The Relation of the First Plane of Cleavage and the Grey Crescent to the Median Plane of the Embryo of the Frog.

By

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With Plate XXII.

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The main purpose of our study of the early cleavage of the frog's egg was to find what relation exists between the grey crescent, the first plane of cleavage, and the median plane of the body. We first examined the relation of the position of the first cleavage to the grey crescent in a large number of preserved eggs. Later, in the spring of the year, we determined with living material the relation of the median plane of the embryo to the first plane of cleavage and to the median plane of the grey crescent. The latter results will be given first.

The factors determining the median plane of the embryo in Rana palustris.

Our study of preserved material showed, that while in most cases the first plane of cleavage coincided more or less nearly with the middle of the grey crescent, yet in other eggs it did not, and in a few it even lay at right angles to the median plane of the crescent. This relation made it possible to find out whether the median plane of the embryo follows the first plane of cleavage or the grey crescent.

Each egg was put in a small watch glass having a coat of paraffine on the bottom. Very fine needles were stuck through the jelly and into the paraffine, thus holding the egg without compression in a definite position. An examination showed that the egg held this first position under these conditions. The rusting of the needles did not interfere with the normal development, and was, in fact, of assistance in determining the relation of the plane of cleavage to that of the embryo. In one set, fine glass fibres were used, but proved less satisfactory than the needles. A mark was made on the paraffine indicating the position of the first furrow, and in all but the first set of eggs, a cross was placed opposite the centre of the grey crescent. The eggs used were in the first cleavage stage, or before the second furrow had completed itself, so that there would be no danger of confusing the two. A bunch of eggs was looked over, and those selected in which the first furrow was ninety degrees from the centre of the grey crescent. These eggs were fastened in the paraffine dishes and left until the dorsal lip of the blastopore appeared. Then the needles were carefully removed and the position of the dorsal lip in relation to the first furrow determined. In the first bunch, twenty-eight eggs out of 463 had the first furrow about ninety degrees from the centre of the crescent. When they were examined for the position of the dorsal lip, nine were found either injured, or not yet developed far enough, seven had the blastopore ninety degrees from the first furrow, six in the plane of the first furrow, five forty-five degrees from the first furrow, and one sixty degrees. In this set we were not as careful as later to select only those eggs in which the first furrow was exactly ninety degrees from the centre of the crescent. So that in those eggs in which the dorsal lip appeared ninety degrees from the first furrow, the median plane need not have coincided exactly with the centre of the crescent, and those in which the dorsal lip appeared less than ninety degrees from the first furrow may have more or less nearly coincided with the centre of the crescent. Nevertheless the results show that when the first furrow and the grey crescent did not coincide, there was much discrepancy between the position of the median plane of the embryo and of the first plane of cleavage, and in some cases the median plane of the embryo appeared to coincide with the grey crescent[1]).

The number of eggs with the first furrow at right angles to the grey crescent was remarkably similar in the different bunches, being one in every sixteen in the first, one in every fourteen in the

[1]) In one in which the centre of the crescent was marked as forty-five degrees from the first furrow, the blastopore appeared forty-five degrees off, thus following the centre of the crescent.

second, and one in twelve in the third. In the second lot, we used the glass instead of needles, and the results were unsatisfactory. In the third lot, we used only those eggs which had the first furrow at right angles to the centre of the crescent, and the crescent side of the egg also was marked. The crescents in this set were much more distinct than in the other sets. Of the thirty-four eggs in this experiment, two had not yet formed the blastopore when examined, three had the dorsal lip in the plane of the first furrow, six ninety degrees away, that is, coincident with the centre of the crescent, three eighty degrees, one seventy-five degrees, one sixty degrees, three forty-five, one thirty, one twenty, and one fifteen degrees away from the first furrow. In two other eggs where the angle between the first furrow and the crescent was only forty-five degrees, the blastopore in one followed the crescent, and in the other neither the crescent nor the first furrow. In every case the blastopore appeared on the crescent side of the egg.

As a check experiment, we fixed twenty-nine eggs of another bunch, in which the middle of the crescent coincided exactly with the plane of the first furrow. In all of these but one, which was accidentally stuck, the blastopore formed in the plane representing both the first furrow and the centre of the crescent. To see whether this bunch was different from the others, we fixed five eggs in which the first furrow was ninety degrees from the crescent; in four eggs the dorsal lip appeared at the centre of the crescent, i. e., ninety degrees from the first furrow, and in one, in the plane of the first furrow.

The Relation of the Planes of Cleavage and the Grey Crescent.

A number of observers, M. Schultze, Roux, Rauber, Eycleshymer, Morgan and Tsuda, and others, have studied the variations in the method of cleavage of the frog's egg; but the precise numerical relation between the different forms of cleavage has not been very throughly examined. This is all the more desirable, since the more recent work of Schultze, Moskowski, Kathariner, and Morgan has shown the presence of a new factor in the egg's development, namely, the appearance of the grey crescent on the side of the egg which corresponds to the region where the future anterior part of the embryo will develop. It is true that this same region was sufficiently identified by Roux, as that part of the egg at which the white reaches its highest point; this condition being due, as he thought, to the oblique position assumed by the egg after fertilization; the egg turning toward that side at which the spermatozoon entered, in consequence of which the yolk-hemisphere of the opposite side is lifted somewhat above the primary equator of the egg. This wedge-shaped region (crescentic in surface view) is the region in which, in many eggs, the grey crescent appears. The change in color that takes place at this time is due to some rearrangement taking place in the interior of the egg. It has been shown by MORGAN that this grey crescent does not appear in eggs that are kept constantly in motion from the time they are taken from the oviducts, through the fertilization and early segmentation periods. Although an embryo forms on these rotated eggs this result need not necessarily mean that when in other cases the crescentic region in question is produced it may not then become the predominant factor that determines either the future of the first cleavage, or the median plane of the body of the embryo, or both. We undertook, in the first place to examine in several bunches of eggs, in which the grey crescent was particularly well developed, the relation of the cleavage planes to the grey crescent, and in the second place, to examine the relative sizes of the four upper cells in the eight-cell stages in their relation to the crescent.

Eggs of Rana palustris in the eight-cell stage were preserved in 3% formalin. The grey crescent remained evident, although not quite with the same clearness as in the living egg. Out of a hundred and ninety-seven eggs, the crescent could be seen in all the four-cell stages, and in all but twenty-two of the eight-cell stages. In these twenty-two eggs, it is assumed in our calculations that the grey crescent corresponds to the »white crescent«, i. e., that portion of the yolk-hemisphere that lies nearest to the point of meeting of the first and second furrows. This region can also be located from the relation of the pigment to the lower point of meeting of the first two cleavage planes. On one side the pigment comes nearer to this point than on the opposite; vis-à-vis, from this black region, the region of the grey crescent is formed. The two main conditions which were examined were the following:

1) The relation of the smallest of the four black cells at the upper pole in the eight-cell stages to the furrow or furrows passing through the grey crescent.

2) The relation of the highest point of the grey crescent to the first and second cleavage furrows.

Out of a hundred eggs examined in the eight-cell stage, the majority had one black cell smaller than the other three black cells, and this smallest cell was always on the side of the grey crescent. In fifty eggs, one furrow cut through the centre of the grey crescent, thus causing two black cells to border on it, as the crescent in these cases seldom extended more than half way round the egg (Fig. 2). Of these eggs, twenty-nine had the smallest cell to the left of the furrow (Fig. 1) (assuming the egg in a position with the crescent towards the observer), eighteen had the smallest cell to the right of the furrow (Fig. 2), while in three, it was impossible to determine whether the right or left cell was smallest, as in both the third cleavage furrow had come in vertically instead of horizontally (Fig. 3). In the majority of cases the smallest cell lies at one end of the »cross furrow« at the top of the egg, i. e. the smallest cell has a pointed and not a truncated upper end (Fig. 2), but this is by no means always the case, since in eight out of forty-seven eggs, the upper end was truncated as in Fig. 1.

In the other fifty eggs out of the hundred examined, the crescent was not divided in the centre by a furrow. These eggs fell into three classes, those in which the two furrows were equidistant from the middle of the crescent (Fig. 4); those in which one furrow lay slightly to the right of the centre of the crescent (Fig. 5), and those where it lay a little to the left of the centre (Fig. 6). In the first class there were sixteen; in the second, sixteen; and in the third, eighteen. Usually portions of three cells bordered on the grey crescent, one being nearer the centre of the crescent. In a few cases, the crescent did not extend half way around the egg, so that only one cell and part of another bordered on the crescent (Fig. 6). In all but seventeen of these fifty eggs, the black cell lying nearest the centre of the grey crescent was the smallest, or at least not any larger than the others. In fifteen of the exceptions, the third cleavage furrow had come in vertically in that cell, so that it was impossible to say which was smallest (Fig. 5). Only two had this centre cell distinctly larger than the surrounding cells (Fig. 7). It was found here also that the smallest cell was more apt to be pointed than truncated. The majority were pointed, but the difference in number was not so great as when the furrow divided the crescent equally, the proportion this time being fifteen to twenty.

So many cases having been found in which the third cleavage furrow came in vertically, the question arose as to whether this is more apt to occur in one cell than in another; and whether it frequently occurs in more than one cell in the same egg. Out of the hundred eggs, thirty-five were found in which one or more cells had the third furrow vertical. The majority had this in only one cell (Fig. 5), seven had it in two (Fig. 3), four in three (Fig. 8) and one in all four cells (Fig. 9). Those eggs that had divided vertically in three or four cells had lost their circular shape, and become slightly elongated (Figs. 8 and 9), and rather flat on top. As to the cell in which vertical cleavage is most apt to occur, the following results were obtained: in the eggs where the furrow cuts the crescent equally, the proportions for different cells were too close to make it possible to lay more stress on one than on the other; but in those where the crescent was not equally divided, the dark cell near the middle of the crescent was most often divided vertically, namely, in thirteen cases out of thirty-eight. The cell opposite this central cell, and the cell to the right side of the central cell came next in order, there being nine of each. In seven cases the cell on the left side was divided vertically. These numbers are too small, however, to show with any probability that one cell is more liable to this sort of division than another.

In fifty of these eggs in the eight-cell stages, the shape of the grey crescent was examined. It varied greatly in length and width. Sometimes the horns were pointed (Fig. 10), and sometimes cut off abruptly at a furrow (Fig. 11), thus producing anything but a crescent effect. But almost always the crescent reached a little nearer the upper pole in one region than in any other. In the few cases where this was not so, the crescent was bisected by a furrow, and the crescent was at any rate slightly wider at that furrow than elsewhere (Fig. 12). This we shall call the highest point of the crescent. Sometimes this highest point was at the middle of the crescent, thus making the crescent symmetrical in shape (Fig. 11), and sometimes it was to one side of the middle, making an unsymmetrical crescent (Fig. 10). Whether symmetrical or unsymmetrical, however, in all but five eggs, the highest point coincided with a furrow. Those five exceptions were eggs in which the crescent was symmetrical, and not divided in half by a furrow. Of the fifty eggs examined, twenty-four had unsymmetrical crescents. All but two of these had the highest point of the crescent coincident with the furrow nearest the middle of the crescent (Fig. 10). Those two having the highest point coincident with the furrow farthest away from the middle of the crescent, were very unsymmetrical in shape (Fig. 14).

The question then arose whether it is the first or the second furrow which coincides with the highest point of the crescent. To determine this, eggs must be examined in which the second furrow has not quite completed itself, so that it can be distinguished from the first furrow. An examination of ninety-seven eggs in this stage showed that the highest point of the crescent might coincide with either the first or the second furrow, but coincided with the first (Fig. 12) much more frequently than with the second (Fig. 11), the proportion being seventy-nine to thirteen. In these eggs, as in the eight cell stages, there were five cases in which the highest point was not coincident with any furrow (Fig. 13). Again there were more symmetrical than unsymmetrical crescents, fifty-three to forty-four. In the symmetrical, the furrow bisecting the crescent might be either the first (Fig. 12) or the second (Fig. 11). In the unsymmetrical, only a few of the eggs had the highest point coincident with the furrow farthest from the middle of the crescent, they were five in number (the coincident furrow in three being the first, Fig. 15, and in two the second, Fig. 14), and in twenty-five cases, the furrow was slightly to the left of the middle of the crescent (Fig. 16), while in seventeen cases, it was slightly to the right (Fig. 10), thus showing the same majority for the left side that appeared in the eight cell stages.

Comparing these observations with those made by Morgan and Tsuda on Rana temporaria, some differences appear. They make no mention of eggs in which the furrow bisects the crescent, and although the eggs they used did not show the grey crescent, we are assuming it to coincide with the highest region of the yolk hemisphere (»white crescent« of Morgan). They say »the first furrow does not seem to cut the pigment zone bilaterally« and »of the four cells into which the egg is divided, one cell has always the greatest amount of pigment«. In Rana palustris, we found 50% of the eggs had the pigment zone (opposite the grey crescent) cut bilaterally, for, when the crescent is cut bilaterally, so is the pigment zone, and this same 50% had two cells containing more pigment than the other two, but no one cell containing the most.

The three types of cleavage that Morgan and Tsuda mention correspond to the three types described here, in which the furrow does not bisect the crescent, but the percentages differ. If type A be those in which one cleavage furrow is slightly to the right of the centre of the crescent (Fig. 5), type B those in which it is slightly to the left (Fig. 6), and type C where the two furrows are equidistant from the centre (Fig. 4), MORGAN and TSUDA found 64 % of A, 25 % of B, and 11 % of C, while here we found 32 % of A, 36 % of B, and 32 % of C.

Another difference is, that, according to MORGAN and TSUDA, in type A it is always the first furrow that is nearest the centre, and in B it is always the second; while we found that of seventeen eggs of type A, five had the second furrow nearest the centre, and of twenty-seven of type B, twenty-six had the first furrow nearest the centre.

The difference in the two sets of observations may be due to their being made on different species, Rana temporaria and Rana palustris; or the bunches of eggs used in the two cases may have happened to have different variations in cleavage. To see whether the difference in species accounts for the difference in result, some eggs of Rana temporaria also were examined. Fifty-four eggs in the eight-cell stage, and forty before the second furrow was complete, were examined. These eggs had been kept in alcohol for ten years and were somewhat shrivelled, and quite hard to work with; also the grey crescent did not show; so these observations are subject to some error. However, every kind of variation present in the Rana palustris was found here, with but one exception, namely, where the third cleavage plane comes in vertically in all four cells. This being so extreme a variation, and only once occurring among a hundred eggs in Rana palustris, it can not be taken into account as showing any marked difference between the two species. But a decided difference in the percentages of eggs following the different types of cleavage was found, as given in the following table:

	R. palustris	R. temporaria
Furrow through centre of crescent	50 %	24 %
Smallest cell left	58	54
Smallest cell right	36	15
Vertical cleavage in right and left	6	23
Crescent not bisected	50	76
Centre cell larger than others	4	5
Furrow to the right	32	32
Furrow to the left	36	22
Furrow equidistant from centre	32	46

	R. palustris	R. temporaria
Eggs with 3rd cleavage vertical	35 %	57 %
in 1 cell	66	55
in 2 cells	20	42
in 3 cells	11	3
in 4 cells	3	0
in »central« cell	40	45
Symmetrical crescent	52	32
Unsymmetrical crescent	48	68
Highest point of crescent coinc. w. a furrow	90	95
- - - - not coinc. w. a furrow	10	5
- - - - coinc. w. 1st furrow	80	67
- - - - - - 2nd -	15	28
- - - - near centre	95	85
- - - - not near centre	5	15

The most striking differences are in the number of those in which the cleavage furrow bisects the crescent, it being only 24 % in R. temporaria while it is 50 % in R. palustris. This shows a much nearer approach to Morgan and Tsuda's results. In both the smallest cell is more often to the left of the furrow than the right, but in R. temporaria a larger percent have both right and left cells divided vertically, so that it can not be told which cell of the upper four is the smallest. In the eggs where the furrow does not bisect the crescent, the smallest cell is in both species usually the cell at the centre of the crescent, and the percentages of exceptions are almost the same. The percentage of the three types of cleavage, where the furrow does not bisect the crescent, differ in the order in which they come, type B, where the furrow is slightly to the left of the middle of the crescent, being most numerous in R. palustris, while type C, where the furrows are equidistant from the middle as in R. temporaria. In R. temporaria, there are more cases where the third furrow is vertical. Since there are fewer eggs of R. temporaria in which the crescent is bisected, there are fewer with a symmetrical crescent. In both, the highest point of the grey crescent usually coincides with a furrow. In R. temporaria, a few more coincide with the second than in R. palustris, but not nearly so many as with the first. Also a few more eggs have the highest point coincident with the furrow not nearest the middle of the crescent.

These results on R. temporaria more nearly correspond to Morgan and Tsuda's on Rana temporaria. There still remains the same kind of differences, but the numerical value of these differences is less.

In one of the bunches of eggs of Rana palustris that we used in the experiments to determine the relation of the median plane of the embryo to the first cleavage plane and the grey crescent, we noticed some eggs in which the first furrow did not divide the egg into two equal cells. This unequal division occurred in eleven out of one hundred and ten eggs. The grey crescent was partly on both cells in five eggs, and entirely on the larger cell in the remaining six, but never entirely on the smaller.

In this same bunch, the third cleavage came in normally, that is horizontally in all four cells, in only five eggs of forty-four; six had vertical cleavage in one cell, fifteen in two, thirteen in three, and five in four cells. This bunch had been brought to the laboratory before segmentation began and placed in a flat dish. This flattening of the bunch would bring more pressure to bear on the eggs than when the bunch floated in the pond. It is possible that this change in pressure relations may have had something to do with the large percentage of eggs in which the third cleavage came in vertically. It has often been shown that pressure can cause the third cleavage plane to change thus. It may be that all cases of vertical third cleavage are due to pressure changes, but this has not been proved.

Summary.

1) When the first plane of cleavage coincides with the median plane of the grey crescent, the median plane of the embryo coincides with these two.

2) When the first plane of cleavage is at right angles to the median plane of the grey crescent, the median plane of the embryo usually coincides either with the first plane of cleavage or with the median plane of the grey crescent, but sometimes with neither.

3) The dorsal lip of the blastopore appears on the grey crescent side of the egg.

4) In fifty percent of the cases the first plane of cleavage coincides with the median plane of the grey crescent. In about eight and a half per cent of the cases the second plane of cleavage coincides with the median plane of the grey crescent.

In the remaining eggs, the first plane of cleavage lies to one side, or the other, of the middle of the grey crescent, usually near to it.

5) The smallest black cell of the four at the eight cell stage always lies on the crescent side of the egg. When the crescent is bisected by the first cleavage plane, the smallest cell lies in eighteen percent to the right, and in twenty-nine percent to the left. When the first cleavage plane does not bisect the crescent, the smallest cell lies nearest the centre of the crescent.

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Zusammenfassung.

1) Fällt die erste Theilungsebene mit der Medianebene des grauen Feldes zusammen, dann stimmt auch die Medianebene des Embryo mit diesen beiden überein.

2) Steht die erste Theilungsebene rechtwinkelig zu der Medianebene des grauen Feldes, so fällt die Medianebene des Embryo gewöhnlich mit der einen von beiden zusammen, manchmal jedoch mit keiner von beiden.

3) Die dorsale Blastoporuslippe erscheint an derselben Seite des Eies, wie das graue Feld.

4) In 50% der Fälle fällt die erste Theilungsebene mit der Medianebene des grauen Feldes zusammen. In ungefähr $8\frac{1}{2}$% ist dies mit der zweiten Theilungsebene der Fall.

Bei dem Rest der Eier liegt die erste Theilungsebene auf der einen oder der anderen Seite von der Mitte des grauen Feldes, gewöhnlich nahe derselben.

5) Die kleinste der vier schwarzen Zellen auf dem Achtzellenstadium liegt stets auf der Feldseite des Eies. Wird das Feld durch die erste Theilungsebene des Eies in zwei Theile getheilt, so liegt die kleinste Zelle in 18% der Fälle rechts, in 27% links. Theilt die erste Theilungsebene das graue Feld jedoch nicht, so liegt die kleinste Zelle ganz nahe dem Centrum des Feldes.