1911

Jacques Loeb, 1910

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HOW DOES THE ACT OF FERTILIZATION SAVE THE LIFE OF THE EGG?[1]

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THE unfertilized egg dies in a comparatively short time, while the act of fertilization gives rise to a series of generations which, theoretically at least, is of infinite duration. The act of fertilization is, therefore, a life-saving act for the egg. The problem arises, in which way can the spermatozoön save the egg's life?

If the ovaries of a star-fish are put into sea-water the eggs are shed. They are generally immature, and in this condition they cannot be fertilized, either by spermatozoön or by chemical means. If they remain, however, for some time in sea-water, all or a number of them gradually become mature; that is to say, their nuclear mass is diminished by the extrusion of two so-called polar bodies. If immediately after the extrusion of the polar bodies sperm is added, the eggs develop. They can at that period likewise be caused to develop by certain chemical and physical agencies.

Ten years ago I made the following observations: If the eggs are not caused to develop by sperm or by physicochemical agencies, they perish very rapidly. At summer temperature they may die in from four to six hours. The death of the egg manifests itself morphologically in a darkening and blackening of the otherwise clear egg. I found that the death of the egg can be prevented by withdrawing the oxygen, or by diminishing the rate of oxidations in the egg through the addition of a trace of potassium cyanide. The life-saving action of lack of oxygen can be shown in various ways. The maturation of the egg itself depends upon oxidations. If one takes away the oxygen from the immature eggs, or if the oxidations in the immature eggs are inhibited by potassium cyanide, the process

[1] Delivered November 19, 1910.

of maturation does not take place. Maturation is, therefore, also a function of oxidations. The eggs of a female, which were unripe, were divided into two groups: the one group remained in sea-water in contact with oxygen; the other was put into sea-water whose oxygen had been removed by a current of hydrogen. The eggs of the second group remained alive; the eggs of the first group perished in a few hours.

It is not even necessary to drive out the air by hydrogen; one can preserve the life of the unfertilized eggs also by putting large masses of them into a narrow glass tube which is sealed at the bottom. The eggs sink down to the bottom of the tube, and those which are lying near the bottom receive no oxygen, since the oxygen which diffuses from the air through the sea-water is consumed by the uppermost layer of the eggs. On account of this lack of oxygen the eggs lying at the bottom of the tube do not mature and do not perish; hence by withholding oxygen from the immature eggs their maturation and their death are prevented. This might perhaps not be so strange, but the following result is much more strange.

If the oxygen is withheld from the eggs immediately after they become mature their life is also saved. A. P. Mathews has repeated this experiment and obtained the same results. This proves that the death of the mature but unfertilized egg is determined by oxidations. If these oxidations are inhibited death does not occur. When these experiments were published they first caused opposition. This opposition was founded on the application of potassium cyanide in part of the experiments. The objection was raised that the potassium cyanide in these experiments only acted by preventing the development of bacteria. The authors, however, who raised this objection, overlooked the fact that lack of oxygen acts in exactly the same way as the addition of potassium cyanide, and that it is entirely immaterial how lack of oxygen is produced, whether the oxygen is driven out by carefully purified hydrogen or whether the eggs are put together into a large heap, whereby only those lying on the surface of the heap receive enough oxygen.

It is, however, easy to show directly that the above-mentioned objection is incorrect. It is not difficult to put the eggs of the star-fish, without bacterial infection, into sterilized sea-water. The following experiment was tried. The eggs of a star-fish were separated into three parts: one part was put aseptically into a series of flasks with sterilized sea-water; the second part was put into ordinary sea-water without asepsis; the third part was put into sea-water to which a large quantity of a putrid culture of bacteria, that had developed on the dead eggs of the star-fish, had been added. It was found that in all three cases the mature eggs died within the same time. The sterilization of the eggs of the first group was complete, as was shown by the fact that the eggs kept in flasks for two months preserved their form, while the dead eggs in the normal sea-water were destroyed in a few days by the action of the bacteria.

It is, therefore, certain that the death of the star-fish eggs which are not fertilized is not caused by bacteria, but by the process of oxidations in the egg. If no spermatozoön enters the egg, or if the egg is not caused to develop in a chemical way, it perishes very rapidly. If, however, a spermatozoön enters the egg, it remains alive in spite of the fact that the entrance of the spermatozoön causes a relative acceleration of the oxidations in the egg. Warburg found for the eggs of the sea-urchin at Naples that fertilization raises the velocity of the process of oxidations to six times their original value, while Wasteneys and I found that fertilization caused an increase in the velocity of oxidations of Arbacia in Woods Hole to three or four times the rate found in the unfertilized eggs.

How can we explain that fertilization saves the life of the egg? Let us make the following preliminary assumption: The unfertilized egg contains a poison, or some faulty combination of conditions which, if oxidations take place, cause the death of the egg. In the unfertilized but mature egg oxidations take place. The spermatozoön carries among others a substance into the egg which protects the egg against the fatal effects of the oxidations, and allows them even to carry on

oxidations at an increased rate without suffering. We might say that the mature but unfertilized egg is comparable to an anaërobic being for which oxidations are fatal, and that the spermatozoön transforms the egg into an aërobic organism.

If we compare the eggs of different animals, we find great differences in regard to the above-mentioned conditions. The eggs of certain annelids (Polynoe) also perish rapidly if they become mature without being caused to develop, while the eggs of the sea-urchin remain alive for a longer period of time after they have become mature. It has not yet been investigated what determines this difference.

II

The analysis of the process of fertilization by the spermatozoön shows that we must discriminate between two kinds of effects, the hereditary effect and the activating or developmental effect. The experiments on artificial parthenogenesis make it very probable that the two groups of substances: the substances which determine the heredity of paternal characters and the substances which cause the egg to develop, are entirely different. In this paper we are only concerned with the second group of substances, namely, those which cause the development of the egg.

The analysis of the causation of development of the egg by a spermatozoön has shown that the latter acts by carrying at least two substances or groups of substances into the egg. The one of these substances causes the formation of a membrane; the second serves the purpose of rendering the egg immune against the fatal action of oxidations.

I have shown in a number of papers that the essential feature in the causation of development of the egg is a modification of its surface, which in many cases leads to the formation of a membrane. If we cause membrane formation in an unfertilized sea-urchin egg by artificial means, it begins to develop, but it perishes very soon; much more rapidly than if it is not exposed to any treatment. I was able to show that this rapid death of the sea-urchin egg, after artificial membrane

formation, can be prevented either by withdrawing the oxygen from the egg or by inhibiting the oxidations in the egg through the addition of a trace of potassium cyanide. The membrane formation, therefore, causes the rapid death of the egg through an acceleration of oxidations. Warburg has recently shown that the artificial membrane formation in the unfertilized sea-urchin egg causes the same increase in the rapidity of oxidations as the entrance of a spermatozoön.

If we wish to cause the unfertilized eggs to develop to the pluteus stage after the membrane formation, we have to subject them to a second treatment. This may consist in putting them about 15 minutes after the membrane formation into a hypertonic solution of a certain osmotic pressure (for instance, 50 c.c. of sea-water + 8 c.c. $\frac{N}{2\frac{1}{2}}$ NaCl) for one-half to one hour. If, after this time, they are put back into normal sea-water they no longer perish, but develop into normal larvæ. I ventured the hypothesis that the artificial membrane formation causes a rapid increase of the oxidations in the egg and in this way causes it to develop, but that these oxidations lead to the rapid decay of the eggs at room temperature for the reason that the egg contains a toxic substance, or a toxic complex of conditions, which in the presence of oxidations leads to the rapid death of the egg. The second treatment serves the purpose of rendering the egg immune against the toxic effects of the oxidations.

If we first cause the artificial membrane formation in the unfertilized egg by any of the various means which I have described in former papers, and if we afterward treat the eggs for a short time with a hypertonic solution, they develop after being transferred to normal sea-water in the same way as if a spermatozoön had entered them. They reach the successive larval stages, develop into a blastula, gastrula and pluteus, and live as long as the larvæ produced from eggs fertilized by a spermatozoön.

Hence the physicochemical activation of the unfertilized egg of the sea-urchin consists of two kinds of treatment. The one is a change in the surface of the egg which may or may not result in the so-called formation of the membrane. This

change causes the acceleration of oxidations which in my opinion is the essential feature of the process of fertilization. The second treatment consists in abolishing the faulty condition which makes oxidations fatal to the egg. This second treatment may consist in exposing the eggs for about half an hour or a little more to a hypertonic solution. We can substitute, however, for this treatment another treatment, namely, the deprivation of the egg for three hours from oxidations, either by removing the oxygen from the solution or by adding a trace of potassium cyanide to the solution. If, after the treatment with the hypertonic solution for half an hour, or the treatment with lack of oxygen for about three hours, the eggs are put back into normal sea-water they can develop normally into normal larvæ.

We can show that the spermatozoön also causes the development of the egg by two different agencies comparable in their action to the agencies used in the methods of chemical fertilization which we have just described.

For this purpose we must fertilize the egg of the sea-urchin with a sperm different from its own, and for the following reason: The spermatozoön of the sea-urchin enters so rapidly into the egg of the sea-urchin that it is impossible to show that it causes the development of the egg by two different substances.

If, however, we fertilize the sea-urchin egg with the sperm of star-fish, it takes from ten to fifty minutes to cause the membrane formation in the eggs, the reason being that the star-fish sperm can penetrate only very slowly into the egg of the sea-urchin.

It is, as a rule, not possible to fertilize the egg of the sea-urchin by star-fish sperm in normal sea-water. But I found eight years ago that if we make the sea-water slightly more alkaline than it naturally is the eggs of the sea-urchin can be fertilized by the sperm of the star-fish. For the fertilization of the Californian sea-urchin, Strongylocentrotus purpuratus, with the sperm of Asterias, the best results were obtained when 0.6 c.c. of N/10 NaOH were added to 50 c.c. of sea-water. In

that case, with active sperm, in about fifty minutes all the eggs form the typical fertilization membrane.

If one watches the further development of sea-urchin eggs fertilized by star-fish sperm one notices very soon that there are two different kinds of eggs present; the one kind of eggs behave as if they had been fertilized with sperm of their own kind. That means, they segment regularly and develop into swimming blastulæ and gastrulæ. The other kind of eggs, however, act as if they had been treated with one of the agencies which cause the membrane formation in the unfertilized sea-urchin egg; these eggs begin to segment, but at room temperature they slowly perish by cytolysis. If, however, these eggs are treated for half an hour with a hypertonic solution they develop into larvæ.

If one examines the eggs of a sea-urchin which have been treated in an alkaline medium with the sperm of the star-fish, one finds that only a certain percentage of these eggs contain the sperm nucleus, and this percentage seems to be identical with the percentage of the eggs which develop into larvæ. As far as the other eggs are concerned, which only form a membrane and then disintegrate, no sperm nucleus can be found inside of them. I am inclined to draw the following conclusion from these observations: The spermatozoön of the star-fish penetrates very slowly through the surface film of the sea-urchin egg. When it lingers for some time partially imbedded in the surface film, one of the substances of the spermatozoön is dissolved in the superficial layer of the egg and causes the membrane formation. Through the act of membrane formation the further entrance of the spermatozoön into the egg is prevented; since the fertilization membrane is impermeable to sperm. This membrane formation leads to an increase in the rate of oxidations and the beginning of the development of the egg. The latter, however, contains a toxic substance, or a faulty complex of conditions which has to be abolished, before the oxidations necessary for development can take place without the egg being destroyed by them. The spermatozoön carries a second substance into the egg which renders it immune

against the fatal actions of the oxidations. While the membrane forming substance of the spermatozoön may be situated at its surface, or superficially at least, the second substance which transforms the egg from an anaërobe into an aërobe must be situated in the interior of the spermatozoön; since it can only act if the spermatozoön penetrates into the egg. We see in these observations, concerning the fertilization of the sea-urchin egg by the star-fish sperm, a proof that the activation of the egg by the spermatozoön is also caused by two different substances, one of which causes the membrane formation, while the second renders the egg immune against the toxic action of the oxidations. These data support the assumption made above that the life-saving action of the spermatozoön is due to the fact that it carries a substance into the egg which renders the latter immune against the toxic action of oxidations.

III

Seven years ago I found that a number of agencies destroy the fertilized egg much more rapidly than the unfertilized egg. Thus, for instance, while in a pure sodium chloride solution the unfertilized egg of the Californian sea-urchin may be kept alive for several days, the fertilized egg is destroyed in such a solution in less than twenty-four hours. If we use slightly alkaline solutions of sodium chloride the greater resistance of the unfertilized egg is perhaps still more striking. The egg of Arbacia is cytolyzed in a neutral sodium chloride solution in a few hours, while the unfertilized egg may live for a considerably longer period of time. When we put fertilized eggs and unfertilized eggs into hypertonic solutions, we find also that the fertilized eggs suffer much more than the unfertilized eggs. What causes this difference of sensitiveness between fertilized eggs and unfertilized eggs? It is possible that the permeability of the fertilized eggs is greater than that of the unfertilized eggs. While this is probably to some extent true, yet it is not the whole explanation of the difference in the behavior of the two kinds of eggs. I have been able to show for a number

of toxic solutions that their effect can be either completely annihilated or at least diminished if we take the oxygen away from the solution. Thus, for instance, fertilized eggs of the sea-urchin which perish very rapidly in pure salt solutions, or a solution of sodium + calcium, or a solution of sodium + barium, can be kept alive for a considerable period of time in the same solutions if we either carefully remove the oxygen from the solutions, or if we diminish the rate of the oxidations in the eggs by adding a trace of sodium cyanide. In this case we have the direct proof that solutions which are fatal for the egg when the oxidations are allowed to go on are rendered completely, or at least partially, harmless if we stop the oxidations in the egg. Not only the toxic action of salt solutions upon the fertilized egg could be inhibited by the suppression of the oxidations in the egg, but also the toxic action of sugar solutions, or of solutions of alcohol in the sea-water, or of a solution of chloral hydrate.

These observations prove directly that in the presence of certain toxic substances or mixtures of substances the oxidations in the egg lead to its rapid destruction; while a suppression of the oxidation saves the life of the egg.

We, therefore, believe that we may conclude that the rapid death of the unfertilized egg of certain species is caused by the oxidations which take place in these eggs; and that the life-saving action of the spermatozoön consists in the fact that the latter, in addition to the membrane-forming substance, carries a second substance, or group of substances, into the egg which renders the egg immune against the harmful effect or consequences of oxidations.