

THE MECHANISM OF THE ENTRANCE OF SPERM INTO THE STARFISH EGG.*

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(Received for publication, May 24, 1923.)

The fertilization process in echinoderm eggs has been the subject of a great deal of investigation. It is very striking, however, to find so few actual observations on the manner in which the spermatozoon reaches and enters the egg. This may be accounted for by the fact that most workers have relied upon the early researches of Hermann Fol,¹ who first observed the entrance of a spermatozoon into an animal egg. Fol described the process in such detail that even recent text-books comment upon the completeness of his observations. From his researches on the starfish egg, Fol concluded that the presence of spermatozoa in the immediate vicinity causes the egg to respond by forming on its surface a conical elevation which attracts the spermatozoon from a distance. This cone he called the attraction cone. This rather extraordinary interpretation has been generally accepted to account for the arrival of the spermatozoon on the surface of the egg. Among the few who have taken exception to this view is Buller,² who claimed that the egg exerts no directive action and that the spermatozoa accumulate in the jelly surrounding the egg because of their positive stereotropism.

Buller studied only those species whose eggs possess a jelly readily penetrable by its own spermatozoa. The starfish spermatozoa have very blunt heads and are apparently unable to penetrate the very adhesive jelly which surrounds the starfish eggs. They come into

* 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.

¹ Fol, H., *Compt. rend. Acad.*, 1876, lxxxiii, 667; *Mem. Soc. phys. et hist. nat., Genève*, 1879, xxvi, 89.

² Buller, A. H. R., *Quart. J. Micr. Sc.*, 1902, xlvi, n. s., 145.

contact with the outer border of the jelly, in which they appear to become inextricably entangled. Fol himself commented upon this and tried to explain the passage through the jelly of the spermatozoa which succeed in fertilizing the egg, by assuming that the cone which forms on the egg's surface at this time exerts an attraction which in some mysterious way draws the spermatozoon through the jelly to it.

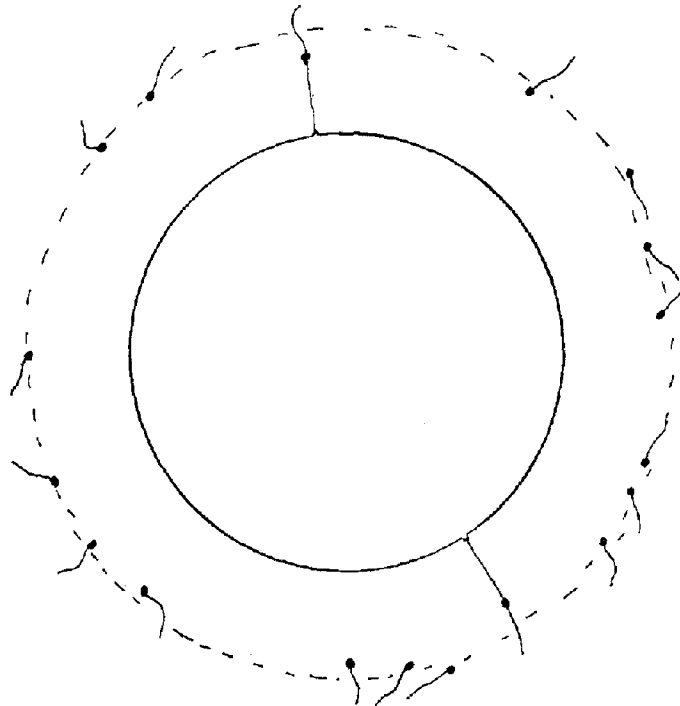


FIG. 1. Diagram of a starfish egg 1 minute after insemination. Spermatozoa are caught in the outer border of the jelly indicated here in outline. Two spermatozoa are advancing through the jelly to the egg and from each extends a visible line to the summit of a cone on the egg's surface.

The results recorded in this paper constitute an attempt to explain the peculiar behavior of the starfish spermatozoon which enables it to migrate through the jelly of the egg.

When an egg is observed within thirty seconds of its insemination, there may be seen, among the many spermatozoa which adhere to the

outer surface of the jelly, one or several spermatozoa whose heads are advancing steadily into the jelly towards the egg (Fig. 1). During its course the head does not deviate from a straight line except when it occasionally gives a spasmodic twitch. Its tail usually trails motionless behind but may sometimes lash feebly to and fro. On allowing one's eye to travel ahead of the spermatozoon to the egg, one will see a

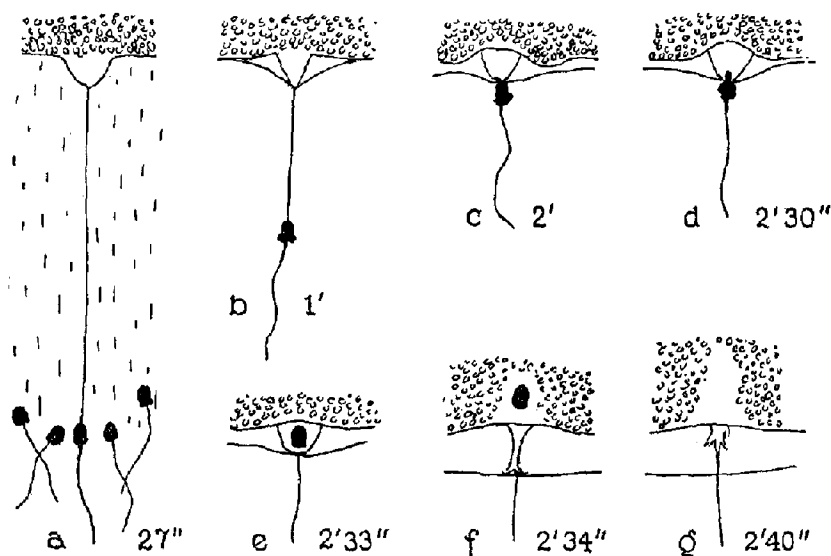


FIG. 2, *a* to *g*. Seven steps in the passage of a spermatozoon through the jelly and into the living egg. The whole process figured here lasts about $2\frac{1}{4}$ minutes. (*a*) 27 seconds after insemination. From the fertilization cone extends a delicate filament to the periphery of the jelly where the tip of the filament has touched a sperm head. (*b*) 30 seconds later. The spermatozoon has been dragged halfway through the jelly. At the base of the cone the fertilization membrane is rising. (*c*) 1 minute later. The sperm head has reached the cone. (*d*) The tip of the sperm head is narrowing as it passes through the fertilization membrane into the cone. (*e*) The head is within the cone. (*f* and *g*) The sperm head is sinking into the egg and the fertilization cone is being replaced by what Fol termed the exudation cone.

conical elevation on the egg, from the summit of which a tenuous but distinctly visible line extends to the head of the advancing spermatozoon. Owing possibly to the extreme tenuity of this line, I have been unable to observe the filament itself except when the cone of the egg

on the one hand and the head of the spermatozoon on the other can be utilized as a guide. The earliest stage at which it has been observed is shown in Fig. 2, *a*, in which it reaches the periphery of the jelly. Evidence is given below to show that this line is a filamentous process which grows out from the summit of a conical elevation on the egg through the intervening jelly.

While the spermatozoon is advancing through the jelly to the summit of the cone a membrane, separating from the surface of the cone at the base of the filament, begins the formation of the fertilization membrane (Fig. 2, *b*). As the spermatozoon approaches still nearer, one may see wave-like quivers which pass over the cone and the surface of the egg bordering the cone so that one gains the distinct impression of a pull on the spermatozoon (Fig. 2, *c*). On arrival at

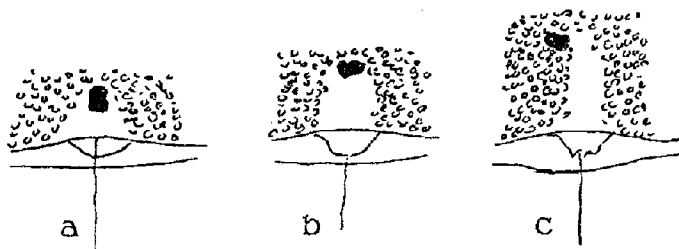


FIG. 3, *a* to *c*. Three stages showing the unusually active behavior of a sperm head after penetrating the egg. Instead of sinking in without apparent motion it wriggled from side to side for one minute and finally worked its way among the cytoplasmic granules to one side of the hyaline pathway.

the summit of the cone there ensues a pause of about thirty seconds during which time the sperm head occasionally gives one or two convulsive twitches. The sperm head then narrows at its tip (Fig. 2, *d*), and lengthens out (*e*), as it slips rapidly through the fertilization membrane to round out again after it has entered the substance of the cone (Fig. 2, *f*). There apparently exists a pore in the fertilization membrane through which the filament previously extended and which is now the only means of ingress for the spermatozoon. The cytoplasmic granules at the base of the cone disappear in front of the spermatozoon to form a hyaline pathway along which it glides into the egg. Occasionally, a sperm head, after having entered the egg (Fig. 3, *a* to *c*), keeps on oscillating from side to side and finally

leaves the pathway prepared for it and crowds in among the closely packed cytoplasmic granules where it gives rise to a typical sperm aster quite out of the usual position.

The egg does not necessarily form one fertilization cone only. If over inseminated it may form several cones at different spots on its surface. Each filamentous process of the cones may have a spermatozoon attached to its tip. We then have the spectacle of several spermatozoa being steadily drawn in toward the egg. If all the spermatozoa start advancing at the same instant and if they reach the surface of their respective cones within a few seconds of each other,

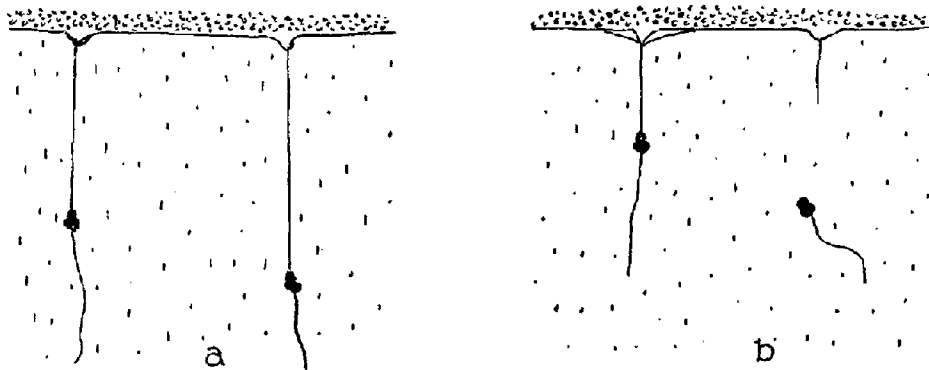


FIG. 4, *a* and *b*. (*a*) Two attached spermatozoa. (*b*) A few seconds later; the more advanced one is pursuing its course while the other has been released and lies motionless in the jelly.

they all pass successfully into the egg. This, however, does not usually occur in healthy mature eggs. One of the cones tends to form before the others and the spermatozoa seldom travel through the jelly at the same rate of speed. Before they are more than half way through the jelly the filaments which are behind hand often lose their spermatozoa (Fig. 4). The released spermatozoa remain inextricably entangled in the jelly while the naked filaments withdraw and their cones sink back into the egg. This reaction is presumably a response emanating from the filament which first came into contact with a spermatozoon.

Another phenomenon also occurs to protect the egg from polyspermy. Beginning at the cone which was the first to secure

a spermatozoon, the egg membrane rises to form the well known fertilization membrane. The rising of this membrane spreads rapidly over the egg. Fig. 5 shows a case in which a delayed filament did not lose attachment to its sperm. The spermatozoon was steadily drawn in until it reached the summit of the cone. By this time, however, the fertilization membrane had spread over the region and proved to serve as a barrier to the entrance of the spermatozoon. The cone spread out at its base and formed accessory elevations on its surface. The fertilization membrane also wrinkled con-

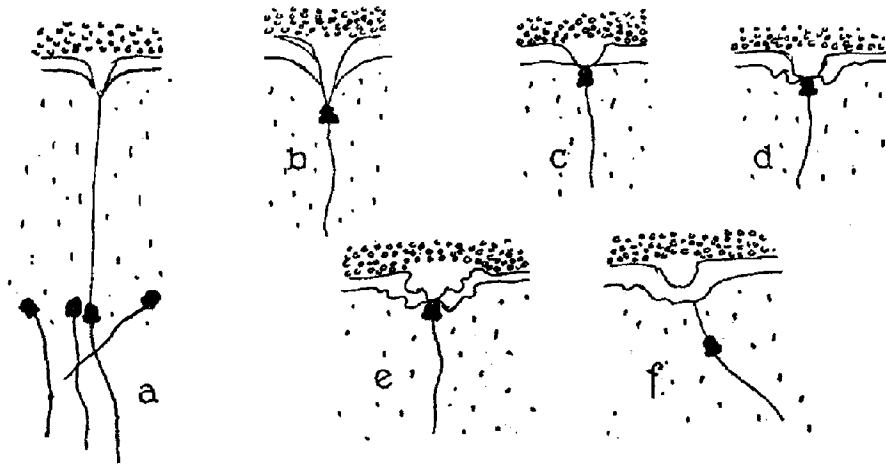


FIG. 5, *a* to *f*. Six stages in the advance of a spermatozoon towards the cone of an egg which has already formed a fertilization membrane from another source. Note in (*d*) and (*e*) the wrinkling of the fertilization membrane and the irregular elevations which form on the cone of the egg. In (*f*) the cone has definitely receded from the fertilization membrane leaving the spermatozoon outside.

siderably but when the cone finally withdrew from the membrane, the spermatozoon was still outside. Such a spermatozoon usually springs away for a short distance in the jelly where it remains permanently motionless and attached to the membrane by a slender thread. The protective action of this membrane was first shown by Fol.

Eggs aged by standing in sea water for several hours lose their protective reaction against polyspermy. In the presence of spermatozoa the surface of an old egg forms numerous cones and the filamentous process from each one quickly secures a spermatozoon. None of the

filaments tend to lose their spermatozoa, which are usually readily taken in, even after the fertilization membrane has been formed.

We have thus far described the formation of cones, all of which happen to lie in direct alignment with particular spermatozoa. This may indicate that the spermatozoon is directly responsible for the

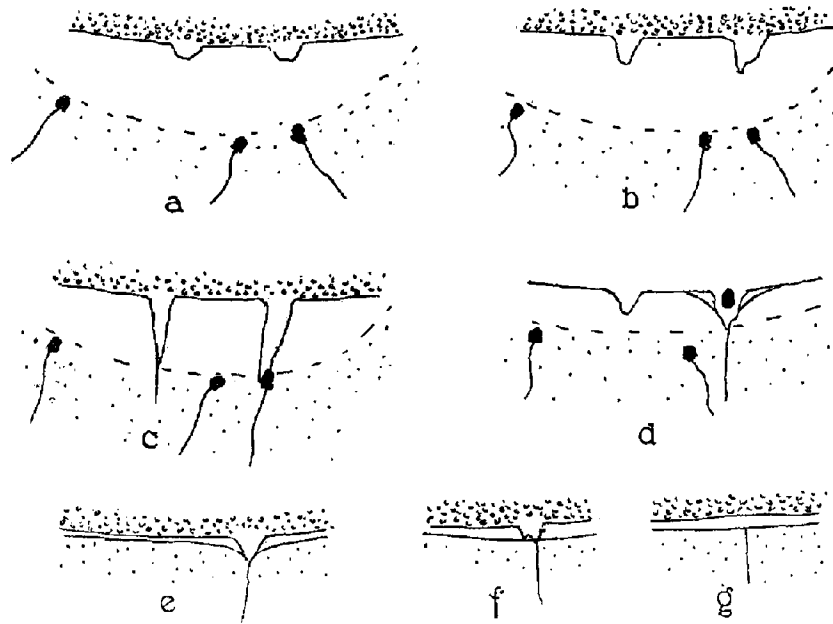


FIG. 6, *a* to *g*. Seven stages in the insemination of an egg from which the investing jelly was pulled away at one place on its surface. (*a*) Two cones have formed on the bared surface of the egg. (*b*) Both cones are growing through the artificial space. (*c*) One of the cones has come into contact with a spermatozoon which lies motionless in the jelly. The other cone has extended a filamentous process into the jelly but has met with no sperm. (*d*) Both cones are receding, one of which has secured a spermatozoon. (*e*) The unsuccessful cone has disappeared. (*f*) The successful cone has been replaced by Fol's exudation cone. (*g*) This also has disappeared in its turn. The gradual obliteration of the artificial space is due possibly to a rounding of the egg upon fertilization.

conical elevation on the surface of the egg just opposite it. That this is not necessarily the case is shown in Fig. 6. Here the jelly was pulled away from the egg at one spot by means of micro dissection needles, in order to see whether a cone would develop a filamentous

process if the jelly be absent. Two cones happened to form in the denuded area. In Fig. 6, *a* and *b*, we see both cones growing out into the space between the egg and the jelly. In *c* they have reached the jelly into which they now extend as filamentous processes. One cone came into contact with a spermatozoon. The other did not. A significant feature is that the contact of the first cone with a spermatozoon is immediately followed by a retraction, not only of the cone successful in securing a spermatozoon but of the other one as well. Both withdrew into the egg, the unsuccessful cone disappearing before the other. We have here a wave of response initiated at the point of contact with a spermatozoon and travelling through the successful cone over the surface of the egg to cause the retraction of both cones.

The following experiment (Fig. 7) indicates what happens when the vitelline membrane and its investing jelly is entirely removed from the egg.

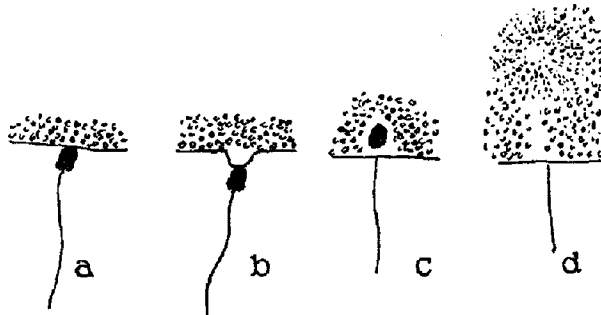


FIG. 7, *a* to *d*. Four stages in the insemination of an egg which was stripped not only of its jelly but also of its membrane. (*a*) A spermatozoon is touching the surface of a completely naked egg. (*b*) A blister-like elevation has formed where the spermatozoon was in contact with the egg. (*c*) The spermatozoon has sunk into the egg. Note the absence of a fertilization membrane. (*d*) 7 minutes after insemination the sperm head has begun to form an amphiaster.

By careful manipulation with the micro dissection instrument an egg was successfully shelled out of its membrane (Fig. 7). The egg rendered naked in this way was inseminated. When a spermatozoon touched its surface (Fig. 7, *a* to *d*) a blister-like elevation quickly formed into which the sperm head was immersed. No fertilization membrane developed. In spite of this the egg segmented in the normal manner, Fig. 7, *d* showing the sperm aster.

The formation of the cone on the surface of the starfish egg is not necessarily a response of the egg to any spermatozoon which happens to come into contact with it. I have frequently observed spermatozoa collide with the surface of unfertilized, naked eggs without causing the egg to respond in any way. After a few seconds, however, a papilla suddenly rises on the surface of the egg and a spermatozoon which is in contact with it sinks in, whereupon the fertilization of the egg is effected.

In conclusion we may summarize the insemination of the starfish egg as follows:

The egg is surrounded by a considerable zone of sticky jelly. The spermatozoa swim about with peculiar spasmodic movements until their heads accidentally hit the outer border of the jelly in which they become caught. Continued lashings of their tails only serve to imbed them more firmly in the jelly or, occasionally, to set some free. The released spermatozoa swim away and show no signs of being attracted by the egg. The heads of the trapped spermatozoa finally come to rest as they are driven into the denser region of the jelly while their tails continue lashing about for many minutes. While this is occurring on the outer border of the jelly, the egg responds to the presence of the spermatozoa by forming one or several hyaline conical elevations on its surface. From the summit of each cone a filamentous process grows out through the jelly until it touches and adheres to a motionless sperm head which happens to lie in its path. An extraordinary reaction now takes place. The filament immediately begins to retract, dragging the spermatozoon with it through the jelly to the summit of the cone on the surface of the egg. At the same instant all the other filaments are similarly withdrawn whether they have each secured a spermatozoon or not. The fertilization membrane rises and spreads from the region of the cone whose filament first made contact with a spermatozoon. When the spermatozoon arrives on the cone it is engulfed by the cone and then passes deeper into the egg, where it gives rise to the amphiaster of the fertilized egg.³

³ Further details of this study will be published in the near future.