CHANGES IN PROTOPLASMIC CONSISTENCY AND THEIR RELATION TO CELL DIVISION.

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I. Periodic Changes in Consistency of the Egg Cytoplasm after Fertilization and during Cleavage.

On fertilization an increase in the viscosity of the semifluid cytoplasm of the sea urchin egg was noticed by Albrecht[1] and recently fully demonstrated by Heilbrunn.[2] Heilbrunn based his conclusions on his observation that a greater centrifugal force is necessary to stratify the cell constituents of an egg after fertilization than before. I[3] have presented evidence, from microdissection studies on the sand-dollar egg and the egg of *Cerebratulus*, that the increase in viscosity is associated with the appearance and growth of the aster.

Upon entrance of the spermatozoon into the egg a diminutive aster makes its appearance as a ball of a jelly-like consistency in the immediate vicinity of the sperm head. This aster, with the sperm nucleus, moves inward as it steadily increases in size until, when its center comes to lie in or near the center of the egg, its radiations extend throughout the whole egg. During this migration the sperm nucleus comes into contact with the egg nucleus. The aster then develops completely around the two nuclei, which fuse to constitute the cleavage nucleus.

The development of the sperm aster in the sea urchin egg is at its height within 10 to 15 minutes after fertilization. This is the

[1] Albrecht, E., Untersuchungen zur Struktur des Seeigeleies, *Sitz-ber. Ges. Morph. u. Physiol.*, 1898, xiv, 133.

[2] Heilbrunn, L. V., Studies in artificial parthenogenesis. II. Physical changes in the egg of *Arbacia*, *Biol. Bull.*, 1915, xxix, 149.

[3] Chambers, R., Jr., Microdissection studies II. The cell aster: A reversible gelation phenomenon. *J. Exp. Zool.*, 1917, xxiii, 483.

time when Heilbrunn informs me he found the egg substance to be of maximum viscosity.

The increase in viscosity of the egg cytoplasm is produced by an influence spreading out in all directions from the center of the aster. While this occurs the central hyaline area of the aster (the hyaloplasmsphere of Wilson) increases in size, and there is strong evidence[3] that this is due to the accumulation of a hyaline liquid which separates out of the semisolidifying cytoplasm and flows in very fine converging streams to the center of the aster. It is possible that this and kindred phenomena give to the aster the appearance of radiations from a common center. The consistency of the cytoplasm incorporated in the aster diminishes in firmness on passing from the interior of the aster to its exterior, being greatest in the region bordering on the centrosphere and least at the periphery.

The disappearance of the sperm aster, in the opinion of the writer, occurs through a process of liquefaction. During the liquefaction the substance of the centrosphere collects into two areas at opposite poles of the cleavage nucleus. The experiments to be described in this paper indicate that shortly before cleavage each of these areas becomes a center around which the cytoplasm commences again to pass into a semisolid state. The radial configuration about these areas constitutes the amphiaster. The comparatively firm consistency that the egg now attains for the second time since fertilization is due to two masses, the two asters, instead of to a single aster as was the case shortly after the entrance of the sperm. The importance of this phenomenon in its bearing on cell division is discussed in the last part of this paper.

Experiment 1.—The consistency exhibited by the protoplasm of the sea urchin egg at various periods from the moment of fertilization until the completion of the first cleavage, was ascertained by careful probing with the microdissection needle.

Immediately after fertilization the cytoplasmic granules readily flow by the moving needle. After the sperm has entered the egg, the sperm aster constitutes a comparatively firm mass which gradually increases in size as it moves to a central position in the egg. When the sperm aster is at its full development the highly viscous state of the cytoplasm is detected by the needle. Illustrations of this

are given in a former paper.[3] The cytoplasmic granules, instead of being readily dislocated by the moving needle, are held as in a jelly, and movements of the needle produce torsions of the entire egg substance. This condition is at its height 10 to 15 minutes after fertilization.

15 to 20 minutes after fertilization, the radiations of the aster begin to fade from view, with a reversal in the cytoplasm of the semi-solid to a more fluid state. The cytoplasmic granules are now easily dislocated by the moving needle. The more prominent radiations disappear first, while the finer ones persist for some time, owing probably to the viscid nature which the cytoplasm always maintains. The liquid substance of the central hyaline area now flows over the nucleus to its two poles, beyond which it often extends. This causes the appearance characteristic of this stage, of a hyaline streak plainly visible in the otherwise granular cytoplasm of the egg. Toward the end of this stage, which lasts for about 20 to 30 minutes, the hyaline substance finally collects into two semispherical masses lying at the two poles of the nucleus.

Shortly before cleavage, about 40 to 50 minutes after fertilization, an increase in firmness sets in, spreading radially from each of the two centers situated at the poles of the nuclear spindle. This constitutes the amphiaster. The egg elongates, the long axis passing through the two centers of the amphiaster. The cleavage furrow now appears and the egg rapidly divides. The time of appearance of the amphiaster until completion of cleavage lasts from 10 to 15 minutes. The increased viscosity of the egg during this amphiaster stage could be more easily demonstrated by the needle in the eggs of *Echinarachnius* and *Cerebratulus* than in those of *Arbacia*.

After completion of the cleavage process, there are indications that the firmness of the cytoplasm persists in the two blastomeres while they are still more or less spherical. Within 10 to 15 minutes after cleavage the two blastomeres crowd up against one another, each assuming a more nearly hemispherical shape. At this stage their cytoplasm is again quite fluid.

These observations demonstrate a pronounced periodicity in the physical state of the egg subsequent to fertilization and during the first cleavage process. In the immature egg the viscosity is high,

after maturation it drops. Upon fertilization it begins to rise again, to reach its maximum with the full development of the sperm aster. The viscosity drops again and continues low until the approach of cleavage. It thereupon rises again to drop only after completion of the first cleavage. Subsequent to the first cleavage the rhythmic appearance and disappearance of the asters within the blastomeres most probably indicate periodic successions of a process analogous to a jellying and liquefying of the cytoplasm.

The segmentation process may thus be explained as consisting essentially in a growth within the egg of two bodies of material through a gradual transformation of the cytoplasm. This transformation is associated with a change in the physical state of the protoplasm, two semisolid masses growing at the expense of the more fluid portions of the cytoplasm.

II. Cutting Experiments on the Segmenting Egg.

If it is true that the segmenting egg consists of two rather firm masses which are most fluid at their periphery, and if the physical state of the protoplasm is not affected in the process, one should be able to cut a segmenting egg into pieces without disturbing the cleavage plane. Cleavage should, therefore, proceed in such a manner as to complete the separation of what remains of the two bodies within each piece. This is what actually happens. Some experiments of Yatsu,[4] the results of which he made no attempt to explain, are in full accordance with mine and bear directly on this problem. Yatsu cut the eggs of *Cerebratulus* which were just beginning to segment (anaphase stage) into nucleated and non-nucleated fragments. He found that the cleavage furrow proceeded in its original plane irrespective of whether the fragments were nucleated or not. In Fig. 1, I have diagrammatically presented some of his results. Fig. 1 *a* represents a segmenting *Cerebratulus* egg being cut in a plane parallel to its long axis and to one side of the daughter nuclei. The original furrow persisted in the non-nucleated fragment (*b*) and

[4] Yatsu, N., Some experiments on cell-division in the egg of *Cerebratulus lacteus*, *Annot. zool. japon.*, 1908, vi, 267.

quickly completed its course in the nucleated fragment (*c*). Somewhat later the cleavage of the non-nucleated fragment (*d*) was also completed. Fig. 1 *e* represents a segmenting egg in which the cut

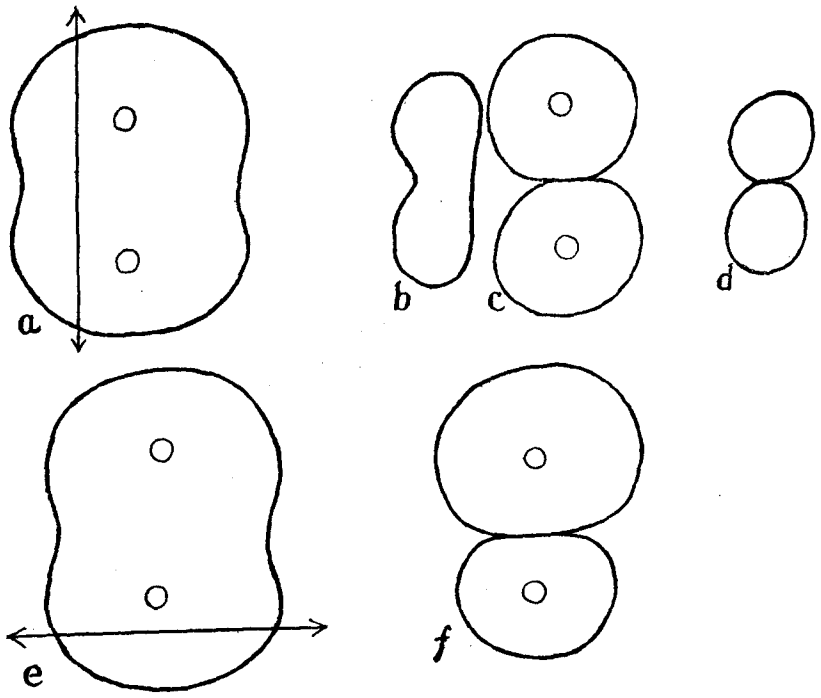

FIG. 1. A diagrammatic representation of Yatsu's results[4] on cutting the segmenting eggs of *Cerebratulus*. The direction of the cut is shown in *a*. The original cleavage furrow completed its course in the nucleated fragment *c* at the same time that it persisted in the non-nucleated fragment *b*. The furrow finally cut through the non-nucleated fragment in *d*. In *e* a cut was made across one end of the segmenting egg. The original furrow completed its course in *f* resulting in two unequal blastomeres.

was made at one end of the egg at right angles to its long axis. The original furrow persisted so as to divide the mutilated egg into two unequal blastomeres (*f*).

My cutting experiments were carried out mostly on the starfish egg, as sea urchins were very scarce during the summer of 1918.

The mature starfish egg averages 0.16 mm. (*i.e.* 160 μ) in diameter. The needles used for dissection averaged 10 μ in thickness at about 1 mm. from the tip and tapered gradually from there to a point far below 1 μ. With such a needle one can make a puncture or a clean cut through the egg in any desired spot or plane without causing apparent disturbance in the protoplasm of the egg. For cutting purposes glass needles as shown in Fig. 2 were used.[5] As the egg lies suspended in a hanging drop the end limb of the needle (Fig. 2 *a*) is set in such a way as to push the egg against the cover-slip. Constriction of the egg is produced by a continued upward pressure of the needle until the egg is cut in two. The operation does not neces-

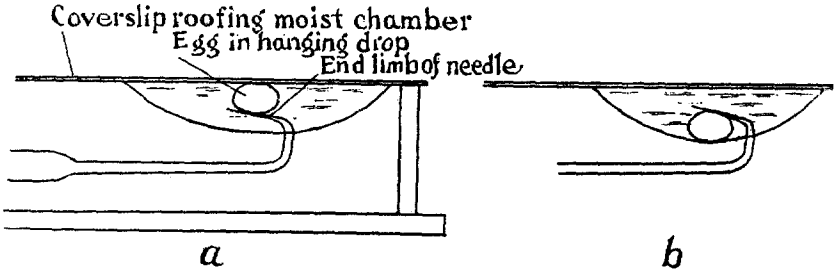

FIG. 2. Methods used for cutting an egg in two. *a*, side view of moist chamber magnified to show needle in position with its end limb so placed as to compress an egg between it and the cover-slip. Continued pressure of the needle cuts the egg in two. *b*, a second method of cutting an egg by bringing the end limb of the needle down on the egg so as to press the egg against the lower surface of the hanging drop.

sarily destroy the fertilization membrane which envelops the egg. The egg may also be cut in two on bringing it (Fig. 2 *b*) between the end limb of the needle and the lower surface of the hanging drop. Lowering the needle out of the drop in such a way as to give to the egg a rolling motion cuts the egg cleanly in two. This second method is not as satisfactory as the first for cases where one wishes to preserve the spatial relations of the egg contents, as the rolling motion produces churning movements within the cell.

Experiment 2.—(Figs. 3 to 7.) An *Asterias* ovum just beginning to segment and with the amphiaster in full development was cut

[5] Chambers, R., The microvivisection method, *Biol. Bull.*, 1918, xxxiv, 121.

in two in a plane diagonal to the cleavage furrow. The fresh surfaces caused by the cutting form films which prevent reunion of the pieces. The egg was in this way cut into two pieces each consisting of egg substance lying on both sides of the cleavage furrow.

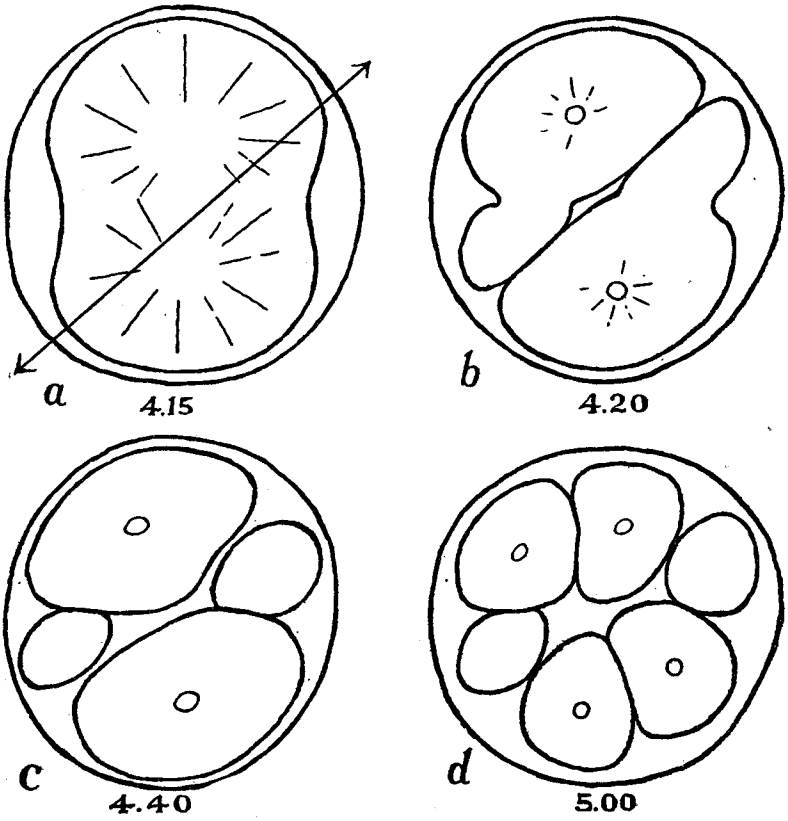

FIG. 3. Effect of a diagonal cut through an *Asterias* ovum beginning to segment in which the cut did not disturb the physical state of the ovum. *a*, operation performed at 4.15 p.m. *b*, 4.20 p.m., persistence of cleavage furrow in the original plane. *c*, 4.40 p.m., non-nucleated fragments pinched off. *d*, 5.00 p.m., nucleated fragments have segmented.

On one occasion the operation was performed on twelve eggs. In nine cases the original cleavage plane was maintained so that each piece pinched off a non-nucleated fragment normally belonging to the other blastomere. Two of them are illustrated in Figs. 3 *a* to *d* and 4 *a* to *d*.

In one case the cut was made at 4.15 p.m. (Fig. 3 *a*). 5 minutes later the cleavage furrow had progressed in the original plane (Fig. 3 *b*). At 4.40 it had completed its course so that each piece was divided into a small non-nucleated and a large nucleated fragment

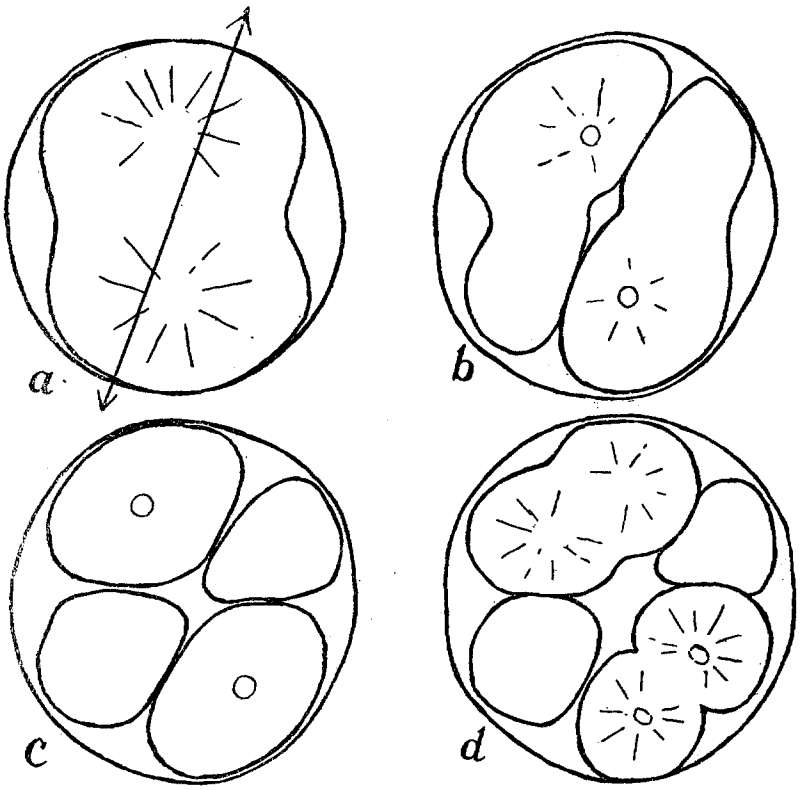

FIG. 4. Similar operation to that shown in Fig. 3 except that the diagonal cut is more nearly perpendicular to the cleavage plane with the result that larger non-nucleated fragments are pinched off by the cleavage furrow.

(Fig. 3 *c*). At 5 p.m. each of the two nucleated fragments or blastomere remnants had divided once (Fig. 3 *d*). 1 hour later they had divided once again. By the next morning the egg developed into a double blastula with the two non-nucleated fragments lying as inert masses within the fertilization membrane.

Fig. 4 *a* to *d* illustrates a similar case in which the non-nucleated masses are considerably larger than those depicted in Fig. 3. The similar behavior of one of the first two blastomeres in an egg is shown in Fig. 5 *a* and *b*.

In the remaining three cases the astral radiations faded out during the operation (Fig. 6 *a*). The original segmentation furrow gradually filled up and disappeared (Fig. 6 *b*) and each piece assumed the appearance of a normal blastomere. The nucleus then shifted so as to occupy a more central position in what one may term the reconstructed blastomere and further segmentation proceeded as if the ovum had not been operated upon (Fig. 6 *c* and *d*). This procedure always

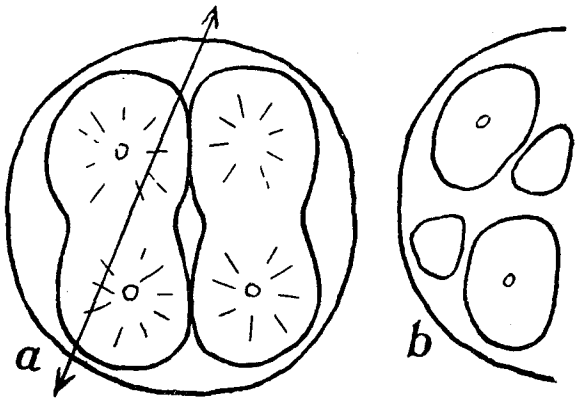

FIG. 5. Effect of a diagonal cut through one of the first two blastomeres of an *Asterias* ovum. *a*, egg showing direction of cut. *b*, cut blastomere a few minutes later.

occurred when the ovum was consciously rolled during the operation so as to produce a disturbance evidenced by a churning movement of the egg constituents.

A similar instance in the case of an *Arbacia* egg is shown in Fig. 7 *a* to *c*. A piece was cut from one pole of the amphiaster egg. In the process the piece was cytolyzed. The amphiaster in the remainder of the egg disappeared to reappear again in a new position with the result that two equal sized blastomeres were formed.

That mechanical disturbances may cause a reversal of a solid to a fluid state has already been shown.[3] This would make all the

protoplasm on each side of the cut merge into a single fluid mass. The nucleus then comes to occupy a central position. Normal mitosis takes place with the formation of an amphiaster and cleavage

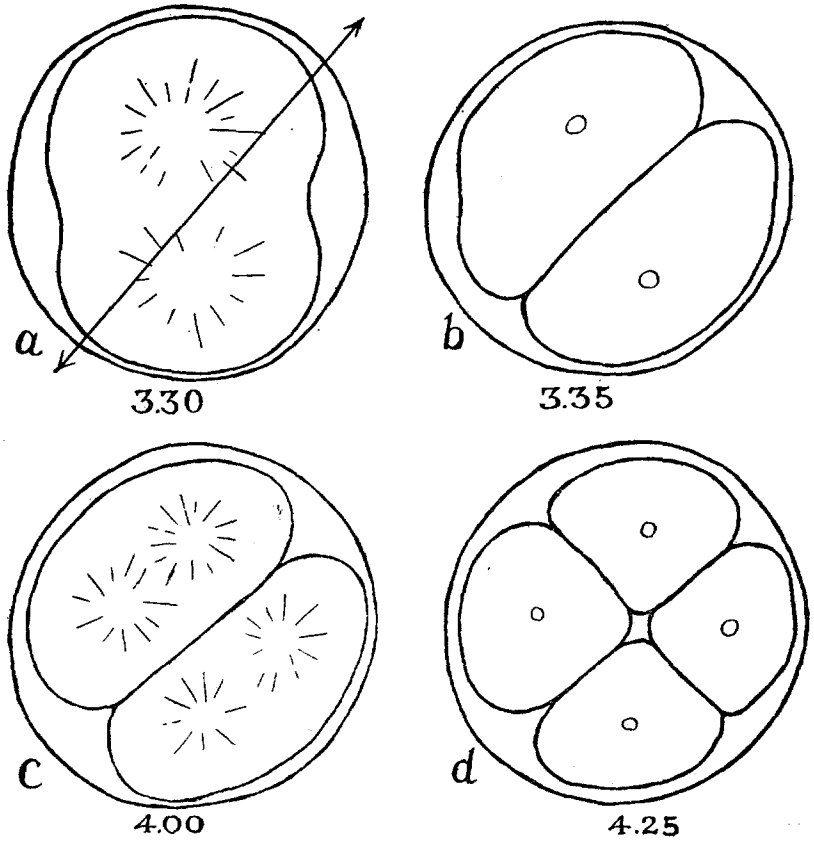

FIG. 6. Effect of a diagonal cut through an *Asterias* ovum in which the cut brought about a change in the physical state of the egg. *a*, operation performed at 3.30 p.m. *b*, 3.35 p.m., original cleavage furrow beginning to be obliterated. *c*, 4.00 p.m., an amphiaster formed in each of the two pieces produced by the cut. *d*, 4.25 p.m., four celled stage in which one cleavage plane was produced by the needle and the other by normal fission.

proceeds along the equator where the boundaries of the two asters are contiguous.

In the nine cases, in which the original cleavage plane persisted after the cutting process, the semisolid state about the two astral

centers was not disturbed. Each of the two pieces resulting from the cut, therefore, consisted of two unequal semisolid masses separated by a fluid area corresponding to the equator of the original egg. As this fluid area is incorporated into the two masses a furrow appears

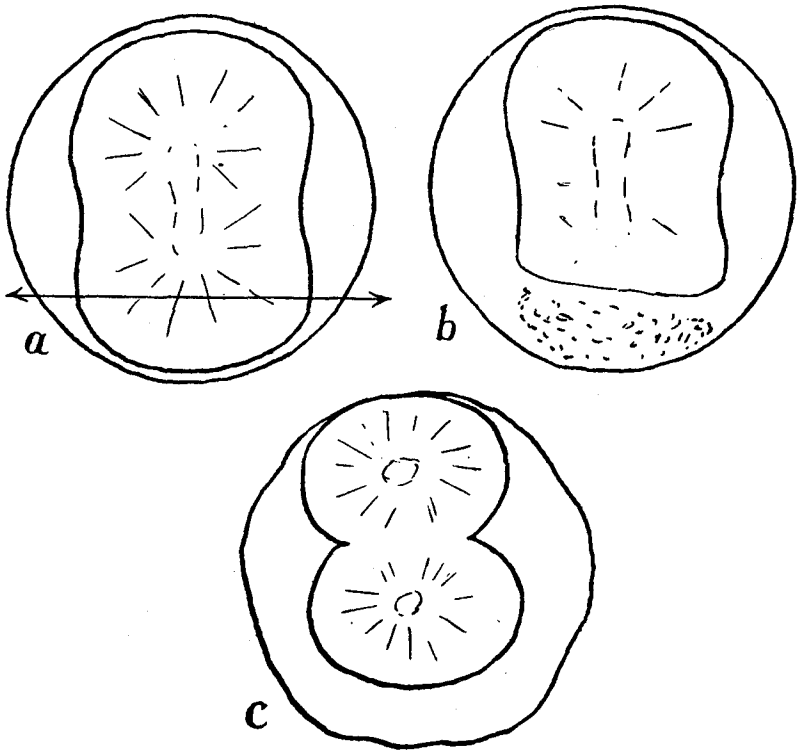

FIG. 7. Effect of cutting off a piece from one pole of an *Arbacia* ovum in the amphiaster stage. *a*, direction of cut. *b*, the piece cut off cytolized. The original shape of the remainder of the egg persisted for some time as the ovum of *Arbacia* is less pliable than that of *Asterias*. *c*, the reappearance of a new amphiaster resulting in the formation of two equal blastomeres.

which separates each piece into a larger nucleated and a smaller non-nucleated body.

The operated eggs were kept under observation until the gastrula stage, indicating that the operation had not destroyed the capacity of the egg for further development.

The following experiments are supplementary to the second. In all of them the results obtained are explicable on the basis of the existence, during cleavage, of reversible changes in the consistency of the cytoplasm.

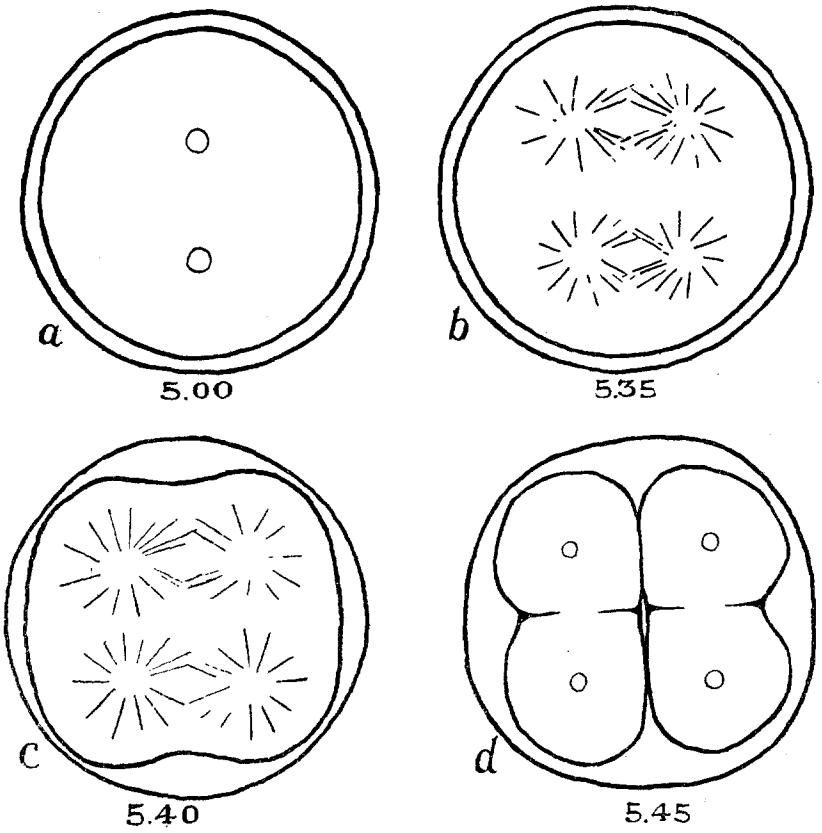

FIG. 8. Development of an *Asterias* ovum manipulated with a needle so as to suppress the first cleavage furrow. *a*, 5.00 p.m., disappearance of the amphiaster and obliteration of the cleavage furrow. *b*, 5.35 p.m., appearance of two amphiasters. *c*, 5.40 p.m., change in shape of the ovum with appearance of second cleavage furrow ahead of the first. *d*, 5.45 p.m., ovum cleaving into four blastomeres. (The ovum developed into a normal embryo.)

Experiment 3.—(Fig. 8.) In this case the first segmentation furrow was prevented from forming by tearing at the equator whenever it made its appearance. The progressive changes within the egg were

undisturbed. As soon as the amphiaster disappeared there was no longer a tendency for the furrow to form (Fig. 8 *a*). The two nuclei now lay in a fluid cytoplasm. Within half an hour after the suppression of the first segmentation furrow, an amphiaster developed about each nucleus preparatory to the next division. The two amphiasters lay side by side but remained distinct from one another, no connecting radiations being formed (Fig. 8 *b*). The formation of the two amphiasters resulted in the transformation of the egg substance into four semirigid bodies, the four asters. Cleavage furrows now extended into the fluid regions between the asters and divided the egg almost simultaneously into four blastomeres. The furrow corresponding to the second cleavage started to form and cut through the egg about a minute ahead of that of the first (Fig. 8 *c* and *d*).

This experiment may throw light on the nature of the segmentation in ova in which several nuclear divisions follow one another with no outward manifestation of the segmentation of the egg. After a certain period the ovum breaks up simultaneously into as many blastomeres as there are nuclei. This is the normal method in certain *Actinozoa* and can be artificially produced in many eggs by exposing them to various reagents, notably hypertonic solutions.[6,7]

The solidification associated with the aster formation divides the egg cytoplasm into a number of bodies each surrounding a nucleus. Between successive divisions the cytoplasm reverts to a more fluid state but its viscid nature may suffice in preventing the merging of neighboring areas. After a varying number of nuclear divisions with accompanying solidification periods furrows suddenly appear between these bodies and the ovum tends to break up at once into separate blastomeres. A differentiation of this type may possibly have taken

[6] Loeb, J., Investigations in physiological morphology. III. Experiments on cleavage, *J. Morph.*, 1892–93, vii, 253. Norman, W. W., Segmentation of the nucleus without segmentation of the protoplasm, *Arch. Entwcklngsmechn. Organ.*, 1896, iii, 106. Wilson, E. B., Experimental studies in cytology. I, *ibid.*, 1901, xii, 529. Lillie, R. S., Fusion of blastomeres and nuclear division without cell division in solutions of non-electrolytes, *Biol. Bull.*, 1902–03, iv, 164.

[7] Wilson, E. B., Experimental studies in cytology, II and III, *Arch. Entwcklngsmechn. Organ.*, 1902, xiii, 353.

place in the unsegmented *Chætopterus* embryos experimentally produced by Lillie.[8]

Experiment 4.—(Fig. 9.) Fig. 9 *a* to *e* depicts the case of an egg with the cleavage furrow just beginning in which the diagonal cut was incomplete so that the two pieces remained connected at one end of the cut. The original furrow persisted for a time during which it deepened considerably. 30 minutes after the cut had been made

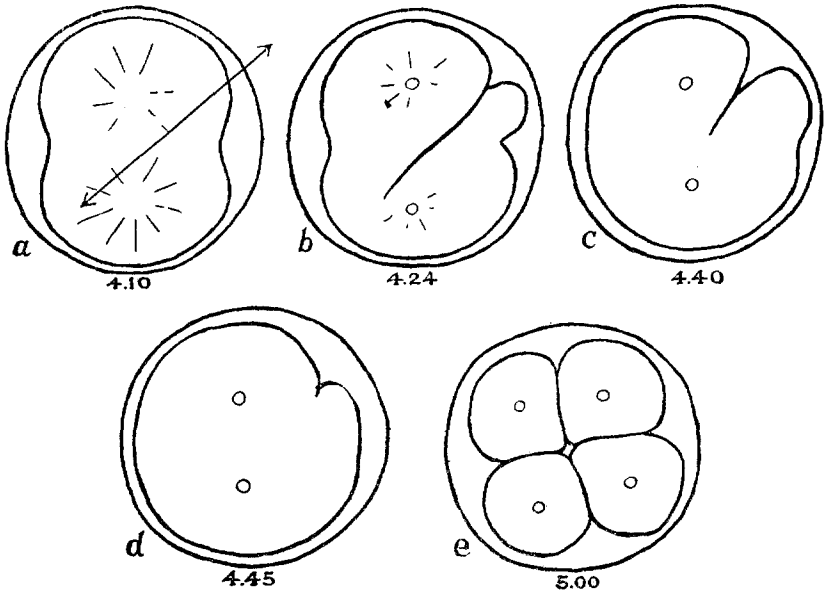

FIG. 9. Effect on an *Asterias* ovum of a deep cut which did not persist. *a*, operation performed at 4.10 p.m. *b*, *c*, and *d* show the egg respectively at 4.24, 4.40, and 4.45 p.m. Both the cut and the cleavage furrow disappear together with a reversal of the ovum from a semisolid to a more fluid state. *e*, 5.00 p.m., the ovum has divided into four normal blastomeres. (The ovum developed into a normal embryo.)

no sign of astral radiations were present and both the original segmentation furrow and the cut produced by the needle were being obliterated (Fig. 9 *c* and *d*). At 5 p.m. the egg had divided into four apparently normal blastomeres (Fig. 9 *e*) and was only slightly

[8] Lillie, F. R., Observations and experiments concerning the elementary phenomena of embryonic development in *Chætopterus*, *J. Exp. Zool.*, 1906, iii, 153.

behind the normal controls. By the next morning it had developed into a swimming blastula not to be distinguished from the normal controls.

The obliteration of the cut and of the furrow is consequent to a reversal of the egg cytoplasm from a semirigid to a more fluid state. The film projecting into the egg gradually merges into the liquid

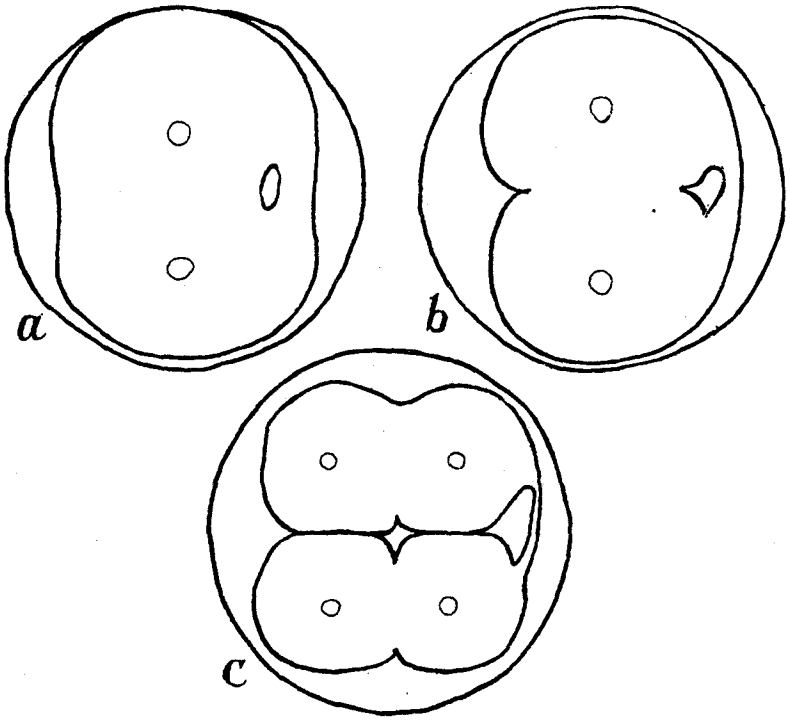

FIG. 10. Successive stages of an *Asterias* ovum showing persistence of a puncture made below the first cleavage furrow as it is beginning to form.

cytoplasm surrounding it and surface tension forces finally overcome the deformation of the egg. The egg now proceeded to divide into four blastomeres as in Experiment 3.

Experiment 5.—(Fig. 10.) This experiment demonstrates a peculiar property of the equatorial region during the formation of the cleavage furrow. A tear was made through the egg below the segmentation furrow (Fig. 10 *a*). The hole produced by the tear

remained open. The cleavage furrow continued its course beneath the hole leaving an outer margin as a bridge of protoplasm which connects the two blastomeres (Fig. 10 *b*, *c*). After several divisions of the egg the bridge thinned down in its middle until it broke through and the resulting strands were gradually drawn into the blastomeres from which they had projected.

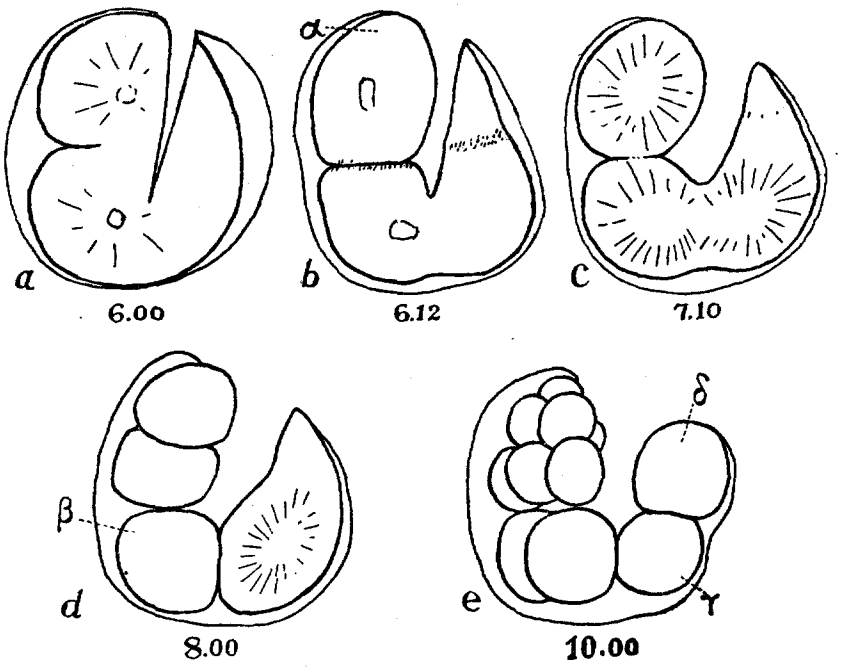

FIG. 11. Effect on an *Arbacia* ovum of a deep cut which persisted. For description of the results see text. Pigment granules collect in plane of original furrow.

Experiment 6.—(Fig. 11.) This experiment was performed on an *Arbacia* egg. An incomplete cut was made almost perpendicular to the cleavage furrow but to one side of the daughter nuclei. The furrow on the side away from the daughter nuclei became obliterated (Fig. 11 *a*). On the other side it continued its original course resulting in the pinching off of the nucleated Blastomere α (Fig. 11 *b*). The nucleus in the remainder of the egg shifted its position only slightly and

the amphiaster (Fig. 11 *c*), forming about it, resulted in a second unequal cleavage with the formation of Blastomere β (Fig. 11 *d*). The projecting piece of the egg above the obliterated furrow remained quiescent during these divisions and not until after the third unequal cleavage resulting in the formation of Blastomere γ in Fig. 11 *e*, did it become incorporated in Blastomere δ.

In this experiment the cut was probably made in the egg when the process for the first cleavage was too far advanced for the egg to retrace its course. The gash was therefore not obliterated and a very peculiar condition resulted in a succession of advances of the cleavage process about the gash. Blastomere α, being the earliest formed, segmented ahead of its fellows (Fig. 11 *d*). Blastomere β came next (Fig. 11 *e*). Unfortunately before Blastomeres γ and δ divided the egg died.

It is significant that Blastomere δ is larger than γ as evidently the former finally incorporated the hitherto inactive part of the egg that lay above that part of the original first cleavage furrow which lay on the right side of the gash (Fig. 11 *b*).

III. Concerning the Mechanism of Cell Division.

The changes in shape that an echinoderm egg undergoes during cleavage can be in part understood on the assumption that the astral formation is a solidifying process. It has long been known that at the time of cleavage the eggs of echinoderms, many worms, mammals, etc., become elongated,[9] the cleavage furrow forming in a plane at right angles to the long axis of the egg. As the furrow deepens, each resulting blastomere tends to assume the shape of a sphere (Fig. 12 *a*).

Nobody, however, has thus far been able to explain the cause of this elongation. The observations recorded in this paper may explain this phenomenon. The two spheres of solidification grow at the expense of all but possibly a small peripheral part of the fluid egg substance. The combined diameters of the two fully formed semisolid spheres are greater than the original diameter of the egg,

[9] Hertwig, O., Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies, *Morph. Jahrb.*, 1876, i, 347. Gurwitsch, A., Morphologie und Biologie der Zelle, Jena, 1904.

and hence the egg must elongate. After elongation the surface of the egg seems to tear in the plane separating the two semisolid spheres. The periphery of the two asters of the amphiaster stage never becomes so firm as their interior. This may account for the observation of von Erlanger,[10] confirmed by Spek,[11] who described peripheral currents in the rapidly dividing nematode egg. In this egg peripheral currents flow from the two poles toward the equator and from there inward to the center of the egg. Spek suggests that such currents exist in all dividing eggs, and that they are easily visible in the nematode egg because of the great rapidity with which it segments. Conklin[12] described an inward flow of granules at the equator of the dividing *Crepidula* egg, and I[3] have observed a similar current, although a very slow one, in the sand-dollar egg.

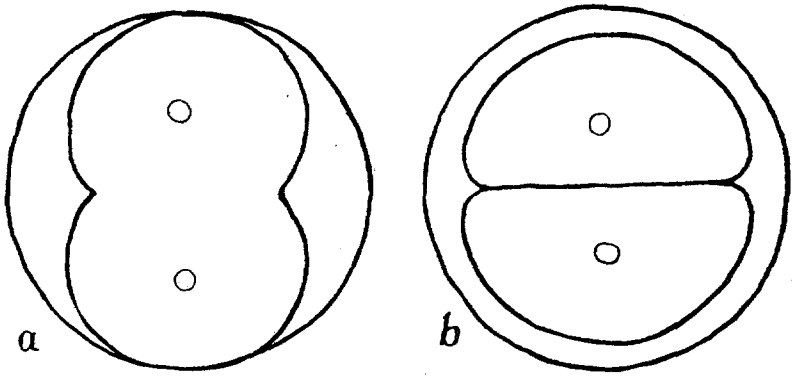

FIG. 12. Change in shape of an *Asterias* ovum (*a*) before and (*b*) after completion of the first cleavage furrow.

Immediately after cleavage both of the two blastomeres are more or less spherical; but later, when they become more fluid, they are pressed against each other so as to be flattened at the plane of contact.

[10] von Erlanger, R., Beobachtungen über die Befruchtung und ersten Teilungen an den lebenden Eiern kleiner Nematoden, *Biol. Centr.*, 1897, xvii, 152, 339.

[11] Spek, J., Oberflächenspannungsdifferenzen als eine Ursache der Zellteilung, *Arch. Entwcklngsmechn. Organ.*, 1918, xliv, 5.

[12] Conklin, E. G., Protoplasmic movement as a factor of differentiation, *Marine Biol. Lab., Biol. Lect.*, 1899, 69.

Wilson,[7] in producing binucleate eggs by artificially obliterating the first cleavage furrow, noted that when this was caused by shaking, the resulting binucleate eggs retain the elongated shape (Fig. 13) characteristic of the egg in cleavage. During the ensuing pause (corresponding to the completion of the first cleavage and when

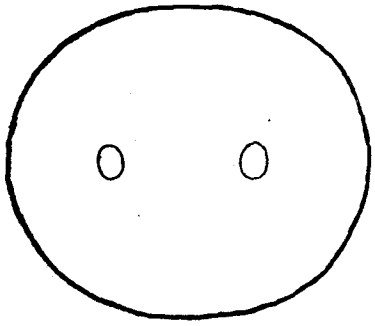

FIG. 13. Copy of Fig. 58 from Wilson[7] of *Toxopneustes* ovum immediately after shaking which caused obliteration of the first cleavage furrow.

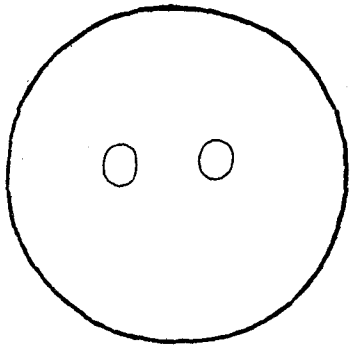

FIG. 14. Copy of Fig. 34 from Wilson[7] of *Toxopneustes* ovum in which obliteration of the first cleavage furrow was produced by exposure to ether.

the astral radiations fade out preparatory to formation of a new amphiaster system) the egg becomes more nearly spherical. Evidently the shaking does not necessarily produce a reversal of the semisolid astral system to the more fluid state. As soon, however, as this occurs (in the ensuing pause) the egg resumes its spherical shape.

Wilson noted that the suppression of the cleavage furrow can also be produced by placing eggs, during their anaphase stage, in a 2.5 per cent ether solution. The astral radiations disappear and the resulting binucleate egg at once resumes the shape of a sphere (Fig. 14). This phenomenon may be comparable to the experiments illustrated in Figs. 6, 8, and 9 where the obliteration of the astral radiations follows a precocious reversal of the cytoplasm to the more fluid state. The suppression of the furrow in these cases seems to be primarily effected by the change in the physical state of the egg substance which, on reverting to a more fluid state, merges into a single spherical mass.

CONCLUSIONS.

1. The development of the amphiaster is associated with the formation of two semisolid masses within the more fluid egg substance.

2. The elongation of the egg during cleavage is possibly produced as a consequence of the mutual pressure of these two growing semisolid masses.

3. The division of the egg into two blastomeres consists essentially in a growth, within the egg, of two masses of material at the expense of the surrounding cytoplasm. When all the cytoplasm of the egg is incorporated in these two masses cleavage occurs.

4. After a certain period of time the semisolid masses revert to a more fluid state. In the eggs studied this normally occurs after the cleavage furrow has completed the separation of the two blastomeres. The formation of the furrow, however, may be prevented in various ways, upon which the egg reverts to a single spherical semifluid mass containing two nuclei.

5. An egg mutilated during its semisolid state (amphiaster stage) may or may not revert to a more fluid state. If the more solid state is maintained, the cleavage furrow persists and proceeds till cleavage is completed. If the mutilation causes the egg to revert to the more fluid state the furrow becomes obliterated and a new cleavage plane is subsequently adopted.

6. The nuclei of eggs in the semifluid state are able to alter their positions. In semifluid mutilated eggs the nuclei tend to move to positions which may assure symmetry in aster formation and cleavage.