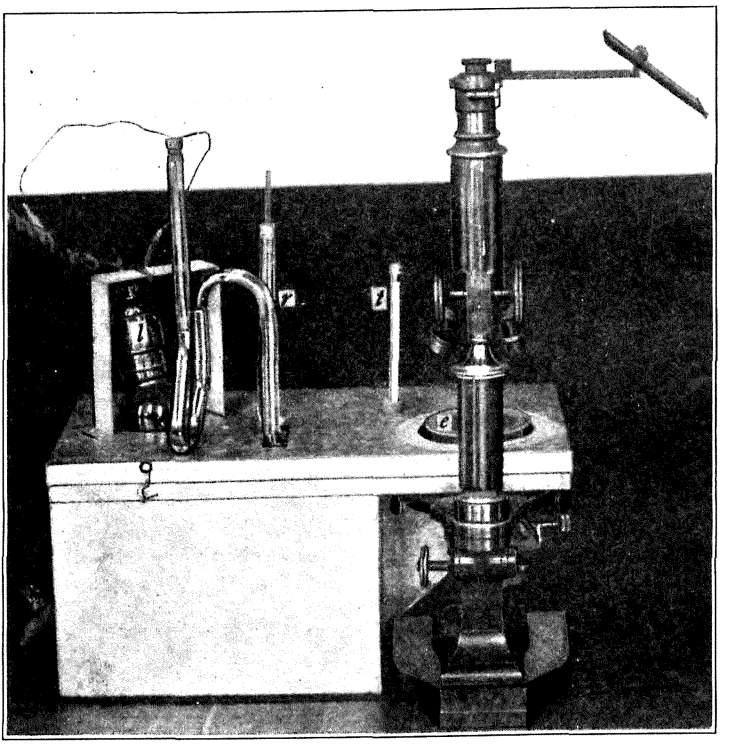

FIG. IV. Microscope-stage incubator used in studying the living egg.

over the free hand method: (1) the place to be injured is easily located; (2) the injury is made with mechanical precision; and (3) consequently the results obtained for any given set of operations are practically constant.

In connection with the experimental work as well as with the study of sections it is important to make direct observations on the

developing egg. In order to do this it was necessary to devise a *microscope-stage incubator,* a photograph of which is shown in Fig. IV. This apparatus is so constructed that it can be used with either a binocular or compound microscope, and, in case the latter is employed, camera drawings can be made of the object under observation.

The water in the incubator is heated by an incandescent lamp (*l*) controlled by an electric thermoregulator (*r*), which can be adjusted so that any constant temperature may be maintained in the region of the egg-cell (*e*).[8]

To study the developing egg, a hole is made in the shell and the blastoderm thus exposed is covered with fresh albumin. It is then placed in the egg-cell and nearly surrounded with sterile physiological salt solution, and the whole dish is covered with a thin glass plate. In this moist chamber eggs develop normally at least for several hours (in one case for thirty-three), and I have been able to study them, not only during the entire period of gastrulation, but also during many cleavage stages.

III. Gastrulation.

A. Study of the Developing Egg.

The individual variation in the development of pigeon eggs amounts ordinarily to about two hours, although in some cases it may reach as high as five hours. Owing to this variation it is difficult to set exact time limits to the process of gastrulation. In general, however, it may be said to occur between thirty-four and thirty-seven hours after fertilization. This conclusion is based on the fact that the youngest and oldest stages of gastrulation are usually found in eggs taken thirty-four and thirty-seven hours respectively after fertilization, and is further supported by the data gathered from a study of the developing egg. This does not mean that gastrulation in a given egg lasts for three hours. Indeed, in all probability not over two and a half hours elapse between the involution of the posterior margin and the closing of the blastopore.

[8]For a description of this incubator, see the Biol. Bull. for May, 1908, Vol. XIV, No. 6.

In order to understand fully the process of gastrulation, it will be necessary to consider somewhat in detail a series of stages covering a period of at least thirteen hours preceding the involution of the margin. Indeed, a knowledge of the entire history of cleavage is necessary; for all these early stages may be said to be preparatory to gastrulation. It does not fall within the scope of this paper, however, to consider these earlier periods. They have been studied and described by Miss Blount ('07). According to her account the supernumerary sperm nuclei disappear between ten and twelve hours after fertilization, and the marginal cells then "open peripherally and the periblast becomes organized with nuclei derived from the cleavage nucleus." From this time on the blastodisc increases in diameter by the addition of cells from the marginal and central periblast, cells which are "individualized" about the periblastic nuclei.

In the study of surface views of the developing egg, the changes observed between twenty and twenty-eight hours after fertilization are not very noticeable, for during the greater part of this period the blastoderm appears as a white opaque disc, there being no differentiation into areas opaca and pellucida. The disc, however, is not of equal opacity in all places, for the central region is more opaque than the marginal zone, these two parts gradually merging into each other. From the twentieth to the twenty-fifth hour[9] the margin of the disc is very irregular and gradually fades out into the surrounding zone of white yolk, which, for the most part, constitutes the "marginal periblast." From the twenty-fifth to the twenty-eighth hour the margin gradually becomes more regular and distinct, and at the same time the central opaque region increases rapidly, almost doubling its diameter. By the twenty-ninth hour the margin is still more regular and distinct, and the circumference of the disc is almost a circle (Fig. V, *A*).

Between the twenty-ninth and thirty-first hours the entire disc becomes more uniformly opaque, that is, the marginal region becomes thicker. This condition lasts but a few minutes, for almost immediately a small area lying just posterior to the center of the disc

[9]Throughout this paper the age of the egg will be designated by the number of hours that have elapsed after fertilization has taken place.

gradually becomes less opaque (Fig. V, *B*). This eccentrically lying region is the beginning of the area pellucida, and is brought about by the development of the subgerminal cavity, together with the thinning-out of that portion of the disc lying directly above this cavity. At first the boundary between the areas opaca and pellucida is very indistinct. In fact, this is more or less true throughout the entire period of gastrulation, and it is not until just a few hours before the egg is laid that a sharp differentiation between these two areas is established—a condition characteristic of the unincubated blastoderm.

Within forty-five minutes after its appearance, the area pellucida has practically doubled its diameter (Fig. V, *C*), this expansion taking place most rapidly toward the posterior margin. During the next two hours and a half the changes consist in an extension of the processes just described (Fig. V, *D-F*). In some cases the area pellucida extends almost to the posterior edge of the blastodisc, while in others it is difficult to determine from surface views the exact condition of this margin. Under high magnification, however, *the posterior edge of the disc is seen to differ from the rest of the margin, in that it does not blend into the surrounding yolk, but ends rather distinctly.*

At about thirty-four hours there occurs the most significant change yet observed. It is the appearance of an *indentation at the posterior edge of the blastodisc. This bay is the beginning of the gastrula-invagination, and often takes the form of a distinct marginal notch* (Fig. V, *G*). The edge of the disc included within the limits of the bay is to be regarded as the dorsal lip of the blastopore, and, owing to a slight opacity in this region, stands out in sharp contrast to the rest of the margin. During the next half hour the blastoporic margin changes from that of a notch to that of a broad shallow bay (Fig. V, *H*), finally becoming straight (Fig. V, *I*). This straight margin then becomes slightly rounded and less opaque (Fig. V, *J*), and at the same time the rest of the blastodermic edge is sharply defined. This change in the contour of the margin is due to the origin of the *region of overgrowth,* a structure that will be understood better from a study of sections.

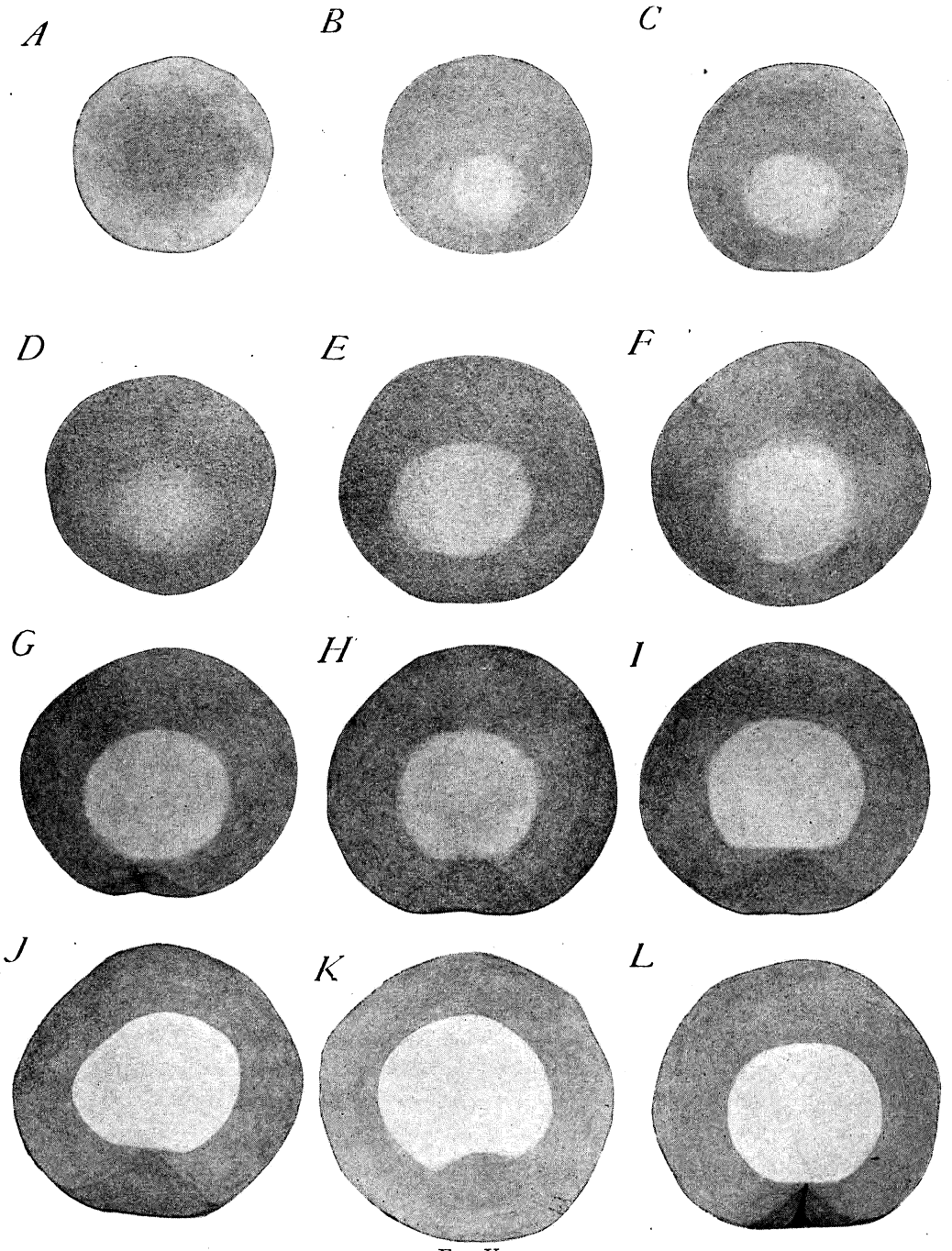

FIG. V.

Except in "rare" cases, all traces of the blastoporic bay are lost by the thirty-seventh hour, and the circumference of the blastoderm is again a circle. The only difference between the anterior and posterior halves of the blastoderm is found in the slightly opaque area lying between the areas opaca and pellucida at the middle of the latter half (Fig. V, *K*), and even this opacity usually disappears by the time the egg is laid, that is, by the forty-first hour. Hence the surface view of a freshly laid egg will give one no indication of the morphological difference existing between these two regions of the blastoderm.

Throughout the period of gastrulation the entire blastoderm grows less opaque—a change due to the progress made by the thinning-out of the blastodisc.

In Fig. V, *L* is shown one of two cases that have been observed in these studies, and that are of the greatest interest. Both of these blastoderms show a white opaque streak extending across the dorsal lip of the blastopore from the area pellucida to the posterior margin. This streak is narrow next the pellucid area, but posteriorly it becomes broader and its lateral edges are continuous with the right and left margins of the dorsal lip. The streak represents the line of fusion of the halves of the dorsal lip, for, as we shall see, these halves are moving from a lateral into a median position and

FIG. V. This figure shows a series of drawings made by the aid of camera outlines from the developing egg. *A-D* are all from a single egg, and E-K from another. *L* is from a free hand sketch of a blastoderm taken about thirty-six hours after fertilization. The other drawings were made at the following periods: *A*, 29 hours; *B*, 31 hours; *C*, 31 hours 45 minutes; *D*, 32 hours 25 minutes; *E*, 33 hours 30 minutes; *F*, 34 hours; *G*, 34 hours 15 minutes; *H*, 34 hours 45 minutes; *I*, 35 hours 30 minutes; *J*, 36 hours 15 minutes; *K*, 37 hours 15 minutes. Owing to the individual differences in the development and size of various blastoderms at a given time, any one of the above surface views would not necessarily correspond to that of another egg taken at the time indicated. The two eggs from which these sketches were made were selected because they gave the appearance ordinarily met with at these times, as determined by the continuous study of several eggs throughout the period covered. It is not unusual to find a blastoderm, taken as much as five hours earlier than that figured in *B*, showing a pellucid area. All the sketches are × 12.

uniting along the middle line of the future embryo. This process of "concrescence" is operative in all cases, even though there is no perceptible streak in the majority of blastoderms. The question of concrescence will be considered in connection with the section on experimental studies.

B. Study of Sections.

a. Pregastrular Stages.

In the study of sections, it will suffice to begin by describing a blastodisc in what I shall term a late cleavage stage. A median longitudinal section (Fig. 1, Pl. I) shows that the blastodisc is thinner directly above the "Nucleus of Pander" than in other regions, except at the margin where it may be but one cell thick (Fig. 1, *a*). This thin marginal condition corresponds to the less opaque marginal zone seen in surface views from the twentieth to the twenty-eighth hours, and is brought about, as Miss Blount has shown, by the manner in which the blastodisc is increasing in diameter. External to the margin are periblastic nuclei about which cells are formed and added to the edge of the disc (anterior end of Fig. 1). This process may continue to such an extent that a row of several cells will be seen in section. This is not always the case, for periblastic nuclei are also present in the yolk lying directly beneath the thin margin; and about these nuclei, cells are organized and added upward to the disc, so that the margin may become more than a cell thick (posterior end of Fig. 1). Directly above the Nucleus of Pander, between the white yolk and the deeper cells of the disc, is the fissure-like segmentation cavity (*sc*), and between the edge of this cavity and the margin of the disc is a zone, in which the cells are open below to the white yolk. This region is more or less of a syncytium, in which cell boundaries are either wanting or very indistinct. It exists around the entire margin of the disc, and constitutes *the zone of junction.*[10]

[10]In my preliminary paper I used the term germ wall to designate this zone, but for the sake of unity it has seemed advisable to employ the term zone of junction instead. There is no objection to using this term to designate the entire zone at this stage, at least so long as one bears in mind the fact that the inner part of this zone is potential germ wall.

In connection with this stage (Fig. 1) it remains only to call attention once more to the thinness of the blastodisc above the segmentation cavity (*sc*). While there is some evidence in favor of the view that this thin condition existed from an early cleavage stage, yet, in the light of subsequent events, it lends itself to another interpretation, namely, that it is the beginning of a thinning-out process which will eventually succeed in producing a one-layered condition of the segmentation cells. In other words, all the cells of the segmented disc finally arrange themselves into an epithelial-like structure, the primary ectoderm. This interpretation for Fig. 1 receives support also from a study of several slightly younger stages, which show the disc to be from three to five cells thick in the central region.

As we have seen in surface views, the thinning-out does not begin exactly in the center of the disc (Fig. 1), but slightly posterior to this place, and then spreads in all directions but with more rapidity toward the posterior margin. This thinning-out evidently brings about a rapid centrifugal expansion of the disc, for there is no other period in the early history of the blastoderm in which there is such a rapid increase in the surface area, as occurs during the time when the thinning is at its maximum.

Coincident with the thinning-out, but not connected with it, another important process makes its appearance, that of the interruption of the posterior zone of junction. This interruption is associated with the degeneration of the periblastic nuclei beneath the zone of junction. The presentation of the facts upon which this conclusion is based must be deferred until more advanced stages have been described.

Let us consider next a series in which the progress of the thinning-out as well as that of the interruption of the zone of junction can clearly be seen. A median longitudinal section of such a stage is shown in Fig. 29, Pl. IV. The blastoderm from which this photograph was made is considerably in advance of that of Fig. 1 and would correspond to stage *D*, Fig. V. In addition to the progress made in the thinning-out and the interruption of the posterior zone of junction, the more important changes are (1) the great increase in the number of cells, and (2) the extension of the segmentation

cavity, which we may now call the subgerminal cavity.[11] At the posterior end (Fig. 29, *p.*) the blastoderm is only a single cell thick, but towards the anterior it gradually increases in depth. Although the anterior fourth of the disc is at least six cells deep, yet distinct layers can not be made out, but the cells are more or less loosely arranged. In the enlarged drawing of the anterior end the details

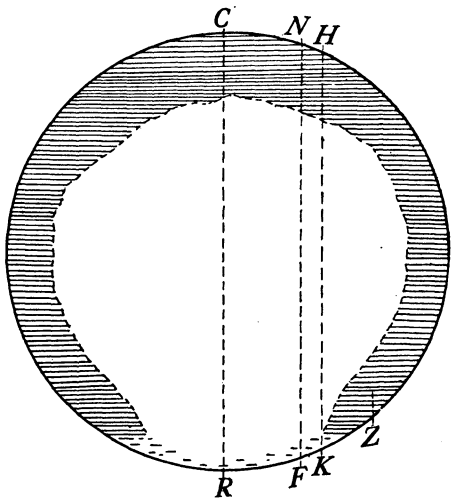

FIG. VI. A diagrammatic reconstruction from camera drawings of the sections of a blastoderm taken about thirty-three hours after fertilization. The lines *CR*, *NF* and *HK* are the planes of sections of Figs. 29 (or 2 and 3), 4 and 5 respectively. The zone of junction (*z*) is all but completely interrupted at the posterior margin. × 27.2.

of the zone of junction are shown (Fig. 2, Pl. I, *z*). Cells in every stage of formation are present, and at *ce* is one completely formed about a periblastic nucleus, and toward the center are several others undergoing the same process. The whole region from the letter *z* to the left end of the drawing is a syncytium—a region containing many periblastic nuclei.

[11]Although the term subgerminal cavity is here used in the sense in which it is usually employed, namely, to designate an enlarged segmentation cavity, yet it should be said that from the standpoint of comparative embryology, it has little or no significance.

At the posterior end of this same section (Fig. 3) an entirely different condition is found. Aside from the thinness of the margin, the almost entire disappearance of the zone of junction is the most characteristic feature. The only remnants of it are the degenerating periblastic nucleus (*pn*) and the single cell which is about to arise from the yolk (*ce*). For a considerable distance on either side of the posterior portion of this section the only normal periblastic nuclei visible are the few which are in the last stages of acquiring distinctly outlined cell limits. All other nuclei are in some phase of degeneration. About twenty-five sections to either side of the median line, however, the uninterrupted zone of junction is again found. In Fig. 4, which is taken twenty sections to the right of the center, two normal nuclei are forming cells about them (*ce*), and to the left of these there is a completed cell. Two degenerating nuclei are also present (*pn*). Five sections farther to the right, the first indication of a true zone of junction is found (Fig. 5). The zone here is very narrow, but still farther to the side it becomes much wider (see Fig. VI).

From this time on the thinning-out of the blastoderm and the interruption of the posterior zone of junction make rapid progress, until about thirty-one to thirty-three hours after fertilization, when the zone is completely interrupted for a distance of 70-80 degrees (Fig. VII) (cf Fig. V, *F*). Comparing Fig. 29 with that of a longitudinal section of such a blastoderm (Fig. 30), it is apparent that the condition of the latter has been brought about as the result of processes already described in connection with the former, and consequently, the section ends posteriorly in a thin free margin (Fig. 14), with the zone of junction entirely wanting. In passing forward, however, one finds a gradual increase in the thickness of the blastoderm (Fig. 30). The subgerminal cavity, which has increased both in depth and extent, is occupied by many segmentation cells, which, for the most part, lie in a row near the floor of the cavity. The position of the cells is purely an artifact—a condition produced during fixation; for a study of this section under high power reveals the fact that the upper contour of every nucleated cell or group of cells lying in the cavity exactly corresponds to the under

contour of the overlying cells. This is especially clear in the photograph at the point marked x as well as in other parts of the blastoderm. Hence, if it were possible to view this section in the living condition, the subgerminal cavity would be seen to contain few or no nucleated cells; for all these cells would then be crowded up

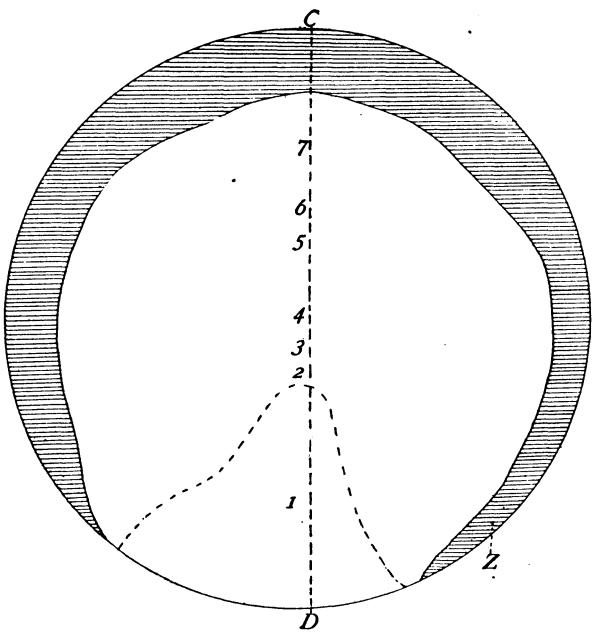

FIG. VII. A diagrammatic reconstruction of a blastoderm taken thirty-one hours after fertilization. It is farther advanced than the majority of eggs at this time. Numbers 1, 2, 3, etc., represent the regions of the blastoderm which are one, two, three, etc., cells deep, respectively. The broken line around "1" indicates the region where the depth is approximately one cell. The plane of the section for Fig. 30 is slightly to the left of line *CD*. × 27.2.

against the under surface of the blastoderm. Their present position within the cavity shows that they have loosened and sunk down during fixation. There would be, however, in the living condition, a few large non-nucleated yolk masses (m), as the present position of these bodies indicates that they have arisen out of the yolk lying beneath the floor of the cavity.

Since, in preparing sections, it is impossible to avoid entirely this artifact, it is important to recognize its significance.[12] A failure to do so might easily lead one to believe that after the completion of the primary ectoderm there would be many cells within the subgerminal cavity to form a "loose layer," and thus to attribute to this latter a possible origin of the gut-entoderm (delamination theory).

It is at about this stage of development (Fig. VII) that the initial step in gastrulation occurs, but before taking up that part of the description I must digress in order to make clear the probable significance of the method by which the avian blastoderm thins out. In this connection one naturally turns to the field of comparative embryology for suggestions, and here, if I mistake not, much evidence is found for an explanation of this interesting process. It will be necessary, however, to call attention to some well known facts in embryology, even at the risk of being somewhat tedious.

First of all, we may refer back to a holoblastic egg such as that of the primitive vertebrate Amphioxus. Here blastulation consists merely in an epithelial arrangement of the blastomeres to form a hollow sphere, and only the slightest difference in size exists between the blastomeres of the vegetative and those of the animal hemisphere—a difference, perhaps, anticipatory of a meroblastic condition.

In the egg of Petromyzon, which has greater meroblastic tendencies than the preceding but is still holoblastic, Hatta ('07) describes and figures a thinning-out of the upper hemisphere that begins approximately in the region where gastrulation is soon to appear and proceeds anteriorly, thus finally resulting in a one-layered condition of this hemisphere. The process, however, is not finished until just before the completion of gastrulation. He believes that this differentiation is brought about by the deeper cells pushing in between

[12]Many fixing fluids have been tried in an endeavor to overcome this artifact, but even in the best fixed series a few cells drop down, showing that they were not tightly wedged in between the upper cells. In one case I have succeeded in fixing an egg in an inverted position, and in this the subgerminal cavity is practically free from nucleated cells.

the more superficial ones. He says, "in the part where differentiation is going on, the cells of the outer row and those of the inner rows are found pushing between one another, and the layer of such condition passes over gradually into the part which has already become a true epithelium."[13] The expansion of the upper hemisphere necessarily brought about by this differentiation plays an important rôle in gastrulation.

Hatta points out the homology existing between the blastulation of Amphioxus and the differentiation of the micromeric layer into an epithelium in Petromyzon. He contends that since it is incorrect to speak of a "blastula" stage in Amphioxus before the blastomeres "are converted into the form of an epithelium," so in Petromyzon it is correct to speak of blastulation only when differentiation of blastomeres into an epithelium has begun. In regard to the latter form he writes, "In this case blastulation, as indicated by differentiation of the blastomeres into an epithelium, should be looked upon as being much delayed; it is still being carried on during the whole period of the gastrulation and is finished only a little earlier than the latter process. In other words, the two processes, blastulation and gastrulation, overlap each other to a great extent in the period of their occurrence. The prime cause of this belated mode of development is indisputably due to delay of segmentation on account of an enormous accumulation of yolk within the ovum."[14]

Without referring to the various eggs showing intermediate conditions, we may consider next the thinning-out in an egg in which the accumulation of yolk within the ovum is carried almost to the extreme, that is, in a meroblastic egg such as that of the Selachian *Torpedo* (Zieglers, '92), or *Pristiurus* (Rückert, '99). In *Torpedo* Ziegler figures and describes a "blastula" stage in which the posterior portion of the blastodisc is differentiated into a single-layered epithelium, while anteriorly it gradually increases in depth and the cells are not arranged in the form of an epithelium. At this stage, invagination of the thin posterior margin begins and soon after, the differentiation (thinning-out) extending both anteriorly

[13] *Loc. cit.*, p. 24.
[14] *Loc. cit.*, p. 35.

and laterally reduces the entire central region of the blastodisc to a single layered epithelium. Concerning this extension of the differentiation, he writes as follows: "Die epitheliale Schichte ist jetzt in ziemlich gleichmässiger Weise an der ganzen Oberfläche des Blastoderms zur Ausbildung gekommen (Fig. 2). Offenbar sind also die Zellen, welche in dem früheren Stadium (Fig. 1) den dickeren Theil des Blastoderms bildeten, in dieses epitheliale Blatt eingetreten, indem die tieferen Zellen sich aufwärts nach der Peripherie bewegten und sich dem Epithel einordneten; daher nahm die epitheliale Schicht beträchtlich an Ausdehnung zu und in Folge dessen hat das Blastoderm jetzt eine grössere Länge und Breite und ist ein Umstülpungsvorgang am Hinterrande des Blastoderms eingeleitet worden."[15]

Almost the same words could be employed in describing the changes which occur in a pigeon's blastoderm after it has reached a stage corresponding to that shown in Fig. VI. Hence, the process of thinning-out of the avian blastoderm, as well as that of the selachian, is to be regarded as homologous with the process of blastulation in Amphioxus and Petromyzon. There will be, undoubtedly, a wide difference of opinion as to the advisability of using the term "blastulation" to describe this process; for the term blastula has been employed for stages which cover a wide period of development. Ordinarily, however, it is used to designate that stage of development just preceding the gastrula-invagination—a stage in which the segmentation cavity is more or less enlarged. The result is that the so-called blastulæ of the various vertebrates have not the same morphological value.

As to when one should call the pigeon's egg a blastula, will depend on the criteria adopted. Using the term as it is variously applied among the different vertebrates, one might speak of a blastula from the eight-cell stage to the beginning of invagination, and, adopting Hatta's suggestion, even to the end of gastrulation. It is obvious, therefore, that the term could be used only in the most general way. I prefer to avoid it altogether, and for that reason, shall

[15] *Loc. cit.*, p. 58.

simply speak of the thinning-out process, by which I mean the differentiation of the cleavage cells into a single layered epithelium above the enlarged segmentation cavity (subgerminal cavity).

In this connection I must speak of the probable reason why the thinning-out process affects the posterior region of the blastoderm first. I have come to regard this as signifying that the posterior region is farther advanced in its differentiation than other parts. This interpretation is in harmony with the general law for the early development of the embryo, namely, that differentiation progresses from the head end backward. As we shall see later, it is in the posterior central part of the blastoderm that the head of the embryo will arise.

b. Gastrulation Stages.

(1) Invagination.

If the thinning-out were completed before the invagination began, the interpretation of the steps of gastrulation would be greatly facilitated. But such is not the case, for immediately following a stage such as shown in Fig. 30, the initial step in gastrulation occurs. This consists in the rolling under of the free posterior margin of the blastoderm. The reconstruction of a blastoderm in which the involution has just taken place is shown in Fig. VIII, and a surface view of a corresponding stage is seen in Fig. V, *G*. In this egg (Fig. VIII) the zone of junction is not essentially different from that seen in Fig. VII, except at the anterior inner margin, where a portion of it has given rise to a partial germ-wall (*gw*). The numbers scattered over the figure indicate the relative depths of the various regions. Thus in the central area, the blastoderm is thinned-out to one or two cells, while the marginal parts are much thicker, varying from two to four cells. In the extreme posterior is shown the region covered by the invaginated entoderm (*E*).

The posterior portion of an oblique section passing through the region of invagination is represented in Figs. 32 and 15. At the extreme posterior is a cavity (Fig. 15, *c*) which is bounded above by the vitelline membrane and below by the yolk, or ventral lip of the blastopore. In reality the cavity is but a portion of the blasto-

pore (*b*), which passes beneath the dorsal lip (*d*) to become the archenteron (*ac*). Directly above the archenteron is the invaginated entoderm (*e*), and just in front of the anterior limit of this is a portion of the subgerminal cavity (*sg*), above which the blastoderm is two cells thick, but anterior to which it is three thick. Owing to the obliquity of the plane of section, the wrong impression is given as to the condition of the blastoderm directly in front of the central

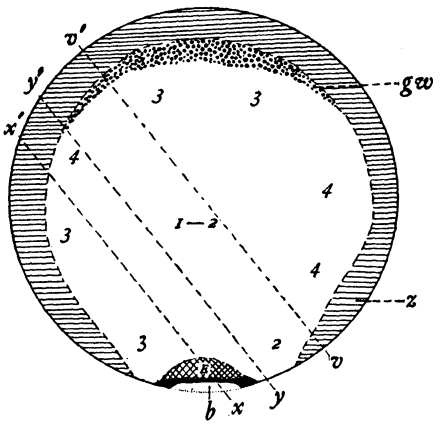

FIG. VIII. A diagrammatic reconstruction of a blastoderm taken thirty-four hours after fertilization, or seven hours before laying. Invagination has just taken place and the entoderm (*E*), a tongue-like process, is starting to grow forward through the subgerminal cavity. As indicated by the numbers, the blastoderm is thinned out to one or two cells deep in the central part, while around the anterior and lateral margins it varies from two to four deep. The anterior inner edge of the zone of junction is differentiated into a germ wall. In this, as in the other reconstructions, the ectoderm is not represented.

part of the invaginated region. If the series had been cut parallel to the longitudinal axis of the future embryo, a median section would have been diagrammatic in clearness, that is, it would show what we should expect to find in case of a true involution. The condition of the central part of the blastoderm, however, can be inferred from the photograph shown in Fig. 49. On either side of the invaginated region the posterior ends of the sections terminate with thin free margins (Fig. 16), and differ from those in the invaginated area, therefore, in having no cavity posteriorly.

I have said above that invagination takes place by a turning or rolling under of the free margin. It is important to show that there is a plain rolling under, and the following facts are offered as proof. First, as regards the morphological evidence; I think it is clear from the above description that this line of proof strongly supports the conclusion. There is no other explanation for the appearance of a cavity just beyond the posterior margin (Fig. 15, *c*) than that it was brought about as the result of the rolling under of the edge and of the simultaneous forward growth of the involuted cells.

This conclusion can be tested experimentally; for an injury made on the edge of the thin posterior margin (Fig. IX) just previous

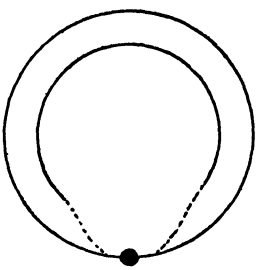

FIG. IX. Scheme for the operation in Experiment I.

to gastrulation ought to be carried down beneath the blastoderm during the course of further development, that is, it ought to be found in the entoderm.

Experiment I.

The operation was made thirty-three and one-fourth hours after fertilization, and the egg was then incubated for three and three-fourths hours. The result of the injury is shown in the posterior end of the median section (Fig. 66). There is a distinct dorsal lip, in which the deeper portion shows the cells affected by the operation. In the vitelline membrane, a short distance posterior to the dorsal lip, is the break made by the operating needle (at *op*). All of the injured cells are found in the entoderm, while the ectoderm is well differentiated almost back to the end of the section. Just ante-

rior to the dorsal lip the entoderm is almost wanting (Fig. 67). In an uninjured blastoderm at a corresponding stage of development the entoderm in this region is very thick (see Fig. 37). It is clear

TABLE I.

	Ser.	Age.	Antero-post. diameter.	Trans. diameter.
Late pre-gastrular stages....	304	31 hrs.	2.857 mm.	2.857 mm.
	427	31 "	3.333 "	3.333 "
	346	32 "	3.428 "	3.428 "
	411	33 "	3.411 "	3.411 "
	326	34 "	3.809 "	3.809 "
Gastrulation stages.....	394	34 "	3.333 "	3.619 "
	342	34 "	3.285 "	4.238 "
	368	34 "	2.571 "	2.667 "
	370	34 "	3.428 "	3.524 "
	372	34 "	2.761 "	3.142 "
	190	35 "	2.860 "	3.333 "
	409	35 "	2.400 "	2.857 "
	164	36 "	2.860 "	3.333 "
	178	36 "	2.857 "	3.333 "
	188	36 "	2.860 "	3.048 "
	254	36 "	2.857 "	3.048 "
	330	36 "	2.952 "	3.333 "
	362	36 "	3.000 "	3.500 "
	406	36 "	2.400 "	2.857 "
	176	37 "	2.660 "	2.857 "
Early post-gastrular stages..	162	37 "	3.247 "	3.333 "
	256	37 "	3.333 "	3.429 "
	390	37 "	3.333 "	3.429 "
	332	38 "	3.428 "	3.428 "
	316	39 "	2.762 "	2.762 "
	166	40 "	3.524 "	3.524 "
	382	40 "	3.524 "	3.524 "

therefore, that while such an operation destroys most of the cells that are to give rise to the entoderm, yet the posterior margin is still capable of forming a rounded dorsal lip.

Measurements taken on the living eggs also can be interpreted in support of this view; for such data show that previous to and following gastrulation the blastoderm is approximately circular, while during gastrulation the antero-posterior diameter is always shorter than that of the transverse (Table I). This is what we should expect in case the margin actually involuted.

Owing to individual variation in the size of different blastoderms at the same stage of development, it is impossible to determine, from the above table, whether the antero-posterior diameter is actually shorter after the beginning of gastrulation than just preceding the

TABLE II.

	Egg 448.			Egg 440.		
	Time.	Ant. Post.	Trans.	Time.	Ant. Post.	Trans.
Pre-gastrular stages	6.00	2.857 mm.	2.857 mm.	6:00	3.510 mm.	3.510 mm.
Gastrulation stages	6.30	2.762 "	2.953 "	6:15	3.333 "	3.570 "
	7.00	2.857 "	3.047 "	6:45	3.451 "	3.689 "
	7.30	2.953 "	3.095 "	7:30	3.510 "	3.748 "
	8.30	3.142 "	3.238 "	8:15	3.570 "	3.748 "
Post-gastrular stages	9.30	3.242 "	3.242 "	9:15	3.808 "	3.808 "

same, or whether it is only relatively shorter in comparison with the transverse diameter. If it can be shown that the former alternative is the true one, then the evidence for a "plain rolling under" of the margin will be well nigh conclusive. This I have been able to do by studying the living egg and taking measurements of the same blastoderm at different periods of its development. In the above table are given the data from such measurements taken on two eggs.

Finally, the above interpretation for the origin of the entoderm is in harmony with the views of a large majority of the investigators who have worked on other groups of vertebrates. It is with the fish, however, that the most interesting and instructive comparisons are to be drawn. The large size of the selachian ovum,

together with the fact that this form is a more generalized type, would seem to indicate that the development of the avian egg ought more nearly to approach that of the selachian than that of the teleostean ovum, and so far as the thinning-out is concerned, it does; but as regards the involution of the margin it more closely resembles the teleostean type. Thus, Agassiz and Whitman ('84) state that in Ctenolabrus there is a "plain rolling under, or involution, as an initiatory step in the formation of the ring." However, they regard it more correct to describe the process "as an ingrowth, due both to a rapid multiplication of cells, and also to the centrifugal expansion of the ectoderm."[16] The ingrowing under layer in the pigeon's blastoderm with its free inner edge is in many respects comparable to the "ring" in the teleostean blastoderm, and is, therefore, to be regarded as a highly modified germ-ring. It is, of course, only a partial ring, in that but a small part (at most an arc of 70-80 degrees) of the margin invaginates, while in the ordinary teleost an invagination occurs around the entire margin. In the egg of the Toad-fish (Batrachus tau), however, we have an interesting modification of the germ-ring, a condition which can be understood best by quoting a part of the summary of Miss Wallace ('99), who has described the development of this ring. She writes as follows: "In the egg of Batrachus there is a centripetal growth of cells at the embryonic pole, the ingrowth having a voluted outline in sections. Around the remainder of the blastoderm there is not even the appearance of an invagination, but only a slight thickening due to an ingrowth of cells from the ectoderm, and a few loose cells which may represent a true germ-ring found as a layer in ordinary forms. The peripheral thickening gradually fades out, first at the anterior pole, until the last remnant is found in a few cells lying beneath the ectoderm, forming a linear streak from the posterior end of the embryo to the lip of the closing blastopore."[17]

We have in the egg of the Toad-fish a condition intermediate between such a form as Ctenolabrus and the Pigeon. The eggs of these three forms represent a series in which the differences in

[16] *Loc. cit.*, p. 68.
[17] *Loc. cit.*, p. 12.

development are measured by the relative quantities of yolk accumulated within the ovum. Thus in the Ctenolabrus egg, which contains the least amount of yolk, invagination occurs around the entire margin of the blastoderm, but stronger at the embryonic pole; in the Batrachus egg, which contains much more yolk than the preceding, there occurs only a slight thickening about the greater part of the margin as "the initiatory step" in invagination, this thickening soon disappears, and at the embryonic pole alone is there a true germ-ring formed; and finally, in the Pigeon egg, which is loaded to the extreme with yolk, invagination occurs at the "embryonic pole" only, the greater part of the margin lacking even "the initiatory step."

(2) *Middle and Late Gastrulation Stages.*

The entoderm after reaching a stage such as shown in Fig. 32 continues to grow forward through the subgerminal cavity as a tongue-like process. At the same time the thinning-out progresses anteriorly and laterally, ordinarily with sufficient rapidity to keep ahead of the advancing entoderm. This results in most blastoderms in the formation of a space just in front of the anterior limit of the entoderm. This space is but a part of the subgerminal cavity that is free from segmentation cells, the latter having passed upward into the differentiating ectoderm. In some few cases, however, no space is found and in such it is impossible to determine the anterior limit of the entoderm.

The posterior end of a median longitudinal section, in which the length of the invaginated layer equals about one third of the diameter of the blastoderm, is shown in Fig. 34. Only a part (about one-third) of the above mentioned space is included in the photograph. The ectoderm above the space, as well as posterior to it, is not yet differentiated into a single layer, but here and there the lower segmentation cells are seen apparently crowding in between the upper ones. A group of such cells is shown at *s*. The dorsal lip of the blastopore is much thicker than in Fig. 32, and the method by which it increases will be discussed in another connection. It

is sufficient to state here that in all probability it is not brought about alone by the multiplication of cells *in situ*.

A section lateral to the median line shows essentially the same conditions as Fig. 34, except that the entoderm does not extend so far anteriorly (Fig. 33, *e*).

The question must naturally arise in the reader's mind as to whether or not the upper layer is still rolling under at the posterior margin to give rise to the lower layer. The appearance of sections would seem to indicate that it is (*e. g.*, Fig. 33). The question can be tested, however, by experimentation, for if a rolling under is occurring, cells disturbed by an injury made on the extreme posterior margin of the dorsal lip, ought to be found later in the entoderm.

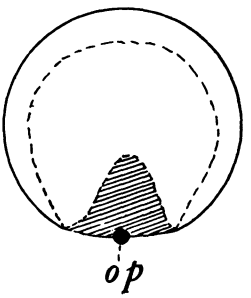

FIG. X. Scheme for operating in Experiment II.

Experiment II.—The scheme for such an operation is shown in Fig. X, and the result in Fig. 50. The injured cells are found immediately associated with the entoderm. This is especially clear in a transverse section through the affected region (Fig. 51). There is no evidence of an injury either in the ectoderm or mesoderm, and hence we must conclude that the affected cells have been brought to their present position by an actual rolling under of the posterior margin. Although this operation has been repeated several times with the above result, yet the position of the injury in the entoderm may vary in an antero-posterior direction; but this variation is easily accounted for by the fact that one can tell in the living egg only approximately the extent to which invagination has progressed.

If an injury be made in the same manner as above on slightly

older blastoderms the affected region is not found in the entoderm, but in the ectoderm and mesoderm, showing that the involution has ceased, and the further extension of the entoderm is now brought about by an ingrowth, in which cell division and the centrifugal expansion of the ectoderm play an important rôle. The latter two processes are doubtless factors in the extension of the entoderm throughout the entire period of involution, but they are not so conspicuous during the earlier stages of invagination.

We must now pass to a series in which gastrulation may be said to have reached its height, and one in which several structures and processes hitherto unnoted must be considered. The reconstruction of this series appears in Fig. XI. The tongue-like process of entoderm (*E*), the dorsal lip of the blastopore (*D*), the germ-wall (*GW*), and the zone of junction (*z*) were considered in connection with Fig. VIII, but they have all undergone important changes. Thus, the germ-wall extends almost around the whole inner margin of the zone of junction, and on the lateral margins its cells extend into the subgerminal cavity, within the edge of the area pellucida. This extension of cells is not due to an ingrowth from the inner edge of the germ-wall, but rather to the spreading of the subgerminal cavity by the liquefaction and fragmentation of the underlying yolk.

The changes in the dorsal lip consist in the growth of the right and left halves toward each other and their simultaneous fusion in the middle line, that is, in the plane of the longitudinal axis of the future embryo. A blastoderm in which the line of fusion was seen in surface view is shown in Fig. V, *L*. The movement of material is participated in by the more lateral parts of the margin, namely, the horns of the zone of junction, and as they move toward the median line they are at the same time being carried centrifugally by the expansion of the blastoderm, and in this way the fused halves of the lip are gradually being enclosed within the inner edge of the zone. The question of this movement of material will be fully discussed in connection with the description of experiments performed to throw light on the method by which the embryo arises.

The region of overgrowth (*O*), which is represented in the figure

as a crescent-shaped area extending around the anterior and lateral margins, is a structure hitherto not noted. It arises, however, at an earlier period than this, and consists in the outgrowth of the marginal cells beyond the zone of junction.

Besides the entoderm and the germ-wall cells (at the sides) there are many large yolk masses within the subgerminal cavity, and also a few of the lower segmentation cells that have sunk down from the

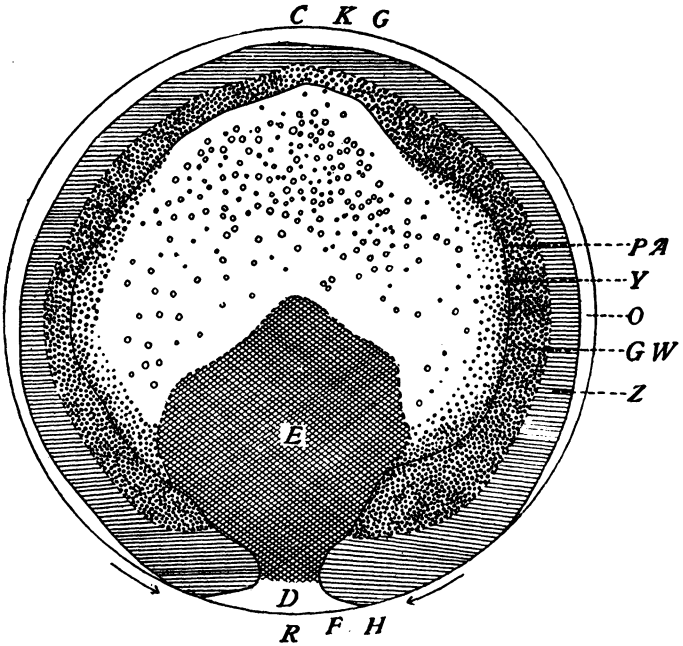

FIG. XI. A diagrammatic reconstruction of a blastoderm taken thirty-six hours after fertilization, or five hours before laying. It represents the ectoderm as transparent. *O*, region of overgrowth; *Z*, zone of junction; *Y*, germ-wall cells beneath which the subgerminal cavity has spread; *D*, dorsal lip of the blastopore; *PA*, outer boundary of the area pellucida; *E*, region covered by the invaginated or gut-entoderm. Lines drawn through *CR*, *KF*, and *GH* represent the planes of the sections illustrated in Figs. 35, 40, and 41 respectively. The anterior margin of the entoderm as here represented is only the average for the different lengths of entoderm as measured in the sections, from which the reconstruction was made. The arrows at the posterior margin indicate the direction of movement of the halves of the dorsal lip. × 27.2.

under surface of the differentiating ectoderm. That part of the subgerminal cavity lying beneath the entoderm (*E*) is to be regarded as the archenteron, which communicates with the blastopore by a narrow passage situated just below the dorsal lip (*D*). At its union with the deeper cells of the lip, the entoderm is very thick, but gradually thins out anteriorly, ending with a thin irregular margin slightly beyond the center of the blastoderm.

The changes described above can be made clearer by a study of longitudinal sections. Thus in the photograph of a median section (Fig. 35) the various regions are easily recognized, and in the enlarged drawing of the posterior end (Fig. 19) the dorsal lip is seen to be composed of compact cells, all of which are completely delimited by cell-walls. Directly above the lip there is no distinct ectoderm, but anterior to the point *u* it is well differentiated and in only a few places (*s*) are lower segmentation cells crowding upward into it. The entoderm at its union with the deeper cells of the lip is five cells thick, but anteriorly gradually decreases, finally ending with a free margin (Fig. 35, *e*—to the left). At this stage the entoderm can not be said to be a distinct layer, for its cells are arranged more or less into groups. In the archenteric cavity (*ac*), which lies between the entoderm and the yolk, are several large yolk masses, some of which are in the act of rising from the floor. Posteriorly the archenteron communicates by a narrow passage with the blastopore.

The conditions presented in this section very much resemble those of a corresponding section from a teleostean blastoderm in which invagination is well advanced. Thus Miss Wallace's ('99) Fig.5, Pl. III, not only compares very favorably with Fig. 19 in the appearance of the dorsal lip, but also as regards the method by which the entoderm grows forward; for I consider it better to describe the entoderm as now advancing anteriorly by a multiplication of its cells and their gradual arrangement into a single layer. This view is in accord with the account for Ctenolabrus as given by Agassiz and Whitman ('84). At this stage the main difference between the teleostean and pigeon blastoderms is that in the former the ectoderm is from three to five cells thick at the embryonic pole, while in the latter it is but one cell thick (Fig. 37). This difference

is doubtless to be accounted for by the fact that the teleostean embryo is precociously formed, that is, as compared with the formation of the avian embryo.

The conditions at the anterior end of this section (Fig. 18) are entirely different from those at the opposite end. First of all, the differentiation of the ectoderm into a single layered epithelium is not complete, for in many places the lower segmentation cells are crowding upward against its under surface, although some of them have sunk down into the cavity (*s*), but aside from these few the subgerminal cavity is entirely free from nucleated cells anterior to the fore end of the entoderm (*e*). There are found in the cavity only large yolk masses, some of which are disintegrating (*dm*).

The germ-wall is not well differentiated in this section, but in the sections to either side it is clearly defined.

The region of overgrowth (*o*) is a wedge-shaped process extending out from the zone of junction. The earliest stage in which this region has been observed is illustrated in Fig. 27, and is characterized by having no periblastic nuclei either beneath it or external to it, and by having a fine granular area just beneath its under surface.[18] This region arises when the thinning-out is at its maximum and at first is three or four cells thick, but later becomes reduced to a single layer of cells (Fig. 28).

The phyletic significance of this region is not clear. On the one hand, it might be compared to the overhanging margin of the selachian blastoderm, and thus be regarded as showing a tendency toward a "peripheral gastrulation." Its appearance in an unincubated chick blastoderm would favor this view (Fig. 65). On the other hand, the fact that it first arises at the anterior margin and is not a continuation of a dorsal lip (Fig. XI), would indicate that it was not comparable to the margin of the selachian blastoderm. The answer to this question, however, turns upon the view one takes as to the extent of the blastopore. I cannot agree with those in-

[18]In the series shown in Fig. 10 but a single nucleus was found beneath the overgrowth (Fig. 25), and this one had doubtless arisen from the nucleus lying below the zone of junction when that region formerly occupied the margin of the blastoderm.

vestigators (Haeckel, Balfour, Goette, and others), who have maintained that the entire margin of the avian blastoderm is to be regarded as the blastopore, for the evidence furnished by my material is conclusively in favor of the view that but a small part of the margin is the blastoporic region. The rest of the margin (overgrowth region) I regard therefore as a specialized region, rather than as a place where the upper germ-layer bends under to become continuous with the lower layer.

The three regions, overgrowth, zone of junction, and germ-wall, are all concerned in the spreading of the blastoderm over the yolk. Since the region of overgrowth has no periblastic nuclei either beneath or external to it, its spreading over the yolk can not be due to the addition of cells from the periblast, unless it be indirectly from the zone of junction. However, the fact that its cells are undergoing rapid division makes it almost certain that the spreading of the overgrowth is due to the multiplication of its own cells. This conclusion is strengthened by the fact that the cells are digesting the underlying yolk as indicated by the fine granular area.

As the overgrowth travels peripherally over the yolk, it is followed by the zone of junction, which in turn is differentiating, from its inner edge, ectoderm above and germ-wall below (see anterior end of Fig. 35). The first two regions seldom have greater widths than those in this series (cf. Figs 18 and 28), and hence the germ-wall is continually increasing in width. The subgerminal cavity is also increasing in diameter, but at a slower rate, and in this widening of the cavity there are left around its margin cells which were previously embedded in the yolk. These cells (Fig. XI, Y) constitute the under loose layer of the area opaca, and later enter into the formation of the yolk-sac entoderm, and, according to Ruckert, '06, also contribute to the vascular tissues. The inner edge of this lower layer becomes united to the free margin of the invaginated entoderm, when the latter spreads over the subgerminal cavity sufficiently to meet it. The first place for this union to occur is necessarily at the postero-lateral regions, and the last place is at the anterior end of the cavity.

Sections taken slightly to either side of the median line are of interest, in that they have vacuoles or cavities in the dorsal lip

(Figs. 38 and 39). The position of the cavities suggests that they are probably to be regarded as the remains of the cavity that was formed between the upper and lower layers when the former turned under to give rise to the latter. I might suggest that there is another possibility, namely, that such cavities are the homologue of "Kupffer's Vesicle."

Fig. 40, which is from a section taken through the plane *KF* of Fig. XI shows the tip of the right horn of the zone of junction

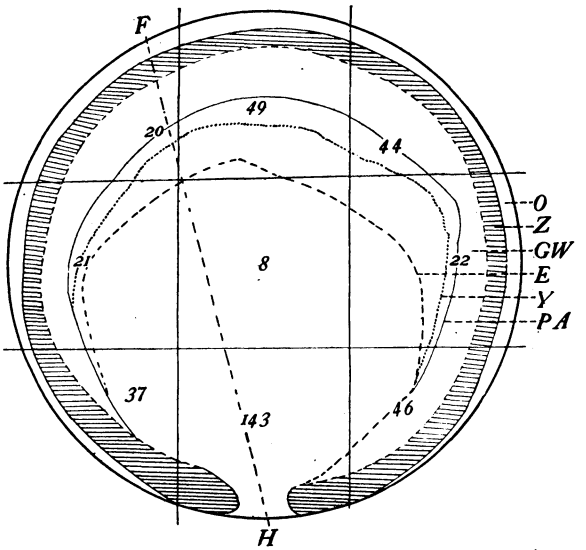

FIG. XII. Reconstruction of a blastoderm taken thirty-six and one-fourth hours after fertilization. Lettering is the same as in Fig. XI, *FH*, plane of section represented in Figs. 20 and 21. The numbers within the areas formed by the four intersecting lines indicate the number of degenerating periblastic nuclei in these areas. × 27.2.

and the lateral part of the dorsal lip. The length of the lip in section becomes less and less in passing laterally, finally disappearing altogether. Thus in Fig. 41 it is no longer present, and the margin is occupied by the zone of junction, inside of which is a region whose position would lead one to call it germ-wall, but it is probably more correct to regard it as a portion of the lip that has already been enclosed within the horns of the zone. Passing still farther to the

side one finds the posterior margin becoming less thick and gradually taking on the syncytial condition characteristic of the anterior and lateral parts of the zone of junction. However, it is not until one has reached about 45 degrees to either side of the median line that the posterior margin is found to be reduced to the average thickness of the rest of the edge.

Closing of the Blastopore.—It was stated above that the entoderm grows forward through the subgerminal cavity. The source from

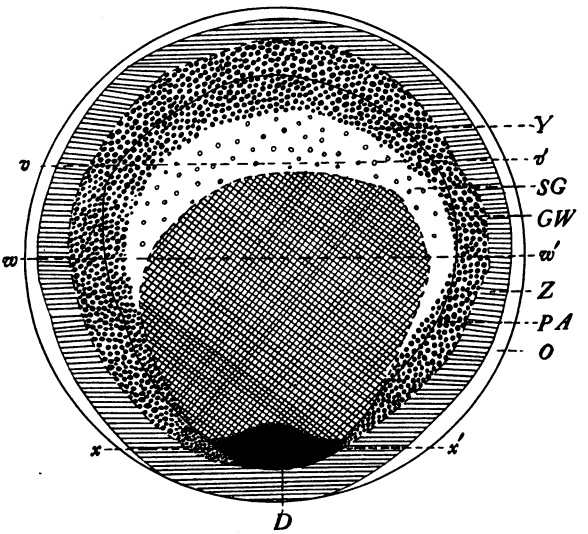

FIG. XIII. Reconstruction of a blastoderm taken thirty-five and one-fourth hours after fertilization. The blastopore has just closed, and the zone of junction completely encircles the blastoderm. *D*, is the enclosed dorsal lip. vv', ww', and xx' are the planes of the sections illustrated in Figs. 24, 23, and 22 respectively. × 27.2.

which it draws its material for this forward growth is, of course, the thick dorsal lip. This results in producing either one of two conditions in the lip at the time the blastopore is closed. On the one hand, the entoderm may have grown forward to such an extent as to have produced a very perceptible diminution in the lip before the closing occurs. Such is the case in the blastoderm represented in Fig. XII, as is apparent from the median section (Figs. 20 and

On the other hand, in the majority of blastoderms there is no apparent reduction in the lip preceding the closing, but it remains quite as thick as that of Fig. 37, even after being entirely enclosed within the zone of junction. This can be made out from a series of transverse sections of the blastoderm shown in Fig. XIII. Thus in the section taken through xx′ the entoderm is at least five cells thick, and passes over gradually into the region of the zone of junction (Fig. 22). Above the entoderm a distinct ectoderm is differentiated, but slightly posterior to this it can no longer be distinguished, and still farther back is found the zone of junction, which completely encircles the blastoderm (Fig. XIII, Z). Anteriorly the entoderm rapidly thins out, the cells being arranged in groups (Fig. 23), which become less and less thick, finally disappearing altogether, so that only a few cells are found (Fig. 24).

Interruption of the Posterior Zone of Junction.—We are now in a position to consider the interruption of the zone of junction. It was stated above that the interruption was associated with the degeneration of the periblastic nuclei in the region of the posterior zone. Abundant evidence is found for this statement in the study of any egg taken either just before or during gastrulation. Thus in Figs. 8-13 is shown a series of nuclei in various stages of degeneration. The first indication of the breaking down process is found in the increase in the size of the nucleus. In this condition the nuclei do one of two things. In some cases, they stain intensely (Fig. 8) and apparently the nuclear membrane breaks down directly, leaving the chromatin lying free within the yolk (Fig. XIV, *A*). In the large majority of cases, however, they continue to increase in size and at the same time their capacity for stains gradually diminishes, until it is difficult to study them at all after the use of hæmatoxylin. When they have increased to a volume equal to many times that of an average normal nucleus, they begin to divide (rarely into equal parts—as in Fig. 10), or rather portions are pinched off from the sides of the nucleus (Figs. 9 and 11)—the process continuing until the entire nucleus is reduced to small fragments (Figs. 12 and 13). Finally one sees among the yolk spherules only clear spaces, which indicate the places previously occupied by these fragmenting nuclei.

Abnormal yolk or periblastic nuclei are found in many meroblastic eggs, especially those of the fish. Several of the nuclei figured by Raffæle, '98, for Belone, greatly resemble what I have observed in the Pigeon, and in the eggs of Squalus also are found many such nuclei. So far as I am aware, no one has described the complete fragmentation of the periblastic nuclei in the bird's egg, although Harper, '04, observed abnormal ones in early stages of the pigeon's egg. He regarded these as sperm nuclei, but in the light of Miss Blount's, '07, work, they are doubtless to be considered as periblastic nuclei, and are therefore undergoing this disintegrating process.

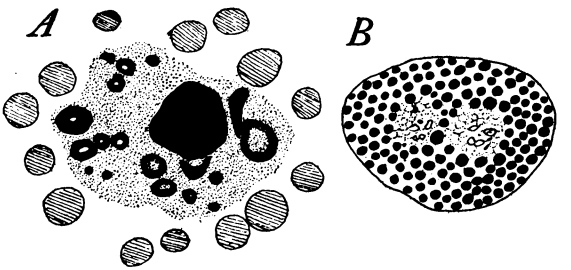

FIG. XIV. *A* is from the blastoderm shown in Fig. XII. It shows the chromatin lying free in a finely granular area among the yolk spherules—the nuclear membrane having disappeared. *B* is a yolk mass in which are two fragmenting nuclei. This mass had arisen from the yolk lying beneath the archenteron, and doubtless had taken up two degenerating nuclei which were in the central periblast. × 250..

The position of these nuclei within the egg is of importance; for in the main they are located in the region where the zone of junction is being interrupted. In the blastoderms illustrated in Figs. VI and VII they are found mainly in the yolk lying beneath the posterior margin—the rest of the edge being almost wholly free from them, in some cases entirely so. Later, when invagination begins, they are found around the greater portion of the margin (*e. g.*, in the series shown in Fig. VIII), and still later, they may be seen in all parts of the edge, but not in such abundance in the anterior half of the blastoderm as in the posterior, except during late gastrulation, when practically all of the nuclei beneath the archenteron have completely disappeared. In some few cases, however, even in late gastrulation,

there are many degenerating nuclei found in the yolk lying beneath the floor of the archenteron, but in such the nuclei are in the very last stages of disintegration (Fig. XIII). The absence of yolk nuclei beneath the archenteron is not characteristic of the birds alone, for in some of the Selachians also the same condition is found (*e. g.*, in Torpedo and Squalus).

In the interruption of the posterior zone of junction we have another line of comparison with the teleostean development; for this process is but the separation of the blastoderm from the underlying periblast. The comparison will become all the more obvious when we shall have shown experimentally that approximately that portion of the margin of the avian blastoderm, beneath which the zone of junction has disappeared, enters into the formation of the embryo. In other words, in the teleost the entire margin of the blastoderm separates from the periblast, and this whole margin (germ-ring) concresces to form the embryo; whereas, in the case of the bird, only about seventy to eighty degrees of the margin of the blastoderm parts company with the periblast, and just about this portion of the posterior edge is concerned in the process of concrescence.

Since abnormal nuclei are found as early as fifteen hours after fertilization (Harper, '04), there would seem to be some doubt regarding the possibility of such nuclei being instrumental in bringing about the interruption of the posterior zone of junction. Furthermore, I have found degenerating yolk nuclei in eggs taken several hours after gastrulation. Nevertheless, there is certainly no period, aside from that of gastrulation, in which they are in such abundance; and in addition to this, they are present mainly where the interruption takes place. The fact that such nuclei later are found gradually extending anteriorly around the margin, would only indicate that there was a tendency for the entire margin of the blastoderm to separate from the periblast.

c. Postgastrular Stages.

In eggs taken slightly later than the preceding, the entoderm is found not only to have grown farther forward, but also to have spread to the sides, so that its lateral margins have become united with the

inner edge of the germ-wall (Fig. XV). Hence, in transverse sections of the majority of blastoderms taken at this time, the entoderm will appear to be an outgrowth from the inner edge of the germ-wall. Fortunately, in not a few blastoderms the union between the invaginated entoderm and the germ-wall does not take place until about the time the egg is laid, and in such deferred cases it is easy to distinguish the lateral edge of the entoderm (Fig. 48), and thus to demonstrate that the gut-entoderm, at least in its lateral parts, does not receive elements from the germ-wall. Can the same be said

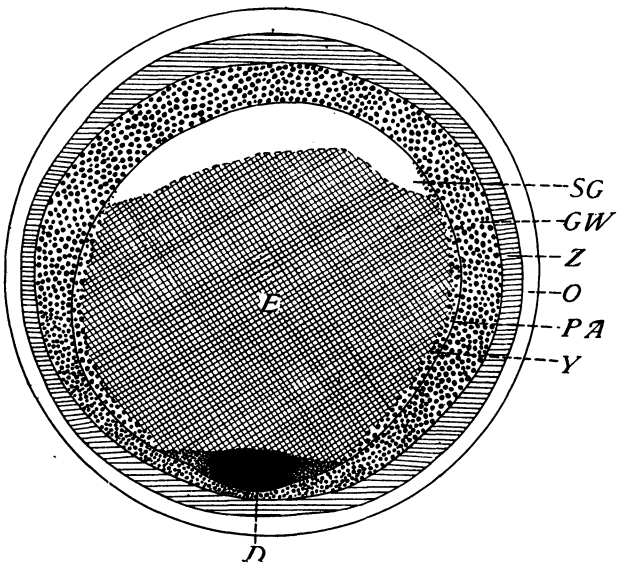

FIG. XV. Reconstruction of a blastoderm taken thirty-eight hours after fertilization. This blastoderm is approximately in the same stage of development as that of an unincubated hen's egg. × 27.2.

of its anterior and posterior parts? In regard to the former we can answer in the affirmative without hesitation; for in every blastoderm taken from the time of midgastrulation until three or four hours after incubation, there is found between the anterior limit of the entoderm and the germ-wall, a portion of the subgerminal cavity in which there are practically no nucleated cells. Indeed, in many blastoderms there are no cells, not even yolk masses (Fig.

45), and yet it is clear from measurements of such that the distance between the inner edge of the anterior germ-wall and the entoderm is growing less at the same time that the entoderm is increasing in length (Table III).

Concerning those cases in which cells (other than entoderm) and yolk masses are found in the cavity, more must be said. Many writers have described these elements in the chick blastoderm, and as far back as 1874 Goette figured them as arising from the yolk lying beneath the floor of the cavity, that is, from the central periblast. Recently Hertwig ('03) has described them, and in speaking of those that lie between the entoderm and the floor, he writes as

TABLE III.

Ser.	Age.	Length of Blastoderm.	Length of p. area.	Length of Entoderm.	Length of "Space."*
394	34 hrs.	2.190 mm.	1.547 mm.	0.119 mm.	1.428 mm.
342	34 "	2.261 "	1.666 "	0.595 "	1.071 "
254	36 "	2.667 "	2.071 "	1.476 "	0.595 "
256	37 "	2.976 "	2.262 "	1.786 "	0.476 "
332	38 "	3.190 "	2.215 "	1.786 "	0.429 "
377	40 "	3.219 "	2.310 "	1.905 "	0.405 "
341	44 "	4.048 "	2.238 "	2.143 "	0.095 "
195	46 "	4.809 "	2.619 "	2.619 "	0.000 "

* By the term space is meant the distance from the anterior limit of the entoderm to the inner edge of the exterior germ-wall.

follows: "Zwischen ihm und dem Dotterboden liegen in der Urdarmhöhle zerstreut einzelne kugelige Embryonalzellen, darunter auch grössere, dotterhaltige Kugeln, die Megasphären von His. Letztere haben nicht den Formwert einer Zelle, da Kerne auf keine Weise in ihnen sichtbar zu machen sind, wie von Gasser ('84) und anderen Beobachtern festgestellt worden ist. Sie sind daher nur vom darunter liegenden Dotter losgelöste, kugelige Ballen, die wohl allmählich zur Ernährung der Zellen der Keimblätter aufgebraucht werden. Auch im Raum zwischen den beiden Keimblättern kommen

wenige vereinzelte Zellen vor."[19] I fully concur with Hertwig's views regarding the fate of the non-nucleated yolk masses, for one can examine scarcely a series in which some evidence of their disintegration is not found. The manner in which these masses break up is of interest, in that the fragments often resemble cells. Thus, above and to the left of the mass at *dm,* Fig. 47, are smaller masses that have broken off and become spherical—a process probably comparable to the phenomena of surface tension. These smaller masses in turn continue to subdivide until the cavity may become crowded with very fine particles (Fig. 44) which in this state are doubtless taken up by the cells. The yolk masses therefore play no rôle in the formation of the primary germ layers, except, of course, indirectly as nutriment. The contention of Balfour ('73) that they may become nucleated by the formation of nuclei *de novo* from yolk spherules would scarcely accord with the views of modern cytologists.

Again, in regard to the significance of the nucleated elements within the cavity, I agree with Hertwig, who thinks that in numbers they are far too few to be of any importance in the formation of the germ layers. On making counts of these elements, I was surprised to find that in blastoderms such as shown in Fig. 47, less than two per cent of them are nucleated, and that even in this small number many of the nuclei show signs of degeneration (Fig. XIV, *B*). Not infrequently the cytoplasmic portion of such elements disintegrate, leaving the nucleus lying free within the cavity (Fig. 52, *n*). In other cases neither the cytoplasm nor nucleus breaks down at first, but the latter multiplies at the expense of the former until a solid mass of nuclei is formed (Fig. 55). Sooner or later these nuclei go to pieces.

These abnormal nuclei are to be accounted for by the fact that some of the yolk masses in arising from the central periblast (Fig. 46) naturally take up the periblastic nuclei, which, as was shown above, are degenerating. Their presence is in no way necessary to the formation of the masses, as is evident from the fact that the large majority of the masses are non-nucleated.

The phenomenon of yolk mass formation is only an index to the

[19] *Loc. cit.*, p. 858.

process of digestion, by which the blastoderm is securing its nourishment, and is doubtless similar to the phenomena of degeneration or fragmentation of the yolk that has been described by many workers on practically all of the vertebrate ova (Barfurth in the Teleosts; Dean in the Chimæroids; Stahl in the Reptiles; Ruge and Born in the Amphibians; Pflüger in the Mammals; Brunn and others in the Birds).

There are a few small cells within the cavity that are still to be accounted for (Fig. 53). These may come from two sources: either they are lower segmentation cells that have failed to get into the differentiating ectoderm, or they are wandering entoderm cells (Gasser, '82). If they come from the latter source and are later taken into the entoderm, no further consideration is necessary; but if they are to be regarded as coming from the former source, we may justly ask, Why is it that when an egg is fixed in an inverted position during the differentiation of the ectoderm, no cells are found in the cavity? Whatever be their source, they are too insignificant in numbers to be of any great importance.

So far, we have considered the question of whether or not the invaginated or gut-entoderm receives cells from the anterior or lateral parts of the germ-wall, and on the whole the evidence favors the negative; but in regard to the relation of the entoderm to the posterior germ-wall, further considerations are necessary. It was stated above that as a result of the manner in which the blastopore closes, the dorsal lip comes to lie within the margin of the blastoderm. Hence, in longitudinal sections, the entoderm, while ending anteriorly with a free margin (Fig. 42), appears to arise directly from the posterior germ-wall. This apparent union of the entoderm with the posterior wall is only secondary, and the greater part of the mass of cells here belongs to the dorsal lip. This is most obvious immediately after the closing of the blastopore, when the ectoderm is not differentiated from the underlying mass (Fig. 26).

Although Balfour ('82) and many other investigators, working on the unincubated hen's eggs, have noted that the entoderm is incomplete anteriorly and united to the germ-wall posteriorly, yet Nowack ('02) was the first to clearly state that the entoderm was

to be regarded as growing forward. He, however, not having studied the earlier stages, naturally supposed that the entoderm was an outgrowth from the posterior germ-wall, and thus missed the key to the origin of this germ-layer.

During the course of further development the entoderm completely penetrates the subgerminal cavity (Fig. XV, *SG*), and at the same time the mass of cells (lower cells of the dorsal lip) at its posterior border thins out to a single layer, thus showing that these cells contribute to the entoderm in its forward growth.

IV. Experimental Studies.

While many of the foregoing conclusions were first deduced from data gathered in a study of sections, yet they are of such a nature that experimental tests can be applied readily. Only a few of the many experiments that have been performed can be offered at this time, and these are selected, not because they are of any more interest than the others, but rather because they throw light on that mooted question, "How does the vertebrate embryo arise?" The two views that have been held by students of vertebrate embryology in regard to this question are too well known to need any discussion here. Both theories have been defended by able workers, but too often the attempt has been made to support the one to the exclusion of the other. This has been especially true of those who hold to the theory of differentiation.

The results obtained by experimental investigators have not been uniform. In the main, writers have been willing to admit that only a modified form of concrescence is found in the formation of the embryo. In the few desultory experiments (Assheton, '96, Kopsch, '02) that have been made on the chick blastoderm only negative results have been found. This failure to secure positive evidence is due to two causes. In the first place, the technique has not been sufficiently refined. Thus, Assheton used sable hairs which he inserted in the unincubated blastoderm on either side of the axial line on the boundary between the areas opaca and pellucida. The results were negative, as one might expect; for who would suppose that the force exerted by the movement of materials in the delicate

blastoderm could be sufficient to overcome the resistance offered by the hair held above by the vitelline membrane and below by the yolk, even, indeed, if such material did not merely flow around the obstructing hair. In the second place, both Kopsch and Assheton were operating at a time when concrescence either had ceased altogether, or its influence was waning. Thus in Kopsch's ('02) experiment VII the operation was made after twelve hours of incubation, at a time when the bulk of the axial material had been marshalled from a lateral into a median position for a period of at least twelve to fifteen hours. It is obvious, from the above morphological data, that any experiments from which we could hope to gain any insight into the part played by concrescence, must be made during gastrulation, for concrescence and gastrulation are but different phases of the same process.

If the avian embryo is the product of concrescence and the right and left parts of the dorsal lip represent the homotypical halves of the future embryo, then injuries made on the posterior margin at different distances from the median line during early gastrulation, ought later to appear at different levels in the embryo, that is, an injury made at 10° from the median line ought to appear nearer the head region than one made at 45°. Furthermore, such injuries ought to affect only that half of the embryo which corresponds to the side of the dorsal lip injured. The progress of concrescence can be tested by operating on successively older stages. Thus, the following sets of operations will be described: Set A, on early gastrular stages; Set B, on late gastrular stages; and Set C, on unincubated and early incubation stages.

Set A—On Early Gastrular Stages.

Experiment III.

An injury made in the middle of the dorsal lip slightly within the margin (Fig. XVI, *a*) is later found in the cephalic region of the embryo, greatly disturbing the material of what is later to become the primary fore-brain (Fig. 59, *op*). While the section through this injured region shows the affected cells to be situated slightly to the right of the median line, yet the entire head-fold is disturbed

(Fig. 57). The result of such an operation leaves but two alternatives with reference to the position of the embryonic primordium at the time when the injury was made. Either we must suppose that this primordium was situated in the exceedingly small space between the operated region and the posterior margin (Fig. XVI, *a*), or that its right and left halves lay along the lateral margins, and were gradually brought together by concrescence. That the latter alternative is the correct one will become obvious from the results of the following experiments.

Experiment IV.

In this experiment the operation was made ten degrees to the right of the median line, the needle being set so that the outer edge

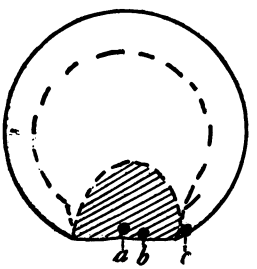

FIG. XVI. Scheme for operating in Experiments III, IV, and V.

of the resulting injury coincided with the margin of the blastoderm (Fig. XVI, *b*). After thirty-six and three-fourths hours of incubation the injury was found on the right neural fold in the mid-brain region (Fig. 63). Although the left neural fold is slightly distorted, yet the section shows with great clearness that the affected cells are found only on the right side (Fig. 62). All the structures characteristic of this region in a normal embryo, are here found well developed. As we should expect, the mesoderm and chorda are uninjured, for when the operation was performed these structures were not yet present in the head region.

Experiment V.

Passing now to the experiment in which the operation was made forty-five degrees to the right of the middle line (Fig. XVI, *c*), we

find that the injury is situated seven sections anterior to the posterior end of the resulting embryo (Fig. 71 *op*), that is, the injured cells have been moved from a lateral into a median position. In the sketch of the transverse section through the affected region (Fig. XVII) the mass of cells is seen to be located slightly to the right side. While the needle destroyed a considerable portion of the primitive streak material, yet the blastoderm has apparently recovered from the injury, with the mass of affected cells separated from the blastoderm proper.

The apparent recovery of the blastoderm from the operation is to be explained by the fact that the injury, being made so far to the side, affected a region less highly differentiated than in the case of the operation at ten degrees. That is what one would expect,

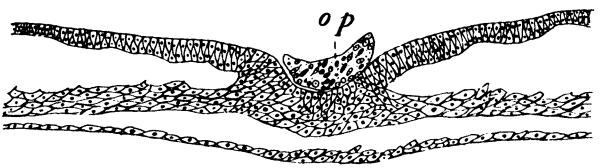

FIG. XVII. Transverse section through the injured region of the embryo shown in Fig. 71. See text for description. × 95.

for just as at any given period of the early development, the anterior portion of the embryo (or primordium) is in a higher state of differentiation than the posterior.

The results of the above experiments (III-V) show very clearly that the axial portion of the embryo arises from material previously situated in the right and left halves of the dorsal lip, material brought together by the process of concrescence. These experiments have been repeated several times, both in the manner described above, and with certain variations. Thus an operation made on the lip at twenty degrees either to the right or to the left of the axial line, is later found at the level of the anterior somites. In this paper we are not concerned, however, in analyzing the exact morphological value of the different parts of the blastoporic lip, but rather in showing that the avian embryo is the product of concrescence.

If the theory of concrescence is correct, it is obvious from these

experiments that similar operations made at a later period of development should be found located more posteriorly in the resulting embryo. Thus in a late gastrulation stage, an injury made in the middle of the dorsal lip just anterior to the posterior margin, should be found later not in the fore-brain, as in Experiment III, but at a point situated more posteriorly to that region. Furthermore, if an injury be made on the margin to the side of the axial line at such a stage, it ought later to appear in the corresponding side toward the posterior end of the embryo. The results of such operations are shown in the following set of experiments.

Set B. On Late Gastrular Stages.

Experiment VI.

The scheme for this operation appears in Fig. XVIII, *a*. It would be equivalent to injuring the posterior margin of such a blastoderm as that shown in Fig. V, *J*. The result of the operation is shown in Fig. 54, in which the injury is seen to lie at the level

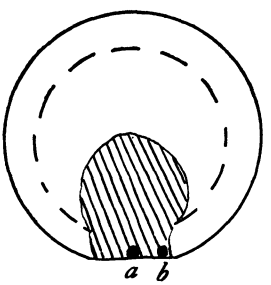

Fig. XVIII. Scheme for operating in Experiments VI, VII and VIII.

of the tenth pair of somites. Posterior to the affected region there is found a normal primitive streak, which has not yet differentiated into the posterior axial portion of the embryo. The transverse section (Fig. XIX) shows a mass of dead cells lying between the separated halves of the neural tube, and a few scattered dead cells lying just above the entoderm, which is intact. The notochord is situated on the right side.

It would seem from this result that although the halves of the dorsal lip have been unable to coalesce in the region of the injury,

yet they are capable of giving rise to the normal structures characteristic of this region. Posterior to the injury, however, they have succeeded in fusing and forming the primitive streak material.

Experiment VII.

If an operation be made similar to the preceding, but at a slightly later period, it is not found in the body of the embryo, but at the extreme posterior end (Fig. 64, *op*).

Experiment VIII.

In this experiment the injury was made twenty degrees to the right of the axial line (Fig. XVIII, *b*) at thirty-six hours after fertilization, that is, at a stage corresponding to the one shown in Fig. V. *J*. The egg was then incubated for forty-eight hours. The injury is

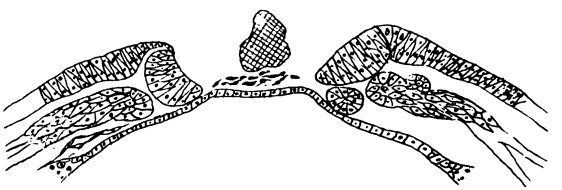

FIG. XIX. Transverse section through the injured region of the embryo shown in Fig 54. × 95.

located on the right neural fold, at about one-third the distance from the posterior end to the first mesoblastic somite (Fig. XX, *op*). The left neural fold is uninjured. The result of such an experiment admits certainly of no other explanation than that the mass of affected cells has moved from a marginal into an axial position.

SET C. ON UNINCUBATED AND EARLY INCUBATED STAGES.

In this set of experiments I shall endeavor to show that not all of the embryonic material has been brought into a median or axial position at the time when the egg is laid; but that it lies to either side, on the boundary between the areas opaca and pellucida. The presence of a slightly more opaque spot in this region has already been noted in connection with the study of surface views (Fig. V,*K*),

as well as in the study of sections (Fig. 43.) Furthermore, Koller ('79 and '81) has figured and described for the unincubated chick blastoderm a thickening in this region.

Experiment IX.

If a very small injury be made on the boundary between the two areas in line with the axis of the future embryo (Fig. XXI,*b*), it is

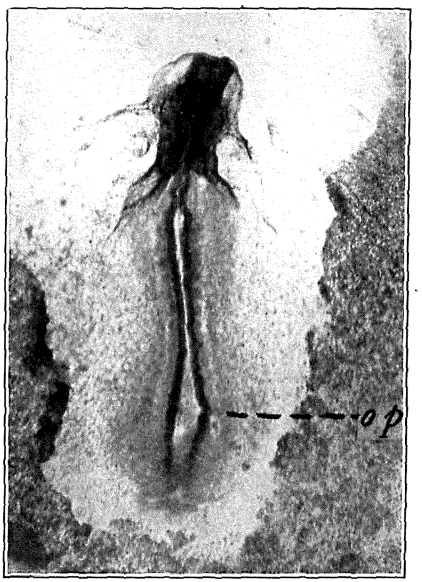

FIG. XX. A photograph of the embryo described in Experiment VIII. The injured cells are seen at *op* on the right neural fold in the region of the open myelon. The primitive streak is bifurcated at the posterior end.

found later some distance from the posterior end of the embryo (Fig. 60, *op*). Such an operation destroys a considerable portion of the primitive streak material in the region over which it extends (Fig. 58), but for twenty-five sections posterior to the injury a normal primitive streak is found. The point of interest in this experiment lies in the question regarding the source of the material from which the tail of the embryo is developed. The material must lie just posterior to the pellucid area, or to either side of the axial line on

the boundary between the two areas. If from the former source, the material would be disturbed by an injury made in the area opaca just posterior to the pellucid area; but if from the latter source, it would be affected only by operations made to the side of the axial line on the boundary between the two areas.

Experiment X.

The operation was made just posterior to the pellucid area (Fig. XXI,*a*). The injury has in no way affected the embryo (Fig. 56), but lies posterior to it in the area opaca. Assheton ('96) has performed a similar experiment on the chick blastoderm, using a sable hair instead of the needle. He also found the embryo uninjured. From the result of this operation it is evident that the material out of which the tail of the embryo is differentiated does not lie just posterior to the pellucid area.

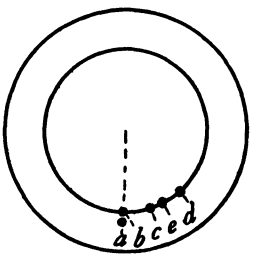

FIG. XXI. Scheme for operating in Experiments IX-XIII.

Experiment XI.

If the injury be made on the boundary between the two areas twenty degrees to the right of the middle line (Fig. XXI,*c*), it is found later drawn into the side of the embryo (Fig. 68) a short distance from the posterior end. In the transverse section through the operated region, it is seen that just half of the axial material is affected (Fig. 61). This is of the greatest importance, because it shows that the tail end of the embryo is formed by the concrescence of material lying to either side of the middle line. The limit to which material extends laterally is shown in the following experiments.

Experiment XII.

This experiment is similar to the preceding, except that the operation was made 30 degrees to the right instead of twenty (Fig. XXI,*e*), and in the resulting embryo the injury is found in the right side of the posterior end of the primitive streak (Fig. 69).

Experiment XIII.

An injury made still farther laterally (Fig. XXI,*d*) does not affect the embryo (Fig. 70), but is found in relatively the same position as that in which it was performed.

The results obtained from this set of experiments can leave no doubt concerning the presence of portions of the dorsal lip which lie between the boundary of the two areas, and which have not yet completely fused together at the time when the egg is laid. The manner by which this region is established has been considered in connection with an earlier stage (Fig. XV). It is this structure doubtless that Koller ('79) has described for the unincubated chick blastoderm, and which is often called *Koller's crescent.* In the pigeon I have never observed a "crescentric groove" as described by Koller,[20] and furthermore this region does not give rise to the entire primitive streak, but only to the posterior part. Even then it usually completely disappears three or four hours before the primitive streak becomes visible.

V. Discussion and Summary.

Discussion.

Throughout the foregoing pages the term gastrulation is employed to designate the process of invagination by which the gut-entoderm takes its rise, together with the concomitant phenomenon of concrescence. It may therefore seem to be used in a sense that does not

[20]I refer here to the presence of a groove during the early hours of incubation. It is true that in later stages (*e. g.*, Fig. 20) one sometimes finds the posterior end of the primitive streak bifurcated, but these must be regarded as much delayed cases, and are similar to those figured by Schauinsland ('03) for the sparrow.

accord with general usages; as, *e. g.*, in the case of the lower vertebrates, Amphioxus and the fishes, where the gut-entoderm, mesoderm, and chorda are all said to be involuted at the same time in the form of the primary entoderm.

This objection, however, completely disappears if the primitive streak formation is regarded as a part of the gastrular phenomenon. That such an explanation for the primitive streak formation is fully justified is obvious when considered in the light of comparative embryology. Thus in Amphioxus all of the chorda and mesoderm are derived from the primary invaginated layer. In Amphibians the posterior part of the mesoblast is formed about the lips of the blastopore, and is often spoken of as the "peristomal mesoblast," in contrast to the more anterior portion, or "gastral" mesoblast. In this case, no one hesitates to consider the whole process in Amphibians as that of gastrulation, because the "gastral" and "peristomal" mesoblast are directly continuous with one another. In the case of birds, all of the mesoblast is derived from the primitive streak, that is, it is all peristomal mesoblast. In the bird, therefore, the transition from a gastral to a peristomal mesoblastic formation has gone a step farther than in the case of the Amphibians. We have shown experimentally that as the gut-entoderm is being involuted, the concrescence of the halves of the dorsal lip is also taking place. Furthermore, there arises later from this fused region the primitive streak, or mesoblast. It is evident, therefore, that the invagination of the gut-entoderm and the primitive streak formation are but different parts of the same process, namely, that of gastrulation. The occurrence of a short period (from shortly after the closing of the blastopore to the appearance of the primitive streak), during which one cannot distinguish, either by sections or by surface views, the primitive region, in no wise invalidates the above comparison.

Hertwig ('03) has divided the process of gastrulation in the amniota into two parts or phases. He, however, had little else to offer for his first phase in the bird other than Duval's work—a work over which he himself casts a shadow of doubt as to correctness, as is evident from the citation given in the first part of this paper.

It is not my purpose to enter into a discussion of the whole ques-

tion of concrescence, for several able papers dealing with the problem have appeared from time to time since His first clearly stated the theory (Semper, '76; Whitman, '78 and '83; Rauber, '76; Kollmann, '85; Ryder, '85; Minot, '90, and others). It is sufficient to state here my conclusion, that concrescence is the method by which the avian embryo takes its rise. The conclusion is supported not only by experimental evidence, but also by the structure of such blastoderms as shown in Fig. V,*L*, as well as by that of the rare ones (Whitman, '83). In fact, it matters not from what angle we approach the problem, the conclusion is the same, namely, that the axial material of the avian embryo is derived from the fused lateral parts of the blastoporic lip.

The anterior limit to which concrescence is operative in the formation of the avian embryo is another problem, but it would seem, from the result of Experiment IV, that at least that portion of the embryo which lies posterior to the primary fore-brain is formed by concrescence. This is in accord with the experimental results of Peebles ('04) and Kopsch ('02); especially those of the latter, who maintains that all of the embryo except the pre-chordal head area arises directly from the primitive streak material (that is, from material that is formed by concrescence).

In this paper I have endeavored to establish two main points with reference to avian development: (1) that the gut-entoderm is formed by invagination; (2) that concrescence is the method of embryo formation. If I have been successful in establishing these two points, it follows that the early development of the birds can be brought into complete harmony with that of other vertebrates; for although differences do exist, yet they are those for which comparative embryology has an explanation. Indeed, in the avian development the differences have been brought about very largely as a result of the enormous accumulation of yolk within the ovum. Even concrescence itself has been made necessary as a result of this accumulation, and for that reason it is a process that is to be regarded as coenogenetic rather than as palingenetic. If concrescence is considered as a secondary process, we ought not to expect to find it in the embryo formation of those vertebrates that have ova practically

wanting in yolk; and as matter of fact a majority of the investigators on the development of Amphioxus maintain that there is no concrescence, as does Conklin ('05) also for the Ascidians. Eycleshymer ('02), as a result of his experimental studies on the Amphibian egg, concludes also that concrescence is a secondary process. He says, "that in those Amphibia which approach most nearly the holoblastic type, as Rana, Bufo, Acris, and Chorophilus, the greater portion of the embryo is formed through differentiation *in situ* and overgrowth, concrescence being confined to a limited region at the caudal end of the embryo. In those forms like Necturus in which there is a marked meroblastic tendency, due to the relative increase in the amount of yolk, a lesser extent of the embryo is formed through differentiation *in situ,* while there is a corresponding increase in the extent of the embryo formed through concrescence, or coalescence of the lateral margins of the blastopore." Again, in his concluding paragraph he writes that "there is every reason for maintaining that differentiation *in situ* is the primitive method of embryo formation, concrescence being a secondary process which has progressed *pari passu* with the increase of yolk material."[21]

Owing to the close affinities existing between birds and reptiles, we should expect to find many points of comparison in their modes of development. Although many writers have pointed out the similarities existing between the two modes, yet, judging from the results obtained in the study of the pigeon, it would be of the greatest interest to be able to trace the origin of the "Primitive Plate of Will" to the margin of the blastoderm, and thus to establish a more exact comparison between the two forms.

Summary.

The main points brought out in this paper may be stated in the following brief summary:

1. Gastrulation in the pigeon's egg is preceded by the thinning out of the thickened blastodisc. The thinning-out process begins at about twenty-one hours after fertilization, and consists in the crowd-

[21] *Loc. cit.*, p. 353.

ing upward of the lower segmentation cells in between the superficial ones, finally reducing the entire central region to a single layer—the primary ectoderm. The thinning-out begins slightly posterior to the center of the disc and then spreads in all directions, but with more rapidity toward the posterior margin. The thinned-out central region is the beginning of the *area pellucida.*

2. Between thirty and thirty-three hours after fertilization the *zone of junction,* or the region where the marginal cells are open to the white yolk or periblast, becomes interrupted for a distance of seventy to eighty degrees at the posterior margin. Hence, this margin of the blastoderm now ends with a free edge. The interruption is but the separation of the blastodisc from the underlying periblast, and has associated with it the degeneration of periblastic nuclei.

3. At about thirty-four hours after fertilization (or seven hours before the egg is laid) there occurs the gastrula-invagination. This consists in the rolling under of the free posterior edge of the blastoderm, together with the simultaneous forward growth of the involuted cells. The invaginated cells are arranged in the form of a tongue-like process, which finally penetrates the subgerminal cavity (enlarged segmentation cavity). It does not reach the anterior limit of this cavity until from three to four hours after the beginning of incubation.

4. Immediately after the gastrula-invagination occurs, the rounded posterior margin thickens up; in part by the multiplication of the cells *in situ,* but mainly by the movement of material from the right and left halves of the dorsal lip, which come together and coalesce in the middle line—a process to be regarded as a form of "concrescence."

5. The median region formed by the coalescence of the lips of the blastopore is the primordium out of which the primitive streak develops. Since the primitive streak gives rise to the mesoderm and chorda, its formation is to be considered as a part of gastrulation.

UNIVERSITY OF CHICAGO, JUNE, 1908.

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COMMON REFERENCE LETTERS USED IN THE FIGURES.

a, anterior end of the blastoderm.
ac, archenteric cavity.
b, blastopore.
cc, individualizing cells.
d, dorsal lip of the blastopore.
e, invaginated or gut-entoderm.
E, region covered by gut-entoderm.
ec, ectoderm.
gw, germ-wall.
m, yolk masses.
np, Nucleus of Pander.
o, region of overgrowth.
p, posterior end of the blastoderm.
pn, periblastic nucleus.
s, segmentation cells.
sc, segmentation cavity.
sg, subgerminal cavity.
z, zone of junction.

DESCRIPTION OF FIGURES.

PLATE I.

FIG. 1. A median longitudinal section of a blastoderm taken twenty-one hours after fertilization, or twenty hours before laying. × 117.

FIG. 2. A portion of the anterior half of a median longitudinal section from a blastoderm taken about thirty-three hours after fertilization, or eight hours before laying. It is not so far advanced in its development as blastoderms usually are when taken at this time. See text for description. × 161. (See also Figs. 29 and VI).

FIG. 3. A portion of the posterior half of the same section as preceding. Compare these two figures as to the condition of the zone of junction. × 161.

FIG. 4. Posterior end of a section, twenty sections to the right of the preceding. Note especially the cells organizing about the periblastic nuclei at "*ce*," and also the two degenerating periblastic nuclei (*pn*). × 365.

FIG. 5. Posterior end of a section, five sections to the right of the preceding. This shows the tip of the right horn of the zone of junction. × 365 (see also Fig. VI).

FIG. 6. A group of six (three shown in the section) cells organized about periblastic nuclei, which are in the central periblast. × 657.

FIG. 7. A normal periblastic nucleus which was introduced for comparison with the following figures (8-13). × 886.

FIGS. 8-13. Various stages of degenerating periblastic nuclei. × 886.

FIG. 14. Posterior end of a longitudinal section taken slightly to the left of the median line. It shows the thin epithelial-like margin just before invagination occurs. At "*m*" is one of the few yolk masses that are found at this stage. × 259. (See also Figs. 30 and VII).

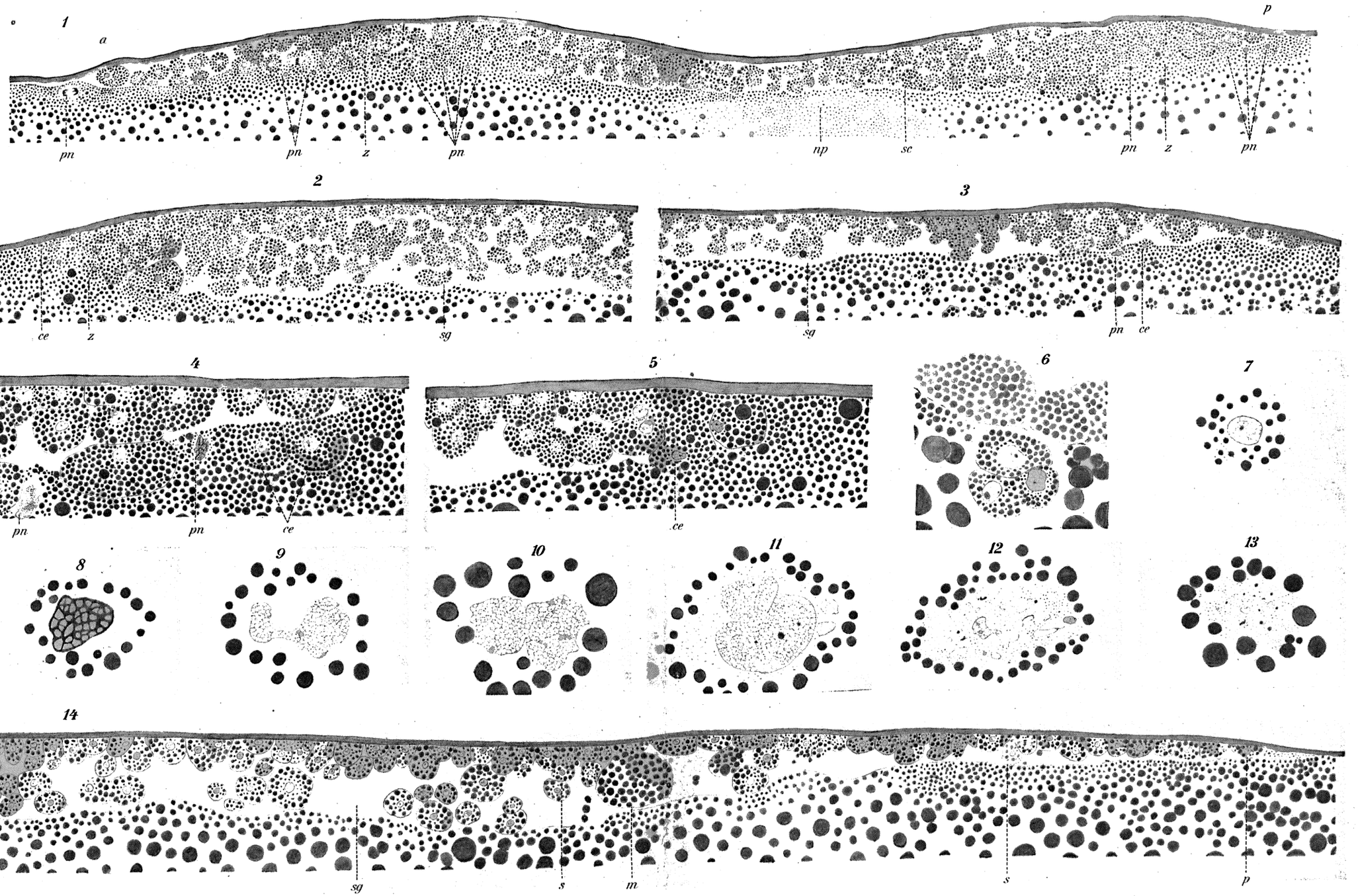

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PLATE II.

FIG. 15. Posterior end of an oblique section from a blastoderm taken thirty-four hours after fertilization, or seven hours before laying. The yolk is cracked and as a result granules are found in the cavity (*c*) just posterior to the dorsal-lip. See text for description, and also Fig. VIII. × 339.

FIG. 16.—Posterior end of a section taken through y-y', Fig. VIII. The section ends with a thin free margin. × 259.

FIG. 17. Posterior end of a section slightly to the right of the one represented in Fig. 14. It shows how a considerable cavity may develop beneath the margin before invagination begins. × 259.

FIG. 18. Anterior third of a section taken in the plane passing through *CR* of Fig. XI. The thinning-out is in the last stages, and at the points marked "s" a few cells have loosened and sunk down. Otherwise the subgerminal cavity contains no nucleated cells—only large yolk masses are present, some of which are disintegrating (*dm*). See text for description, and also Figs. 35 and 36. × 259.

FIG. 19. Posterior third of the same section as preceding. *u*, union between the deeper cells of the dorsal-lip and the entoderm. See text and Figs. 35 and 37 for description. × 259.

FIG. 20. Anterior part of a section taken in the plane passing through *FH* of Fig. XII. Three large degenerating periblastic nuclei are shown (*pn*), and at "y" are the cells which constitute the inner margin of the germ-wall. The zone of junction is too far to the left to be seen in the figure. × 259.

FIG. 21. Posterior part of the preceding section. See text. × 259.

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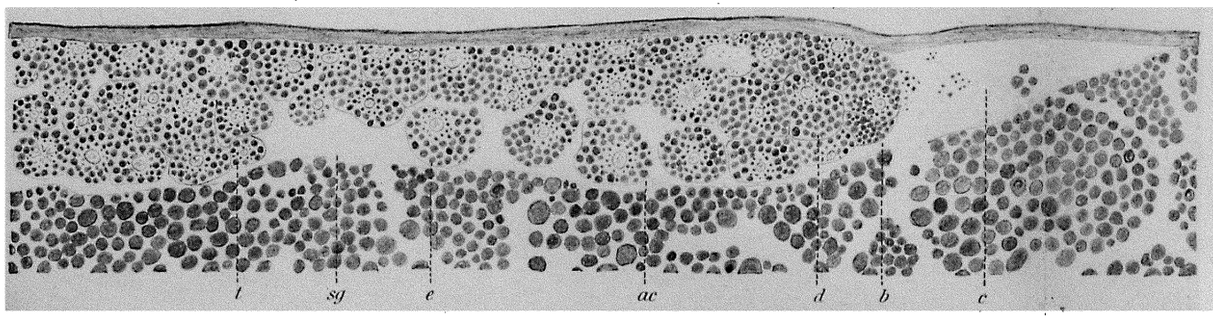

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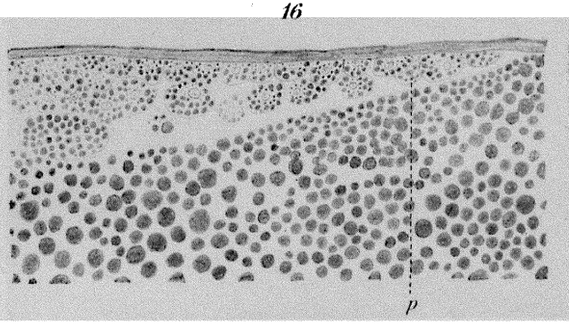

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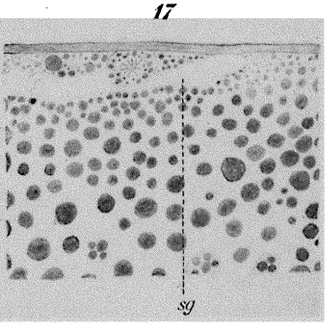

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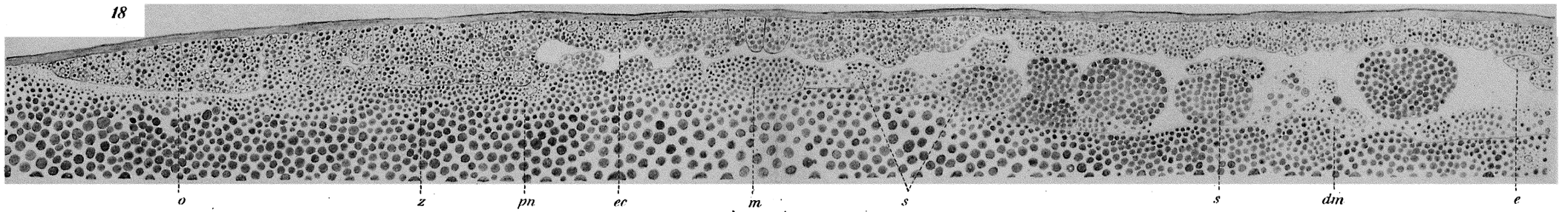

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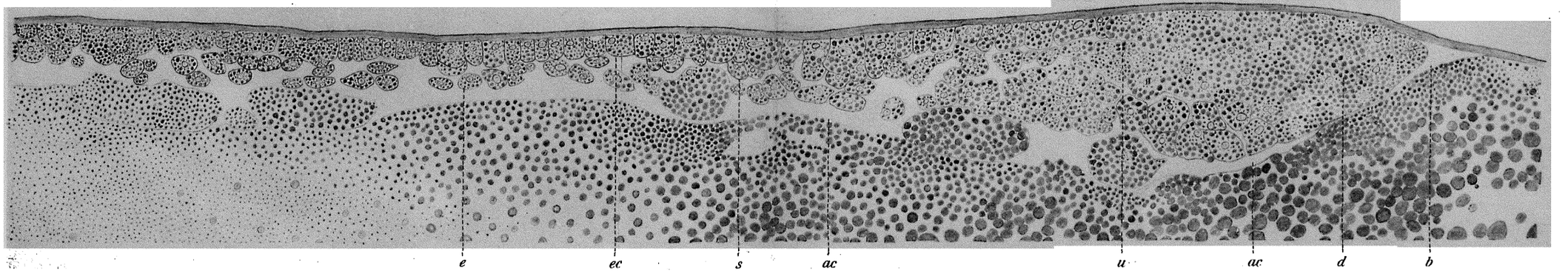

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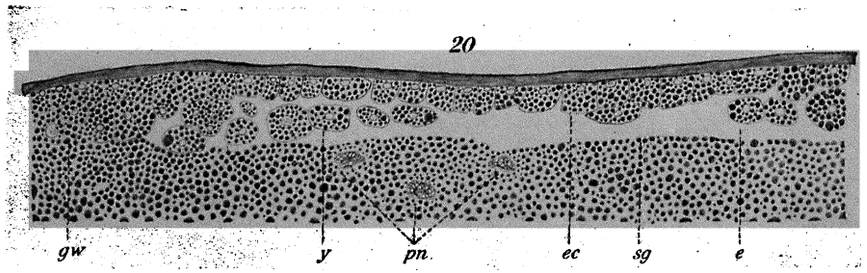

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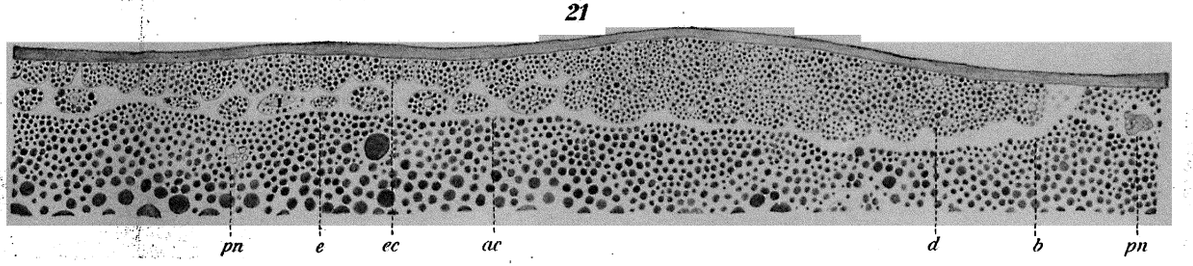

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PLATE III.

FIG. 22. Right half of a transverse section through the plane *xx'* of Fig. XIII. × 259.

FIG. 23. A portion of the right side of a section passing through *ww'* of Fig. XIII. × 259.

FIG. 24. A portion of the central part of a section passing through *vv'* of Fig. XIII. × 259.

FIG. 25. A part of the region of overgrowth from the series which is reconstructed in Fig. XI. It shows a large periblastic nucleus which has moved down from the edge of the blastoderm. This is the only periblastic nucleus in the series that was found either beneath or external to the region of overgrowth. × 657.

FIG. 26. Posterior portion of a median longitudinal section from a blastoderm taken thirty-seven hours after fertilization, or four hours before laying. At "*d*" is shown the dorsal-lip of the blastopore, which has been enclosed within the zone of junction. It is doubtful whether the region marked "*gw*" should be regarded as germ-wall. × 152.

FIG. 27. Left side of a median transverse section from a blastoderm taken about thirty-five hours after fertilization, or six hours before laying. It shows the beginning of the region of overgrowth at "*o*." × 138.

FIG. 28. Posterior end of a longitudinal section from a blastoderm taken four hours after incubation. Introduced to show the condition of the region of overgrowth at this time. × 138.

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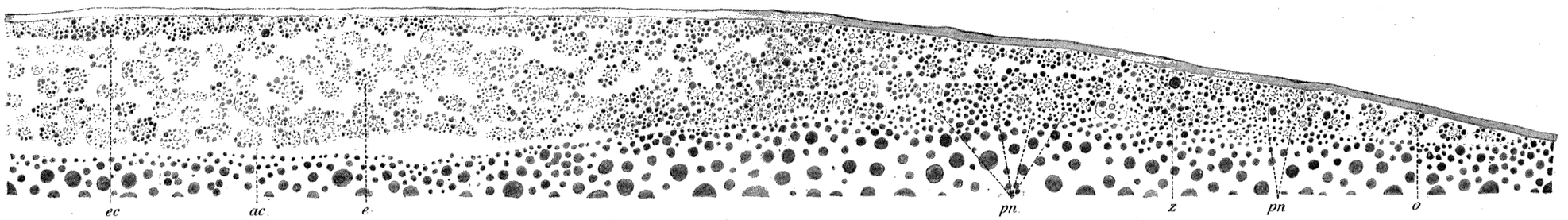

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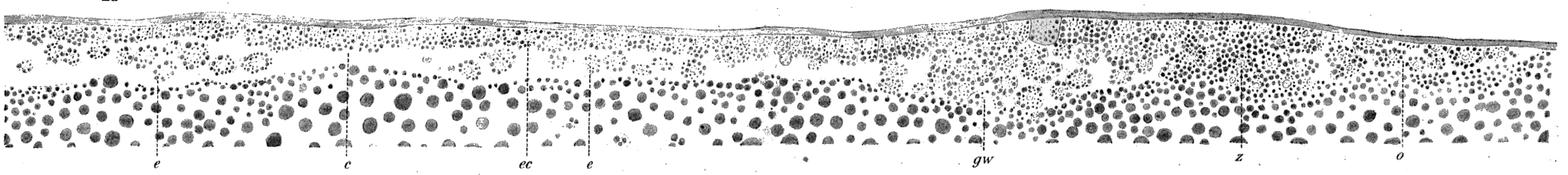

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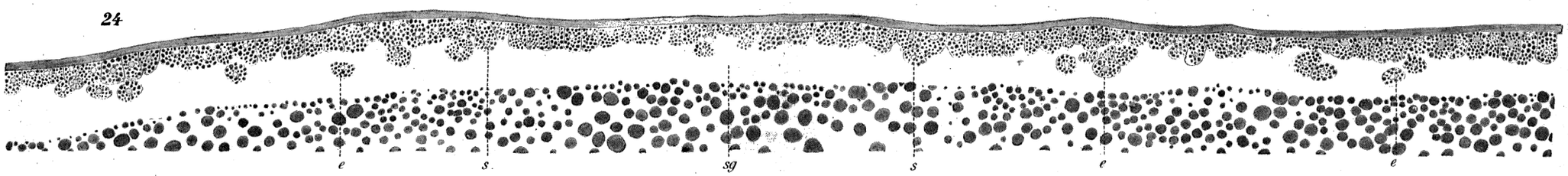

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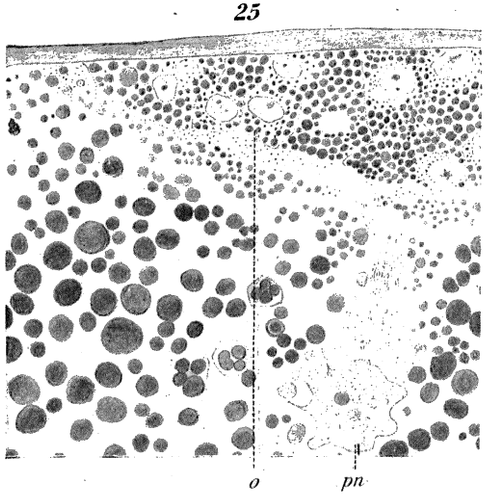

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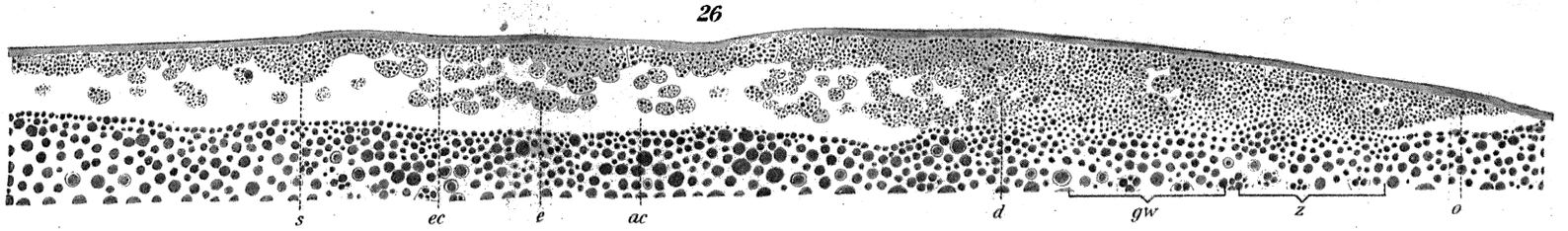

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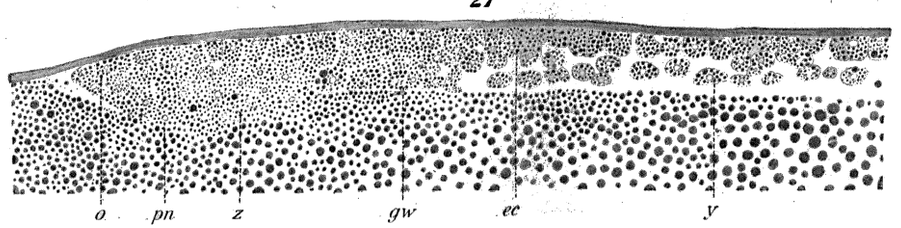

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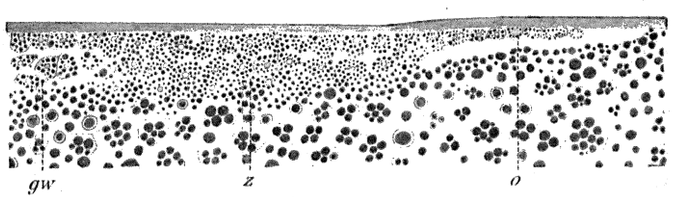

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PLATE IV.

All the photographs in Plates IV-VI are from the sections of the blastoderms, and the prints were made directly from these negatives without any retouching. The Zeiss apo., 8 and 16 mm. lenses, and compensating ocular 4 were used with camera draw varying from 12 to 20 inches. The magnification is given in each case.

All of the photographs in Plates VII-X were made directly either from the whole mount preparations or from the sections, with various combinations of lenses. In each case the magnification is given.

FIG. 29. A median longitudinal section taken through *CR* of Fig. VI. Note especially the gradual increase in the depth of the blastoderm in passing from the right (posterior) to left (anterior). × 143.

FIG. 30. Longitudinal section taken slightly to the left of the line *CD* of Fig. VII. The thinning-out is farther advanced than in the preceding section, and at the point marked "x" is clearly shown the cells that have loosened and sunk down during fixation. Otherwise the subgerminal cavity would contain only a few non-nucleated yolk masses. × 117.

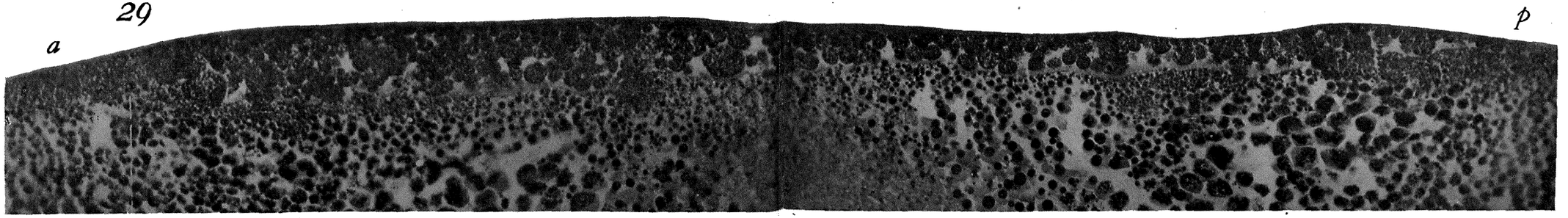
FIG. 31. An enlarged portion of the posterior end of the preceding photograph. × 301.

FIG. 32. Posterior end of a section taken through plane x-x′ of Fig. VIII. See text for description. × 366.

FIG. 33. Posterior end of a longitudinal section, seven sections to the left of the median line, from a blastoderm taken thirty-four hours after fertilization, or seven hours before laying. The anterior limit of the entoderm is shown at "*e*." × 193.

FIG. 34. The median section of the same blastoderm as the preceding. The length of the invaginated entoderm is necessarily greater than in Fig. 33. × 193.

29



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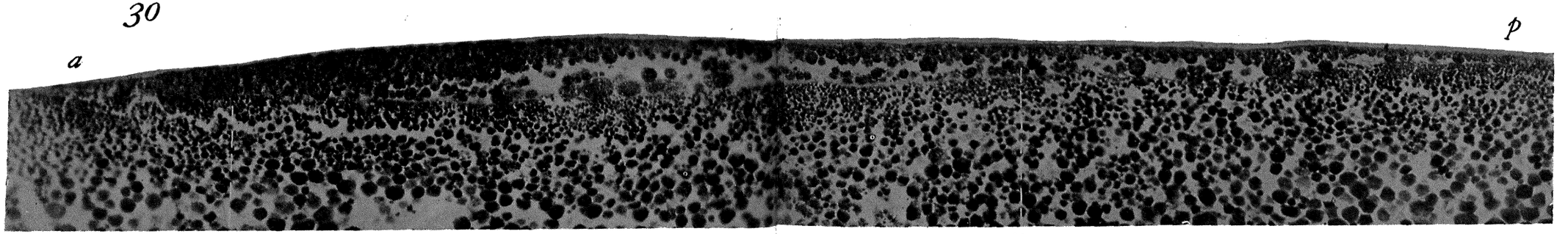

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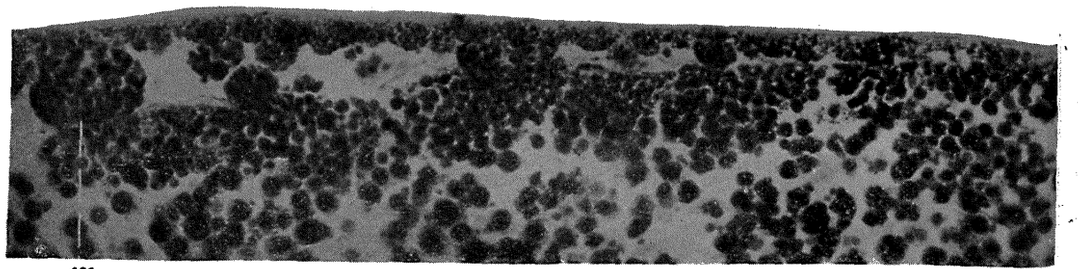

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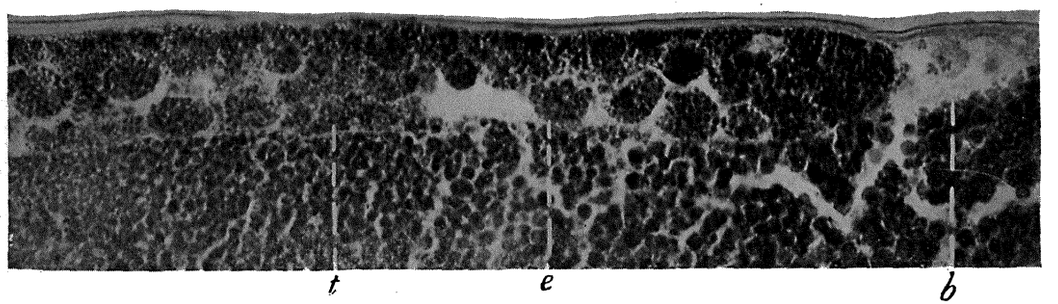

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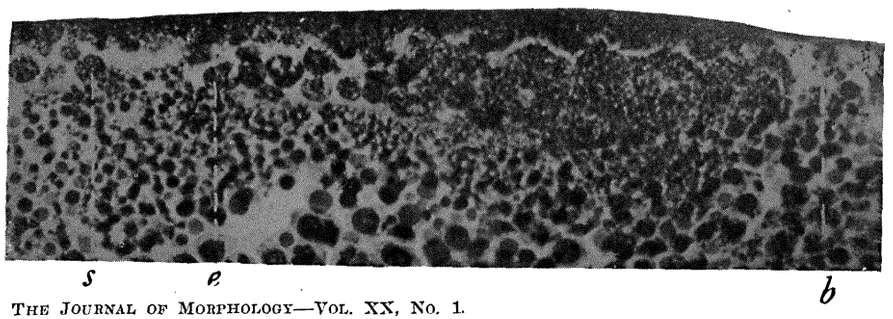

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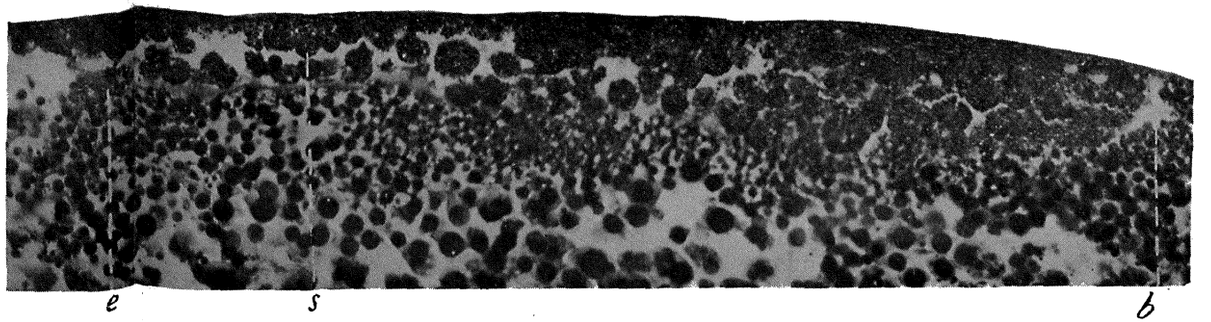


PLATE V.

FIG. 35. A median longitudinal section taken in the plane *CR* of Fig. XI. See text for description. × 107.

FIG. 36. Enlarged anterior end of the preceding. × 245.

FIG. 37. Enlarged posterior end of Fig. 35. × 245.

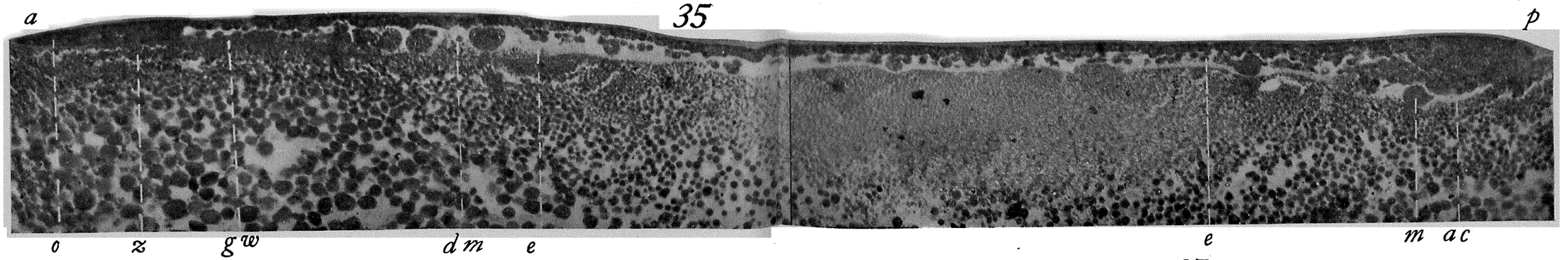
FIG. 38. From a section, four sections to the left of the one represented in Fig. 35. The cavity in the thick dorsal lip is, perhaps, the remains of the space that was formed between the upper and lower layers when the former turned under to give rise to the latter. × 245.

FIG. 39. From a section, two sections to the right of the one represented in Fig. 35. It shows the same conditions as the preceding. × 245.

FIG. 40. Posterior end of a section taken through *KF* of Fig. XI. The tip end of the zone of junction (*z*) is shown, and also the lateral portion of the dorsal lip of the blastopore. × 245.

FIG. 41. Posterior end of a section taken through *GH* of Fig XI. There is no overhanging margin (dorsal lip) in this section. × 245.

35



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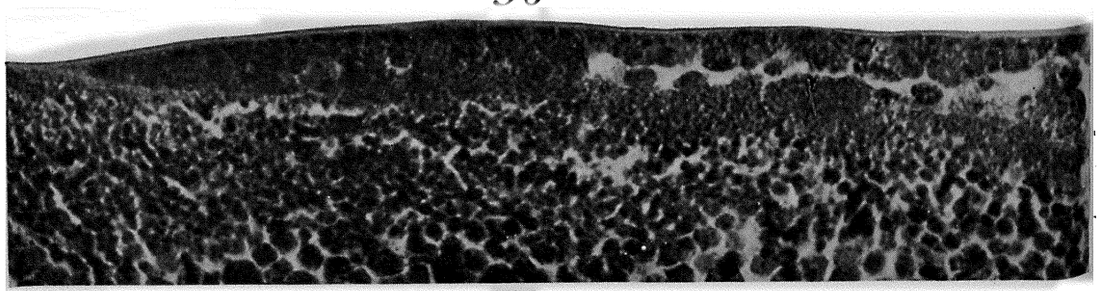

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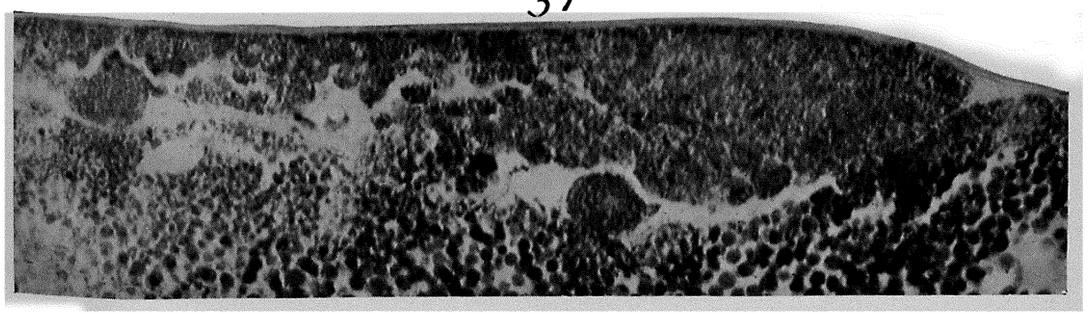

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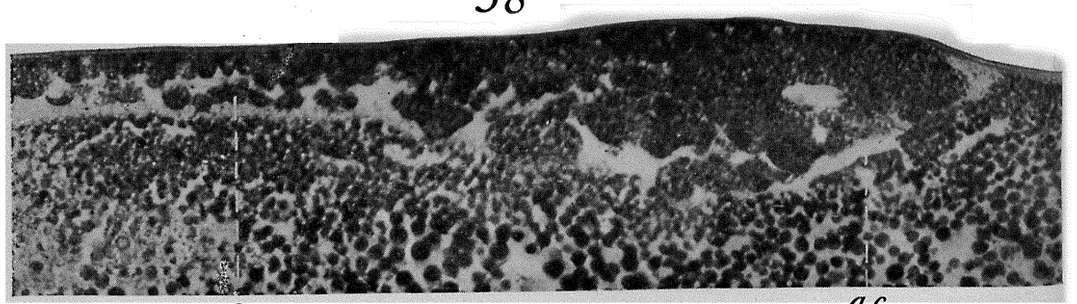

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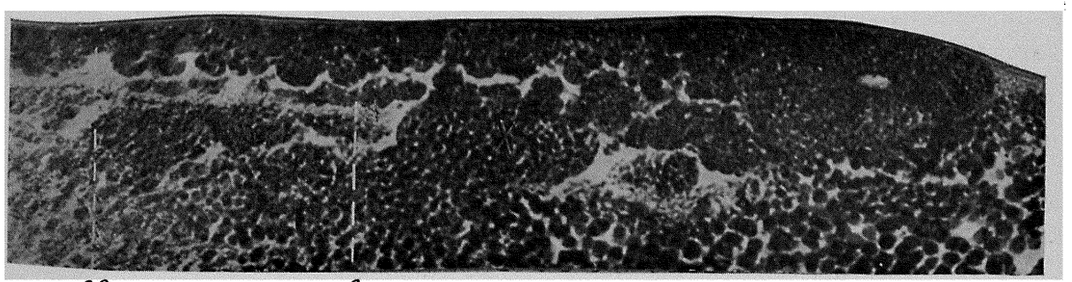

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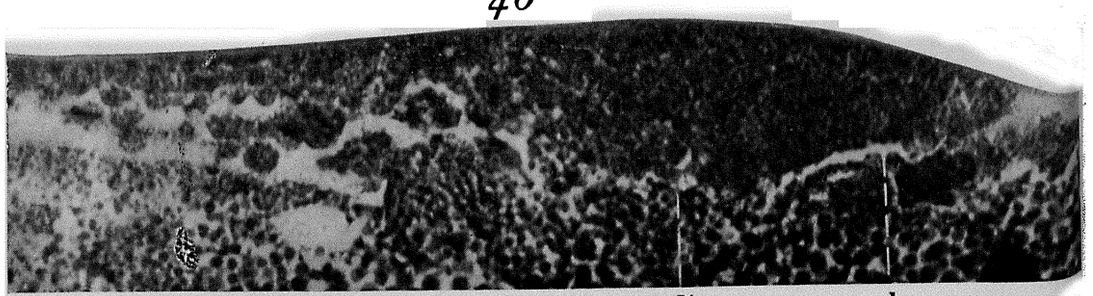

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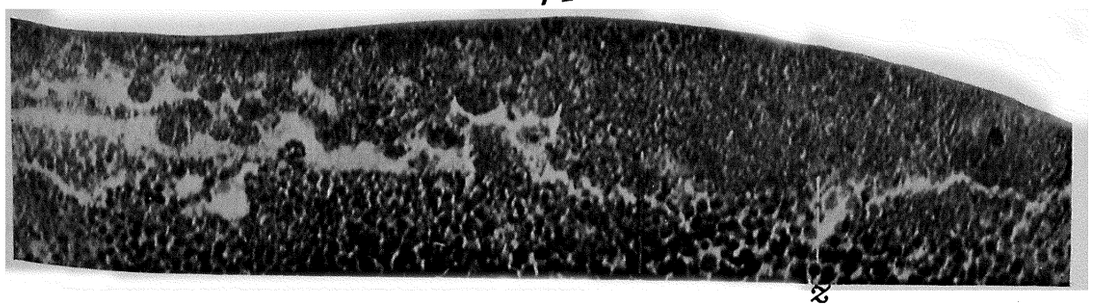


PLATE VI.

FIG. 42. A portion of the anterior half of a median longitudinal section from the blastoderm represented in Fig. XV. At "*e*" is the anterior limit of the entoderm. ×120.

FIG. 43. A portion of the posterior half of the preceding section. × 120.

FIG. 44. The enlarged central portion of a section from the same blastoderm as Figs. 42 and 43. Note especially the epithelial character of the ectoderm, the grouping of the entoderm cells, and the granular contents of the cavity. × 184.

FIG. 45. From a median longitudinal section of an unincubated egg. It shows the anterior limit of the entoderm in its forward growth. The remains of the subgerminal cavity (*sg*) is entirely free from yolk mass. × 245.

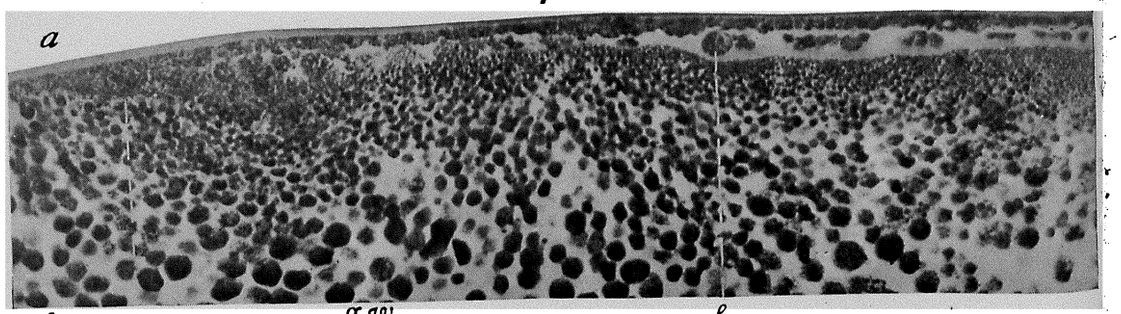
FIG. 46. The central part of a longitudinal section from a blastoderm taken three hours after incubation. It shows the fragmentation of the yolk lying beneath the floor of the cavity. These yolk masses (*m*) are non-nucleated. × 246.

FIG. 47. The anterior portion of a longitudinal section from a blastoderm taken forty hours after fertilization. The remains of the subgerminal cavity not yet penetrated by the entoderm (*e*) is full of yolk masses, some of which are undergoing fragmentation (*dm*). × 245.

FIG. 48. The right side of a median transverse section taken one hour after incubation. The lateral edge of the entoderm is shown at "*e*" and the inner margin of the germ-wall at "*y*." The space between these two points can be followed along the entire right side, showing that the fusion between the lateral margin of the entoderm and the inner edge of the germ-wall had not yet taken place. × 245.

FIG. 49. The central part of a section taken through v-v′ of Fig. VIII. At "*s*" is shown a segmentation cell that has loosened and sunk down from the underside of the ectoderm. × 245.

42



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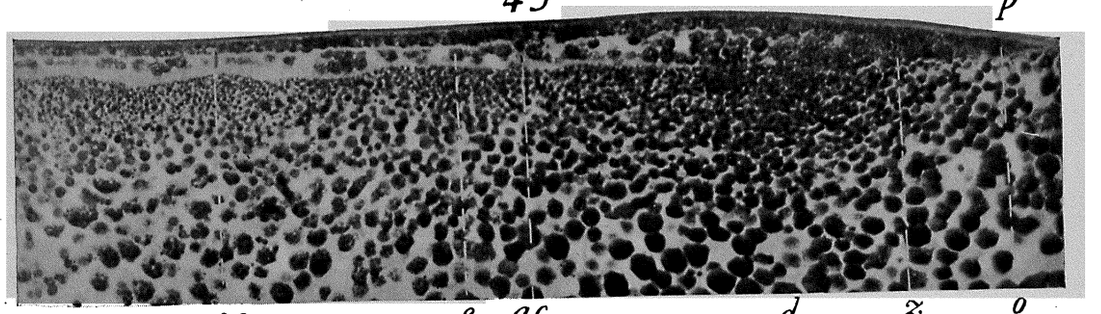

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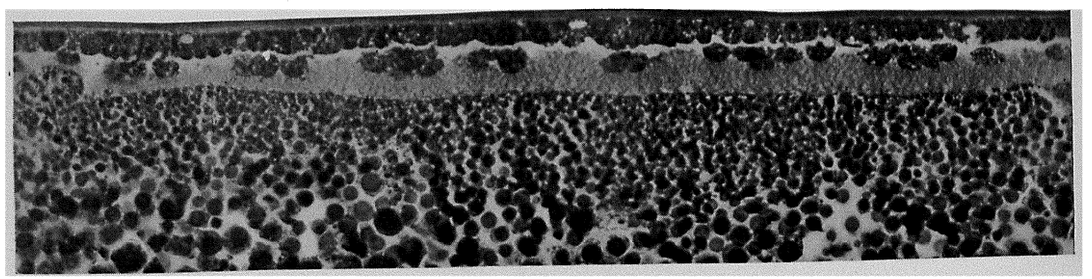

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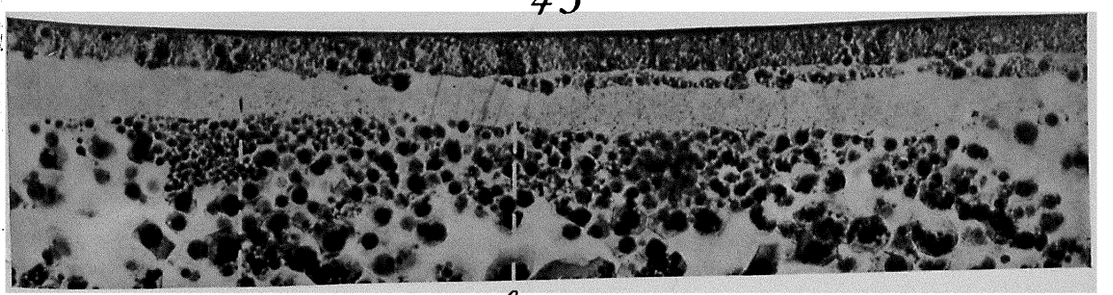

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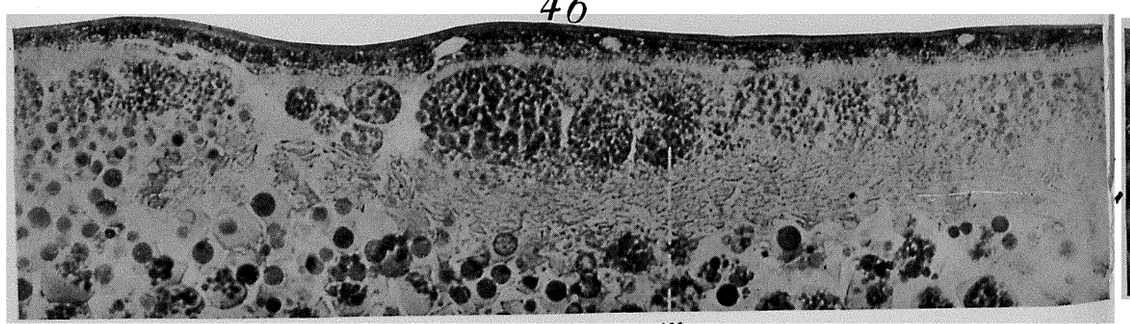

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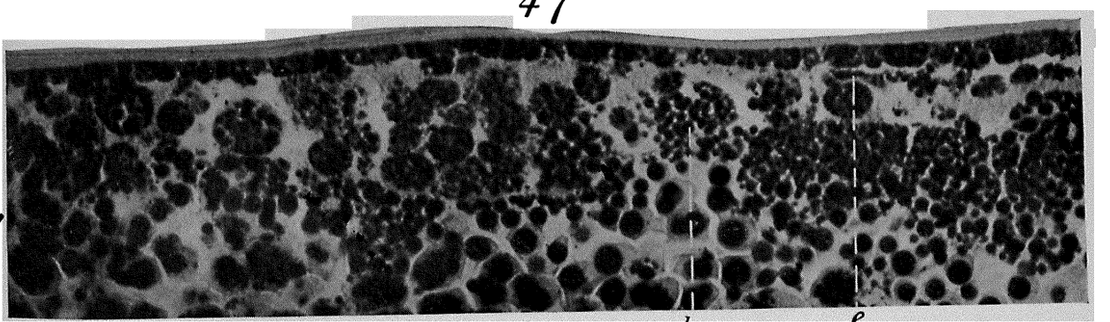

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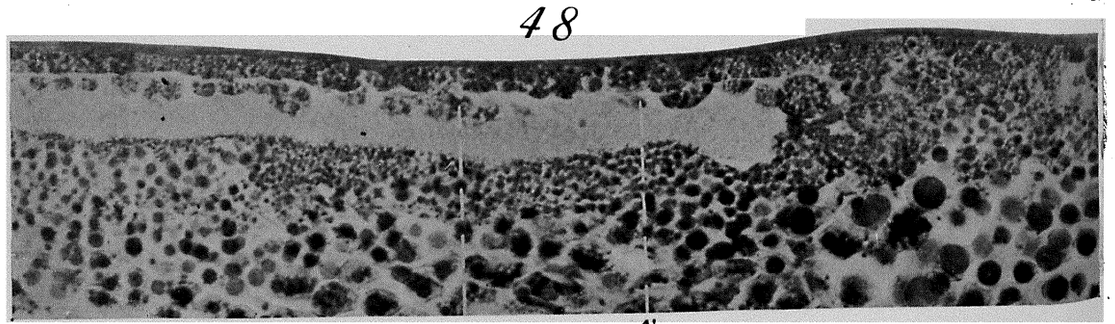

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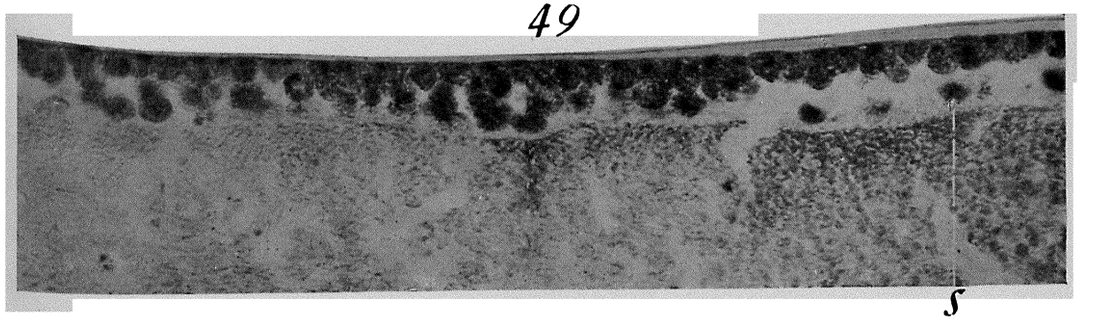


PLATE VII.

FIG. 50. This embryo shows the result of the operation described in Experiment II (page 93). The injury was made on the posterior edge of the dorsal lip, thirty-five and three-fourths hours after fertilization. The egg was then incubated for forty-nine hours. The anterior end of the embryo is normal in every way, and nineteen pairs of somites are developed. The depth to which it was necessary to focus the microscope in order to obtain an image of the injured material in the entoderm can be seen by the fact that the somites are out of focus. × 30.

FIG. 51. A transverse section through the injured region of the preceding embryo (Fig. 50, op). The affected cells lie in the entoderm. × 95

FIGS. 52, 53, and 55 are all from an unincubated blastoderm. Fig. 52 shows the entoderm and part of the ectoderm above, and a "free nucleus" at *n* lying on the floor of the cavity, which contains many small granules. × 688.

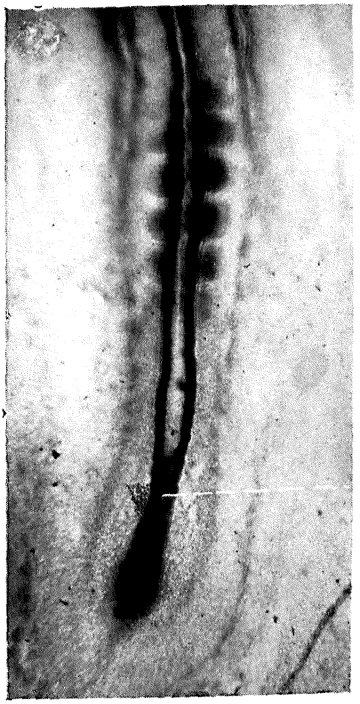
FIG. 53. It shows two small nucleated cells (at *s* and *s*), which are doubtless wandering entoderm cells. There are also many large yolk masses in the cavity. × 500.

FIG. 54. This shows the result of the operation in Experiment VI. The injury was made thirty-five hours after fertilization, and the egg then incubated for forty-eight hours. There are twelve pair of mesoblastic somites present, the development being slightly retarded. × 20.

FIG. 55. This shows a large multinucleated yolk mass, in which most of the nuclei are degenerating. × 500.

FIG. 56. This shows the result of the operation in Experiment X. The injury was made on an unincubated blastoderm, and the egg was then incubated for twenty-three hours. The embryo is normal in every way. × 25.

50



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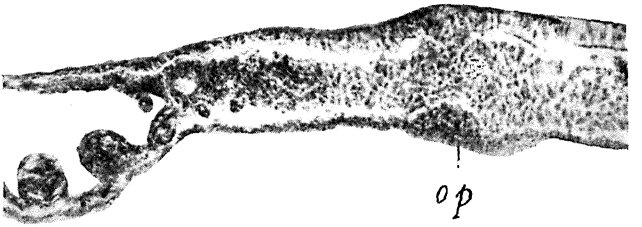

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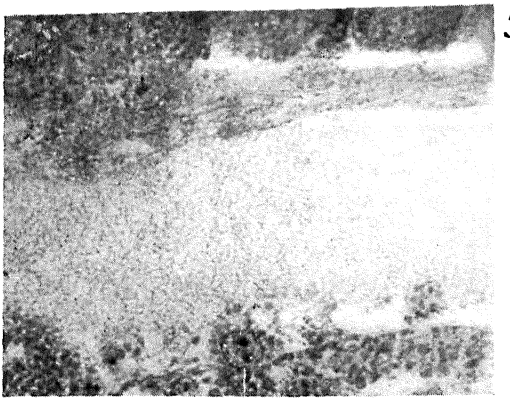

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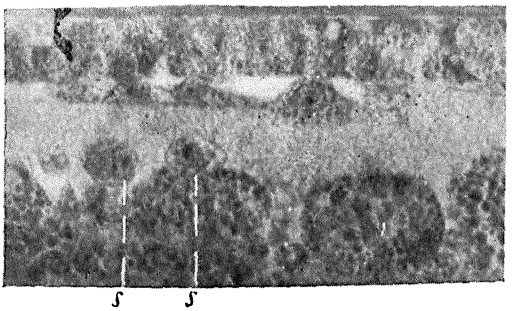

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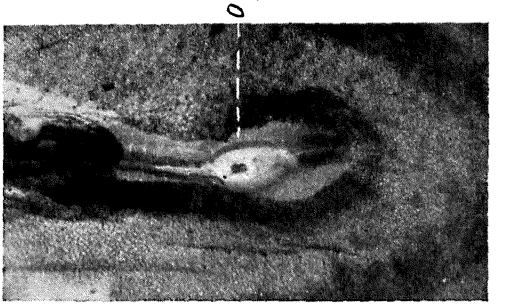

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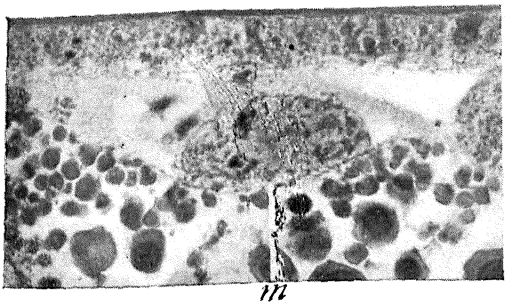

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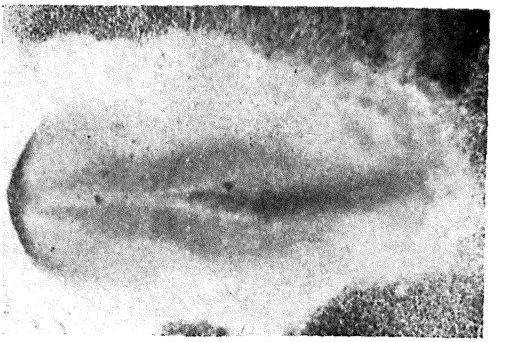


PLATE VIII.

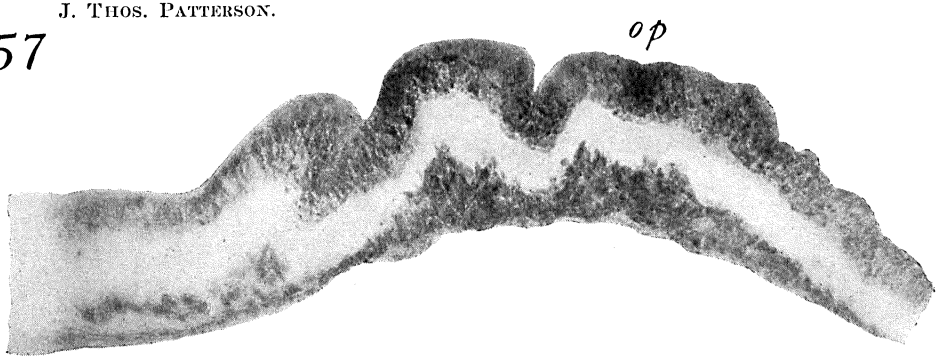
FIG. 57. Transverse section through the injured head-fold of the embryo shown in Fig. 59. The main group of injured cells is at *op*. × 95.

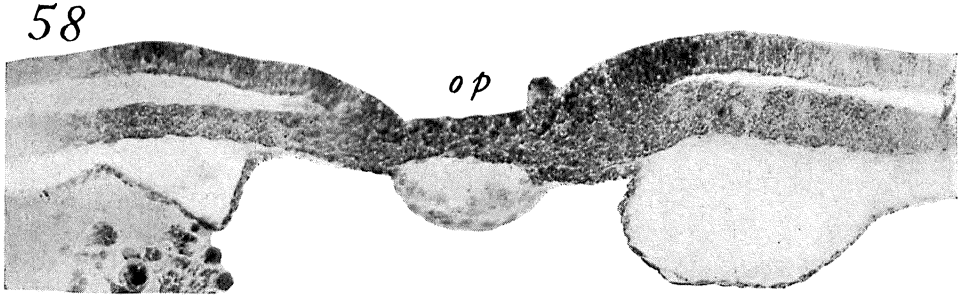
FIG. 58. Transverse section through the injured region of the embryo shown in Fig. 60. The needle has destroyed a considerable portion of the primitive streak material, and has also disturbed the underlying entoderm. The folding of the lateral portions of the entoderm is an artifact. × 95.

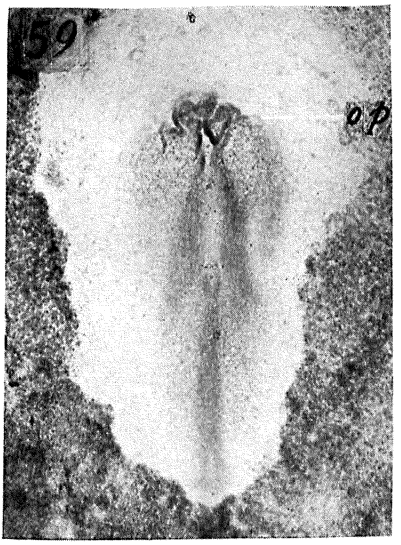
FIG. 59. This embryo was operated on thirty-four and one-third hours after fertilization, and then incubated for thirty-four hours. The injury was made in the center of the dorsal lip at a distance from the posterior margin equal to about half the width of the needle (see Fig. XVI, *a*). Posterior to the head fold the embryo is normal in every way. × 21.

FIG. 60. The operation was performed on a freshly laid egg, which was then incubated for twenty-six and three-fourth hours. The injury was made on the boundary between the areas opaca and pellucida, in line with the axis of the future embryo (see Fix. XVI, *b*). There are twenty-five sections posterior to the injury that show a characteristic primitive streak structure. × 21.

FIG. 61. Transverse section through the injured region of the embryo shown in Fig. 68, Pl. X. Just one-half of the embryo has been affected by injury. × 95.


57
op


58
op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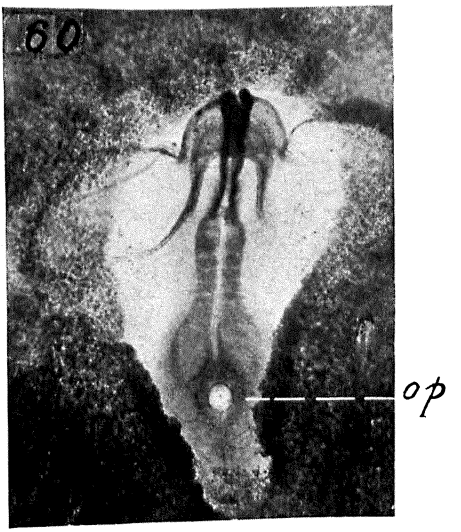
op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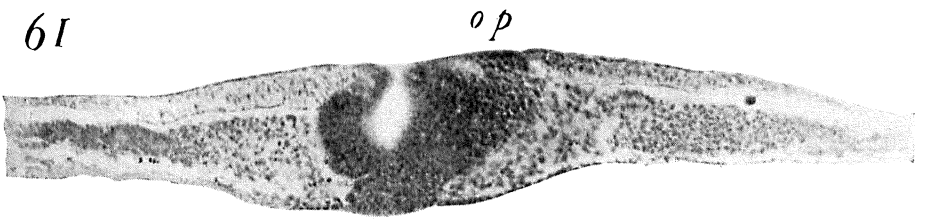

61
op

PLATE IX.

FIG. 62. Transverse section through the injured region (mid-brain) of the embryo shown in Fig. 63. Only the right neural fold is affected by the operation. × 87.5.

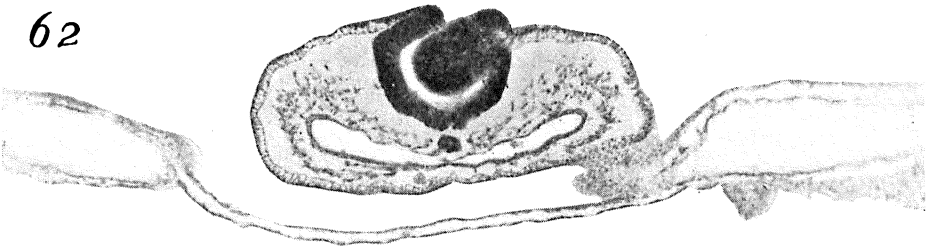
FIG. 63. This embryo shows the result of an operation made ten degrees to the right of the median line, in the margin of the dorsal lip. The injury was made thirty-four and three-fourth hours after fertilization, and then incubated for thirty-six and three-fourth hours. × 20.

FIG. 64. This shows the result of the operation for Experiment VII. The injury was made thirty-six and three-fourth hours after fertilization, and the egg was then incubated for thirty-six hours. The operation was made just after the closing of the blastopore (see Fig. V, *K*). × 21.

FIG. 65. This shows the left side of a transverse section of an unincubated hen's blastoderm. The figure is introduced to show the rounded condition of the region of overgrowth, which is raised up from the yolk. I am indebted to Professor George Lefevre for his generosity in sending me the series from which this photograph was made. × 128.

FIG. 66. Posterior end of a median section from the blastoderm described in connection with Experiment I (see text for description). × 120.

FIG. 67. A portion of the same section taken just anterior to the preceding. × 120.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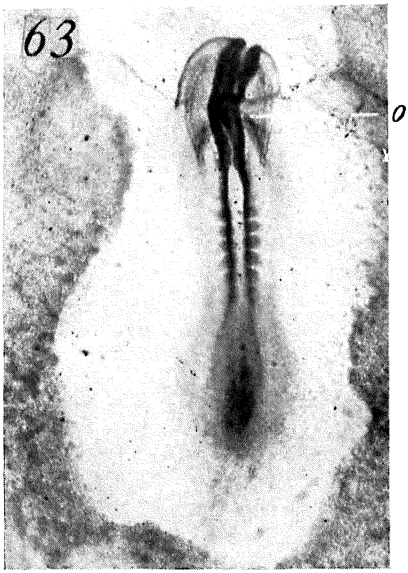
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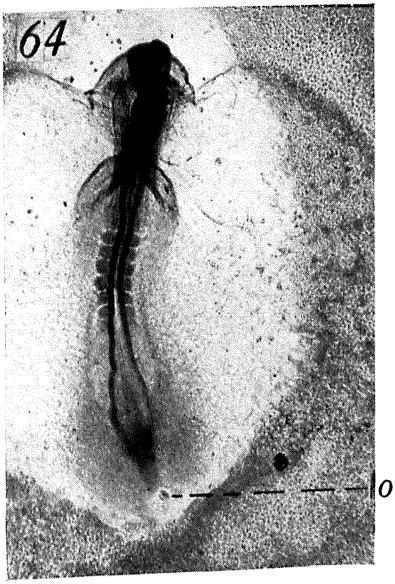
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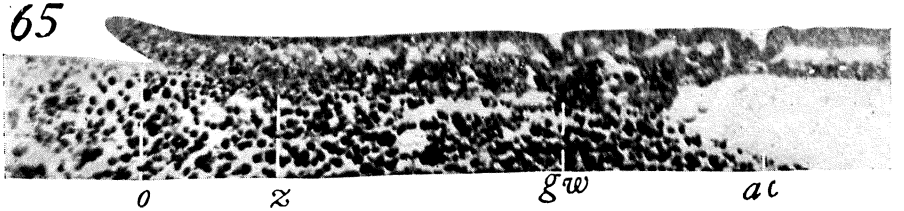
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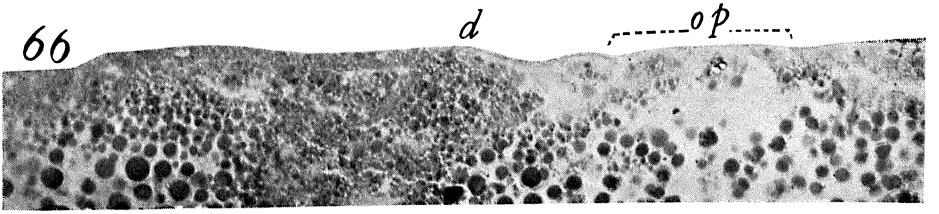
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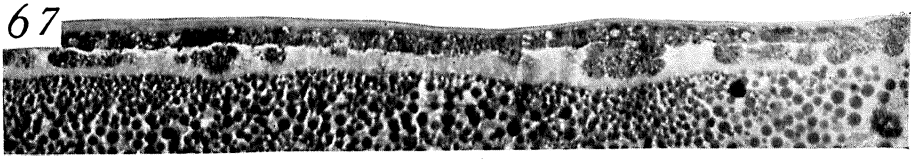

67

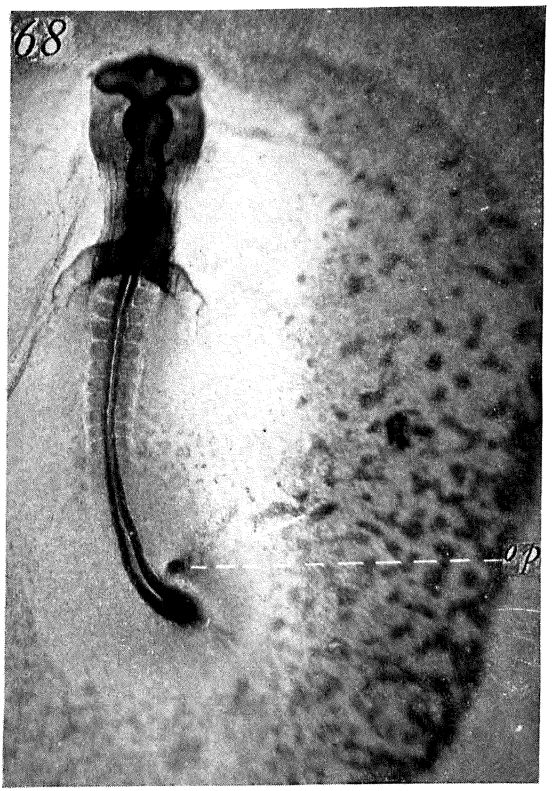
PLATE X.

FIG. 68. This embryo shows the result of an injury made on an unincubated blastoderm, at about twenty degrees to the right of the median line on the boundary between the areas opaca and pellucida. The egg was incubated for forty hours. The arrow shows the path traversed by the mass of injured cells, as indicated by the small groups of dead cells. The posterior end of the embryo is bent to the right. The curvature is due doubtless to unequal growth of the cells on the two sides. (For a transverse section through the injured region of this embryo, see Fig. 61). × 20.

FIG. 69. The operation was of the same nature as the preceding, except that it was made about thirty degrees to the right of the axial line instead of twenty. The egg was incubated for twenty-four and one-half hours, and the injury is situated slightly more posteriorly than in the preceding experiment. × 25.

FIG. 70. In this embryo the injury was made between three and four hours after incubation had begun, at about forty-five degrees to the right of the axial line on the boundary between the areas opaca and pellucida. The egg was then incubated for thirty-six hours. The embryo is normal in every way and the injured spot is in the vascular area, about half way between the *sinus terminalis* and the pellucid area. × 18.

FIG. 71. The injury in this blastoderm was made on the posterior margin forty-five degrees to the right of the median line. The operation was performed thirty-three and one-half hours after fertilization, and the egg was then incubated for thirty-six hours. The group of injured cells has been brought into the axis of the embryo. × 21.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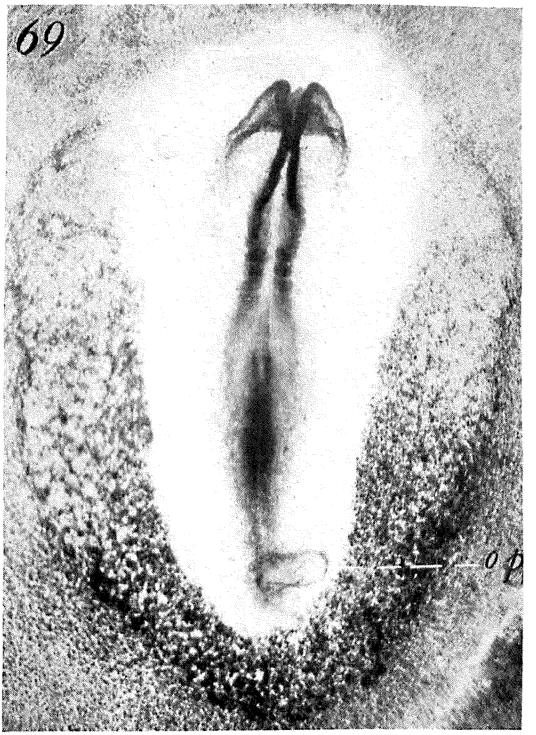
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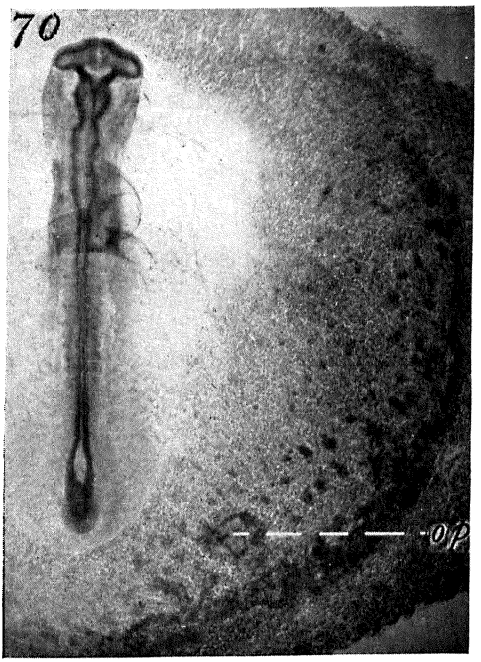
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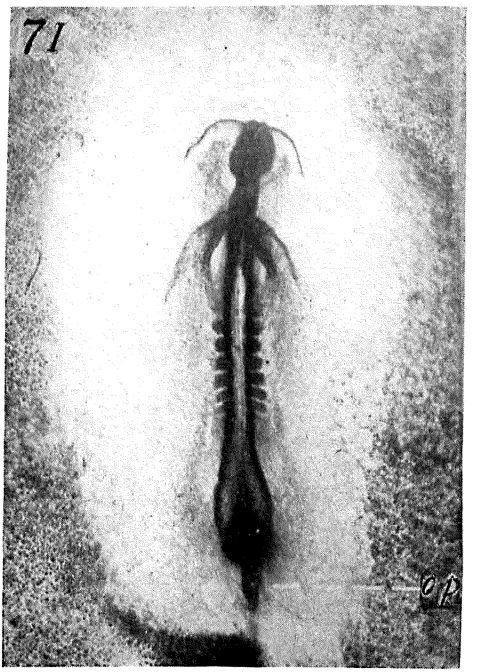
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