The Orientation of the Frog's Egg.

By

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With Plates 24 and 25.

I.

THE classical experiments of Pflüger on the segmenting frog's egg, and the important conclusions drawn by Roux from a study of the same egg, have made it very desirable to have an accurate knowledge of the relation existing between the early segmenting egg and the position of the embryo with respect to the egg.

The interpretation of certain embryos in which the blastopore has failed to close, recorded by Roux and Hertwig, will likewise depend on the normal position of the embryo on the egg. Pflüger, Roux, and Hertwig have come to the conclusion that the embryo forms over that portion of the unsegmented egg which is normally directed downwards, i. e. over the white hemisphere. Schultze supports the old view, that the embryo lies on the upper or black hemisphere.

Pflüger based his conclusion on the evidence obtained by actually following the dorsal lip of the blastopore in its migration over the white hemisphere. Roux based his conclusion on evidence obtained by destroying definite portions, both of the segmented and unsegmented eggs.

Hertwig's conclusions were based on the evidence furnished by certain abnormalities, while Schultze's conclusion rests on a study of normal development.

It seemed to me at first, from a study of eggs developing normally, that it was impossible that the embryo should lie entirely over the white hemisphere. Schultze pointed out that Roux's earlier results are contradictory in themselves, and I had reached the same conclusion from a careful reading and re-reading of Roux's earlier papers. I was prepared, therefore, to find some truth in each view, and expected to find the embryo forming partly over the black, partly over the white hemisphere. I was then not a little surprised to find that our studies led to the conclusion that the embryo is formed over part of the white hemisphere of the egg. In the main point, therefore, I am in agreement with Pflüger and Roux, although not entirely so, for I hope to be able to show the extent of the white hemisphere of the unsegmented egg, covered by the blastopore, to be somewhat different from that affirmed by Pflüger and Roux.

Our work in relation to the orientation of the embryo has covered the ground somewhat more extensively than that of any previous author, since we have made use of the methods employed by all of them.

Our results will be considered under three headings:

1st. Normal development and location of blastopore.

2nd. Results obtained by injury to definite portions of the early embryo.

3rd. Results obtained from embryos whose development had been modified by artificial means.

A word of personal explanation ought to be added. The senior author is responsible for Sections I, III, IV, and V of the present paper. The work recorded in these was done in the spring of 1893.

Section II is the record of the results obtained by Umé Tsuda while a student in the Biological Laboratory of Bryn Mawr College. This work was done during the winter of 1891-2; the account written in the spring of 1892. Only

very slight alterations have been made in this portion preparatory to publication.

II.

In studying a series of eggs of the early stages of Rana temporaria from the segmentation period to the beginning of the formation of the blastopore, a few points in regard to the peculiar development of the pigment and the orientation of the dorsal lip of the blastopore have been noted, and are here given briefly.

The eggs, which had been previously hardened and preserved in 80 per cent. alcohol, were studied chiefly by surface views with a dissecting microscope. The study of the segmentation of the early stages only verified previous accounts. The first cleavage furrow divided the egg into two equal parts; the second is at right angles to it; the third or horizontal furrow is much nearer the upper or pigmented pole, thus forming four small pigmented cells in the upper and four large cells in the lower hemisphere.[1] The four cells of the upper half then each divide, thus forming eight cells; but the division from this point becomes quite irregular, both in the upper and lower halves of the egg. I found a number of eggs in what clearly seemed a twenty-four cell stage—eight cells in the lower and sixteen in the upper; but I could not verify the fact in the living egg.

A curiously abnormal egg of eight cells was found where the horizontal or third furrow was entirely wanting, and the furrows of the next division, which would normally have divided the egg into sixteen cells, had cut through from the upper pole, reaching down about two thirds of the distance to the lower pole. On sectioning the egg eight nuclei were found corresponding to the number of segmentation furrows. No

[1] I have found a number of probably abnormal eggs from one lot in which the first furrow divided the egg into unequal parts, one large and the other much smaller. Also a number of eggs of the four-cell stage, where neither the first nor second furrow had met at the lower pole.

nuclei were found in the yolk portion of the lower half of the egg, which would normally have been separated from the upper cells by the third furrow.

In addition to the division of the cells of the segmenting egg from the surface a delamination of the cells begins about the thirty-second cell stage. The horizontal and vertical sections at this stage show elongation of the nuclei at right angles to the plane of division. In the sixty-fourth cell stage the delamination can be easily seen to have taken place by the dissection of an egg under a hand-lens or a dissecting microscope.

A careful study of the segmentation of the cells around the lower pole in the advanced segmentation stages has shown that the greatest irregularity exists.

In many cases, however—and I have reason to believe in nearly all cases,—the cells lying nearest the lower pole, and especially the four cells which are around the point where the first and second furrows intersect, remain larger than the surrounding cells. In the later stages of many eggs I have distinctly made out four cells, which I think are without doubt the ones grouped around the lower pole. Figs. VII, VIII, IX, where cells marked *b* and *a* are much smaller than the surrounding ones, might seem to oppose such a conclusion; but in the later stages, at the time of the formation of the blastopore (figs. X, XI, XII), there is a certain regularity in the grouping of the larger size cells around the point which, from other indications, I should judge to be the lower pole; and hence I believe that such a cell as cell *a*, fig. VIII, though smaller at this particular stage than the cells surrounding it, does not develop so rapidly later on. I have not been able to section the eggs at these stages to find out what relation the real size of the cells bears to the apparent size from surface views. At present I see nothing against the hypothesis that the portion of the egg around the lower pole in the late as well as in the early segmentation stages is the most retarded portion of the developing egg. The group of cells that remain largest always bear a certain relation in position to the pigment that leads me to

believe that this is undoubtedly so, and as yet I have seen no indication that would tend to a contrary conclusion.

It has been noted that with the splitting off of cells from the upper corner of the yellow cells of the lower hemisphere new ectoderm-cells are formed, and it had been generally supposed that with this growth a continuous formation of pigment took place, the black pigment gradually growing down over the whole egg. I have found that the growth of pigment does not in any way correspond to the growth of new ectoderm-cells, but, on the contrary, there seems to be a great variation in the amount of pigment found in various lots of eggs of the same stage procured at different times. Some eggs of the two- or four-cell stage have the pigment covering more than two thirds of the egg, while others at this point of development have only a black cap of pigment extending down to the third furrow. However, all the eggs of the same stage from one cluster, and hence laid by the same frog, are alike in the quantity of pigment. The amount of pigment in the egg seems a variation dependent on conditions previous to the beginning of segmentation, and due to individual difference in the adult frog. There is, of course, some formation of new pigment in the later segmentation stages, and a most marked and rapid change in the amount of pigment formed at the time of the first appearance of the blastopore.

In all the eggs, from the earliest stages up to the blastopore, there is one marked peculiarity of the pigment. There is always a greater deposit of pigment on one side of the egg than on the other; and if we judge the exact position of the lower pole from the crossing point of the first two segmentation furrows, we find that the pigment is not only denser, but it comes down much nearer to the lower pole on one side than on the other. As this was found to be the case in greater or less degree, and in some eggs very markedly, in all the stages up to the beginning of the blastopore, and, moreover, in eggs procured at several different times and places, I judged it could not be an accidental variation.

I have tried, therefore, to find out—

I. Whether the pigment bore any fixed relation to the first furrow, and by this to follow out approximately the first furrow in the later stages.

II. The relation of the dorsal lip of the blastopore to the pigment and to the first furrow.

I have examined a large number of eggs in the early stages, in order to ascertain the exact appearance of the pigment, and to compare it with the later stages. When the egg is looked at on the under and non-pigmented side, with the lower pole turned uppermost, the line of pigment, extending, as it does, nearer to the lower pole on one side than on the other, has a crescentic outline. The pigment zone or band is not usually visible on the opposite side (see fig. VI). The same crescent-shaped appearance of the pigment is easily followed in the later stages up to the formation of the blastopore, at which time there is a rapid growth of pigment downward towards the lower pole. In order to ascertain what relation the first furrow bears to this crescent-shaped band of pigment, I examined 119 preserved eggs, as well as a number of living ones, in the stage when the second furrow had begun to come in from the upper pole and was about to meet the first furrow on the lower side. I made my observation on the eggs just before the two sides of the second furrow had met and intersected the first furrow at the lower pole, but when they were near enough to meeting, so that the point of the lower pole could be approximately judged. In this way I was able to distinguish the first furrow from the second, which would not have been possible after the second cleavage was completed, and at the same time I could guess approximately the position of the lower pole, the meeting-point of the first and second furrows.

The first furrow does not seem to cut the pigment zone bilaterally; nor does it, on the other hand, always divide the egg into a lighter and a darker half. Out of the 119 eggs examined, in 76 cases the first furrow cut through a little to one side of the central point of the crescent, only approximately dividing the pigment.

Figs. A, B, and C show diagrammatic views of the lower

pole of different eggs. The line *m n* represents the first furrow, and *a*, *b*, *c*, *d*, the four cells formed by the first and second segmentation furrows. In Fig. A the dotted line *x y* passes through the centre of the crescent-shaped pigmented area, and divides the egg symmetrically. The first furrow lies to the right of it.

FIG. A.

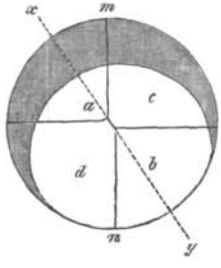
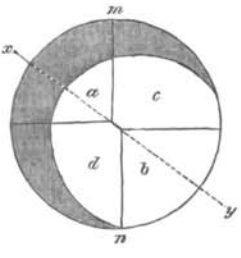
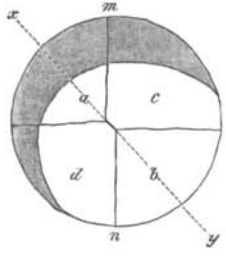


FIG. B.



In 30 cases out of 119 the second furrow seemed to divide the pigment more equally (see Fig. B), and the second furrow is a little to the left of the imaginary line of symmetry *x y*.

FIG. C.



In the remaining 13 cases the pigment seemed to extend as much on one side as on the other, and the line *x y*

in this case would be as near to the first furrow as to the second (Fig. c). It will be seen on examining the above diagrams that the apparent variation of pigment in the three cases depends on very slight differences. A little shifting of the pigment or an addition to one side or the other would change Fig. A to Fig. C or B. Judging by numbers, A would seem to be the more typical one.

One thing remains obviously unchanged in all the eggs. Of the four cells into which the egg is divided, one cell, *a*, has always the greatest amount of pigment; and cell *b*, which is opposite to it on the other side of the egg, is the lightest. This is true in every case, and these two opposite sides can be distinguished in eggs far advanced up to the end of segmentation. The cells marked *c* and *d* are intermediate, being neither so dark as *a* nor so light as *b*.

The pigment on the darker side of the egg not only extends much farther down, but I have observed on dissecting the egg in the upper hemisphere that in some cases the pigment extends inward almost to the centre of the egg (blastula), and to about twice the depth of the opposite side.

The interesting point in connection with the two opposite, the darker and the lighter, sides of the egg, on which I have dwelt at such length, is that the less densely pigmented half of the egg very early in the segmentation shows signs of a more rapid development and growth than the darker and pigmented side. This is true of the cells of the upper hemisphere as well as the lower. Moreover it is on the side that shows this advance in development that the dorsal lip of the blastopore makes its appearance.

I first noticed the unequal growth of the cells in an egg of about ninety-six cells, sixty-four in the upper and thirty-two in the lower half. There was a decided retardation of one side of the egg. In later stages the lighter half seems often two stages ahead of the other side. Figs. IV, V, show the unequal development in the early stages. Figs. IV, V (camera drawings), are surface views of an exceptionally fine egg, in which the unequal development is seen to what might appear an ex-

aggerated degree. Very few eggs at this early stage in the development show such a marked difference of the two halves. In some eggs of this stage the segmentation seems equally advanced on both sides, but these are rather the exception than the rule. I have never found a single case in any stage where the pigmented side was in any way in advance of the lighter side. On the contrary, the reverse is true in almost every egg towards the close of segmentation, and in most cases a superficial glance will reveal the fact plainly.

Sections of the egg parallel to the third furrow show the cells smaller over one hemisphere than over the opposite, and prove at least that there is an unequal development of two sides of the egg, and that the difference which exists between them is no superficial one.

A careful examination of the early blastopore stages of the egg with reference to the pigment and to the unequal development shows conclusively that the blastopore makes its first appearance on the less pigmented and further developed side of the egg, and, moreover, at a short distance only from the group of large cells around the lower pole.

I have examined over a hundred eggs at this stage, and my best observations have been made on eggs in which the development of the yolk-cells, as compared with the rest of the egg, was retarded, so that the outlines and size of the cells, as well as the unequal development of the two sides, could be plainly seen by surface views with a dissecting microscope. In some eggs it was very easy to follow out the outline of the yolk-cells around the lower pole after the formation of the blastopore, though the development was often too far advanced to make this out satisfactorily. But wherever I could follow out the cells it was plain that the region around the blastopore was in advance of the opposite side. In most cases it is difficult also to orient the egg as regards the pigment after the appearance of the blastopore, though I had a number of specimens where this could be done. Towards the close of the segmentation period pigment rapidly forms over

the area where the blastopore is about to appear, so that a line of dark pigment is distinctly seen in sharp contrast to the lighter cells lying next to it. The change is so rapid that it is often impossible to orient the sides of the egg. In some cases, however, when the blastopore has only just appeared, and before the pigment increases to any extent, it is easy to see that the blastopore is forming on the previously lighter side of the egg, as well as on the side which is segmenting most rapidly. In spite of the dark pigment formed just above the blastopore there is often a distinct light area on one side of the arc. This area probably corresponds with the cell marked *b*, the least pigmented cell, which lies opposite to the centre of the pigment crescent and opposite to the dark cell, *a*, in the diagrammatic figures *A*, *B*, *C*.

Figs. x—xiii are views of favorable specimens, and show distinctly a cluster of large cells, presumably those around the lower pole. Fig. x is before the appearance of the blastopore. The crescent-shaped area of pigment is distinctly seen, the pigment coming much nearer the group of large cells on one side than on the other. It is on this pigmented side that the cells are largest. In the centre are a large cell and three smaller ones, which probably are the four cells nearest the lower pole. The unequal segmentation is also shown in fig. xi, where the blastopore has already formed on the side where the cell division is more advanced. In fig. xii there are four cells distinctly larger than the surrounding ones, between which probably run the first and second furrows. It will be noted how much nearer the pigment approaches these cells on the side marked *m* than on the opposite side, where the blastopore appears. To the right and left of the blastopore the pigment is less dense than on the opposite side, though it is rapidly forming just above it. If it is granted that the four cells are around the lower pole, and that this is the point where the first and second furrows intersect, the exact relation of the blastopore to the lower pole can be easily ascertained. Fig. xiii is a side view of the same egg, in which the position of the supposed lower pole is shown. It is very near

the line of pigment on one side, as we should expect, while the blastopore on the opposite side is less than one third of the distance from the lower to the upper pole.[1]

It has been almost conclusively proved by previous experiments and observation that the plane of the first furrow in the case of the frog divides the egg into halves corresponding to the right and left sides of the embryo; and this study of the blastopore does not contradict, but would tend to confirm the fact. Although the arc of the blastopore is often not opposite the centre of the crescent of pigment (as it is in fig. XII), this is easily accounted for by the distribution of the pigment as shown in Figs. A, B, C (text). If we suppose the second furrow rather than the first to cut through the centre of the crescent (Fig. B), we should have the pigment much as in fig. XI, allowing for the formation of some new pigment.

III.

The eggs of two species of frogs were used for the experiments recorded below. Eggs of Rana temporaria were found on the morning of March 25th. These had not as yet segmented. The eggs of another species (not determined) were brought to the laboratory on April 4th. These had just begun to segment. Since much of the experimental work was done on these eggs, it was first necessary to find out whether the facts recorded in the last section were also applicable here.

A study of representative stages showed the same distribu-

[1] It has been thought by some investigators that the blastopore formed much higher up in the egg, but it needs only a superficial study to show that this at least is impossible. The cells within the blastoporic ring are non-pigmented and yolk-cells. A study of the surface view of the early stages shows that the pigment from an early stage often extends down on one side two thirds of the side, and on the other one half of that side. The blastopore forms lower down than the pigmented area, and this would make it at least halfway down the egg from the upper pole, and much below the plane of the third furrow. We see in fig. XIII that if the lower pole, as I have attempted to show, is marked by the large cells the blastopore appears below even the equator of the egg.

tion of pigment as found in the eggs of Rana temporaria, but the eccentricity in its distribution in respect to the axis of cleavage was greater than in eggs of Rana temporaria. The egg looked at from above (with one pole of the axis turned directly upwards) showed on one side a distinct white crescent, as seen in fig. I.

The most interesting fact is that in the thirty-two-celled stage a very decided irregularity of the segmentation spheres of the upper portion of the egg is to be found.

This is readily seen in the three figures of the same egg drawn in figs. I—III. The first of these (fig. I) shows the egg from above; eight cells lie along a line (four on each side) that corresponds presumably to the first cleavage plane. These upper eight cells are all approximately the same in size in this egg. The eight cells forming the zone around the egg below the upper eight, and which are sister cells with the latter, show a difference in size on opposite sides of the egg, as shown in figs. II, III. The difference may be seen from above, but still better by a study of the opposite (lateral) sides of the egg.

The lighter side of the egg is shown in fig. III, in which the border line of black pigment extends only for a short distance over the side of the egg. The dark side of the egg is drawn in fig. II, and here the pigment extends much further into the lower hemisphere. On the light side of the egg (fig. III) the cells of the second and third zones are smaller than the corresponding cells on the opposite side of the egg (fig. II). Unfortunately the four-, eight-, and sixteen-celled stages of these eggs were not preserved, so that I am unable to say how far back this difference in the two sides may be present.

This led to a re-examination of the eggs of R. temporaria. Here I found that at the eight-celled stage in most eggs one of the four upper cells is somewhat smaller than its upper vis-à-vis. It was also found that this smaller cell is the cell nearest to the highest point reached by the white crescent; therefore it must have come from that cell (now) of the lower pole that contains the least pigment. At the sixteen-celled stage those cells on the side of the egg nearest the upper limit

of the white are also smaller than those opposite to them. Sections of the hardened eggs, made with a scalpel through the plane of these smaller cells and their opposites, showed that these cells are not only smaller superficially, but in the third dimension as well.

Undoubtedly, then, from the eight-celled stage onwards the distribution of larger and smaller cells on the dark and light sides of the egg is present, and I have been able to push back a step farther the differences noted for the later stages in the preceding section.

Whether or not a still more careful examination of very favorable material would find the same difference present in the four-celled stage I am unable to say, but it seems not improbable that such a difference exists.

A study of the method of gastrulation of the egg of the unknown species shows that the first traces of the blastopore appear on the light side of the egg within the white cells. Presumably the pigment has here also extended farther over the sides of the egg than at first. The outlines of the cells in the region of the blastopore are at first polygonal. Dark pigment appears in the walls of the cells, producing the dark line seen in surface view. Certain of the cells pull in from the surface, leaving only their outer small pigmented ends exposed. These cells subsequently pull in all together to form the beginning of the archenteron by invagination. The cells dorsal to the blastopore become narrow and elongated from above downwards. The light cells, below the point of invagination, retain their polygonal outline.

The changes that take place in the overgrowth of the dorsal lip of the blastopore will be recorded below. First let us examine the embryo when first outlined on the egg. Sometimes the outlines of the medullary folds may appear before the yolk-plug has entirely disappeared—at other times not until after this change has taken place. Careful measurements of the embryo at this time show that the embryo anterior to blastopore covers in length about one third of the periphery of the egg. The relative length of the embryo

to the egg is shown in Pl. 25, fig. 5. In some cases the embryo measures a little more than one third of the periphery, in others a little less. Very quickly after the appearance of the medullary folds the embryo increases in length, and the proportions of the egg change, so that in order to determine accurately the length of the medullary folds as compared with the egg they must be measured when their outlines are just indicated by darker pigment.

The suckers appear in front of the medullary folds, and arise at about the same time: a dark line of pigment marks their position. This crescentic line of pigment—the beginning of the suckers—is not quite halfway around the egg from the blastopore, i. e. it is nearer to the dorsal lip of the blastopore than to the ventral.

Experimental Investigation.—Loss of time and material was caused at first by attempts to do experiments that proved to be impossible; also many results were valueless, because the eggs experimented upon were not watched continuously. I cannot too strongly emphasise this point, that unless each egg is carefully followed, from the moment of injury to the time of preservation, the results become uncertain and of little value. I have seen an injured point completely heal over, and the extra-ovate of Roux plough a long furrow over the surface of the developing egg; consequently any conclusion drawn from the end result without a knowledge of the intermediate stages would lead to error, and I cannot but think that some of Roux's earlier experiments that seem to be so contradictory may have been caused by some such changes.

Futile attempts were made to remove as much as half the yolk and protoplasm from the fertilised egg. Such eggs collapsed completely. Equally unsuccessful were attempts to add the yolk removed, by means of a hypodermic syringe, to another egg.

At the two-celled stage, just as the four-celled stage was beginning, attempts were made to suck out with a syringe all of the protoplasm from one hemisphere, in order to determine

whether the remaining hemisphere would develop a perfect half-sized embryo or half an embryo. Many eggs went to pieces, both during and subsequent to the operation. Others partially rounded up and continued to develop, but the greater number of these died later. The few embryos that formed the medullary folds were very imperfect, but, as each egg stuck had not been carefully followed during the stages of segmentation and gastrulation, I hold these results to be valueless. They show, however, I believe, the possibility of carrying out the experiment successfully.

In several eggs at the eight-celled stage one of the black cells was killed by pricking, so that its contents ran out. Such eggs developed, and in the blastular stage defects were found in the black hemisphere. In other eggs one of the white cells was stuck, and, later, defects were found either in the white hemisphere or just within the edge of the dark area. In these cases no record was kept of the position of the particular cell removed; hence the results are of little or no value, and I think the same statement will apply to the similar experiments of Roux. Now that it seems to be possible to recognise, even in the eight-celled stage, the relationship between particular blastomeres and definite portions of the later embryo, more successful results ought to be obtained.

The consistency of the yolk in the eggs of the two species is different. That of R. temporaria is more fluid, and the egg collapsed more easily than in the other case. Owing to this difference the eggs of the unknown species were far more favorable for experiment, and the following results were made on these eggs.

In order to determine the extent of overgrowth of the lower pole by the blastopore a large number of experiments were made by slightly sticking the white cells below the blastopore. By using a very fine and sharp needle an exceedingly small injury could be made, so that only a few small cells protruded from the surface of the egg. These, however, gave a definite landmark for orientation. The determination of the extent of overgrowth by injury to the lower cells has a great advan-

tage, it seems to me, as compared with the more common method of injuring the upper or black cells.

Owing to the great thickness of the lower wall of yolk-bearing cells there is no chance of breaking into the segmentation cavity or archenteron. As the white cells seem to be the more passive cells during development, injury to them has less serious consequences for the developing embryo.

The eggs were stuck at the time when the blastopore first appeared, and a sketch made in each case to indicate the distance of the point of injury from the blastopore. A series of these eggs were prepared in which the injuries were at varying distances from the dorsal lip of the blastopore that had just appeared. A series of figures were drawn from time to time to show the relations between blastopore and point of injury. Moreover duplicates of each lot were followed. Inasmuch as all the experiments gave similar results, I think any doubt as to abnormality caused by the operation is removed.

If the egg (embryo) be turned with its white area uppermost at the time when the blastopore first forms, so that the blastopore just appears above the horizon, it will be found that the white area does not cover quite a hemisphere of the egg. A border of dark pigment appears around the periphery of the white, as shown in outline by Pl. 25, fig. 9. The primary pole of this white area (hemisphere) lies not quite in the centre of the white, but nearer to the side where the blastopore has appeared, as shown in figs. 1, 3, and 4. The "centre" of the white area does not, therefore, correspond with the "lower pole."

The experiments here recorded were made on Rana, sp.?

The × shows the point where the egg was stuck.

Experiment I (figs. 10—12).—Egg in which the blastopore had just appeared. Pricked at 4 p.m. on opposite side of white area, i. e. nearly a hemisphere away from blastopore. At 8 p.m. the blastopore has become more arched, and the distance between the point injured and the dorsal lip of the blastopore is much less than at first. The dotted line running out from the ends of the blastopore marks the rather sharp

line of separation of the black and white, and also indicates the subsequent line of invagination of the remainder of the blastopore. It will now be seen (fig. 11) that the point of injury lies just outside of the pigmented line. At 12 midnight the blastopore had grown much smaller (fig. 12), and the point of injury was outside of the blastoporic rim. The point of injury is now at less than half its former distance from the dorsal lip of the blastopore.

Experiment II (figs. 13, 14).—Egg at blastula stage, had been kept overnight on ice to retard rate of development. At 9 a.m. the blastopore had appeared. Egg stuck in white at a point not quite so far from the blastopore as in the last case. At 4.30 p.m. the outlines of the whole blastopore to be seen, but the point of injury, as before, is still outside of the blastoporic rim, and is nearer to the dorsal lip of the blastopore than at first.

Experiment III (figs. 15, 16).—In this egg the blastopore appeared at first as a vertical pigmented line (fig. 15), which soon extended laterally into the usual crescent. The point of injury was nearly the diameter of the egg from the blastopore. At 4.30 p.m. (fig. 16) the crescentic blastopore was much nearer to the point of injury. Later stages not followed.

Experiment IV (figs. 17—19).—Stuck at 8 a.m. at far edge of white, fig. 17. Blastoporic crescent already formed. At 4.30 p.m., fig. 18, dorsal lip of blastopore much nearer to defect. At 8 p.m. circular outline of blastopore present, and defect lies just within the edge of the blastopore.

Experiment V (figs. 20, 21).—Stuck at 4 p.m. quite near to the blastopore. Blastopore had already formed a crescent. At 8 p.m. the dorsal lip of blastopore had nearly reached the defect.

Experiment VI (figs. 22, 23).—In this experiment the lower pole was not stuck until the circular outline of the blastopore was formed (fig. 22). The dark line of the crescent marks the dorsal lip of blastopore. The dotted line marks

the boundary line, between black and white, ready to invaginate. The injury was made nearly in the centre of the blastoporic plug, somewhat nearer to the dorsal lip. In a later stage, when the yolk-plug is smaller, the defect still lies near the centre of the yolk-plug (fig. 23). It seems relatively a little nearer to the dorsal lip than at first. The blastopore, therefore, must close in after its circular outline is formed at a nearly equal rate from all points. In this case the injury was so small that the overgrowth of the blastopore could not have been in the least retarded.

The experiments recorded above are taken from a series of twenty-one recorded cases, and will serve as types for the rest. All the results point unmistakably to the conclusion that there is an overgrowth of the lower white cells by the lips of the blastopore. Moreover the experiments show the extent of overgrowth of the blastopore and the relative amount of overgrowth of the dorsal and ventral lips respectively.

Examining the results more in detail, we find, if we assume the point of injury to be a fixed point, that the dorsal lip of the blastopore moves over the white to the extent illustrated in fig. 24. This figure is made from data of fig. 10, &c., keeping the point of injury in the same position. The diameter of the circle (representing the outline of the egg) equals 27 mm. The distance between the blastopore and the injury equals 24 mm. From 4 p.m. to 8 p.m. the dorsal blastoporic lip has moved through 8 mm., and is therefore now 17 mm. from the defect. At 12 p.m. the distance travelled through since 8 p.m. is 7 mm. The dorsal lip is now 10 mm. from the defect. So far the blastopore has passed through 15 mm. of the 24. As the defect lies outside of the point of closure of the blastopore by 2 mm., the blastopore now measures 8 mm. Assuming that from this time onwards the blastopore grows together at an equal rate towards its centre, the dorsal lip will pass over about 4 mm. more of the white. In this time the dorsal lip has moved through 20 mm. of the white area. The ventral lip has passed through 4 mm.

The region in front of the blastopore covered by the over-

growth (20 mm.) is less than the diameter of the circle (27 mm.). Comparing this with the length of the medullary folds when they first appear, the area overgrown is found to be somewhat less in length. If we deduct from the length of the embryo the thickness of the medullary folds at their anterior border, we find that the length of the two regions corresponds almost exactly. In other words, the connection around the anterior end of the medullary folds lies just in front of the point where the blastopore first formed, and the area overgrown by the dorsal lip equals the length of the medullary folds between the anterior connection and the blastopore.

A few corrections should be made; the measurements just given apply only to the flat surface, while the embryo lies over a spherical surface. As the measurements of the overgrowth and the measurements of the embryo are both projections into the same plane, no gross error will come into the calculation. The rate of overgrowth is not quite the same in all the observations, but approximately so. Even the extent of overgrowth is variable, and we have seen that the length of the embryo formed is also variable.

The first overgrowth of the dorsal lip of the blastopore is more rapid than the later growth; that is, the approach to the point of injury is faster at first. After the blastopore has completed its circular outline the process of overgrowth (or withdrawal) of the yolk-plug is much slower.

I have assumed the point of injury to be the fixed point, and the approach of the blastopore to be due to the movement of the latter. We might have assumed that the overgrowth was due to a forward movement of the whole of the white area passing beneath the blastoporic lip. The end result would be the same in either case, the process different. It is not an easy question to decide, but to any one following the process in the living egg it will be clear that the change is due to the movement of the blastopore lips, and not to the white area. The condition of the cells in the white area points to a relative stability and inertness, while the reverse is true for the dorsal lip of the blastopore. The method of invagination of the

anterior, lateral, and posterior edge of the blastopore points to the same conclusion.

I believe, however, that the details of the actual process of concrescence of the blastopore has not as yet been accurately worked out. The migration of cells that takes place during the process has not been determined. Whether or not the dorsal lip rolls in as it grows over, or whether its exposed edge always carries the same cells, has not been shown. Both experimental and structural evidence must be brought to bear on the problem before its solution will be possible.

A series of ten experiments were made by sticking the embryo (when the blastopore first appeared) at the apex of the black pole. Other experiments involved sticking at the apex of the black and white in the same egg. The latter experiment ought to settle the question as to what portion was the active agent in the overgrowth. Unfortunately the experiments did not give satisfactory results, nor were the results uniform. Injury to the delicate roof of the segmentation cavity may have helped to produce poor results. Failure to find in the later stage the point injured, shifting of the extra-ovate if large, and the difficulty of determining the exact apex, may all have had a hand in the matter. Only two such embryos are drawn, although other as definite records were also obtained.

Experiment VII (fig. 5).—Egg when blastopore had just appeared was stuck at apex of black pole. When the medullary folds appeared the injury was found on the ventral surface of the body, as shown by the × in the figure. The defect was at about equal distances from the anterior end of the medullary folds and from the blastopore.

Experiment VIII (fig. 6).—Injured as in last. Defect appeared at point 180° from blastopore, therefore some distance in front of the anterior end of the medullary folds.

Both of these results show that the embryo does not form over the black pole, but why in these cases the defects are at such different distances from the blastopore I do not know.

Review of Literature.

There are certain statements made in the papers of Pflüger, Roux, Schultze, and Robinson and Assheton that bear directly on the results given above. Pflüger records that in one set of eggs the blastopore first appeared at 6 a.m. At 11 a.m. the blastopore was broader, with the corners turned down. The blastopore had left the equator of the egg and approached to the lower pole. At 12.30 p.m. the blastopore was semicircular, and had pushed further towards the lower pole. At 1 p.m. it was circular, and now it lay at the opposite point of the white hemisphere from which it had started.

An examination of the relation of the pigment shows that the egg as a whole has had no part in this overgrowth of the lower pole, i. e. no rotation of the whole egg has taken place.

At 2.15 p.m. the yolk-plug was smaller, and the blastopore has continued to move in the same direction. At 4.15 the blastopore is narrower still, and its diameter equals about one eighth the diameter of the egg; it has moved even further, and is in the region of the equator of the egg, but at the opposite point of the equator from which it started in the morning.

These observations point conclusively to the view that "the opening of Rusconi passes from a point on the equator lying in the meridian of the egg over to the opposite point of the equator through the lower white hemisphere, and the egg-axis during the period has not changed its position."

The arc travelled is not quite 180°, but is certainly more than a right angle,—variable, however, in different eggs. The overgrowth is due to a process of invagination.

From 4.15 p.m. till 7.45 p.m. the egg as a whole rotates in the opposite direction along the same meridian. Due to this true rotation more than one half of the (new) upper hemisphere is covered by those cells that overgrew the blastopore, and which therefore have a lighter colour than the cells of the primary upper hemisphere. From this clearer portion in front

of the anus of Rusconi develops the anlage of the central nervous system.

Pflüger adds, "In order to avoid a misunderstanding I must say that I do not by any means believe that the whole anlage of the central nervous system is a derivative of the white hemisphere. Since the lighter substance of the white hemisphere is directly continuous with the lighter substance of the black, it is possible that the anterior portion of the medullary plate corresponding to the brain, and even to the upper portion of the neck, may form in the black hemisphere."

There are two statements only in the foregoing account from which I should dissent. In the first place it seems reasonably certain that the blastopore does not originate in the equator of the egg, but at some distance below it. In the second place Pflüger believes that the blastopore, as it encroaches on the yolk-plug, moves as a whole further along the meridian of migration. This means that after the ventral invagination of the blastoporic rim has formed, the ventral lip still moves upwards towards its nearest equatorial point. This migration of the whole blastopore is stated in the text, and is definitely shown in the series of diagrams drawn to illustrate the process of overgrowth.

I have attempted to show that the overgrowth of the dorsal lip itself is sufficient to account for the length of the medullary folds; also that the posterior lip of the blastopore, after its formation by invagination, closes by a forward growth. There is, therefore, no evidence for such a migration as Pflüger supposes, and if the circular blastopore after its formation does move further upwards it must be due to a slight rotation of the egg as a whole in this direction. But the statement that it does continue to move must be re-examined in living eggs.

It is difficult to give any adequate summary of Roux's results. In his later papers he is not always consistent with his earlier views. Schultze's damaging criticism of some of Roux's earlier conclusions Roux has not answered, although

he has ably replied to other parts of the criticism at great length. It is needless, however, to criticise Roux without repeating his experiments. This, no doubt, will come in time, and it is somewhat surprising that so little has been done by other workers along the lines laid down by Roux. We may here confine our criticism to those points that are connected with the present ground covered. We may pass over the experiments of Roux in which one of the first eight cells was killed in order to determine its position in relation to the embryo. These experiments, as Schultze says, contain direct contradictions.

In Roux's paper published in the 'Breslauer Aerztliche Zeitschrift,' No. 6, March 22nd, 1884, it is stated, "Eine weitere hierher gehörige Beobachtung machte ich an den Eiern vom Wasserfrosch (Rana esculenta, s. viridis). Bei dieser Species stellt sich die Eiaxe nicht senkrecht sondern der Art schief ein, dass bei der Ansicht von oben neben dem hier braunen oberen Pol an einer Seite noch ein mondsichelförmiger Saum des hier gelbweissen unteren Poles zum vorschein kommt. Die erste Furchungsebene steht wie bei R. fusca senkrecht, ist aber so orientirt, dass sie dieses Bild symmetrisch theilt, wies blos möglich ist, wenn sie zugleich durch den höchsten Punkt der gelben Randsichel und durch die schief stehende Eiaxe hindurch geht. Durch die schiefe Einstellung der Eiaxe zur Richtung der Schwerkraft wird hier also auch schon die Richtung der ersten Furchungsebene und mit ihr die Richtung der künftigen Medianebene des Embryo noch vor der Theilung bestimmt.

"An diesjährigen Eierstockeiern von Rana escul. sah ich trotz der noch mangeln den Entwickelungsfähigkeit schon diese Einstellung beim Schwimmen im Wasserglas eintreten. Sofern die gleiche Einstellung reifer Eier im Wasser sich nach der Befruchtung nicht ändern, würde hier also schon im unbefruchteten Eie die Lage der Medianebene und das Oral und Aboral neben dem Dorsal und Ventral bestimmt sein; womit alle Hauptrichtungen des Embryo bereits vor der Befruchtung gegeben wären."

Schultze maintains the same view for the brown frog, but Roux, in a later publication, denies this for this species.

From Roux's conclusions, published in the 'Archiv für Mikros. Anat.,' vol. xxix, 1887, the following quotation is taken:

1. The unfertilised frog's egg has determined one main axis of the median plane of the embryo. This results from the bipolar arrangement of the yolk material, and corresponds to the direction of the egg-axis passing from the black to the white pole, i. e. to a ventro-dorsal direction of the real, a cephalo-caudal direction of the virtual, embryo.

2. From the innumerably different meridional planes which can pass through the egg-axis, that one corresponds to the median plane of the embryo that lies in the direction of the copulation of the two pronuclei.

3. The plane of copulation of the pronuclei is not in any predetermined meridian, but may be determined by localised [artificial] fertilisation.

4. The side of the egg where the sperm enters forms the ventro-caudal side of the embryo; the opposite side corresponds to the dorso-cephalic.

In the body of the same paper Roux says that his experiments show conclusively that there does not exist a latent bilateral construction of the frog's egg. He further adds that in this year he "was fortunate enough for the first time to follow out with success the process of fertilisation in Rana esculenta, and to observe that in this species a peculiar typical change of position of the egg-axis takes place, i. e. the black hemisphere sinks down 20°—30° towards the side of entrance of the spermatozoon."

Hence the first line of cleavage that passes through the upper pole of the egg and the line of the entering spermatozoon would also pass through the apex of the white crescent. Logically no fault can be found with this ingenious explanation; but how explain Roux's earlier observation, that unfertilised eggs of Rana escul. also show the white crescent? Moreover the distribution of the pigment in the unfer-

tilised normal egg seems to be such as not to allow a secondary orientation described by Roux. While, therefore, we cannot deny or refute Roux's statement at present, it seems to me that this point must be carefully examined by other workers before its acceptance will be possible.

In 1888 Roux records the results of new experiments "with improved methods" to determine the relationship of the embryo to egg-axes. If the blastula were injured at the apex of the black pole the defect was found on the ventral side of the embryo. Roux says that he had previously found that if the blastula was stuck at the equator on the blastopore side the defect appeared in the middle of the medullary folds, and he had concluded that the head half of the embryo was formed in the upper half of the egg, i. e. the embryo was placed vertically. The researches of this year (1888) show that this defect was not a primary phenomenon, but that it represented a later change where a "reparation" had taken place.

Roux injured the first anlage of the dorsal lip of the blastopore, and found the defect to lie in the cross-connection at the anterior end of the medullary folds. Injury to the blastula or young gastrula at one side of the equator produced a defect in the middle of the medullary folds. Injury to the young gastrula at a point opposite the gastrula crescent produced defects in the caudal region. Injuries in the middle of the white area gave no defect in later embryo.

These experiments of Roux's are of great importance, for if true they show the method by which we must regard the blastopore to be closed. I shall return to this in the final section when speaking of the general problem of concrescence.

Roux concludes that the embryo lies over the lower hemisphere, and that the dorsal lip of the blastopore moves through 170°. His figures ('Anat. Anzeiger,' 1888) show the head end of the embryo near that point of the equator at which the embryo first appeared. The anterior connection of the medullary folds lies just above the equator upon the black hemisphere. From this region the embryo stretches over the lower pole for 170°.

In these figures I believe Roux represents the early embryo as extending over too great an extent of surface of the sphere. Moreover, it seems, as I have said, most probable that the blastopore does not start at the equator of the egg, but some distance below that circle.

Schultze's conclusion that the embryo lies over the black hemisphere may be dismissed, as it is completely contradicted by well-determined facts.

Finally, Robinson and Assheton make certain statements as to the method of closure of the blastopore that call for notice. Apparently at the outset they have orientated the embryo wrongly, for they state, "The segmentation cavity has a roof which ultimately becomes the anterior wall of the gastrula; for the anus, which marks the posterior end of the embryo, appears at the opposite pole of the ovum,—that is, in the floor of the segmentation cavity." Again, they say, "For during the formation of the blastopore the epiblast does not grow over the yolk-cells enclosing them by a process of embolic invagination." This statement is intended to apply rather to the extension of the epiblast over the sides of the embryo, and as such is perfectly correct. But, in addition to this process of delamination, there is a decided and extensive overgrowth, as we have seen, of the dorsal lip of the blastopore enclosing the yolk embolically. Further, the statement of Robinson and Assheton that no portion of the archenteron in the anura is formed by invagination is certainly incorrect, as I hope to show in a later paper.

They continue, "According to some former accounts, to which we have made reference above, the anus of Rusconi has been said to diminish in size by the gradual coming together of each portion of the blastoporic rim simultaneously. This we believe to be incorrect. The anus of Rusconi gradually diminishes in size by the concrescence of the ventral part of the lateral lips." In their diagrams, to show the method by which the ventral lip of the blastopore comes together, they show the right and left sides applied to one another, and subsequently fused. Later they say, "We infer . . . . that the

anus of the frog, although apparently a new perforation, is really a reopening of the temporarily closed portion of the original blastopore." Now I doubt exceedingly whether concrescence of the ventral lip of the blastopore takes place in any such way as the authors' diagram indicates. The cells from the sides may come later to the middle line, but not by a process of apposition of the latero-posterior walls of the blastopore. Rather the cells stream or migrate to the median line below the surface, while the surface grows continuously from behind forward. Hence we cannot speak of a reopening of the blastopore, as nothing was left behind to reopen; but we must speak of a perforation at the point where the blastoporic lips first began to concresce in a sense different from that used by the authors.

IV.

Roux and Hertwig have given accounts of embryos in which the blastopore had failed to close. Roux figures an embryo with a hemisphere of white exposed, and the embryo lying as a thickened zone around the border line between the black and white hemispheres. Hertwig has not figured such extensive exposures of yolk, but describes stages with varying amount of blastopore unenclosed.

I attempted to produce such embryos artificially, and after a great number of attempts that gave no favorable result found at last a method that made it possible to produce such embryos at will.

Embryos in which the blastopore had just appeared were put into the following solutions, with the results recorded:

Hydrochloric acid, $\frac{1}{20}$ per cent.	Died.
Sodium hydroxide, $\frac{1}{20}$ per cent.	Some died, in others the blastopore closed.
Corrosive sublimate, $\frac{1}{20}$ per cent.	Died.
Curari (weak solution)	Nearly normal.
Quinine, ·02 gr. to 500 c.c. H_2O	Normal.
Morphine ,, ,,	Nearly normal.
Strychnine (only partially dissolved)	Normal.
Alcohol, 10 per cent.	Developed very little.
,, 5 ,,	More than last, but died (unclosed blastopore).
,, $2\frac{1}{2}$,,	Developed partially (closed).
Sodium chloride (3 grms. to 500 c.c. H_2O = $\frac{1}{4}$ strength of sea water	Gave the desired result. Blastopore open.
,, ,, $\frac{3}{8}$,, ,,	Normal.
,, ,, $\frac{1}{2}$,, ,,	Died.

Of the solutions given only one gave the desired result, although the alcoholic solutions seemed to have a similar tendency. The series in which ·6 per cent. salt was used produced the embryos to be described below. This happened both for the frog's and the toad's eggs, and was repeated with similar results. Success depends on using exactly the right amount of salt. Too much kills; too little does not affect the embryo. To ensure success a series of trials should be made approximating to the ·6 per cent. solution.

In a second series of experiments the recent suggestion of Herbst was followed. Embryos were placed in solutions of salts of barium, calcium, sodium, and potassium. Three sets of each solution were used, one stronger, one weaker, and one the same strength as the ·6 per cent. solution.

Although in some of these solutions embryos with large blastopores were produced, no particular relation was found between the formation of abnormalities and the series of compounds used. The best results were again in the sodium chloride.

Fig. XIV shows an embryo as seen from in front. A narrow pigmented line marks the position of the suckers. Between this and the white a thick fold of ectoderm marks the anterior end of the medullary folds. The fold continues on each side

along the border line between the black and white hemispheres. These lateral medullary folds can be traced for only a short distance.

Between the anterior connection of the medullary folds and the white is found a small plate of ectodermal cells. The same embryo seen from below is shown in fig. xv. We see that the extent of white exposed corresponds to the whole of the lower white area,—in fact, to somewhat more of the lower hemisphere than would be finally enclosed by the normal blastopore; for in the normal egg the far edge of the white, where it shades off into the black, does not normally become involved in the closure of the blastopore. This was shown definitely in the experiments made by sticking the lower pole, and is corroborated by the fact, that in these abnormal embryos the far side of the large blastopore contains much more pigment than does the ventral surface of the yolk-plug of the normal embryo. Hence any statement as to the extent of the white closed over by normal embryos, based on these abnormal embryos, will give an erroneous conclusion. This, I believe, Roux has drawn.

The embryos produced in the salt solution may be examined at each stage of their development, and the exact method by which the blastopore forms be followed out. This gives a decided advantage over the haphazard finding of embryos already formed. In watching such embryos one sees that the blastopore extends from its point of origin differently in these embryos than in normal embryos. Instead of the corners of the blastopore extending downwards to produce a deep crescent or horseshoe-shaped outline, they extend laterally around the border line between black and white. Hence results, I believe, a more extensive enclosure of the lower hemisphere than under normal conditions.

In fig. xvi is drawn another embryo, differing from the last only in the greater extent of the medullary plate lying in the black hemisphere. This is due without doubt to the greater extent to which the dorsal lip of the blastopore has crept over the white. Figs. xvii and xviii are drawings of embryos where the overgrowth of the dorsal lip has been carried farther

than in the last case, so that not only the anterior connection, but also the anterior end of the medullary folds, lie on the black portion of the egg. This embryo shows conclusively that the extent of closure of the blastopore is far more than the normal, for if the embryo really had covered so much of the sphere as the whole of the white area, and as much of the black as the anterior end now occupies, it would have covered nearly two thirds of the sphere, and not one third, as in the normal embryo.

Fig. XIX is from an embryo in which the dorsal lip of the blastopore has grown over the lower pole to the extent indicated by the medullary folds. When looked at from below—i. e. with the white area turned up—we see still a large exposure of white, but the posterior extension of the blastopore is not completed, again verifying the statement made above, that the exposure of the white is in these eggs abnormally extensive.

In fig. XX is drawn the posterior end of an embryo much more advanced than the last. Quite a large exposure of yolk is present, but not nearly so much as in the other cases. Anterior to the blastoporic plug the medullary folds have met to form a closed tube. Posterior to the blastopore, as seen in the figure, a deep groove is present, and this groove is formed by the posterior medullary folds. The ventral lip of the blastopore has, therefore, grown over the white to the extent indicated by the medullary folds. Whether this forward growth of the ventral lip is unusually extensive I do not know, nor have I any records to show whether in the earlier stages so much of the white was enclosed as in the preceding cases; but, judging from the length of the embryo and from other facts, I think we may safely conclude that the area enclosed was less.

Other embryos, with still less exposure of white, need not be figured at present; Hertwig's description seems to cover such cases.

Serial sections were made through these embryos. In the embryo shown in figs. XIV, XVI, &c., sections add little knowledge to that formed from surface views. The most noticeable

structure is the large archenteron that begins at the edge of the black in the mid-dorsal line, and extends as far forwards as the level of the suckers. The medullary folds in these embryos have not as yet rolled in to form the (half) nerve-cords. Longitudinal section of the embryo drawn in fig. XIX shows that at the ventral lip of the blastopore only a very slight depression is present.

Conclusion.—His supposed concrescence of the Vertebrate embryo to take place by apposition of the sides of the germ ring, and due to this process the embryo grew posteriorly.

Balfour believed this to be untrue, and that the posterior end of the embryo grew in length by a process of intussusception in front of the last segment of the body.

Roux's experiments by sticking the border between black and white point directly to a process of concrescence of some sort. If Roux's experiments are accurate we must suppose that the cells that will later form the central nervous system are already laid down along the black-white border. These cells must come to the middle line as the blastopore gets smaller. The closing of the blastopore from before backwards would then be due not to a backward extension of all of the material of the dorsal lip over the yolk, but would take place by new tissue coming up to the middle line from the sides and placing itself with or behind the cells already present in the dorsal lip.

I should not regard this, even if it took place, as apposition in the strict sense of the word, nor is it intussusception in Balfour's sense. It would not be intussusception because new tissue is coming in continually from the sides to mingle or mix with the cells already present and multiplying in the dorsal lip of the blastopore. Nor would it be apposition in His's sense, because the lateral borders of the blastopore are not laid down side by side, since the blastopore does not close by actual apposition of its lateral rim. I shall not here attempt to formulate a theory of overgrowth, but merely to point out the apparent bearing of the evidence furnished by this experiment of Roux. It will, I think, be possible to de-

termine experimentally exactly the process that takes place in the dorsal lip of the blastopore, and then we shall be prepared to formulate more definitely a theory of overgrowth.

BRYN MAWR, PA., U.S.A.,
May 22nd, 1893.

DESCRIPTION OF PLATES 24 & 25,

Illustrating Messrs. T. H. Morgan and Umé Tsuda's paper on "The Orientation of the Frog's Egg."

FIGS. IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, Rana temporaria.
FIGS. I, II, III, XIV, XV, XVI, XVII, XVIII, XIX, XX, Rana, sp. ?

PLATE 24.

FIG. I.—View of 32-celled stage from above.
FIG. II.—View of same stage from dark side.
FIG. III.—View of same stage from light side.
FIG. IV.—View of blastula (about 150 cells), dark side.
FIG. V.—View of same, light side.
FIG. VI.—Four-celled stage, lower pole; *M*—*N*, first furrow.
FIG. VII.—About 100-celled stage, lower pole.
FIG. VIII.—About 100-celled stage, lower pole.
FIG. IX.—Earlier stage than last.
FIG. X.—View of lower pole of egg at end of segmentation.
FIG. XI.—Early blastopore stage. Pigment has somewhat shifted its earlier distribution, lower pole.
FIG. XII.—Early blastopore stage, lower pole.
FIG. XIII.—Early blastopore stage, side view.
FIG. XIV.—Embryo with unclosed blastopore, seen from in front.
FIG. XV.—Same from below.
FIG. XVI.—Embryo with unclosed blastopore, seen from in front.
FIG. XVII.—Embryo with unclosed blastopore, seen from in front.

FIG. XVIII.—Same as last, side view.

FIG. XIX.—Embryo with large blastopore, seen from dorsal side.

FIG. XX.—Embryo with large blastopore, seen from behind.

PLATE 25.

FIG. 1.—Diagram normal egg, lower pole.

FIG. 2.—Diagram same egg, side view.

FIG. 3.—Diagram normal egg, lower pole.

FIG. 4.—Diagram same egg, side view.

FIG. 5.—Diagram to show defect × produced by sticking apex blastula.

FIG. 6.—Diagram to show defect × produced by sticking apex blastula.

FIG. 7.—Diagram normal embryo to show length of medullary folds, side view.

FIG. 8.—Diagram same embryo to show length of medullary folds, dorsal view.

FIG. 9.—Diagram to show lower white area when blastopore appears.

FIG. 10.—Diagram egg stuck opposite edge of white from blastopore, see text.

FIG. 11.—Diagram same, later stage, see text.

FIG. 12.—Diagram same, later stage, see text.

FIG. 13.—Diagram egg stuck far from blastopore, see text.

FIG. 14.—Diagram same, later, see text.

FIG. 15.—Diagram egg stuck far from blastopore, see text.

FIG. 16.—Diagram same, later, see text.

FIG. 17.—Diagram egg stuck nearer to blastopore, see text.

FIG. 18.—Diagram same, later, see text.

FIG. 19.—Diagram same, later, see text.

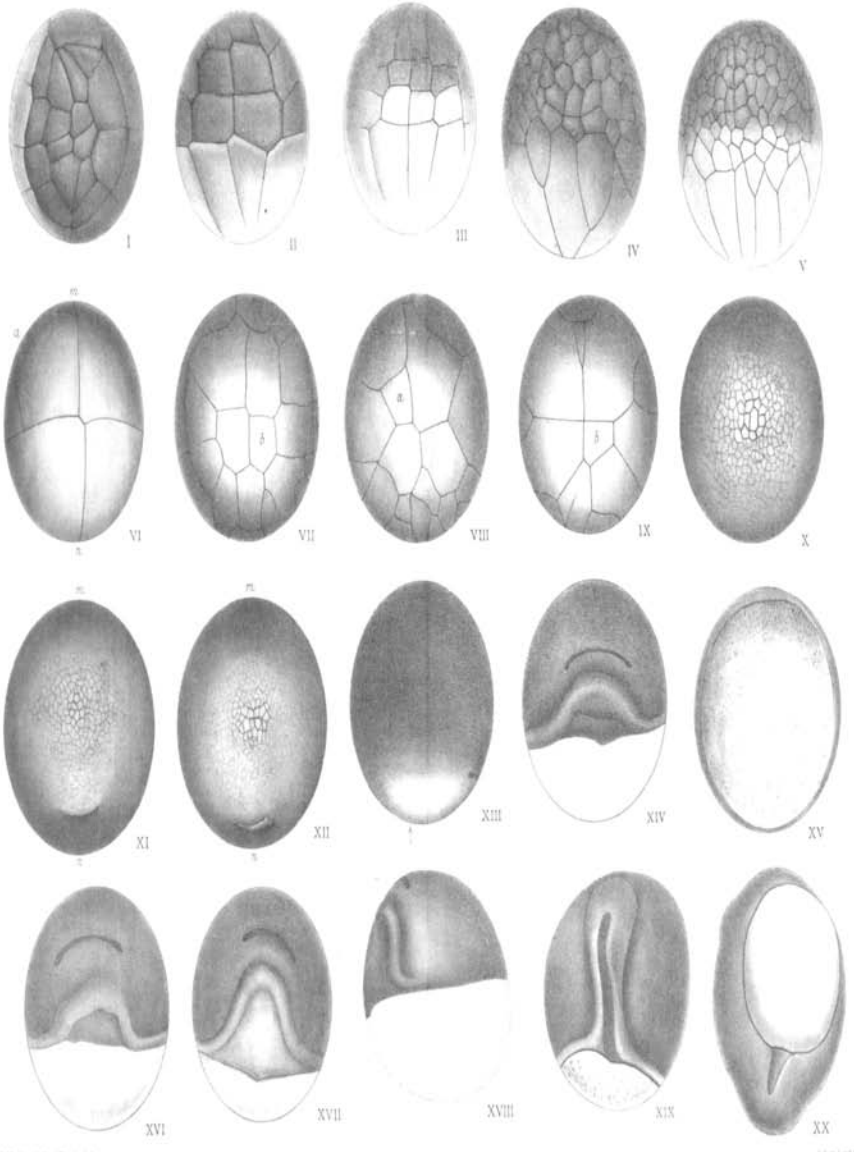
FIG. 20.—Diagram egg stuck near blastopore, see text.

FIG. 21.—Diagram same, later, see text.

FIG. 22.—Diagram egg stuck centre of early blastopore, see text.

FIG. 23.—Diagram same, later.

FIG. 24.—Diagram with injury taken as a fixed point × to show relative advance of dorsal lip of blastopore.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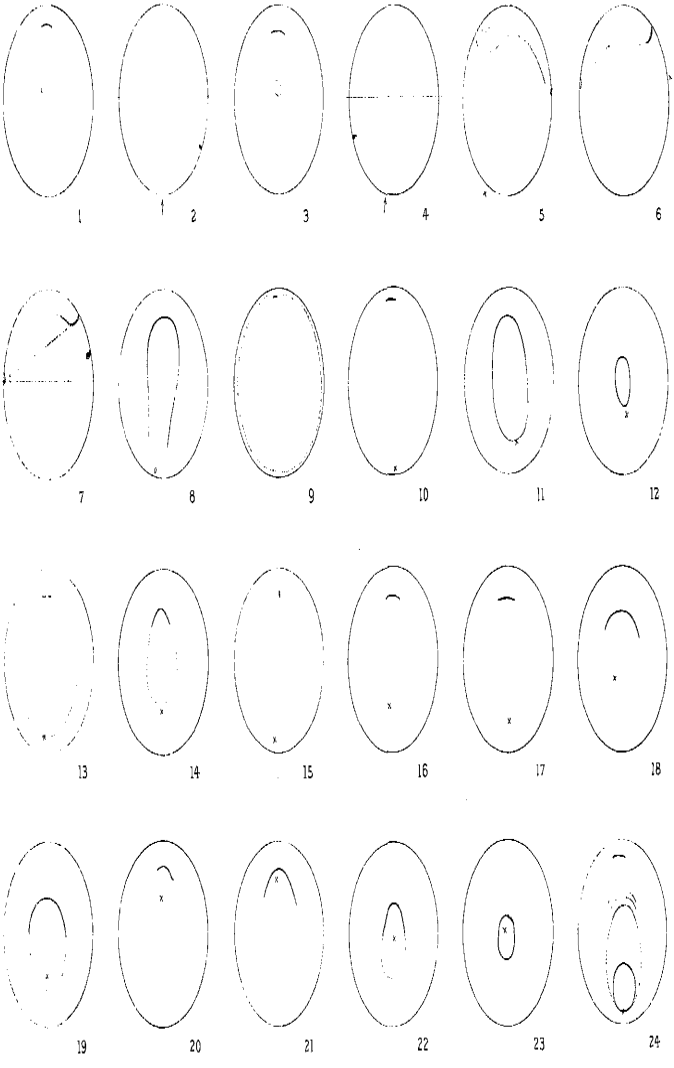
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