

# A COMPARISON OF THE DEVELOPMENT OF NUCLEATE AND NON-NUCLEATE EGGS OF ARBACIA PUNCTULATA

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A further study has been made of the development of the non-nucleate half-eggs or parthenogenetic merogones of *Arbacia punctulata*, especially in comparison with similar nucleate half-eggs or fertilized merogones. A study has also been made of the cytological details as shown in sections of fixed material, and of the reaction with the Feulgen technique. In order to have a better understanding of the development of the parthenogenetic and fertilized merogones (red halves), a further study has been made of the fertilized nucleate half-eggs (white halves) in comparison with similar whole eggs both normal (uncentrifuged) and centrifuged. Differences in the development of these eggs and half-eggs are caused not only by differences in nuclear content, whether both ♂ and ♀ nuclei are present, or only one, or none at all, but also by the shape of the eggs and differences in cytoplasmic content caused by the centrifugal force.

This comparative study is presented in a series of photographs arranged especially for comparison, and the reader is requested to study the plates which are almost self-explanatory.

## MATERIAL AND METHODS (PLATE I)

The normal *Arbacia* egg when centrifuged (3 minutes at 10,000 × g) stratifies, elongates, becomes dumb-bell shape and then breaks into halves; these halves with further centrifuging elongate and break into quarters. This is shown in Plate I (Photographs 1–11), and has been described in previous papers (1932, 1936). The important facts are that the nucleus always goes intact to the light pole under the oil

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## EXPLANATION OF PLATES

The photographs are all of living eggs, except those in Plate VIII, which are of sections. The photographs of Plates I to VI (except 64) are all as nearly as possible of the same magnification, approximately 250×. The photographs of Plate VII and 64 are also all to the same magnification, approximately 60×. The photographs of Plate VIII were taken with an oil immersion lens and magnified approximately 400× as presented, except 130 and 131, which are magnified about twice that amount. The times given under each photograph refer to the time after fertilization or activation at 23° C.



ARBACIA PUNCTULATA

CENTRIFUGED EGG & FRACTIONS

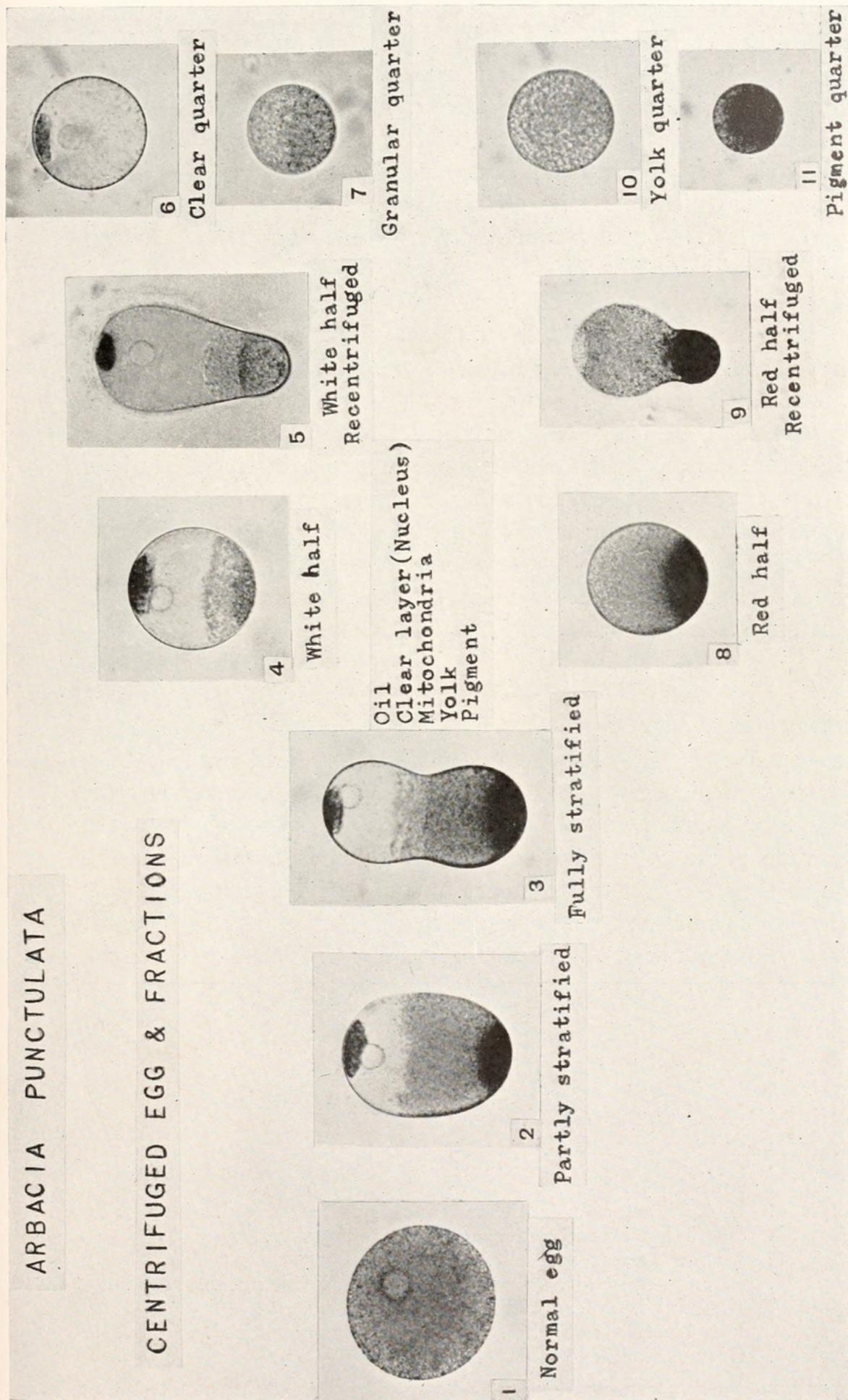


PLATE I

Centrifuged egg and fractions

The actual sizes (diameters) are as follows: whole egg 74  $\mu$ , white half 62  $\mu$ , clear quarter 56  $\mu$ , granular quarter 40  $\mu$ , red half 56  $\mu$ , yolk quarter 52  $\mu$ , pigment quarter 32  $\mu$ . (See 1932 paper.)



cap, and that the halves and quarters are uniform in size, but differ from each other in cytoplasmic content as well as in size. All of the halves and quarters can be fertilized; all will throw off a fertilization membrane and at least begin development. When the elongate whole egg and the elongate halves are fertilized, they become "set" and retain their shape. If left for an hour or so in sea water, unfertilized, they become spherical and the granules partially redistribute. The white and red halves develop much better if allowed to stand an hour or so before fertilizing them.

The whole eggs, both normal (uncentrifuged) and centrifuged, will develop parthenogenetically if treated for 20 minutes with a hypertonic salt solution made by boiling sea water to half its volume or by adding 30 grams of NaCl per liter of sea water. The white (nucleate) halves develop parthenogenetically with practically the same treatment, and the red (non-nucleate) halves also develop to a certain stage. It is of interest that a *hypotonic* solution will cause parthenogenesis as well as a *hypertonic* solution; the immersion of the sea-urchin egg for about a minute in distilled water will cause activation (Schücking, 1903); but the eggs of *Arbacia punctulata* develop only to the amphiaster of the first cleavage and only rarely cleave with this treatment.

#### STUDY OF DEVELOPMENT

##### *Whole Egg, Centrifuged then Fertilized (Plate II, 12-15)*

The elongate centrifuged whole egg, when fertilized immediately, develops as shown in Photographs 12-15. The chief points of interest are that the cytoplasmic materials remain partially segregated and the first cleavage plane comes across the short axis in a rather definite position, so that the first two cells are unequal. The pigmented cells are throughout the cleavages, generally larger than the unpigmented. Micromeres have not been observed. In spite of the peculiar cleavages,

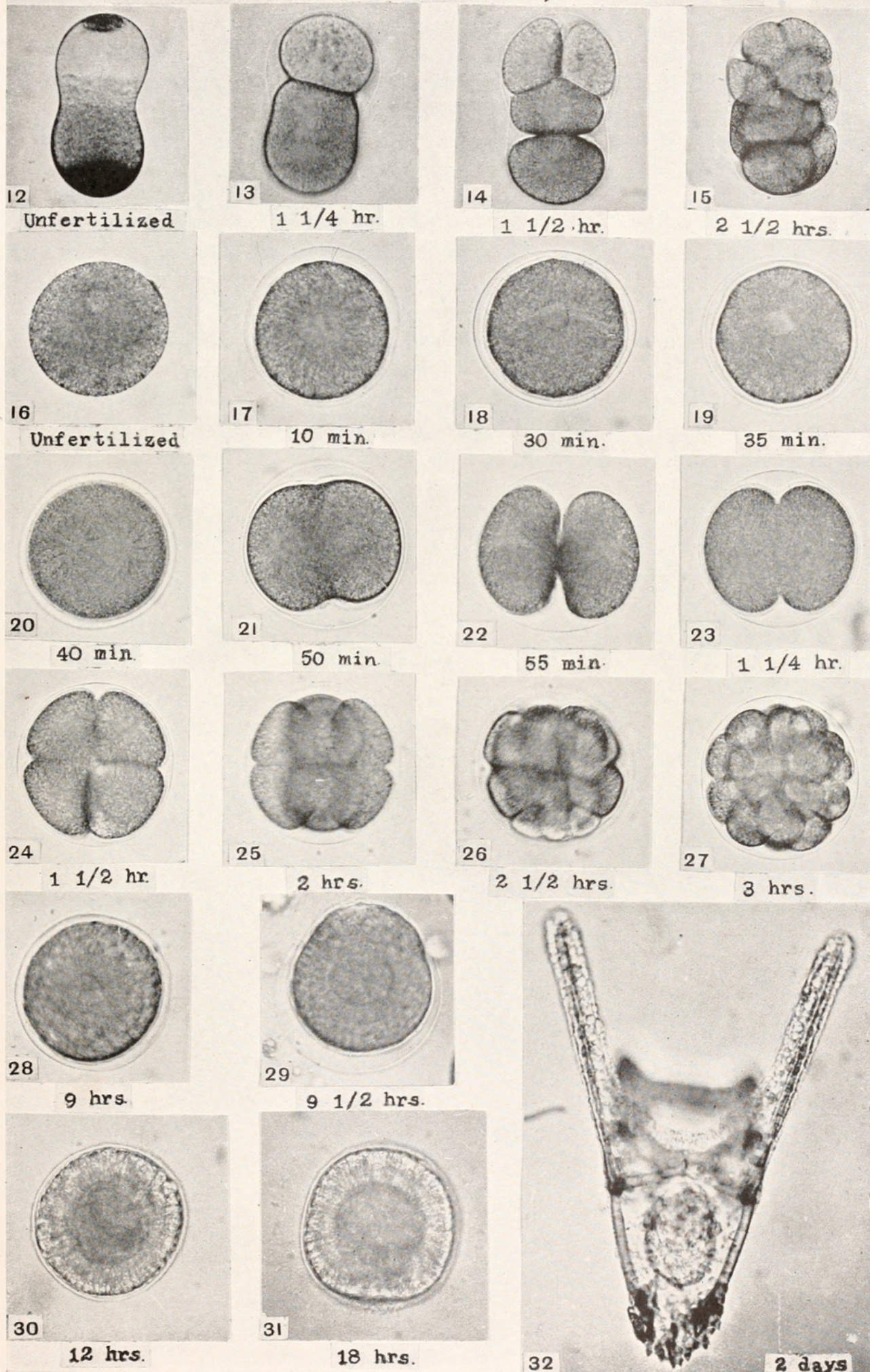
#### PLATE II

##### Centrifuged and normal egg

- Photographs 12-15. Centrifuged egg, fertilized immediately.
- Photographs 16-32. Normal (uncentrifuged) egg.
- Photograph 17. Monaster stage.
- Photograph 18. "Streak" stage.
- Photograph 19. Nuclear membrane just broken.
- Photograph 20. Amphiaster.
- Photograph 22. Immediately after first cleavage; cells well separated.
- Photograph 23. Just before second cleavage; cells close together.
- Photograph 26. Micromere stage; 12 cells.
- Photograph 28. Blurring caused by swimming of blastula inside membrane.
- Photograph 29. Hatching from fertilization membrane.
- Photograph 30. Late blastula; note cilia. The animal was narcotized.
- Photograph 31. Gastrulation has begun. Note cilia.
- Photograph 32. Well-formed pluteus. Notice lattice-like skeleton in arms.



# CENTRIFUGED & NORMAL EGG, FERTILIZED





which result in slipper-shaped blastulae, plutei are formed which are normal in every respect except for the concentration of pigment in certain areas. Usually the pigment is near the oral end, but it may be in any position, as originally described by Lyon (1907).

*Normal (Uncentrifuged) Whole Egg, Fertilized (Plate II, 16-32)*

Some stages in the development of the normal *Arbacia* egg are shown in Photographs 16-32. The especial characteristics are that the first three cleavages are equal and that at the next cleavage micromeres are formed, small colorless cells, giving a definite 12-cell stage (Photograph 26). A peculiarity following the first cleavage is that the two cells are at first widely separated (Photograph 22) and later become pressed together, probably owing to the formation of the next mitotic figure (Photograph 23). The blastulae hatch out from the fertilization membrane when having some 500-600 cells (computed from photographs),<sup>1</sup> and they have only a small blastocoel. The skeleton of the *Arbacia* (*punctulata* and *pustulosa*) pluteus is the lattice type like that of *Sphaerechinus granularis* and unlike the simple rods of *Paracentrotus lividus*, *Parechinus microtuberculatus* and *Strongylocentrotus dröbachiensis*.<sup>2</sup>

*Whole Egg, Parthenogenetic*

Both the normal (uncentrifuged) and the elongate (centrifuged) egg will develop parthenogenetically similarly to the fertilized egg but more slowly. Plutei have been obtained in both cases.

<sup>1</sup> This agrees fairly well with Morgan's (1895 *a, b*) estimate of 500-526 cells for *Sphaerechinus granularis* and 1,000 for *Echinus microtuberculatus*, and MacBride's (1914) figure of 808 for *Echinus esculentus*, at the time of hatching. The number of cells computed for *Arbacia* would represent nine cleavages (2<sup>9</sup>).

<sup>2</sup> A very interesting study of these skeletons with regard to systematic relationships of the adults is given by v. Ubisch (1932).

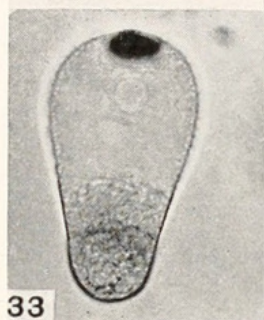
PLATE III

White half, fertilized

- Photographs 33-36. Elongate white half.
- Photographs 37-53. White half after standing an hour after centrifuging.
- Photograph 38. Monaster stage. Monaster does not show well, owing to lack of heavy granules.
- Photograph 39. "Streak" stage. Streak does not show so well as in normal whole egg, but the enlarged nucleus is plainer.
- Photograph 40. Nuclear membrane just broken.
- Photograph 41. Amphiaster.
- Photograph 43. Immediately after first cleavage; cells well separated. Cf. 22.
- Photograph 44. Before second cleavage; cells close together. Cf. 23.
- Photograph 49. Blurring caused by blastula swimming inside membrane.
- Photograph 50. Hatching from fertilization membrane.
- Photograph 51. Late blastula.
- Photograph 52. Gastrula.
- Photograph 53. Well-formed pluteus. Note lattice-like skeleton in arms. Cf. 32.



# WHITE HALF, FERTILIZED



33

Unfertilized



34

1 1/4 hr.



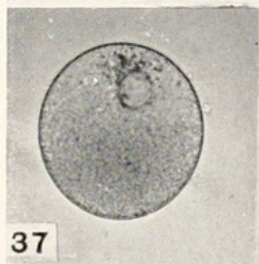
35

1 1/2 hr.



36

4 hrs.



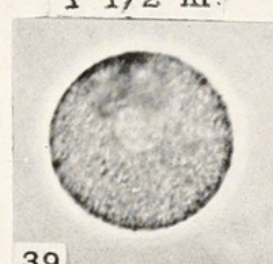
37

Unfertilized



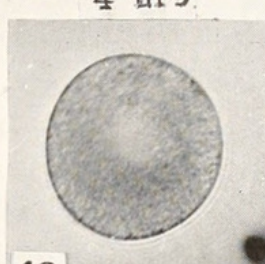
38

10 min.



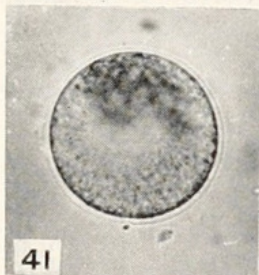
39

30 min.



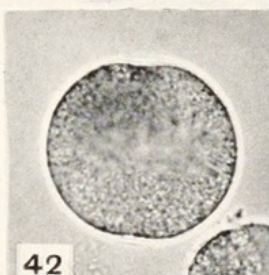
40

35 min.



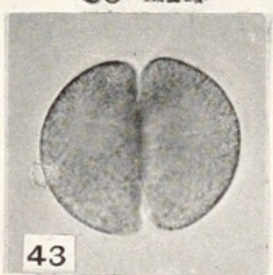
41

40 min.



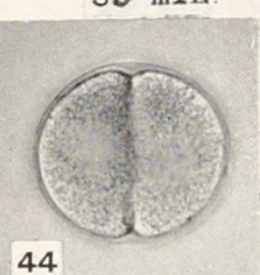
42

50 min.



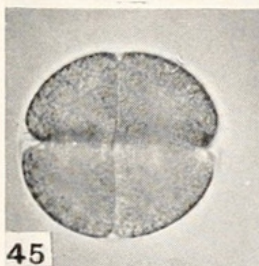
43

55 min.



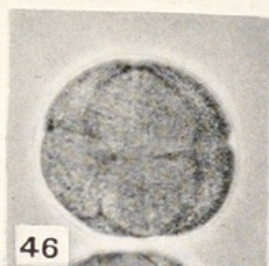
44

1 1/4 hr.



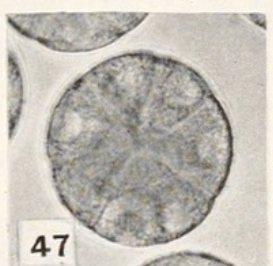
45

1 1/2 hrs.



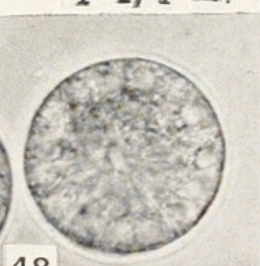
46

2 hrs.



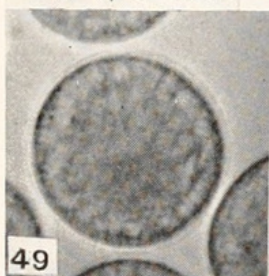
47

2 1/2 hrs.



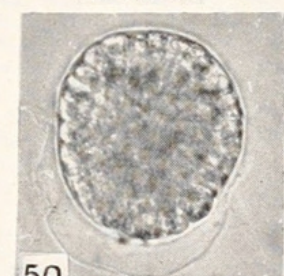
48

5 hrs.



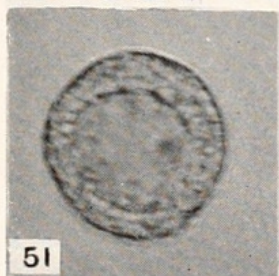
49

9 hrs.



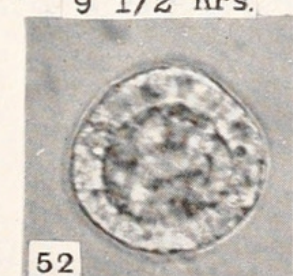
50

9 1/2 hrs.



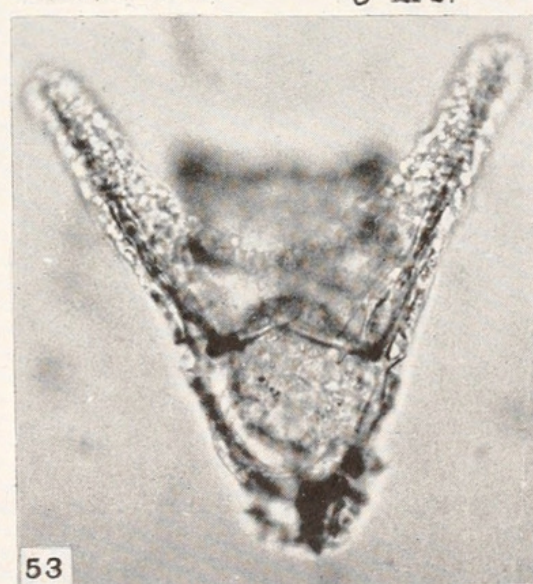
51

15 hrs.



52

24 hrs.



53

4 days



*White Half-egg (Plates III and IV)*

The development of the white half-egg, made elongate by further centrifuging (20–30 minutes at  $10,000 \times g$ ) and immediately fertilized, is shown in Photographs 33–36. It will be noted that the first cleavage plane goes across the short axis and divides the egg unequally (Photograph 34); it is usually in a position corresponding to that in the elongate whole egg (Photograph 13). The subsequent cleavages are similar in the two cases, and slipper-shaped white blastulae are formed (Photograph 36).

The development of the white half which has been allowed to stand for an hour or so till the granules are more evenly distributed, and then fertilized, is shown in Photographs 37–53. This half-egg lacks all the red pigment and most of the yolk granules and yet it cleaves and develops quite like the normal whole egg. After the first cleavage, the two cells are at first well separated and then come close together (Photographs 43, 44) as in the normal egg. Micromeres have not been observed, but they would be difficult to be sure of on account of the small size of the cells, and the lack of color contrast. Plutei have been raised, normal in every respect except for size and lack of pigment (Photograph 53). After a few days, pigment granules appear. The skeleton is of the lattice form typical of the pluteus from the whole egg.

Well-stratified spherical white halves as they occur immediately after having been centrifuged off from the whole egg (Photograph 54), will also develop when fertilized. The first cleavage plane comes in usually through the oil cap (Photograph 55), as it does in whole eggs which have been well stratified but not elongated; this was observed

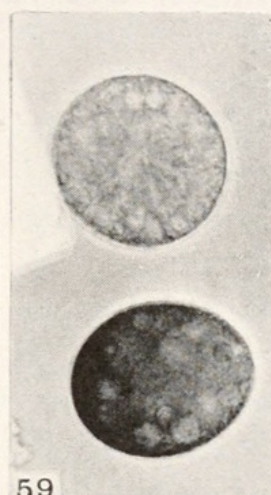
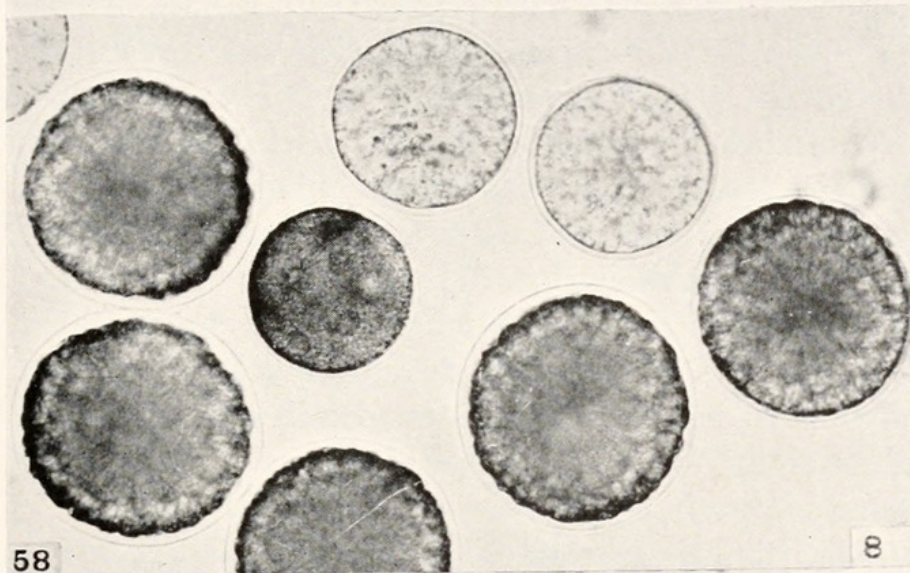
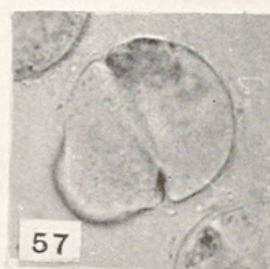
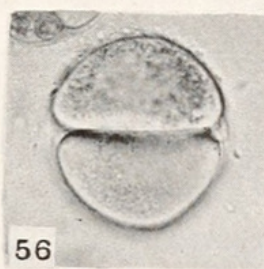
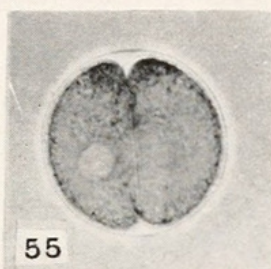
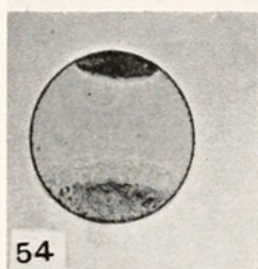
## PLATE IV

## White half fertilized, continued

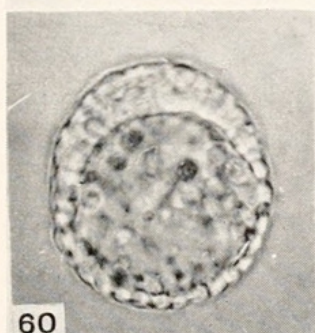
- Photograph 54. White half, immediately after removal from centrifuge.  
Photograph 55. First cleavage through oil cap.  
Photograph 56. First cleavage parallel with stratification.  
Photograph 57. First cleavage diagonal.  
Photographs 58, 59. White halves, together with whole eggs and red halves, fertilized, to show comparative development in same lot of eggs. Higher magnification of lot similar to 116.  
Photographs 60–64. Abnormal development of white halves. Cf. Photographs 11–14 of 1940 paper.  
Photograph 60. "Dauerblastula."  
Photograph 61. Blastula with primitive triradiate spicule.  
Photograph 62. Blastula with abnormal skeleton.  
Photograph 63. Abnormal pluteus without arms.  
Photograph 64. A group of abnormal plutei from one batch, together with one normal whole pluteus from the same batch of eggs.



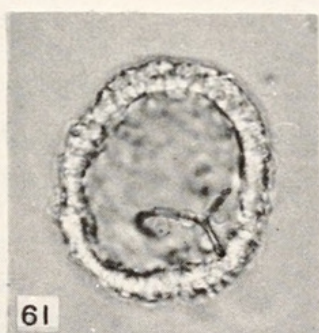
# WHITE HALF, FERTILIZED



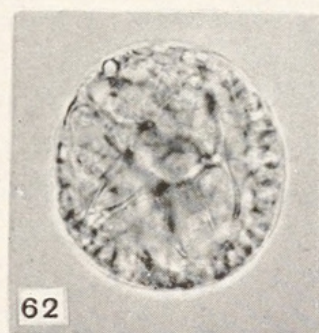
8 hrs



3 days



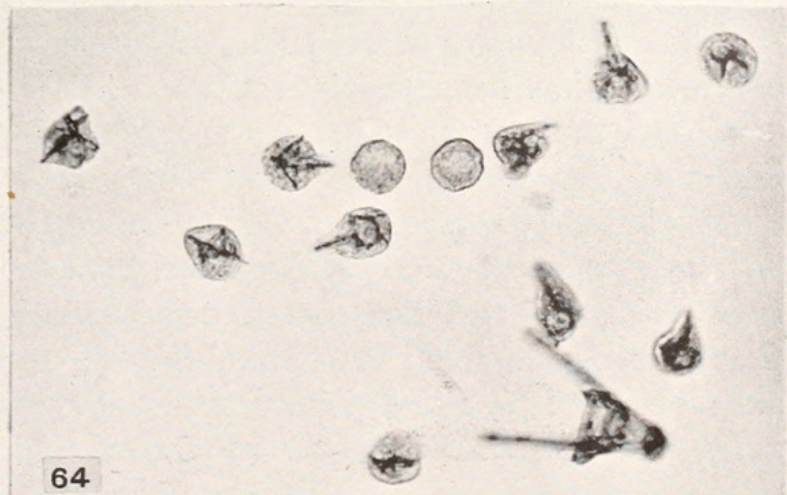
3 days



3 days



3 days



3 days



for the whole egg in the first centrifuging experiments of Lyon (1907). He also observed that the first cleavage plane may come in, less frequently, parallel with the stratification, or at an angle. This is true also of the spherical white halves (Photographs 56–57).

Although many of the white halves develop into normal plutei, some develop abnormally. Among the abnormalities are permanent blastulae (“Dauerblastulae”), blastulae with primitive triradiate spicule or with imperfect skeleton, and abnormal plutei with skeleton and no arms (Photographs 60–64). In a recent paper (1940), it was shown that white halves obtained by centrifuging after fertilization may develop into abnormal blastulae and plutei quite similar to these, but in this case no normal plutei occur. Compare photographs 60–63 of this paper with 11–14 of the previous paper (1940). There seems to be no constant percentage of normal development in any one batch of white half-eggs, but certain whole batches develop much better than others, owing probably to better experimental conditions. In some batches, all are abnormal (Photograph 64; one normal pluteus from whole egg present for comparison).

The white half-egg will also develop parthenogenetically, and give rise to a white pluteus similar to that obtained from the fertilized white half.

*Red Half-egg, Fertilized; Fertilized Mero gone (Plate V)*

The development of an elongate red half-egg, obtained by centrifuging a little longer (5 minutes at 10,000  $\times$  g) and in a slightly denser sugar solution, and immediately fertilized, is shown in Photographs 65–68. This egg has only the ♂ nucleus. The first cleavage plane is across the short axis and divides the egg unequally (Photograph 66), as in the elongate whole egg and white half. The following cleavages are likewise similar (Photographs 67, 68).

The development of the spherical red half is given in Photographs 69–88, and has been previously described (1932). The fertilization membrane and ectoplasmic layer are thicker than in the white halves. Fairly regular cleavages may take place, but with the pigmented cells usually larger (Photographs 73–77). A blastula, with small blastocoel is formed, and this emerges from the fertilization membrane in quite typical fashion and becomes free-swimming (Photographs 78, 79). Complete development into plutei is rare, and only a few normal or almost normal plutei have been obtained (Photograph 80).<sup>3</sup> In some cases, the fertilization membrane breaks during cleavage, and the cleavage cells spread out (Photographs 81–84). Also, the cleavage planes are apt to be omitted after nuclear division, so that multi-

<sup>3</sup> A number of absolutely perfect small plutei with lattice-like skeletons have recently (July 29) been obtained from fertilized red halves.



nucleate forms are common, or forms in which a few cleavage planes occur (usually in the lighter portion) and many nuclei in the uncleaved portion (Photographs 84–88). It seems likely that the great amount of heavy granular material interferes with the cleavage planes, and this may be responsible for the difficulty in raising these eggs to full development.

*Red Half-egg, Parthenogenetic; Parthenogenetic Merogone (Plate VI)*

The red half-eggs, though having no nucleus, can be activated artificially, by means of hypertonic sea water (see under "Material and Methods"), and they develop quite like the fertilized red halves, to a certain stage. They develop best if activated just after centrifuging, even if elongate. This may be due to the fact that the surface membrane is stretched and thinner and thus perhaps more permeable, so that the surface changes take place more readily. The development of the elongate egg is given in Photographs 89–92, and is similar to that of the elongate red half-egg, fertilized (Cf. Photographs 65–68).

The development of the spherical red half is shown in Photographs 93–112. The fertilization membrane and ectoplasmic layer are thick (Photograph 94) as in the fertilized red half. It will be noticed that a clear sphere is present a little later (Photograph 95), resembling the nucleus in the fertilized merogone; whether there is a definite membrane around it, is difficult to determine, though there seems to be a phase boundary. The monaster stage (Photograph 96) is common and striking, though the monaster is, in fertilized eggs, associated with the male nucleus which of course here is absent. Amphiasters are frequently seen and the cleavage plane may come in between the two asters in typical fashion (Photograph 97). The first cleavage plane may divide the egg equally, in any relation to the stratification, though more usually it is parallel with the stratification (Photographs 97, 98). Successive cleavages may be fairly regular, and a many-celled blastula formed (Photographs 99–101) exactly as in the fertilized red half (Cf. Photographs 73–77). This emerges in typical fashion from the fertilization membrane (Photograph 102), but has never developed into a pluteus. Some 500 cells have been counted in the cellular blastulae (Photograph 103), which is approximately the number of cells in a normal blastula at hatching (See p. 170, and footnote 1). There seems to be no blastocoel. The blastulae do not swim actively, though they move or are moved slightly. If they have cilia, they are short and irregularly distributed. Some of these organisms have lived for four weeks, and were still viable; they did not increase in size but were rather, smaller, and the pigment disappeared. (Photograph 104).



Just as in the fertilized merogones, the fertilization membrane frequently breaks during cleavage, and the cells become loosely arranged (Photographs 105–108; cf. 81–84). Also cleavage planes may be omitted, and multi-astral forms occur (Photographs 109–111), similar to the multi-nucleate forms of the fertilized merogone (Cf. Photographs 85–88). In both types, some of the cleavage planes come in and some are omitted, especially in the pigment portion (Photograph 108, right egg; cf. 84). In some batches of red halves, multi-astral forms occur spontaneously soon after activation. These are probably similar to the “artificial astrospheres” of Morgan (1896, 1899, 1900), and the “cytasters” of Wilson (1901) in parthenogenetic whole sea urchin eggs. In some batches there occur many blastulae in which very small clear spheres are observed, sometimes associated with the asters (Photographs 109, 111, 112). These certainly resemble small nuclei, and the similarity of these supposedly non-nucleate red halves to the nucleate (fertilized) ones is indeed striking (Cf. Photograph 112 with 88). These spheres may be re-formed nuclei without chromatin; the chromatin could hardly appear *de novo*, and no chromatin could be detected in stained preparations.

Many substances have been added to the sea water in an effort to obtain further development of the parthenogenetic merogones beyond the blastula. The substances were added in varying amounts before, after and during centrifugation, before and after activation. It was thought that possibly the substances might penetrate better while the

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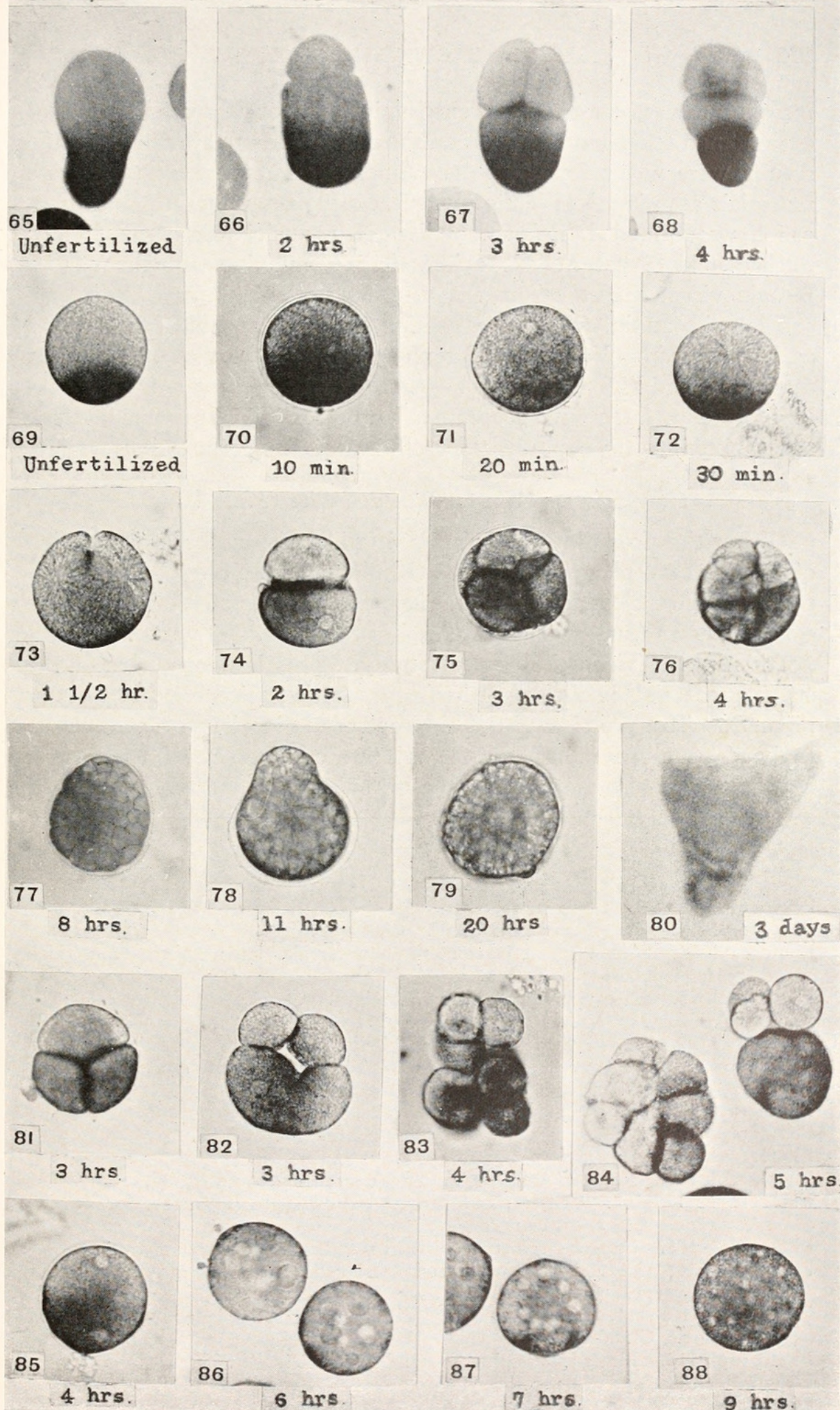
PLATE V

Red half, fertilized (fertilized merogone)

- Photographs 65–68. Elongate red half.
- Photographs 69–88. Spherical red half.
- Photograph 70. Soon after fertilization, to show fertilization membrane and thick ectoplasmic layer.
- Photograph 71. Male nucleus.
- Photograph 72. Monaster.
- Photograph 73. Amphiaster. Cell division will come in perpendicular to stratification.
- Photograph 74. Two-cell stage. Division has been parallel with stratification.
- Photographs 75–77. Regular cleavages with cell division.
- Photograph 78. Hatching from fertilization membrane.
- Photograph 79. Free-swimming blastula.
- Photograph 80. Almost normal pluteus.
- Photographs 81–84. Less regular cleavages, with fertilization membrane ruptured so that cells are more scattered.
- Photograph 84, right. Egg in which cell divisions have come in in light part, but not in pigmented part, though nuclear division has taken place.
- Photographs 85–88. Nuclear division without cytoplasmic division. Notice that the nuclei are not uniform in size in any one egg. Many small nuclei are present in Photograph 88.



# RED HALF, FERTILIZED (FERTILIZED MEROGONE)





membrane was stretched in centrifuging. Though some batches of eggs seemed to develop better than the controls in certain solutions, the results were never reproducible. Among the substances tried were materials associated with the nucleus, hormones, vitamins and dyes, as follows:—killed *Arbacia* sperm, living frog nuclei macerated, thymus nucleic acid, yeast nucleic acid, adenine, guanine, uracil, tobacco mozaic virus, *Megatherium* phage, adrenalin, pituitary, theelin, ascorbic acid, auxin, methylene blue, rhodamine, and also glutathione and leukotaxin. Variation of temperature, of concentration of the sea water, and different parthenogenetic agents were also tried. Preliminary experiments have been carried out of injecting some of these substances into the egg, but the technical difficulties are great.

*Comparison of Shape, Rate and Size (Plate VII)*

From the preceding paragraphs, it will be seen that the position of the cleavage plane differs with the shape of the egg. In spherical eggs, the first cleavage plane divides the egg equally. In elongate eggs, it passes usually through the short axis, parallel with the stratification, and divides the egg unequally. This is irrespective of the specific materials in the egg or half-egg, whether mitochondria, yolk or pigment. The position of the cleavage plane in elongate eggs seems to be determined rather by the relative consistency of the layers; it comes in where there are some granules, but not too many, and

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PLATE VI

Red half, parthenogenetic (parthenogenetic merogone)

Photographs 89–92. Elongate red half. Cf. 65–68.

Photographs 93–112. Spherical red half. Cf. 69–88.

Photograph 94. Soon after activation, to show fertilization membrane and thick ectoplasmic layer.

Photograph 95. Clear sphere simulating a nucleus. Cf. 71.

Photograph 96. Monaster.

Photograph 97. Amphiaster. Division will be perpendicular to stratification.

Photograph 98. Two-cell stage. Division has been parallel with stratification.

Photographs 97–101. Regular cleavages with cell division.

Photograph 101. Fine blastula with many cells. Cf. 77.

Photograph 102. Hatching from fertilization membrane. Cf. 78.

Photograph 103. Many-celled late blastula.

Photograph 104. Non-cellular parthenogenetic merogone, 4 weeks old.

Photographs 105–108. Cells somewhat scattered owing to rupture of fertilization membrane. Cleavages less regular and often asynchronous.

Photograph 106. Perfect 4-cell stage without membrane. Note asters.

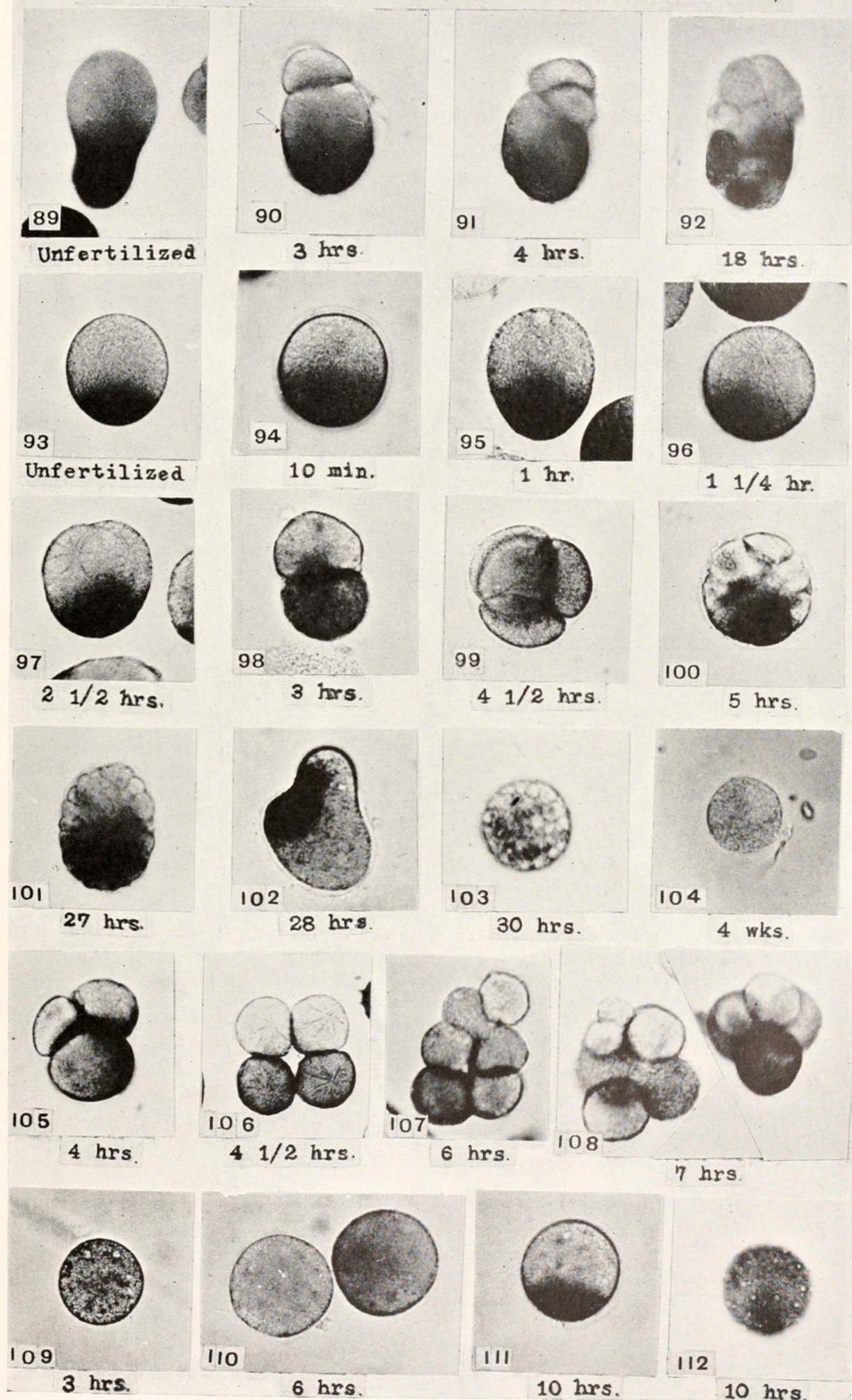
Photograph 108, right. Egg in which cell divisions have come in in light part, but not in pigmented part. Cf. 84.

Photographs 109–111. Multi-astral eggs, with several small spheres associated with the asters. Cf. 85–87.

Photograph 112. Many small spheres resembling nuclei. Cf. 88.



RED HALF, PARTHENOGENETIC (PARTHENOGENETIC MEROGONE)





usually not in the very narrowest part. The cleavage pattern does not, however, seem to affect final development.

The fertilized white halves cleave at the same rate as the whole eggs, if anything a little in advance (Photographs 113–116, 58, 59), and they hatch from the fertilization membrane at the same time. They are slower to differentiate; they are still blastulae when the whole eggs have become plutei (Photograph 117), and they become plutei the following day, but they are smaller than normal ones (Photograph 118). They are at first colorless, but acquire pigment later on. The parthenogenetic eggs, both whole ones and white halves, are slower in cleavage and development than the fertilized ones.

The fertilized red halves (fertilized merogones) cleave more slowly than the normal wholes and white halves. After 4 hours (Photograph 119), they are at about the same stage as the others at 2 hours (cf. Photograph 115). They hatch later, and differentiate still more slowly than the white halves. They are still blastulae after two days (Photograph 120), and become plutei on the following day (Photograph 121).

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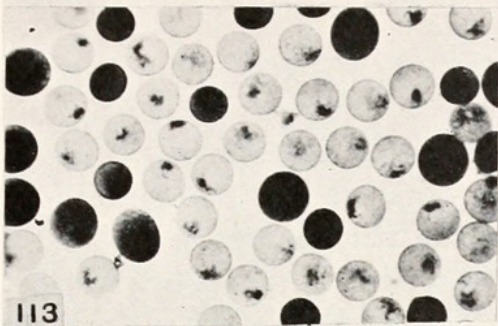
#### PLATE VII

##### Comparative

- Photograph 113. Group of whole eggs, white and red halves to show comparative sizes.
- Photograph 114. Similar group an hour after fertilization. The whole eggs and white halves are in the 2-cell stage, the red halves still uncleaved.
- Photograph 115. Similar group a half hour later. The whole eggs and white halves are in the 2- and 4-cell stage, the red halves still uncleaved. The white halves are slightly in advance of the whole eggs.
- Photograph 116. Similar group 7 hours after fertilization. The whole eggs and white halves are blastulae, the red halves much behind. The inset at right is a group of the whole eggs printed more lightly to show cleavage planes. A group similar to this, more highly magnified, is shown in Photographs 58, 59.
- Photograph 117. The day after fertilization, the white halves are still spherical blastulae, the whole eggs are plutei.
- Photograph 118. The following day, the white halves have become plutei; the small pluteus is from a white half, the others from whole eggs of the same age.
- Photograph 119. A mixed group, printed lightly to show red halves, in 2- and 4-cell stages after 4 hours, similar to wholes and whites after 1½ hours. Cf. 115.
- Photograph 120. The red half still a blastula after two days. A whole pluteus of same age alongside.
- Photograph 121. The red half has become a pluteus the following day. A normal pluteus of the same age is alongside.
- Photograph 122. A pure culture of parthenogenetic merogones, 4 hours after activation. Note the large numbers of cleaved eggs, in 2-, 3-, and 4-cell stages. The normal fertilized egg is many-celled at this time; cf. 27.
- Photograph 123. A parthenogenetic merogone after three days, still a spherical blastula, of same size as previously. A normal pluteus of the same age is alongside.

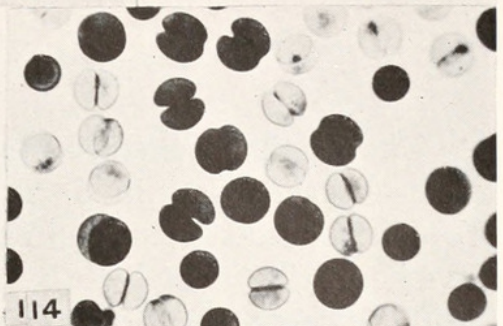


# COMPARATIVE



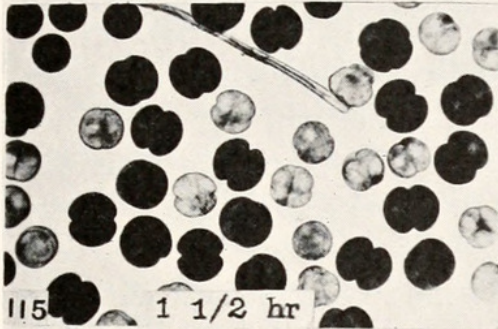
113

Unfertilized



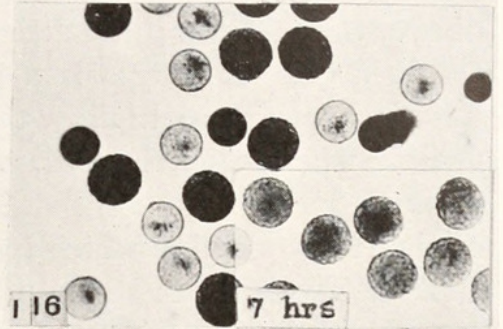
114

1 hr



115

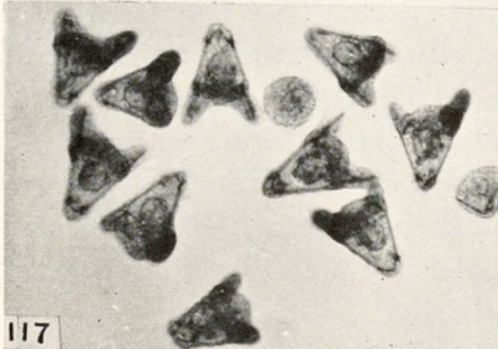
1 1/2 hr



116

7 hrs

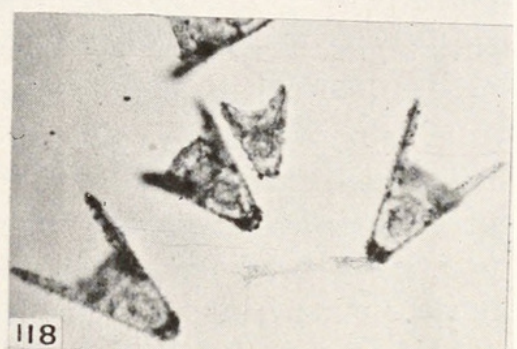
White halves, red halves & wholes



117

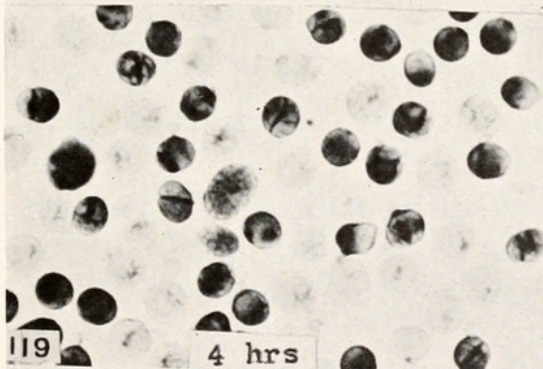
1 day

White half & wholes



118

2 days



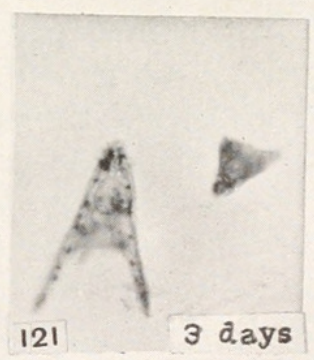
119

4 hrs



120

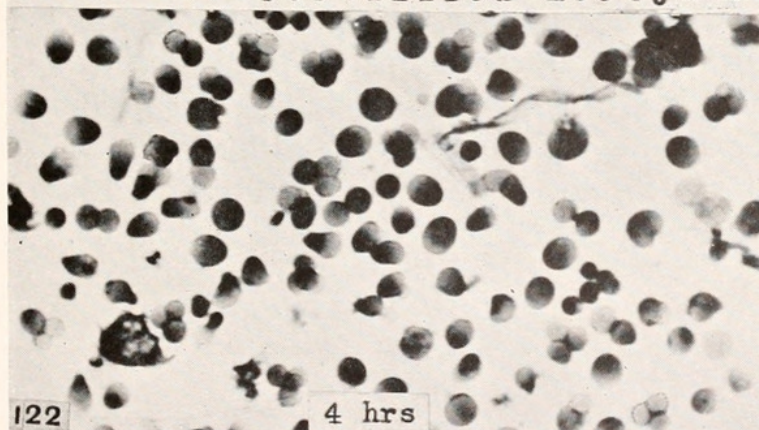
2 days



121

3 days

Fertilized merogone & whole



122

4 hrs



123

3 days

Parthenogenetic merogone & whole



The parthenogenetic merogones develop still more slowly. First cleavage takes place in about three hours, whereas it is two hours for the fertilized merogone, and 50 minutes for the white halves and wholes. In 4 hours they are a little behind the fertilized red halves (Photograph 122). The parthenogenetic merogones usually hatch only after 24 hours, whereas the whole eggs hatch in  $9\frac{1}{2}$  hours. And as stated above, they do not differentiate into plutei (Photograph 123).

As a general comparison of rate of cleavage, the eggs with two nuclei cleave more rapidly than those with one, whether parthenogenetic whole eggs or fertilized merogones. Those with one nucleus cleave more rapidly than those without any. The slow rate of cleavage of the clear quarters which have two nuclei is an exception (Harvey, 1932). Twice the amount of nuclear material in comparison with the cytoplasm, as occurs in the fertilized white halves, does not appreciably affect the rate of cleavage. But as we have seen, these halves differentiate, i.e. become plutei, more slowly. Possibly the lack of the full quota of cytoplasm may be responsible for the delay in differentiation.

The process of cell division and cell multiplication can go on with two nuclei, or one nucleus or none at all. Up to the present time, the parthenogenetic merogone has not gastrulated nor acquired a skeleton; practically no differentiation has taken place. And it may be that nuclear material is necessary for differentiation.

No attempt has been made to compare accurately the nuclear size of the half and whole eggs. A glance at Photographs 59, 74, 78 and 86 will show how variable is the size in a single egg. Also the nucleus in a normal egg changes in size before first cleavage from  $11.5\ \mu$  to  $16\ \mu$ . The nuclei in the clearer portions of the eggs are in general larger than in the pigmented portions in the same size cells.

No special granules seem to be necessary for development since both halves of the eggs, containing certain granules and lacking others, can develop into plutei. The red pigment seems to interfere with cell division but not with final development.

It should be stated that the times given for the various stages are by no means invariable. They depend upon the time of year as well as upon temperature and experimental conditions. This is especially true of the red halves which are very variable, both fertilized and parthenogenetic. Based on eight years of experience with the material, the times given are an average for development, as accurately as could be obtained, for a temperature of  $23^{\circ}\text{C}$ . at the height of the season.



## STUDY OF STAINED SECTIONS (PLATE VIII)

*Stratified Eggs (Photographs 124-127)*

The eggs were fixed immediately after centrifuging, in Bouin's fluid, sectioned and stained with iron hematoxylin, and some were counter-stained with eosin and orange G. The clear layer of the whole egg (Photograph 124), which is optically empty in the living egg, stains blue, and is granular; this is apparently the protoplasmic ground substance, not moved by the centrifugal force used. This was described also in the early paper of Lyon (1907). The yolk stains orange with a rose tinge and the pigment orange. The mitochondria can sometimes be distinguished as a darker bluish band between the protoplasm and the yolk. The oil cap does not show; it is probably dissolved in the fixing or clearing fluid. The white half stains, of course, like the upper part of the whole egg (Photograph 125). The red half (Photograph 126) usually shows a blue cap of protoplasm, though this is obscured in the living egg by granules. In the red half further centrifuged till it is elongate, this cap of protoplasm formed by the further packing of the granules toward the heavy end, is larger (Photograph 127), and it is often seen in the living egg as a clear layer (Photograph 9).

*Eggs Fertilized, Then Centrifuged (Photographs 128, 129)*

When the eggs are fertilized and then centrifuged, the nucleus is always at the light pole, as in unfertilized eggs. At the stage after the nucleus has enlarged and the chromosomes have begun to form, just before the breaking of the nuclear membrane, the chromatin material is thrown to the heavy end of the nucleus as a dense mass (Photograph 128). This does not happen in the unfertilized egg (Photograph 124). The nucleolus of the immature egg is, however, thrown down to the centrifugal end of the germinal vesicle. If such fertilized eggs as mentioned above are left (living) in sea water after centrifuging, the material redistributes within 7 minutes, and normal spindle formation and cleavage follow.

When the fertilized eggs are centrifuged after the spindle has formed, it goes intact (with the forces used) to the light pole (Photograph 129) as found by Spooner (1911). There is no chromatin material left behind either before or after the nuclear wall has broken, as it would be perfectly visible in these stained preparations. There is no possibility, therefore, that the red halves contain chromatin material from the nucleus.



*Chromosome Numbers (Photographs 130, 131)*

The diploid chromosomes in the cleavage figures of fertilized eggs are small and crowded, and are very difficult to count with certainty, as other investigators have also found. The number is between 32 and 38 (Photograph 130). Tennent (1912) gives "about 40" for *Arbacia punctulata*, and Morgan (1927, p. 627) gives 38, quoting Wilson's data of 36-38. The number in the first cleavage of the parthenogenetic whole egg is approximately half the diploid. In Photograph 131, there are 16, but one or two may be missing.

*The Mitotic Figure (Photographs 132-140)*

In the early cleavages of the normal *Arbacia* egg, the spindle and asters are beautifully formed; the asters have rather thin rays. In Photographs 132-134 are given prophase, metaphase and anaphase figures. The mitotic figures of the fertilized white halves are similar to the normal egg. The fertilized merogone has also a spindle and

## PLATE VIII

## Sections

(These photographs have been touched up to bring out details more clearly)

Photographs 124-127. Unfertilized eggs fixed in Bouin immediately after centrifuging, sectioned and stained in hematoxylin, eosin and orange G.

Photograph 124. Whole egg, showing protoplasm above, mitochondrial band, yolk and pigment. The oil cap does not show. Cf. living egg, Photograph 3.

Photograph 125. White half. Cf. living egg, Photograph 4.

Photograph 126. Red half; a protoplasmic layer is at the centripetal pole, not visible in living egg, Photograph 8.

Photograph 127. Red half, centrifuged further. Protoplasmic layer is greater; this is visible in a similar living egg due to greater packing of granules. Cf. Photograph 9.

Photograph 128. Fertilized egg centrifuged just before breakdown of nuclear membrane, showing chromatin material thrown down to heavy pole of nucleus. In the living egg, not centrifuged, this would be Photograph 18.

Photograph 129. Fertilized egg centrifuged at the metaphase, soon before cleavage. The spindle is intact at the centripetal pole. This is the stage shown in the living uncentrifuged egg in Photograph 20.

Photograph 130. Diploid group of chromosomes; 32-38.

Photograph 131. Haploid group from parthenogenetic whole egg; 16, one or two may be missing.

Photographs 132-134. Mitotic figure of normal fertilized egg. Prophase (132), metaphase (133) and anaphase (134). Forty to 45 minutes after fertilization.

Photographs 135-137. Mitotic figure of fertilized red half (fertilized merogone). Prophase (135), metaphase (136) and anaphase (137). Note slender spindle, and thick astral rays. One to 2 hours after fertilization.

Photographs 138-140. Mitotic (?) figures of the parthenogenetic merogone.

Photograph 138. Monaster. Note thick rays. One and  $\frac{1}{4}$  hour after activation.

Photograph 139. Asters present in pairs in 2-cell stage. Note that those in the pigmented part have thicker rays than those in yolk. Four hours after activation.

Photograph 140. Two eggs overlapping, one in amphiaser stage before first cleavage, one in three-cell stage, each cell with a pair of asters. Six hours after activation.

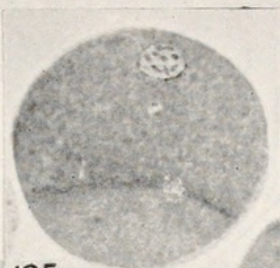


# SECTIONS



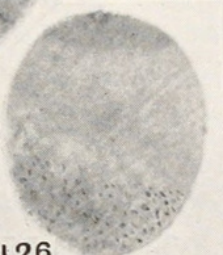
124

Whole egg



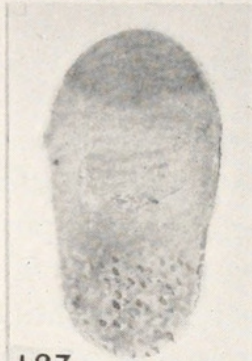
125

White half



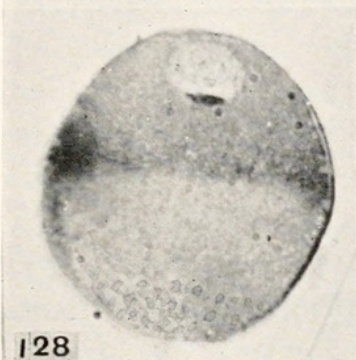
126

Red half

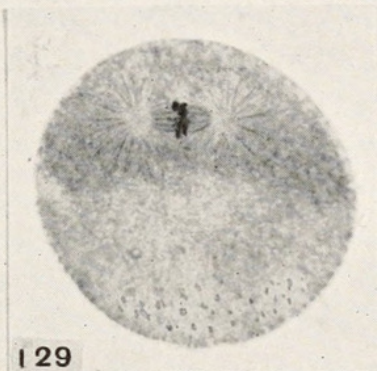


127

Red half



128



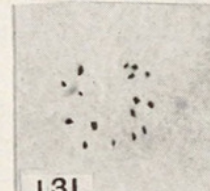
129

Fertilized, then centrifuged



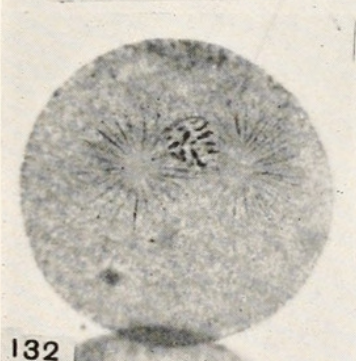
130

Diploid

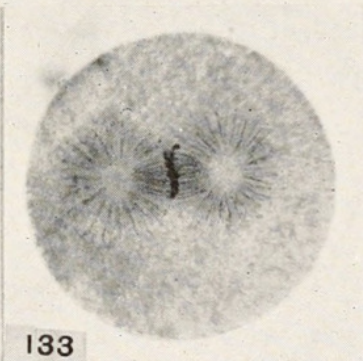


131

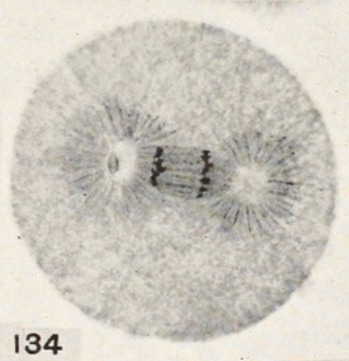
Haploid



132



133

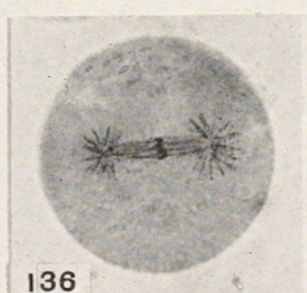


134

Normal whole egg



135



136



137

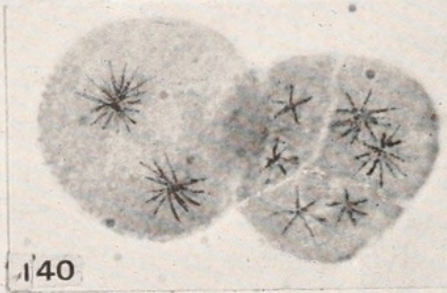
Fertilized merogone



138



139



140

Parthenogenetic merogone



asters; the spindle is much thinner, often curved, and the rays of the asters are shorter and thicker; they are thicker in the pigmented than in the yolk portion. In Photographs 135–137 are given prophase, metaphase and telophase figures of the fertilized merogone. The parthenogenetic merogone has well-formed asters but no spindle; the rays of the asters are even heavier and thicker than in the fertilized merogone and thicker in the pigment area than in the yolk (Photographs 138–140). A large monaster is characteristic of the parthenogenetic merogone (Photograph 138), appearing in the same sequence of events as the monaster which develops in connection with the sperm in fertilized eggs. Later, two asters appear in the same sequence as the amphiaser in the fertilized eggs; the rays may approach each other toward the center, but there is never a well-formed spindle. In later cleavages, the asters may be found in pairs, also without spindles. In Photograph 40, there is one egg with an amphiaser and another egg, overlapping, with three cells, in each of which is a pair of asters. The original of the last photograph was sketched by four different investigators independently, and the sketches all agreed essentially. There is no question that asters are present, often in pairs, in the parthenogenetic merogones, but no spindle and no chromosomes. There is no special granule or centriole in any of the asters in the sea urchin egg.

#### *Feulgen Reaction*

The Feulgen reaction, which is specific for chromatin, is negative for the parthenogenetic merogones. Professor Jean Brachet very kindly helped me with the technique and examination of the eggs. The parthenogenetic merogones showed no red-staining material, whereas the fertilized merogones, prepared in the same way at the same time, showed it very clearly. There is apparently no chromatin material in the parthenogenetic merogones, at least in the early cleavages.

#### SUMMARY

1. A comparative study has been made for *Arbacia punctulata*, especially by means of photographs, of the development of the normal whole egg, the white half, the red half fertilized (fertilized merogone) and the red half parthenogenetic (parthenogenetic merogone), all of these both spherical and elongate. The comparative rate of development is also given.

2. Development of the parthenogenetic merogones is not improved by applying various substances to the outside, such as nuclear compounds, hormones and vitamins.

3. A study of the stratification of the centrifuged egg and its halves as seen in prepared sections has been made.



4. Sections of eggs fertilized and then centrifuged show that at a certain stage, the chromatin material is thrown to the centrifugal pole of the nucleus, and that the spindle goes to the centripetal pole intact.

5. Cytological details have been studied, in sections, of the division figure of the normal egg, of the red half fertilized, and of the parthenogenetic merogone. During cleavage stages, the parthenogenetic merogone has well-formed asters, often in pairs, but no spindle and no chromosomes.

6. The Feulgen reaction is negative for the parthenogenetic merogones; there is no chromatin material.

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