STUDIES ON THE ORGANIZATION OF THE STARFISH EGG.*

By ROBERT CHAMBERS.

(From the Research Division of Eli Lilly and Company, Marine Biological Laboratory, Woods Hole.)

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The following is a preliminary record of operative work on the starfish egg which throws some light on the nature of the fertilization membrane, the interaction between nucleus and cytoplasm, and the relation of the cortex to the interior of the egg.

By means of the microdissection needle it has been possible to show that a morphologically definite membrane closely invests the unfertilized egg, and that it is this membrane which lifts off upon fertilization as the so called fertilization membrane. The description of two methods will suffice to demonstrate this. By carefully pressing an unfertilized mature egg between the surface of a cover-slip and the side of a slender glass needle the egg may be cut in two without tearing the investing membrane. This membrane now becomes apparent, bridging the gap between the two egg fragments and holding them together. Upon the addition of sperm this membrane lifts off as the fertilization membrane, in such a way that the two egg fragments come to lie within a single cavity.

The unfertilized egg can also be slipped entirely out of its investing membrane. Such an egg will undergo normal fertilization and cleave into blastomeres having no investing membrane whatever.

These two experiments definitely show that the normal unfertilized starfish egg is already surrounded by a membrane which, upon fertilization, becomes the fertilization membrane.

The difference in behavior towards sperm of an egg, which has been denuded not only of its jelly but also of its membrane, and one which has not is very striking. In an egg enclosed in its membrane

*The experiments reported in this paper constitute a part of the joint investigation of the mechanism of fertilization in which Dr. G. H. A. Clowes and the writer are engaged.

the spermatozoa quickly crowd about the egg as they are trapped in the jelly surrounding the membrane. In a membraneless egg no crowding of spermatozoa is noticeable and heavy insemination is necessary to bring about fertilization. With such eggs, when a cloud of sperm has been blown upon them, one may frequently observe a spermatozoon swim toward an egg, wander over its surface and then swim away. On the other hand the empty membrane with its investing jelly immediately becomes covered with a halo of active spermatozoa.

The nucleus of the egg cell is a liquid drop surrounded by a morphologically definite membrane. The nucleus may be moved about within the egg with the needle, and can be considerably deformed by pressure. On removal of the needle the nucleus quickly resumes its spherical shape. Tearing the nucleus slightly causes the nucleus to shrink and the nucleolus to disappear; this is followed by a remarkable spread of a disintegrative process which involves the cytoplasm surrounding the nuclear area. In the immature egg, where the nucleus is large, the disintegrative process may extend throughout the entire egg. In the mature egg with a relatively small nucleus the destruction is restricted to a limited area.

The disappearance of the nucleus or germinal vesicle during maturation has been described by several investigators. The nuclear membrane breaks down spontaneously and the nuclear sap spreads slowly throughout the cytoplasm. So long as the nuclear area, aside from the definitive egg nucleus, has not yet mixed with the cytoplasm, I find that a puncture of the area starts up the disintegrative process. When the nuclear sap has entirely mixed with the cytoplasm, any part of the egg, with the exception of the minute egg nucleus, may be torn with impunity. The mere presence of the glass needle in the nuclear sap is not sufficient to start up the disintegrative process. This process occurs only when the nuclear sap is agitated by the needle while the sap is in direct contact with the cytoplasm.

Wilson[1] found in the Nemertine egg that any non-nucleated fragment, prior to the dissolution of the germinal vesicle, is non-fertilizable whereas, any fragment from a mature egg is capable of being fertilized and undergoing cleavage. This I have found to be true also for the

[1] Wilson, E. B., Experiments on cleavage and localization in the Nemertine egg, *Arch. Entwcklingsmechn.*, 1903, xvi, 411.

starfish egg. It is also of interest to note that the fertilizability of the egg fragments is directly connected with the extent of the mixing of the nuclear sap with the cytoplasm in the maturing egg. A non-nucleated fragment, taken from an egg in the early stages of the dissolution of the germinal vesicle, will admit sperm which will undergo several nuclear divisions with, at most, an abortive attempt on the part of the fragment to cleave. When the sap of the germinal vesicle has completely mixed with the cytoplasm, any fragment larger than a certain size limit is capable of being fertilized and undergoing cleavage.

It is well known that immature eggs can be kept in sea water at room temperature for 24 hours or more without disintegrating and that unfertilized mature eggs go to pieces under the same conditions within a much shorter time.[2] The writer has found that nucleated fragments of the two kinds of eggs behave similarly, while non-nucleated fragments act quite differently indicating that the substance which prevents the disintegration is distributed differently in the two eggs. Non-nucleated fragments of immature eggs last for about 4 hours only. Similar fragments of mature eggs last from 8 to 10 hours, or about as long as the mature, nucleated fragments. The substance which prevents the destruction of the egg is apparently in the nuclear sap which, in the immature egg, is confined within the large nucleus or germinal vesicle, while in the mature egg this sap has escaped from the nucleus and spread throughout the entire egg.

The following experiments indicate that the part of the starfish egg which is capable of development is chiefly confined to the cortex of the egg. It was long ago shown by Driesch,[3] Loeb[4] and others that starfish and sea-urchin eggs are highly fluid in that fragments quickly round up into spheres. That the cortex of the mature unfertilized eggs is firmer in consistency than their interior has been

[2] Loeb, J., and Lewis, W. H., On the prolongation of the life of the unfertilized eggs of the sea-urchins by potassium cyanide, *Am. J. Physiol.*, 1902, vi, 305. Loeb, J., Maturation, natural death and the prolongation of the life of the unfertilized starfish eggs (Asterias forbesii) and their significance for the theory of fertilization, *Biol. Bull.*, 1902, iii, 295.

[3] Driesch, H., Entwicklungsmechanische Studien. Der Werth der beiden ersten Furchungszellen der Echinodermentwicklung, *Z. wiss. Zool.*, 1891, liii, 60.

[4] Loeb, J., Ueber die Grenzen der Theilbarkeit der Eisubstanz, *Arch. Physiol.*, 1895, lix, 379.

described by the writer.[5] If the surface of the mature starfish egg be torn with a needle, and the egg then caught at the opposite side and pulled to the edge of the hanging drop, the compression on the egg produced by the shallow water at the edge of the drop will cause the fluid interior to ooze out through the tear, forming a perfect sphere. One may so manipulate the process as to cause the egg nucleus either to remain behind in the cortex (the cortical remnant) or to pass into the extruded sphere.

The cortical remnant is relatively solid and remains more or less enclosed within the egg membrane and its jelly. If left long enough it will eventually round up so as to present the appearance of a diminutive egg surrounded by a collapsed and wrinkled egg membrane.

The material which has escaped from the egg into the sea water is fluid and tends immediately to round up. On tearing with a needle its surface behaves like that of a highly viscous oil drop. These spheres adhere tenaciously to glass and, in the effort to remove them by blowing a current of water against them, they sometimes leave a torn off piece behind. The cortical remnant is readily fertilizable and undergoes normal segmentation. On the other hand, the material which has escaped from the interior of the egg whether nucleated or not, is non-fertilizable. It remains inert until it finally undergoes disintegration. As long as it possesses an intact surface it appears exactly like an egg fragment and will undergo disintegrative changes similar to those of entire eggs, on being torn with the needle. If even a small part of the original cortex is allowed to remain continuous with the sphere it is fertilizable and the more cortical material present the more will the sphere approach normal cleavage.

It is significant that the fluid spheres which escape from the interior of the mature unfertilized egg, whether nucleated or not, withstand disintegration for a much longer period than do fragments, containing cortical material, which have been produced simply by cutting an egg into two or more pieces.

It follows from these facts that the part of the starfish egg chiefly concerned in development lies in its periphery. The interior when separated from the cortex is incapable of developing. On the other hand, an egg containing cortical material alone is able to carry on its usual life activities.

[5] Chambers, R., Microdissection studies. I. The visible structure of cell protoplasm and death changes, *Am. J. Physiol.*, 1917, xliii, 1.