

THE FORMATION OF THE ASTER IN ARTIFICIAL PARTHENOGENESIS.*

By ROBERT CHAMBERS.

(From the Cornell University Medical College, New York.)

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In normally fertilized eggs the development of the aster is attributed to a substance carried into the egg by the spermatozoon. The aster first makes its appearance in the form of diminutive radiations surrounding the neck-piece of the spermatozoon within a few minutes after it has entered the egg. The writer¹ has shown that the formation of the radiations is accompanied by a jellying of the cytoplasm of the egg. The jellying process extends more and more as the aster increases in size and the entire egg becomes involved when the center of the aster comes to occupy the center of the egg.

The formation of the aster is accompanied by an increase in size of a hyaline area in its center. This is Wilson's hyaloplasm-sphere² also called centrosphere and astrosphere by other investigators. The microdissection method has demonstrated that this sphere area is liquid in contrast to the surrounding jellied cytoplasm. The pioneer observers of mitotic division, such as Auerbach, Hertwig, Bütschli and Fol, described the accumulation of a hyaline plasma at the astral centers and suggested that the astral radiations are a result of protoplasmic currents. Later investigators, such as Morgan, Wilson and Conklin, considered this view as the most probable one.

* The experiments, upon which this paper is based, were conducted in the Research Division of Eli Lilly and Company, at the Marine Biological Laboratory, Woods Hole. The experiments constitute a part of a joint research project in which Dr. G. H. A. Clowes and the writer are engaged.

¹ Chambers, R. Microdissection studies. II. The cell aster: A reversible gelation phenomenon, *J. Exp. Zool.*, 1917, xxiii, 483.

² Wilson, E. B., Experimental studies in cytology. I. A cytological study of artificial parthenogenesis in sea-urchin eggs, *Arch. Entwicklungsmech.*, 1901, xii, 529.

The movement of the egg nucleus is possibly also a case in point. As long as the egg nucleus is beyond the confines of the aster it is stationary. As soon, however, as the extending aster reaches it, the nucleus begins travelling toward the sphere in which it finally lies close beside the sperm nucleus. The existence of a centripetal current may be inferred also from the following experiment. In an egg one may occasionally see one or more oil-like droplets 2 to 4 microns in diameter. If one of these droplets be pushed by the needle from the liquid cytoplasm into the periphery of the aster the droplet will move along the rays toward the center.

In view of the above observations it is highly probable that the liquid which accumulates in the center of the aster streams into it from all sides during the jellying of the cytoplasm. It is this streaming which probably occasions the innumerable radiations characteristic of the aster. After the aster has attained its full size the radiations begin to fade from view as the jelly state reverts to a more fluid one. The liquid of the central sphere does not mix with the fluid cytoplasm but separates into two areas, one at each pole of the mitotic figure of the dividing nucleus. Astral radiations now appear about the two areas as the egg cytoplasm jellies again with the formation of two jellied masses instead of one, as heretofore. These grow at the expense of the fluid cytoplasm until all of the cytoplasm of the egg is taken up into two bodies, the two blastomeres of the segmenting egg.

During the rapidly succeeding cleavages of the egg there is always a cap of liquid on the nucleus of each blastomere. With each mitosis this liquid flows around the nucleus to accumulate in two areas at the poles of the mitotic figure. These areas are periodically augmented during the formation of an aster and the ensuing jellying process.

There is every evidence³ that the mechanism of cell division depends upon a readiness of the cytoplasm to pass from a liquid to a

³ Heilbrunn, L. V., Studies in artificial parthenogenesis. II. Physical changes in the egg of *Arbacia*, *Biol. Bull.*, 1915, xxix, 149; An experimental study of cell division. I. The physical changes which determine the appearance of the spindle in sea-urchin eggs. *J. Exp. Zool.*, 1920, xxx, 211; Chambers, R., Changes in protoplasmic consistency and their relation to cell division, *J. Gen. Physiol.*, 1919, ii, 49.

jellied state and *vice versa*. The protoplasm must have its phase relations in a delicately balanced state in order that this may occur. In the egg we have seen that the reversal to a jellied state is probably accompanied by a separating out of a liquid. Something in this liquid may possibly control, in periodic rhythms, the physical state of the protoplasm surrounding it. We may assume that as long as there is a quantity of this substance localized in the egg it can induce aster formation. The idea suggests itself that one purpose of the spermatozoon is to accumulate this substance. In the mature unfertilized egg there is no localized area from which the jelling process may spread. The entrance of a sperm furnishes a focus as it were. Around this focus an aster develops with a steady accumulation of the liquid in its center. This liquid area surrounds the nucleus and puts the egg in a condition similar to that of a blastomere. The process of cleavage then becomes the same in both.

An interpretation dissonant with previous ones concerning the mode of aster formation in artificially parthenogenetic eggs has been recently put forward by Herlant.⁴ Wilson² in *Toxopneustes*, had long ago shown that eggs treated insufficiently with a parthenogenetic agent may form monasters which disappear and reappear in several successive rhythms. Hindle⁵ found this to be true also for the sea-urchin egg, if treated with butyric acid alone. A sufficient treatment, however, of a parthenogenetic agent results in the disappearance of the monaster followed by the appearance of an amphiaster. This results in cleavage of the egg. In the sea-urchin egg, the butyric acid treatment has to be followed by a bath of hypertonic sea water in order that this may occur. The hypertonic treatment often results in the formation of several cytasters in the egg. The cytasters produced by the hypertonic treatment Herlant claimed to be due to dehydrative effects producing spots within the egg cytoplasm about which the asters appear. Herlant assumed that one of these cytas-

⁴ Herlant, M., Comment agit la solution hypertonique dans la parthénogenèse expérimentale (méthode de Loeb). I. Origine et signification des asters accessoires. *Arch. Zool. exp. et gén.*, 1918, lvii, 511; II. Le mécanisme de la segmentation. *Arch. Zool. exp. et gén.*, 1919, lviii, 291.

⁵ Hindle, E., A cytological study of artificial parthenogenesis in *Strongylocentrotus purpuratus*, *Arch. Entwicklungsmech.*, 1910-11, xxxi, 145.

ters connects in some way with the monaster, thus forming the amphia-ster which initiates segmentation. The weakness in this interpretation is the lack of conclusive evidence for the union of the originally independent asters. Neither Wilson nor Hindle ever observed such a phenomenon. All my observations also indicate that the amphia-ster in parthenogenetic eggs arises from a previous single aster just as it does in normally fertilized eggs.

My studies were mainly confined to the egg of the sand-dollar. In its behavior to parthenogenetic agents⁶ the egg is almost identical with that of the sea-urchin which Herlant studied. The absence of pigment and the highly translucent nature of its protoplasm makes the sand-dollar egg an ideal object for observational study.

The mature eggs, normally shed by the female, are placed in butyric acid (2 cc. 1/10 N in 50 cc. of sea water) for 35 seconds. During this treatment the eggs distinctly round up. They are then returned to sea water where, within a few minutes, the fertilization membrane lifts off. After 20 minutes the eggs are placed in hypertonic sea water (5 cc. 2.5 M NaCl in 50 cc. sea water). The eggs shrink slightly in this solution. After 20 minutes the eggs are transferred to a large quantity of normal sea water and the sea water is changed several times to free the eggs from any further action of the hypertonic solution.

Up to this time no change whatever is to be seen in the cytoplasm or in the nucleus. While in the hypertonic solution the cytoplasm appears more granular and opaque than that of an untreated mature egg. However, on the return of the treated eggs to sea water the cytoplasm reverts to its former appearance and to the eye the eggs differ in no respect whatever from unfertilized eggs except for the presence of a fertilization membrane.

It is not until the treated eggs have stood in sea water for several minutes that any cytoplasmic change is to be observed. The first sign of a change consists in the appearance of faintly defined vacuoles about the center of the egg. Within a few minutes they coalesce to form a central clear area of about one-tenth the diameter of the

⁶ Just, E. E., The fertilization reaction in *Echinorachnius parma*. III. The nature of the activation of the egg by butyric acid. *Biol. Bull.*, 1919, xxxvi, 39.

egg. The egg nucleus lies close to or within this area. Gradually rays begin to appear in the jellying cytoplasm about the area. These rays become more numerous and more pronounced until the entire egg is occupied by a large monaster which corresponds exactly with the fully developed sperm aster of a normally inseminated egg. From now on the process is entirely analogous to that of a sperm fertilized egg. During the development of the aster the hyaline central area increases in size and the microdissection needle shows it to be a liquid area characteristic of that of the sperm aster. When the monaster disappears the liquid central area flows around the nucleus now undergoing mitosis and accumulates at the two poles of the nucleus into two polar areas. A jellying process now sets in with these two areas as centers and results in the amphiaster preparatory to the first cleavage of the egg.

In the mode of aster formation the only difference between the sperm fertilized and the parthenogenetic egg consists in the manner in which a liquid separates out of the jellying protoplasm in connection with the formation of the preliminary single aster. In the fertilized egg radiations appear immediately about the sperm-head and the accumulation of the liquid substance is from the beginning through the agency of the ray-like channels of the growing aster. In the parthenogenetic egg several vacuoles first appear in the cytoplasm. These vacuoles collect in the center of the egg after which an aster appears.

The frequent irregularities which obtain in parthenogenetic eggs are apparently due to an incomplete fusing of the vacuoles and to a lack of polarity in the preliminary stages of the aster formation. In undertreatment, or when butyric acid alone is used, a monaster develops as usual. Upon the disappearance of the monaster, the persisting liquid centrosphere, instead of flowing to the two polar regions of the nucleus, remains a single body. With the return of the jellying period a single aster again forms and more fluid accumulates in the centrosphere which increases in size. This process repeats itself several times and segmentation of the egg never occurs.

Eggs treated with butyric followed by a prolonged treatment of the hypertonic solution become abnormal. In cases of this kind the eggs, when returned to sea water from the hypertonic solution,

exhibit vacuoles which, instead of being collected in the center of the egg, are scattered throughout the cytoplasm. Radiations appear about these vacuoles with the result that the egg becomes filled with many small asters. The longer the eggs have been left in the hypertonic solution the more numerous will be the asters, and most if not all of these asters develop independently of one another. Irregularities may occur, even when the vacuoles collect in the center of the egg. In such cases an apparently normal single aster first results. Upon its disappearance, the central liquid area, instead of flowing away from the center into two polar bodies, produces three or four irregular lobes. About each of these lobes radiations appear in the egg cytoplasm producing a multipolar aster. In one instance one such lobe separated itself from the main body and a complete aster formed about it while a multipolar aster formed about the rest of the hyaline area. When the periphery of a multipolar aster reaches the surface of the egg cleavage furrows form between each lobe of the aster so that such eggs may segment simultaneously into three or four or more blastomeres. Asters which form independently of the central area never seem to be large enough to bring about segmentation of the egg into considerable masses. When such asters lie close to the periphery of the egg, furrows often grow in from the surface of the egg enclosing the asters. In this way a superficial type of segmentation results with the pinching off of small masses of the egg. The development of cytasters resulting in a spurious segmentation has already been described by Wilson.²

The first aster appears at about the same time after the acid treatment, irrespective of whether the eggs have been subsequently treated with the hypertonic solution or not. However, with subsequent hypertonic treatment, the reappearance of the radiations following the fading away of the first aster occurs about more than one center. This results in segmentation of the egg. The reaction, therefore, which is peculiar to hypertonic treatment shows up only *after* the disappearance of the first aster. At that time the persisting central liquid area of the aster, instead of remaining as a single centralized mass, separates into two or more bodies with the result that the following reappearance of rays in the cytoplasm occurs as radiations about these bodies. This produces multiple asters. If there be

only two focal points the liquid collects into two bodies, a typical amphiaser then develops, and the egg cleaves into two normal blastomeres.

Aster formation not only consists in a jellying process but also in the separating out of a liquid. The optically visible phenomenon peculiar to the parthenogenetic egg consists in the manner in which this liquid begins to separate out of the egg cytoplasm preparatory to the formation of the preliminary single aster. In the sperm fertilized egg both processes are rapid and occur together, radiations appear immediately about the sperm-head, and the accumulation of the liquid substance is from the very start through the agency of the ray-like channels of the growing aster. In the parthenogenetic egg the jellying process is apparently very slow, and the separating out of a liquid takes place before the cytoplasm is stiff enough to exhibit channels through which the liquid flows to the center. The liquid first collects into several vacuoles and an optimum treatment is necessary to cause these vacuoles to fuse into one body with the subsequent formation of a single aster. Overtreatment causes the appearance of many vacuoles scattered throughout the egg resulting in multiple asters. Undertreatment may result in the formation of a single aster which, however, periodically disappears and reappears as a single aster.

The parthenogenetic treatment, in order to be successful, must not only bring about the separating out of a liquid from the egg cytoplasm, but must also induce polarity within the resulting hyaline area in order to enable it to form two centers about which an amphiaser may develop.