# FURTHER EXPERIMENTS ON NATURAL DEATH AND PROLONGATION OF LIFE IN THE EGG[1]

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1. The unfertilized egg dies in a comparatively short time, while the act of fertilization gives rise to a theoretically at least unlimited number of generations. The death of the unfertilized egg may be called a case of "natural" death of a cell; it is perhaps the only case in which we can feel sure that death is caused by internal "natural" causes and not by avoidable injuries. The act of fertilization is the only one known to prevent natural death.

The velocity with which the unfertilized eggs die differs for the eggs of various species; the mature egg of the starfish dies much more rapidly than the egg of the sea urchin. The writer pointed out that this difference might be connected with a difference in the rate of oxidations in the two kinds of eggs, since he had been able to show that the suppression of oxidations by the withdrawal of oxygen from the sea water or by the addition of a trace of KCN prolongs the life of these eggs. Last year, Loeb and Wasteneys were indeed able to prove a difference in the relative rate of oxidations between the eggs of these two kinds of animals in the sense which is demanded by our hypothesis. The mature unfertilized egg of the starfish has a rate of oxidations which equals that of the fertilized egg;[2] while the rate of oxidations in the unfertilized mature egg of the sea urchin is only about one-fourth or one-sixth of that of the fertilized egg of the same species.

[1] Loeb, Maturation, natural death and the prolongation of life, etc. Biol. Bull., vol. 3, p. 295, 1902. The mechanistic conception of life, 1912.

[2] Loeb and Wasteneys, Arch. f. Entwicklungsmechanik, vol. 35, p. 555, 1912.

2. Our analysis of the process of the causation of development has led us to the result that it requires generally the coöperation of two factors (or substances); one which calls forth the membrane formation (or the change in the cortical layer of the egg); and a second factor which saves the egg from the disintegration with which it is usually threatened after membrane formation. This second—corrective—factor is most conveniently supplied in the form of a short treatment of the egg with a hypertonic solution.[3] The question therefore presented itself to which of these two factors the life-saving effect of the act of fertilization was due.

The natural inference from our former experiences would have been to ascribe the life-saving effect of the act of fertilization to the second corrective factor for the following reason. If we cause artificial membrane formation in the unfertilized egg of the sea urchin, we do not prolong its life but on the contrary we shorten it. Such an egg dies at room temperature in a few hours while without the membrane formation it may live at least for a few days. The artificial membrane formation alone (if it is not followed by the second corrective factor) hastens the death of the unfertilized egg. We can understand the reason for this since the artificial membrane formation accelerates the rate of oxidations in the sea urchin egg to exactly the same amount as the entrance of a spermatozoon (Warburg,[4] Loeb and Wasteneys[5]). By the way of exclusion this seemed to restrict the life-saving effect of the act of fertilization as well as of artificial parthenogenesis to the second corrective factor.

3. Observations which the writer made this winter, however, show that this conclusion is not correct. In his earlier work he had already found that the hypertonic solution is just as effective as a corrective factor if it precedes the artificial membrane formation as if it follows it. The only difference between the two cases is a difference in the time of exposure required. When the artificial membrane formation is called forth first and the eggs are exposed to the hypertonic solution (50 cc. sea water + 8 cc.

[3] Loeb, Die chemische Entwicklungserregung des tierischen Eies, Berlin, 1909. The mechanistic conception of life, 1912.

[4] Warburg, Zeitschr. physiol. Chemie, vol. 66, p. 305, 1910.

[5] Loeb and Wasteneys, Journ. Biol. Chemistry, vol. 14, p. 469, 1913.

2.5 m NaCl) afterwards, the eggs of purpuratus need remain in the solution for only from forty to sixty minutes. If, however, the eggs are put into the hypertonic solution first and submitted to the treatment for membrane formation (e.g., butyric acid treatment) afterwards, they must remain in the hypertonic solution from 90 to 150 minutes. In the case of the egg of Arbacia this treatment in itself would induce artificial parthenogenesis, but in the egg of Strongylocentrotus purpuratus it does in most cases leave the eggs either intact or causes them to segment once or a few times and then to go into a state of rest again. When such eggs are afterwards treated with butyric acid they will develop.

The reason why the eggs must remain longer in the hypertonic solution when this treatment precedes the artificial membrane formation, than when it follows, seems clear if we consider the fact that the corrective effect of the hypertonic solution is weakened or inhibited if we inhibit or diminish the oxidations in the egg. This indicates that the corrective effect is in some way connected with the formation of a product of oxidation in the egg which is not formed in normal sea water. Since the rate of oxidations is from four to six times as great in the egg after the membrane formation as it is before, we can understand why the hypertonic solution brings about the corrective effect so much more quickly in the egg after membrane formation than before.

4. Working on the idea that the corrective effect of the hypertonic solution was due to the formation of a specific oxidation product in the egg it occurred to the writer to test whether the corrective effect of the hypertonic solution was reversible or permanent. Unfertilized eggs of S. purpuratus in which no membrane formation had been called forth were treated with a hypertonic solution and then portions of these eggs were treated after varying intervals of from one hour to three days with butyric acid. In all cases the butyric acid treatment now sufficed to call forth in these eggs a normal development at any time. This shows that the corrective effect produced by the hypertonic solution is irreversible and lasts in the egg as long as the latter lives.

The description of an experiment will illustrate this point. The unfertilized eggs of one female S. purpuratus were put for $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, and $3\frac{1}{4}$ hours into hypertonic sea water (50 cc. sea water + 9 cc. 2.5 m NaCl + KCl + $CaCl_2$). None of these eggs developed into larvae and none of the eggs that had been up to $1\frac{1}{2}$ hours in the hypertonic solution segmented. Of the eggs that had been 2 hours in the hypertonic solution 0.5 per cent went into the two-cell stage but did not develop beyond this; of the eggs that had been $2\frac{1}{2}$ and $3\frac{1}{4}$ hours in the hypertonic solution 10 per cent segmented into the two- or as far as the four-cell stage. Practically all the eggs were intact the next day.

Twenty-two hours later part of each lot of these eggs was treated $1\frac{1}{2}$ or 3 minutes with propionic acid (2.8 cc. $\frac{N}{10}$ acid to 50 cc. sea water) to induce membrane formation. All formed tight fitting membranes. The result was as follows. The eggs that had been treated with the hypertonic solution $\frac{1}{2}$ and $1\frac{1}{2}$ hours disintegrated after the artificial membrane formation. The exposure to the hypertonic solution had been too short to produce a corrective effect. Among those treated for $1\frac{1}{2}$ hours one swimming larva was found the next day. Of those that had been in the hypertonic solution for 2 hours, 25 per cent segmented normally after the artificial membrane formation and developed into perfect blastulae; of those treated with hypertonic solution for $2\frac{1}{2}$ and $3\frac{1}{4}$ hours practically all developed but many of the blastulae were sickly, a sign that the eggs had been a little too long in the hypertonic solution.

Forty-eight hours after the treatment with hypertonic solution a second portion of the eggs that had been exposed $2\frac{1}{2}$ hours to the hypertonic solution was caused to form membranes by the treatment with propionic acid. The eggs had a tendency to stick to the glass but all segmented regularly and developed into blastulae which appeared normal.

Three days after the treatment of the eggs with the hypertonic solution another lot (those that had been two hours in the hypertonic solution) was treated with propionic acid. Those eggs that were still alive formed tight fitting membranes and all began to segment regularly. A few developed into normal blastulae.

On the fourth day all the eggs which had not been treated at all or had only been treated with the hypertonic solution were dead. Those eggs that had been treated with both the hypertonic and the propionic acid were developing and alive.

This experiment was repeated quite often with the same result. It proves that the corrective effect of the hypertonic solution is irreversible and remains in the egg as long as it lives.

5. One word should be said in regard to the fact that the exposure of the eggs to hypertonic sea water which leads in Arbacia quite frequently to the origin of larvae, behaves so differently in the eggs of S. purpuratus. The writer has repeatedly pointed out this difference. His recent investigations have led him to think that this is due to a typical difference in the reactions of the eggs of the two species to the influence of the hypertonic solution. In both species the eggs of only part of the females can be induced to develop by the treatment with the hypertonic solution alone, but the number of females with susceptible eggs is much greater in Arbacia than in S. purpuratus. This year the writer has investigated what constitutes this difference in the behavior of the eggs of different females of purpuratus and found the following fact. The eggs of different females were treated with the same hypertonic solution at the same temperature and for the same time. The eggs of the majority of the females behaved as described in the preceding chapter of this paper. The eggs of some of the females of purpuratus developed into larvae through the mere treatment with a hypertonic solution. The difference between the two groups was this. The eggs which developed formed somewhat atypical membranes (gelatinous films), those that did not develop formed no membranes. The membrane formation did not necessarily occur while the eggs were in the hypertonic solution but often considerably later, while the eggs were already beginning to segment. Those eggs which had begun to segment but did not form the atypical membrane stopped developing and went back into the resting stage again. Those which formed membranes went on developing into larvae. The specific difference between the eggs of different females is therefore a difference in the facility with which the

hypertonic solution causes membrane formation. The fatty acids, if properly applied, cause a typical or an atypical membrane formation in practically every sea urchin egg. The hypertonic sea water, however, causes an atypical membrane formation in the eggs of only a small percentage of the females of purpuratus and a larger though limited percentage of the eggs of Arbacia. As the writer pointed out years ago, the purely osmotic method of artificial parthenogenesis gives better results if the solution is rendered more alkaline.

6. In order to avoid misunderstandings it may be well to point out that in the purely osmotic method of artificial parthenogenesis the causation of development is also due to two factors: a membrane-forming and a corrective factor. The peculiar fact is that one and the same external agency, the hypertonic solution, produces both effects simultaneously. It produces the corrective effect in all cases, if the eggs are left long enough in the solution. It induces membrane formation in a limited number of cases. The eggs develop into larvae only if both effects are produced by the hypertonic solution, otherwise the membrane formation has to be induced by some other agency, for example, the addition of some alkali to the solution or the treatment of the eggs afterward with a fatty acid.

It may be that the membrane formation by the hypertonic solution is always the combined effect of the HO ions and the hypertónic solution, even in neutral hypertonic solutions, where the $C_{OH} = 10^{-7}N$. But this conception may be unnecessary.[6]

7. We are now in a position to answer the question from which we started as to which of the two factors of fertilization (the membrane-forming and the corrective one) was responsible for the saving of the life of the egg. The answer must be that it is the combined effect of both factors. Our experiments have shown

[6] In a former paper the writer had suggested that the HO ions contained in the hypertonic solution might furnish the first factor; this conclusion was reached under the influence of the experiments on purpuratus where a neutral hypertonic solution as a rule never induces artificial parthenogenesis. In Arbacia, however, a neutral hypertonic solution does easily induce the formation of parthenogenetic larvae.

that the corrective effect if once imparted is permanent. If the corrective factor alone were the life-saving factor, eggs treated with a hypertonic solution alone should live indefinitely, or at least much longer than the non-treated unfertilized eggs. This is, however, not the case. In all the experiments the unfertilized eggs of purpuratus treated with the hypertonic solution alone died as quickly as those not treated at all, that is, in three days or less, at room temperature. *For these eggs the artificial membrane formation became a life-saving act;* while the artificial membrane formation had just the opposite effect in sea urchin eggs, to which the corrective effect was not imparted. This admits of only one conclusion, namely that the life-saving effect of the act of fertilization is not due to one but to the combined action of both factors of fertilization.

This harmonizes with the observations on the eggs of such forms as the starfish (Asterina) in which the artificial membrane formation by a fatty acid suffices to call forth development. Not all the eggs, however, will develop into larvae under the influence of artificial membrane formation alone, but only a small percentage. R. S. Lillie has shown that the percentage of eggs of Asterias, which will develop into larvae after artificial membrane formation by heating, can be increased by a subsequent suppression of oxidations in such eggs (which treatment as the writer has shown can also supply the second factor of fertilization). This proves that in the egg of the starfish fertilization is also induced by two factors, but that some of the eggs possess or can produce the second factor normally, while this factor can be produced in the majority of eggs only if they are kept without oxidations for some time. In the sea urchin eggs it is a rare exception if the egg contains the second factor normally, that is, if it can develop into a larva by the mere act of membrane formation. As a rule, the second factor has to be supplied either by a treatment with a hypertonic solution or by a prolonged suppression of oxidations. The mature eggs of the starfish die without exception if they are not fertilized and they die much more rapidly than the unfertilized eggs of the sea urchin; while the mere artificial membrane formation saves at least the life of a small percentage of the eggs of the starfish. All these facts harmonize with the view that both factors of fertilization are required for the life-saving effect of fertilization. A similar situation exists in the case of the eggs of some annelids.

## SUMMARY OF RESULTS

1. Since the unfertilized egg dies in a comparatively short time while the act of fertilization saves the life of the egg, it was proposed to find out which of the two factors of fertilization is responsible for this effect, the membrane formation or the corrective effect (produced in artificial parthenogenesis by the hypertonic solution). In former experiments the writer had shown that the artificial membrane formation alone hastens the death of the unfertilized egg while the treatment of such eggs with a hypertonic solution saves the life of the egg. This would make it appear as if the second factor was solely responsible for the life-saving effect of the act of fertilization.

2. The writer had formerly shown that the treatment of the egg with a hypertonic solution may precede the artificial membrane formation. It is shown in this paper that if the unfertilized eggs of purpuratus have once been treated with a hypertonic solution which in itself will not induce development they will develop at any time after artificial membrane formation has been induced. If the second factor is once imparted to this egg it keeps it as long as it is alive. The hypertonic solution induces an irreversible change in the egg.

3. It is shown that the treatment with a hypertonic solution alone does not prolong the life of the unfertilized egg. Eggs treated in this way live no longer than unfertilized eggs which have not been treated at all. If in such eggs artificial membrane formation is induced they will live indefinitely. For eggs treated with a hypertonic solution the artificial membrane formation becomes a live-saving act.

4. It follows from this that for the prolongation of the life of the unfertilized egg both factors of artificial parthenogenesis, the alteration of the surface as well as the second factor (usually supplied by the treatment with hypertonic solution) are required.