The Polarity of the Centrifuged Egg.

By

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With 9 figures in text, 5 diagrams and plate III.

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The experiments of Lyon (1905,06), Lillie (1906-1908,09), Morgan and Lyon (1907), Morgan (1906,08) on the effects of centrifuging different substances in the egg have brought to the front again in a practical form the question of the role of preformed materials in the egg, versus a predetermined method of action as the essential factor in embryonic development. Our entire conception of the nature of development is involved in this issue. That the problem is far from simple, that we are only at the beginning of the work, and that much that can be done remains to be done, is manifest.

Our immediate problem was to determine first the relation of the micromere formation in the centrifuged egg of the sea urchin to the development of the embryo, and, second, the relation of the micromeres to the sequence of the cleavage planes, for Boveri has shown that in the normal egg the micromeres correspond to the gastrula pole. Lyon did not demonstrate this relation for the centrifuged egg but he did find that altho' the first three cleavages follow the stratification, the micromeres bear no definite relation to the artificial distribution of materials. The location of the micromeres in the centrifuged egg with reference to the unsegmented egg was made possible by the application of Boveri's ingenious method. By means of this method Boveri demonstrated in the normal egg of the sea urchin a funnel-like opening through the egg membrane at the point of its previous attachment to the wall of the ovary. If India ink is rubbed up in
sea-water until a very thick solution is made, the eggs placed in this solution on a slide and a cover slip (supported) be added, the funnel may become evident (Plate III Fig. 8). The jelly-like membrane allows the light from the mirror of the microscope to pass through, except where the granules of ink enter the funnel, these demonstrate its location.

Simple and effective as is this method for the unsegmented egg and for the first three cleavage stages, it has offered very great difficulties when applied in later stages to centrifuged eggs. Gradually the method was modified to meet the special case until finally complete success was obtained. It was found that if the centrifuge is turned rapidly before the eggs have fallen to the bottom of the tube, the membrane is dragged off of most of the eggs, or else so much injured that it breaks off when the cover-slip is put on. It is essential to start slowly until the eggs are at the bottom. It is also advantageous to put some gum arabic solution in the bottom of the tube as Lyon has recommended. After removal from the machine and after the desired stage had been reached the eggs were put with very little water into flat dishes which were placed in a chamber filled with osmic vapor. After 10 to 20 minutes the eggs are fixed. They can then be kept for a day or two and examined at leisure. Sometimes they were placed, after killing, directly in the India ink solution (in sea-water) and kept there until needed.

The funnel is easily demonstrated before cleavage and during the two cell stage. As the membrane swells, however, it becomes increasingly difficult to detect it. Only in very successful preparations can it be found in the 16 cell stage, but when found it is unmistakable.

As shown in Plate III Fig. 8, the micropyle or attachment funnel is broad outside and narrows to a small tube at the surface of the egg. It furnishes the shortest path for sperm to enter, and while, as Boveri has shown, sperm may enter the egg by way of the funnel, fertilization may also occur at any other region.

The first four divisions of the egg of Arbacia may be illustrated by Figs. 1 and 2, and Plate III Fig. 6. The first three cleavages are at right angles to each other, producing eight equal or nearly equal cells, the micromeres are given off at the gastrula pole; the four cells of the opposite hemisphere divide radially and equally. Boveri showed in Toxopneustes that the micromeres are opposite to
the micropyle, and this same relation holds for *Arbacia*. In *Toxopneustes* the polar bodies are given off at the micropyle.

**Cleavage of the Centrifuged Egg.**

As Lyon first showed, the first cleavage of the centrifuged egg is usually at right angles to the stratification (Plate III Fig. 3). The second plane usually lies parallel to the stratification (Plate III Fig. 4) and the third in the third plane of space at right angles to the preceding (Plate III Fig. 6). Since the first cleavages are influenced by the stratification and since the eggs fall at random in the centrifuge, it follows that the location of the embryo, if it has any relation to the cleavage planes, would also be determined by the centrifuging. On the other hand Lyon showed that the position of the micromeres bore no relation to the stratification, but whether the micromeres in the centrifuged egg bear any such constant relation to the poles of the embryo as they do in the normal egg was not determined. Our experiments show that the micromeres in the centrifuged eggs lie opposite the micropyle as in the normal egg. A further analysis of the relation of the cleavage to the formation of the embryo may now be attempted.

As shown in diagram I, the first cleavage in the plane of the paper starting at the centrifugal pole, $C$, passes through the center of the egg. These two points joined give a theoretical axis through which an infinite number of possible planes exist that will divide the stratified materials symmetrically. One such plane will pass through the micromere pole, $M$. Let us suppose that this pole gives the third point for locating the first plane of cleavage. The second plane, at right angles to the first, will also pass through the pole $M$ and be parallel to the stratification. The third plane will be at right angles to both and across the stratification. The micromeres will appear at $M$ and be at the meeting point of the first and second cleavage planes. They will also lie opposite to the micropyle. Here we have the simplest case and the one that appears to be most easily interpreted. The controlling factors of the cleavage would be the direction of stratification and the pole of the egg.
A second possibility is illustrated by Diagram II. If the first division is in the plane of the paper, the second parallel to the stratification, the third at right angles to both, the micromere must lie at the light pole, \( M \), and such cases are not uncommon.

A third possibility appears in Diagram III, where the yolk is opposite to the micropyle. In this case the micromeres should fall in the center of the yolk-pigment pole, and such cases are known.

Diagram II.

Diagram III.

In these three examples the centrifuging has taken place parallel to or at right angles to the axis of the micropylar funnel. In all three, the possibility of forming the micromeres at the meeting point of two cleavage planes exactly opposite to the micropyle is given. But in the great majority of cases it is probable that the point on the surface of the egg that lies opposite to the funnel will be between two points of meeting of cleavage planes. Under these conditions

Diagram IV.

Diagram V.

the relation of the micromeres becomes a matter of doubt. This possibility is illustrated by Diagram IV. Here if the first plane comes in the plane of the paper it will pass through the centrifugal pole and the micromere pole. The second cleavage will lie parallel to the stratification, the third will also cut the layers at right angles the micromeres should lie at the edge of the pigment mass at \( M \), but no two cleavage planes cross at this point. The nearest point of such crossing is at \( X \), and it is here that they will appear.

In the next case, Diagram V, the crossing point \( X \) is more
nearly opposite the funnel than any other, and here the micromeres should appear. Cases of this kind are also found where the micromeres appear at the crossing point of the first and third cleavage planes.

These last two cases will serve to illustrate all other possibilities of this kind. We see that the formation of the micromeres is influenced not only by a definite pole of the unsegmented egg, but is also in part determined by the location or the sequence of the cleavage planes, for the micromeres always lie around the crossing point of two planes. A regulation of some sort must take place during the cleavage to account for these cases. The regulation may be imperfect as shown by the occasional irregularity in the size of the micromeres as well as by their absence, but it is impossible to refer all the results to such abnormalities, because in the first place the majority of eggs do not show them in favorable sets, yet in the majority of cases the point opposite the micropyle must fall outside of a cleavage crossing; and in the second place several cases have been found where normal micromeres were present at a cleavage crossing that lay nearly 45° [one-half of 90°] to one side of the point opposite to the micropyle (Plate III Fig. 9). Such cases warn us against treating this polarity and bilaterality of the egg as a rigid system. Experience teaches the reverse, for we meet constantly with extensive processes of regulation that change or even reverse the polarity.

In the preceding account we have considered those cases in which the first cleavage plane starts at the centrifuged pole. In eggs well centrifuged the majority may be of this sort. But not infrequently the first cleavage plane is parallel to this stratification, as in Plate III Fig. 7. The second and third are then at right angles to the stratification so that the same end result is reached as before. What conditions bring about this change in sequence of the cleavage planes, we have not yet determined. A characteristic 16-cell stage may follow this type of cleavage, and the general principle discussed above appears to apply here also.

Do the Micromeres come from preformed Materials or are they Products of Development?

The appearance of the micromeres at a definite point of the surface, whatever the sequence of the cleavage planes may be, may
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seem to furnish strong evidence in favor of the view that they represent preformed materials that pass at a certain cleavage to one pole of the egg, and become constricted off there. Since they give rise to the mesenchyme, it might be urged that we have here an excellent example of organ-forming materials in the egg. When the micromeres are about to appear the red granules of the polar region move off to the sides as though material from below was being carried to the surface. This fact, too, might be interpreted to mean the movement of preformed material into a given location in the egg. A further fact that might be similarly interpreted is that found in the triple and quadruple abnormal cleavages of the sea urchin egg. When the egg divides at once into four (due to polyspermy), eight micromeres develop, as Driesch first showed. When the egg divides at once into three, six micromeres appear, as I showed later. In both cases the results might be referred to the greater subdivision of the micromere materials.

Opposed to this general conclusion are the following facts: 1) If such material exist prior to its appearance in the micromeres it is not visible and can not be shifted with the centrifuge. 2) The region of the micromere-formation is not affected by the kind of substance thrown into that region from other parts of the egg. It may for instance be filled with yolk or lie at the oil pole, yet the micromeres may be normal. This might mean, however, that the material came from deeper in the egg, and the movement of the spindle towards the surface at the formation period would serve to bring such materials with it, which displace the yolk or oil, etc. there present. There is cytological evidence to show in fact that these materials are displaced from below. The evidence does not show, however, that any particular substance is brought to the surface except that immediately around the spindle. It is probable that the movements of materials here observed may be concerned with the movement of the spindle to the surface rather than with the transportation of preformed materials, for these, if they exist, are not visibly different from other parts of the egg. 3) The adjustment of the micromere formation to the cleavage pattern, when the pattern does not coincide strictly with the polar axis, is a stronger argument against preformed materials, for if such exist they would be shut off in the wrong compartments in some forms of division, and hence could not appear at the polar surface but at best in the center of the group of blastomeres. Such behavior is never observed. 4) The
sequence of the cleavages as will be shown below, bears no relation to the location of the micromeres. They may appear at the meeting points of any of the first three planes. They appear at the fourth division. This fact combined with the last argument (3) indicates a dynamic, rather than a material-shifting, function in their formation.

While none of the preceding arguments is in itself conclusive, collectively they indicate at least that the micromere formation is not due to the extrusion at the surface of preformed materials that existed in the egg prior to cleavage, but rather to the formation of a new region at a certain time in the cleavage, the formation of this region being intimately connected with a preexisting, flexible, polarity. After the micromeres have been produced (and presumably during their production also) they become progressively more and more different from the other blastomeres — at least to judge from their behavior and fate.

Path of the Sperm.

We have assumed in the preceding case that the first cleavage plane is determined by the centrifugal pole and the micromere pole. The latter assumption is arbitrary, and unnecessary in fact, as the sequel shows, for the micromere pole is not in itself determinative as a location point for the micromeres — they form at the nearest crossing.

Our assumption may seem not to be in harmony with an observation of Wilson's which goes to show that the plane of first cleavage passes through the point of entrance of the spermatozoon. If this holds also for the centrifuged egg, the critical points are the centrifuge pole, the center of the egg and the entrance point of the sperm. The micromeres appear, as before, at that crossing that lies more nearly opposite to the micropyle. In reality this conclusion fits in as well with the observations as do the previous assumptions regarding localization of the cleavage planes. Which is the preferable one will turn on the question of whether bilaterality is also present in the egg — fertilization adjusting itself to it — or whether it is later introduced by the point of entrance of the sperm. This point will come up under the next heading.

Gurwitsch (1904) centrifuged the eggs of the frog in order to find out whether the substance of the egg is a mixture or a structure. He argued that if the latter, the heavier particles should pass to one pole and the lighter to the opposite. This in fact occurred. Since
he found that an egg treated in this way would subsequently divide like the normal egg he concluded that the materials of the egg are a mixture and the egg is not a structure, for such a structure would be torn to pieces by driving particles through it with the centrifuge. Gurwitsch's conclusion might seem at first sight to demolish the conception of the organization of the egg unless by organization we mean only a mixture of different substances arranged according to sizes, weights, etc. But organization as used by embryologists, however vaguely, implies more than an orderly mixture. It implies structure in some form or other. The idea of a structure present can be harmonized with the results of centrifuging in one of two ways, either the egg has the power to rebuild or regulate its structure after the solid particle has been driven through it, or else the structure is due to a directive arrangement of its molecules that reasserts itself immediately if disturbed, in a way similar to the rearrangement of the particles of a fluid crystal. On the first alternative we should expect to find a delay in the process of development caused by the repair process, for analogy with regenerative processes in general indicates a considerable time element in repair. On the second hypothesis realignment would occur at once. The latter view of organization appears to fit the facts better.

Moreover when a spindle has formed and a temporary although real structure appears in the egg, the particles are not driven through it. They may pass around the fibres but if caught between or amongst them, they remain held there in place.

The Location of the Gastrula Pole.

Centrifuged eggs in the 16-cell stage were isolated, the location of the micromeres recorded in relation to the distribution of pigment, and the place of inturning of the gastrula noted for the same eggs. In every instance the position of the micromeres corresponded with the gastrula pole.

The relation of the gastrula pole to the sequence of the divisions is shown by the following cases.

In the first case (Textfig. 1 – 2) the first two planes of cleavage are meridional (i.e. through the centrifugal pole), the third equatorial (i.e. parallel to the stratification). The micromeres appeared at the crossing of the first and second planes at the pigmented pole (fourth figure). (Twelve cases observed.)
In the second case the relations are the same (Fig. 1 - 2) except that the micromeres appear at the clear pole (third figure). (Seven cases observed.)

Fig. 1 + 2.

In the third case (Fig. 3 - 4) the first plane is meridional, the second equatorial, the third meridional. The micromeres lie at the crossing of the first and third planes at the pigmented pole (third figure). (Two cases observed.)

Fig. 3 + 4.

In the fourth case the relations are the same, except that the micromeres lie at the light pole (fourth figure). (Six cases observed.)

In the fifth case (Fig. 5 + 6) the first plane is equatorial, the second and the third meridional. The micromeres form at the light pole at the meeting of the second and third planes (third figure). (One case observed.)

In the sixth case (Fig. 5 + 6) the relation would be the same except that the micromeres would lie at the pigmented pole. (No cases observed.)

In the seventh case (Fig. 7) the first plane is meridional, the
second equatorial and the third meridional. The micromeres appear between the pigmented and the clear hemisphere i.e. they lie at the meeting point of the second with either the first or third, which, being impossible to determine. (Seventeen cases observed.)

Fig. 7.

In the eighth case (Fig. 8) the first and second are meridional, the third plane, equatorial. The micromeres appear between the pigmented and the clear hemispheres, i.e. they lie at the meeting point of the third with either the first or second cleavages. (Four cases observed.)

Fig. 8.

In the ninth case (Fig. 9) the first plane is equatorial, the second and third, are meridional. The micromeres appear between the pigmented and the clear hemispheres at the crossing of the first with either the second or the third plane. (Two cases observed.)

Fig. 9.

In regard to the observed number of cases belonging to the different types, it is important to notice that in nineteen cases the micromeres form at the crossing of the first and second planes, as in the normal embryo. In only eight cases do they appear at the
crossing of the first and third and there was only one case where they appear at the crossing of the second and third. In twenty-seven cases the first plane passed through the micromere pole, to only one case where it failed to do so. This fact seems clearly to prove that the original pole is an important factor in determining the position of the first plane of cleavage. The result does not give a decisive answer to the alternative question in regard to the location of the first plane of cleavage so far as the sperm is concerned, because in the first four cases the micromere pole and the centrifugal axis correspond, leaving the third point for the location of the plane undetermined. The entering sperm might fix that point. In the seventh, eighth and ninth cases in which the micromeres form between the pigmented and the clear hemispheres, i.e. at the side it is impossible to tell which vertical plane passes through the micromere pole.

Thus it is clear that the bilaterality is not determined by any particular cleavage plane; for in cases 1 and 2 the third plane cannot be the median plane of the embryo, in cases 3 and 4 the second plane is excluded and in case 5 the first plane cannot determine bilateral symmetry. Modern work has shown that in a large number of forms there is a coincidence between the first or the second plane of cleavage and the median planes of the body. Three interpretations are open. Either the cleavage planes follow a preexisting arrangement of the egg material, or the planes of symmetry follow the planes of cleavage or some such factor as the entering sperm determines both the location of the cleavage plane and the symmetrical relations of the embryo. There is evidence, however, to show that no one of these views can be applied to all cases. The development of eggs by artificial or natural parthenogenesis shows that the entrance of the sperm is not in itself essential to the formation of the plane of symmetry. Our evidence shows that bilaterality is not a function of any particular cleavage plane but may coincide with any one of them, and that a polarity exists that is independent of the arrangement of the gross materials of the egg. Such polarity, however, is not different to all appearances from the directive relations involved in bilaterality or in the dorso-ventral relations. It may seem probable, therefore that a labile stereometrical arrangement of the molecules exists on which the organization of the embryos rests. These considerations incline us to the view that there exist in the molecular constitution of the egg the potential factors of symmetry.

Lillie has recently described an interesting experiment with the
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egg of *Chaetopterus* that seems to him to indicate that bilaterality develops subsequently to fertilization. By means of a high centrifugal force the egg may be broken into fragments. The nucleus usually lies in the hyaline fragments, but since the region from which they come may have occupied any part of the original egg it represents different regions of the ground substance, yet at the first division the same proportionate differences between the first two cells exist which are characteristic for the normal egg. He concludes therefore that bilaterality could not have existed in the egg prior to fertilization in the same sense that polarity exists. This argument does not seem to us convincing. In the first place *Wilson* has shown for the egg of *Cerebratulus* that if pieces are cut off of the egg prior to the disappearance of the germinal vesicle, a proportionate cleavage occurs at the eight-cell stage. This result means that even in those cases where the piece removed corresponds to the micromere or macromere field of the normal egg, no disproportion in the relative sizes of the cells in the partial cleavage occurs. In other words regulation takes place in the direction of polarity in exactly the same sense as it may occur in the antero-posterior plane in *Lillie's* fragments. If as we suppose, the relation is a property comparable to polarity, the two results are alike in every way. Moreover, the isolated blastomeres of the sea-urchin, which develop normal embryos, seem very similar to the hyaline fragments and yet if we applied *Lillie's* argument here the conclusion would be that bilaterality did not exist in the egg till after the 8-cell stage in some cases. Only when bilaterality is conceived as a rigid system, would *Lillie's* argument hold; and numerous facts of experimental embryology show that neither polarity nor bilaterality can be so conceived. If both bilaterality and polarity are expressions of an arrangement of the particles (molecules?) of the bioplasm (ground substance) the system that results from their arrangement must be a labile one, easily susceptible of rearrangement. The regulations shown by many eggs would be an expression of the power of rearrangement of the particles according to the condition of the whole. The epigenetic character of such a rearrangement still holds on the view here maintained.

**Summary.**

1) When the egg of the sea urchin is centrifuged, so that a definite stratification of its substances occurs, the micromeres have been
found to appear without regard to the induced stratification of the materials, hence their formation is independent of the presence of centrifuged materials. Since the eggs fall at random in the machine the induced stratification bears no relation to the axis of the egg. Our problem then was to determine the factor that locates the micromeres, and also the relation of the micromeres in the centrifuged egg to the axis of gastrulation.

2) By means of the micropyle, so called, it has been possible to determine that the micromeres lie in the same position on the centrifuged egg that they occupy on the normal egg, i.e. they lie approximately opposite to the micropyle. Their position is, therefore, independent of any particular cleavage plane, and is referable to the original (not the induced) axis of the egg.

3) The micromeres of the centrifuged egg lie always at the crossing point of two cleavage planes (the first and second, or the second and third, or the first and third). Since the crossing point where they appear is not always exactly opposite to the micropyle a certain amount of regulation must be possible in the formation of the micromeres; for, they appear to be capable of adjusting themselves to some extent to the location of the preceding cleavage planes. The micromeres appear at that crossing point that is most nearly opposite to the micropyle.

4) Gastrulation appears at the micromere pole of the centrifuged egg, as is the case for the normal egg. Gastrulation bears no relation to the distribution of the centrifuged materials; hence the process appears to be independent of any materials in the egg that can be moved, and the differentiation of the embryo appears likewise to be independent of the presence of any of the visible substances of the egg.

Zusammenfassung.

1) Wurde das Seeigelei soweit centrifugiert, daß eine ausgesprochene Schichtung seiner Substanzen eintritt, so wurde ein Auftreten der Micromeren ohne Beziehung zu der so hervorgebrachten Schichtung der Materialien beobachtet; also ist ihre Bildung unabhängig von dem Vorhandensein centrifugierten Materials. Da die Eier in der Maschine ganz beliebig zu liegen kommen, besitzt die herbeigeführte Schichtung keine Beziehung zur Eiachse. Unser Problem war also, zu ermitteln, welche Faktoren den Ort für die Micromeren bestimmen, und welches die Beziehungen der Micromeren im centrifugierten Ei zur Gastrulationsachse sind.

2) Mit Hilfe der sogenannten Micropyle ist es möglich gewesen, zu bestimmen, daß die Micromeren im centrifugierten Ei in derselben Lage sich be-
finden, welche sie im normalen Ei einnehmen, d. h. sie liegen der Micropylie
annähernd entgegengesetzt. Ihre Lage ist daher unabhängig von irgend einer
besonderen Teilungsebene und vielmehr mit der ursprünglichen (nicht der künst-
lich erzeugten) Eiachse in Beziehung zu setzen.

3) Die Micromeren des centrifugierten Eies liegen immer am Kreuzungs-
punkt von zwei Teilungsebenen (der ersten und zweiten, der zweiten und dritten,
oder der ersten und dritten). Da der Kreuzungspunkt, an welchem sie erschei-
nen, der Micropyle nicht immer genau entgegengesetzt ist, so muß bei der Bil-
dung der Micromeren Regulation in gewissem Betrage eintreten können; denn
sie scheinen instande zu sein, sich in einiger Ausdehnung der Lokalisation der
vorhergehenden Teilungsebene anzupassen. Die Micromeren erscheinen an dem-
jenigen Kreuzungspunkt, welcher der Micropyle am genauesten gegenüber liegt.

4) Die Gastrulation tritt am Micromerenpol des centrifugierten Eies auf,
wie es auch beim normalen Ei der Fall ist. Sie besitzt keine Beziehung zur
Verteilung der centrifugierten Materialien; daher erscheint der Gastrulations-
prozeß unabhängig von irgendwelchen Materialien, deren Lage im Ei verändert
werden kann, und die Differenzierung des Embryo erscheint gleichermaßen un-
abhängig von der Gegenwart irgend einer der sichtbaren Eiubstanzen.

Übersetzt W. Gebhardt.

Description of Plate III.

Fig. 1. Normal egg of Arbacia.
Fig. 2. Egg after centrifuging.
Fig. 3. Two cell-stage (dividing) of same.
Fig. 4. Four cell-stage of same, showing second cleavage (equatorial) parallel
to stratification.
Fig. 5. Four cell-stage, showing second cleavage (meridional).
Fig. 6. Eight cell-stage of either type.
Fig. 7. Two cell-stage, with first cleavage meridional.
Fig. 8. Centrifuged egg within membrane to show the funnel.
Fig. 9. Sixteen cell-stage of centrifuged egg, showing funnel and micropyle.
Fig. 10. Sixteen cell-stage with micromeres (too red in figure) at pigment pole.
Fig. 11. Gastrula from this type of egg.
Fig. 12. Pluteus from this type of egg.
Fig. 13. Sixteen cell-stage with micromeres at oil pole.
Fig. 14. Gastrula from this type of egg.
Fig. 15. Pluteus from this type of egg.
Fig. 16. Sixteen cell-stage with micromeres at side.
Fig. 17. Gastrula from this type of egg.
Fig. 18. Pluteus from this type of egg.