On the Hardening of the Fertilization Membrane of Sea Urchin Eggs[1)]

By

Shun-ichi Umezawa and Terukazu Asida

Zoological Institute, Hokkaido University, Sapporo.

(*With* 1 *Text-figures*)

In the fertilization phenomenon of the eggs of marine invertebrates, the formation of the fertilization membrane is one of the most interesting problems. The fertilization membrane of the sea urchin egg is soft and fragile soon after the elevation, but few minutes later hardens gradually. According to Herbst ('93), Goldfarb ('13), C. R. Moore ('16), F. R. Lillie ('21), the membrane can be easily removed by shaking within few minutes after elevation; but afterwards the membrane becomes too rigid to be taken away by means of shaking. Hobson ('32), Chambers ('33) and Chase ('35) have approved this phenomenon with microneedle. According to Hobson ('32) and Sugiyama ('38), in Ca- free or Ca- and Mg-free sea waters the membrane does not harden and gradually sinks back on the egg surface owing to the diffusing of the osmotically active substance existing in the perivitelline space. Hobson thought that the hardening of the membrane is caused by Ca ion in the sea water, and Sugiyama reported that Mg, Sr and Ba ions also have the property to harden the membrane and that the membrane-hardening completes itself within five minutes after insemination in natural sea water.

It is a well known fact that the fertilization membrane is the leakage membrane for water and crystalloids, but not for colloid substances (Loeb '08, Robertson '12, Heilbrunn '15, Runström '28 and Sugiyama '41). From this fact it would be expected that the membrane shrinks in different forms, corresponding to the physical nature of the membrane in hypertonic colloidal solution and consequently, by the forms of shrinkage of the membrane, its physical nature is anticipated to some extent. On

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this standpoint, some experiments on the hardening phenomenon of the fertilization membrane of the sea urchin eggs (*Strongylocentrotus intermedius*) were undertaken at the Akkeshi Marine Biological Station.

Exp. 1. The time-course of the hardening

As the fertilization membrane is permeable to water and crystalloids but not to colloids, the water in the perivitelline space is osmotically taken away in colloidal solutions, causing the shrinkage of the membrane. If it dose not yet harden or retains the elasticity enough, the membrane will shrink without folding as a balloon of gum, while the membrane which has hardened or lost the elasticity will fold in different shapes by the dehydration corresponding to the degree of hardening. Thus, the type of shrinkage of the membrane in hypertonic Arabic gum sea water can be used as an indicator of its hardness. As is shown in Fig. 1, the shrinkage-forms of the membrane in hypertonic Arabic gum sea water can be grouped in four types; spherical (S), rippled (R), wave-like (W) and hollowed (H). S indicates the soft condition retaining the elasticity, and H shows the rigid, and R or W the transitional condition.

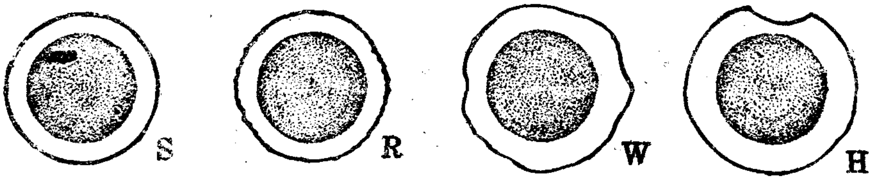

Fig. 1. The shrinkage forms of the fertilization membrane in hypertonic Arabic gum sea water.

To ascertain the time-course of the membrane-hardening the experiment was performed. The eggs [1] were inseminated in natural sea water and then transferred to the Arabic gum sea water [2] at various intervals after insemination. Results obtained were shown in Table 1.

Within one minute after insemination the elasticity of the membrane still remains. Two or three minutes after, the membrane loses elasticity, but not yet completely hardens, because it indicates the wave-like shape. After four minutes, the membrane hollows at one or two points, as a

1) In all of the experiments, eggs completely elevated the membrane within 70 sec. after insemination at 15° to 17°C.

2) The powder of Arabic gum is dissolved by bivalent cation-free artificial sea water. The dehydration effect of various concentrations of gum-solution was examined and it was found that the 0.5% is the most suitable for this purpose.

Table 1. Membrane-shrinkage in Arabic gum sea water (17°C.)

Time after insemination (min.)	Type of shrinkage[1] (10 min. after exposure)
1	S
1.5	S and W
2	W
2.5	W
3	W and H
4	H
5	H
7	H
10	H

depressed ball of pingpong. This fact shows that the membrane becomes rigid.

The same results are obtained in other colloidal solutions, e.g. serum albumen solution.

On the other hand, it is a well known fact that the membrane of the sea urchin egg can be mechanically removed i.e. by shaking for a few minutes after the elevation. With the purpose of comparing the time-courses of the membrane hardening obtained by the methods mentioned above, the following experiment was performed. At various intervals after insemination the eggs were lightly shaken about fifteen times in a test-tube. The results were summarized in Table 2.

Table 2. Removing of the membrane by shaking (17°C.)

Time after insemination (min.)	Condition of the membrane
1	All was removed
2	Most was removed
3	Almost all remained
4	All remained
5	All remained
7	All remained

The results shown in Table 2 clearly indicate that the time-courses obtained by the two methods coincide with each other. Then, it can be safely said that the fertilization membrane hardens in four minutes after insemination at about 17°C. in natural sea water.

Exp. 2. The rôle of bivalent cations on the hardening

Some authors reported that the hardening of the membrane does not occur in Ca- and Mg-free sea water. This fact was clearly ascertained with the next experiment carried out by the colloid sea water method. One minute after insemination in natural sea water, the eggs were trans-

1) About 40 eggs were observed in each case.

ferred to Ca- and Mg-free sea water[1] and then into the 0.5% Arabic gum sea water at various lengths of exposure-time. The results were shown in Table 3.

Table 3. Membrane-shrinkage affected by Ca- and Mg-free sea water (16°C.)

Exposure-time in Ca- and Mg-free sea water (min.)	Type of shrinkage	Diameter of the membrane (μ)
0 (control)	H	130.80
1	S	114.52
2	S and R	114.52
3	S and R	116.15
4	S and R	116.15
5	S and R	118.61
7	S and R	118.61
10	S and R	118.61
30	S, R and W	......

At a glance on the above table it is clear that the membrane does not harden in Ca- and Mg-free sea water. Thus, if the hardening reaction depends only upon the absence of Ca or Mg ion in the medium, the addition of Ca or Mg ion may cause the membrane-hardening.

One minute after insemination, the eggs were washed in Ca- and Mg-free sea water for about twenty seconds and put into artificial sea water, containing, at various degrees, Ca and Mg ions, and five minutes after, the eggs were transferred to 0.5% Arabic gum sea water.

Table 4. Effect of Ca or Mg ion on the shrinkage-form of the membrane (15.5°C.)

Volume of added $CaCl_2$ or $MgCl_2$ solution (0.36 M) to 3 cc. of bivalention-free artificial sea water		Type of shrinkage
$CaCl_2$	0.000 cc.	S and R
	0.001	S and R
	0.005	S and R
	0.01	S, R and W
	0.03	S, R, W and H
	0.05	R, W and H
	0.1	W and H
	0.3	H
	0.5	H
$MgCl_2$	0.0	S and R
	0.5	S and R
	1.0	S, R and W
	3.0	R, W and H
	5.0	W and H

1) This is a modified Herbst's artificial sea water (10 cc. of 0.54 M NaCl + 2.1 cc. of 0.54 M KCl + 1.2cc. of 0.54 M $NaHCO_3$).

From the experiment-results summarized in Table 4, there are no doubtful points as to that Ca or Mg ion is necessary to cause the hardening of the membrane, as reported by many authors, and that Ca ion is more effective than Mg ion in the hardening phenomenon.

Exp. 3. The change of the nature of the fertilization membrane

The effect of Ca- and Mg-free sea water on the membrane which had completely hardened was studied. Five minutes after insemination, the eggs were put into Na-oxalate sea water[1], and after various intervals they were transferred to 0.5% Arabic gum sea water.[2].

Table 5. Effect of Ca- and Mg-free sea water on the membrane completely hardened (15°C.)

Exposure-time in Na-oxalate sea water (min.)	Condition of the membrane	
	Form	Diameter (μ)
0 (control)	H	147.2
1	H	143.9
3	H	147.2
5	H	147.2
15	H	147.2

Judging from the above table, the shrinkage form of the fertilization membrane, which has completely hardened, is not influenced by washing with Na-oxalate sea water. Therefore, it can be clearly concluded that the hardening is an irreversible process.

Now the problem, how long the hardening ability of the membrane is maintained after its elevation, must be tackled. To solve this problem, the following experiments were undertaken. One minute after insemination, the eggs were treated in different lengths of time with Na-oxalate or Ca- and Mg-free sea water, then they were transferred to natural sea water for five minutes,[3] and then put into 0.5% Arabic gum sea water. In Tables 6 and 7 the results are shown.

1) The Na-oxalate sea water was made up of Na-oxalate and Ca- and Mg-free sea water (1cc. of M/3 $Na_2C_2O_4$+10cc. of 0.54M NaCl+2.1 cc. of 0.54M KCl+1.2cc. of 0.54M $NaHCO_3$). When unfertilized eggs were treated with the Na-oxalate sea water and then inseminated in natural sea water, the fertilization membrane elevated and hardened normally.

2) When these eggs were transferred again into natural sea water one hour after, they recovered the spherical shape and developed normally to "blastulae".

3) For the duration of the treatment with natural sea water, 5 minutes is sufficient, because, if the eggs were treated for longer, no difference in the shrinkage form was observed.

Table 6. The effect of Ca- and Mg-free sea water on the soft membrane (15°C.)

Exposure-time in Na-oxalate sea water (min.)	Condition of the membrane	
	Form	Diameter (μ)
0 (control)	H	130.88
1	H	130.88
2	R,W and H	120.70
3	S an R	118.61
4	S and R	118.61
5	S and R	118.61
10	S	118.61
11	S	118.61

Table 7.

Exposure-time in Ca- and Mg-free sea water (min.)	Condition of the membrane	
	Form	Diameter (μ)
0 (control)	H	130.80
1	H	130.80
2	W and H	125.97
3	S, R, W and H	122.70
4	S	114.52
5	S	114.52
10	S	......

As shown in the above tables, the shrinkage-form of the membrane is H type within two minutes after insemination, during which the eggs are immersed in Ca and Mg ions-free medium, and after three or four minutes R, W and S types appear, and finally S type only. In other words, these results clearly indicate that the hardening ability of the membrane is lost, when the hardening process of the membrane is blocked for three to four minutes (at 15°C.) by treatment with Ca and Mg ions-free medium.

Summary

1. The hardening phenomenon of the fertilization membrane of sea urchin eggs (*Strongylocentrotus intermedius*) are studied by colloidal solution method.
2. The fertilization membrane is completely hardened by Ca and Mg ions in four minutes after insemination in natural sea water ; but Ca ion is more effective on the hardening than Mg ion.
3. The membrane hardening is an irreversible change, as the hollowed type shrinkage of the membrane completely hardened is not changed any more by treatment with Na-oxalate sea water.
4. When eggs are exposed to Ca- and Mg-free sea water for more than three minutes at one minute after insemination, the hardening of the

membrane is caused no more by Ca and Mg ions. It may be concluded that a part of the chain of the hardening mechanism of the fertilization membrane is cut off in Ca- and Mg-free sea water within about four minutes after insemination.

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