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ON THE COUNTERACTION OF THE TOXIC EFFECT OF HYPERTONIC SOLUTIONS UPON THE FERTILIZED AND UNFERTILIZED EGG OF THE SEA-URCHIN BY LACK OF OXYGEN.

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During the progress of the experiments mentioned in the previous paper an unexpected and very striking result was obtained, namely, that the toxic effects of hypertonic sea-water are prevented, at least for a long time, if the oxygen is withdrawn from the solution. The toxic effects of hypertonic sea-water had already been observed by me in my earliest experiments on the effects of hypertonic sea-water upon the segmentation of the egg of the sea-urchin.[1] When the fertilized eggs were put into hypertonic sea-water of moderate concentration the eggs at first showed only a retardation or suppression of cellular division, but when they were left too long in such a solution they were killed. The time required for this latter toxic effect was the shorter, the higher the concentration. In my experiments on artificial parthenogenesis I also noticed that the eggs that had been exposed too long to the hypertonic sea-water perished either in the hypertonic sea-water or after they were transferred back to the normal sea-water. In this paper I intend to show that these results were due, primarily, to the presence of oxygen and that in the absence of oxygen these effects are avoided.

[1] Journal of Morphology, Vol. 7, p. 253, 1892.

Eggs were fertilized with sperm and put eleven minutes later into three flasks, each of which contained 100 c.c. of sea-water + 16 c.c. $2\frac{1}{2}$ m $CaCl_2$. One flask was in contact with air, while the other two flasks were connected with a hydrogen generator. The air was driven out from these two flasks before the beginning of the experiment. The eggs were transferred from one of these flasks after four hours and fourteen minutes, from the second flask after five hours and twenty-nine minutes into normal (aerated) sea-water. The eggs that had been in the hypertonic sea-water exposed to air were transferred simultaneously with the others into separate dishes with aerated normal sea-water. The result was most striking. Those eggs that had been in the hypertonic sea-water with air were all completely disintegrated in a way which I will, for the sake of briefness, designate as ''black cytolysis.'' Ten per cent. of the eggs had been transformed into ''shadows'' (white cytolysis). It goes without saying that the eggs that had been in the aerated hypertonic sea-water five and a half hours were also all dead. The eggs that had been in the same solution in the absence of oxygen appeared all normal when they were taken out of the solution, and three hours later—the temperature was only 15° C.—they were all, without exception, in a perfectly normal two- or four-cell stage. The further development was also perfectly normal. They swam as larvae at the surface of the vessel and went on the third day (at the right time) into a perfectly normal pluteus stage, after which their observation was discontinued. Of the eggs that had been five and a half hours in the hypertonic sea-water deprived of oxygen, about 90 per cent. segmented and developed in a perfectly normal way.

Not all the eggs were transferred from the hypertonic sea-water which was deprived of oxygen after four and a quarter hours. Some of the eggs were left in the flask after it had been disconnected from the hydrogen generator and had been exposed to the air. After certain intervals some eggs were transferred into normal sea-water. It was found that within less than two hours all the eggs perished in the hypertonic sea-water that now was in contact with air, while they had remained alive in the same hypertonic sea-water without oxygen. It seems therefore that it is the presence of oxygen which determines this toxicity of the hypertonic sea-water.

In this experiment the specific effects of an excess of Ca and the action of hypertonic sea-water were combined. A mixture 50 c.c. of sea-water + 8 c.c. 2½ n NaCl is less toxic than if the concentration is raised by the addition of $CaCl_2$. Eggs were fertilized with sperm and distributed into two dishes, each of which contained 50 c.c. sea-water + 8 c.c. 2½ n NaCl. One dish remained exposed to air, while the other was deprived of oxygen by means of a current of hydrogen. The temperature was 18° C. and the eggs remained four hours in these solutions. After this time they were transferred to normal (aerated) sea-water. Those eggs that had been in the hypertonic sea-water in the presence of oxygen perished during the next twenty-four or forty-eight hours, while those that had been in the hypertonic sea-water in the absence of oxygen were all alive after the second day and developed into entirely normal plutei. In another experiment fertilized eggs were left in 50 c.c. sea-water + 8 c.c. 2½ n NaCl seventeen hours. Of those that had been exposed for this length of time to the hypertonic sea-water in the absence of oxygen 50 per cent. developed into normal larvae that swam at the surface of the dish after they were taken out of the solution, while of those exposed to the hypertonic sea-water in the presence of oxygen 80 per cent. were dead at the time they were transferred, and of the remaining 20 per cent. only a few reached an early blastula stage. None reached a gastrula stage or rose to the surface of the water.

In another set of experiments the sea-water was made hypertonic by the addition of chemically pure cane sugar. Twenty-eight c.c. 2½ n cane sugar were added to 100 c.c. of sea-water and one dish with such a solution was left in contact with air, while two others were connected with a hydrogen generator and freed from air. Eggs of one female were fertilized with sperm and ten minutes later distributed into the three dishes. The temperature was 12° C. One lot remained in the solution two and three-quarter hours, the other eighteen and a half hours. Eighty per cent. of the eggs which had been in the hypertonic solution for two and three-quarter hours in the presence of air were dead after twelve hours and the eggs which developed were abnormal and died during the first day. The eggs which had been in the same hypertonic solution without oxygen for two and three-quarter hours were practically all alive and developed into larvae which rose to the surface but which—if I am not mistaken—looked like the larvae which develop in a mixture of sea-water and an isotonic solution of cane sugar, *e.g.*, 80 c.c. sea-water + 20 c.c. 5/8 m cane sugar. These larvae continued to develop. The eggs which had been in the aerated hypertonic sea-water for eighteen hours were practically all dead or died in the earliest stage of development, while about 50 per cent. of the eggs which had been in the same solution, but deprived of oxygen, developed into good blastulae and 30 per cent. into poor blastulae, while only 20 per cent. died during the first hour. The observation of these larvae was not continued.

These and similar experiments proved that the toxic effects of hypertonic sea-water can be prevented for some time by lack of oxygen. The next question which presented itself was whether the same is true if hypertonic solutions other than sea-water are used. I shall return to this subject in a subsequent paper and shall confine myself here to the publication of only a few experiments. The eggs of one female were fertilized and then distributed into two flasks, one of which contained 50 c.c. of a 5/8 m slightly alkaline $CaCl_2$ solution, while the other contained 50 c.c. m/2 $CaCl_2$. One series of these flasks had previously been freed from air and a current of hydrogen continued to pass through them. The eggs were left in these solutions one hundred and fifty minutes. The temperature was 15° C. The result was as striking as could be desired. The eggs which had been put in the 5/8 m $CaCl_2$ solution in an atmosphere of air were all dead when first examined after they were transferred to the normal sea-water. The eggs, however, which had been in the 5/8 m $CaCl_2$ solution in an atmosphere of hydrogen were practically all (95 per cent.) normal and began to segment normally and develop into normal plutei. Among the eggs which had been in a m/2 $CaCl_2$ solution in an atmosphere of air 60 per cent. were found dead immediately after they were transferred to normal sea-water and those which were alive segmented abnormally and died during the next twenty-four or thirty-six hours. Those that had been exposed to the m/2 $CaCl_2$ solution in an atmosphere of hydrogen were all alive and developed normally into perfect plutei. This experiment was often repeated with modifications, but with the same result. In order to show that mechanical agitation caused by the bubbling of the current of hydrogen has nothing to do with the result the following experiment may be quoted. The fertilized eggs of the same female were distributed into three flasks, each of which contained 50 c.c. m/2 $CaCl_2$. Care was taken not to bring more than a few drops of sea-water with the eggs into the solution. Through one of the flasks a current of carefully washed hydrogen flowed, through the second flask a current of oxygen was carried, while the third flask remained in contact with air. The temperature was 19° C. The eggs remained in this solution one hundred and ten minutes. At the end of this time they were transferred to normal sea-water. About 98 per cent. of the eggs which had been in the pure $CaCl_2$ solution in contact with air were found dead after the transformation into normal sea-water (black cytolysis). The remaining 1½ to 2 per cent. died during the next day. Those which had been in the $CaCl_2$ solution in the current of oxygen were a trifle better, but the result was practically the same. Those, however, which had been in the $CaCl_2$ solution in the current of H were practically all alive and segmented and developed in an entirely normal way into perfect plutei.

A series of experiments was made with hypertonic NaCl solutions.

The following mixtures of ¾ m solutions were tried:

1. 50 c.c. NaCl
2. 50 c.c. NaCl + 1 c.c. KCl
3. 50 c.c. NaCl + 1 c.c. KCl + 1 c.c. $CaCl_2$
4. 50 c.c. NaCl + 1 c.c. KCl + 1 c.c. $CaCl_2$+5 c.c. $MgCl_2$+0.7 c.c. Na_2SO_4.

Three sets of such solutions were prepared. One set was left in contact with air, while a current of oxygen was sent through a second set and a current of hydrogen through the third set. The eggs of one female were fertilized and ten minutes later distributed among these solutions, in which they remained two hundred and fifteen minutes. The temperature was 16° C. The eggs which had been in the hypertonic solutions in contact with air showed a great difference in their appearance according to the nature of the solution. Those which had been in the pure NaCl or the NaCl + KCl solution were all dead, while of the eggs which had been in the mixture of NaCl, KCl, and $CaCl_2$ about 50 per cent. were alive and began to segment, and of those eggs which had been in the fourth solution (in an atmosphere of air) a still larger number developed. But the segmentation of these eggs was abnormal and almost all died during the first twenty-four hours in an early blastula stage or in even earlier stages, and none reached the gastrula stage. The behavior of the eggs which had been in the same four solutions, but in the atmosphere of hydrogen, was in striking contrast to that of the eggs which had been in the same solutions in the presence of air and whose fate we just discussed. Of course, in the absence of oxygen no nuclear and no cell division is possible, but when the eggs were taken out of the solution they all looked perfectly normal and all segmented perfectly regularly, first into two, then in four cells, and so on. The majority developed into larvae which rose to the surface and reached a perfectly normal pluteus stage. The only difference between the eggs of this set which had been in solutions 1 and 2 and those in 3 and 4 was that a few of the former died, while practically all of the latter reached the pluteus stage, and that the larvae of the eggs, which had been in solutions 1 and 2 (in the atmosphere of hydrogen) had a tendency to stick together.

The eggs which had been in pure oxygen were, if anything, a trifle better than those which had been in contact with air. Those which had been in the pure NaCl solution in an atmosphere of O were all dead when taken out of the solution, while a number of those in the mixture of NaCl + KCl + $CaCl_2$ were still alive and began to develop. Some rose to the surface, but none reached the pluteus stage.

Two facts are noticeable in this experiment, namely, that hypertonic (¾ n) NaCl solutions lose their toxicity (at least for a certain time) when the eggs are kept in the solution in an atmosphere of hydrogen, and second, that, in the presence of air, or oxygen, the addition of 2 molecules of KCl and $CaCl_2$ to 100 molecules of NaCl makes the hypertonic NaCl solution much less toxic.

Those who have followed my papers on artificial parthenogenesis will remember that these experiments followed the discovery of the fact that certain animals (Fundulus) which can live in distilled water, and therefore do not depend upon any salts in the surrounding medium, will die rapidly when put into a pure m/2 NaCl solution, while they can live in this solution when KCl and $CaCl_2$ are added in the proportions mentioned above. May it not be possible that in the pure NaCl solution the oxidations (and possibly other catalytic processes) occur in the wrong direction, while the addition of KCl and $CaCl_2$ turns the oxidations (and other catalytic processes) into the right direction? Further experiments on this subject are in progress.

We have thus far discussed only the prevention of the toxic effects of hypertonic sea-water by lack of oxygen in fertilized eggs. We will now mention a few experiments which show that the same is true for unfertilized eggs with or without membranes.

Unfertilized eggs of the same female were put directly (without having been submitted to the process of membrane formation) into hypertonic sea-water (50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ n NaCl) and were left in the solution one hundred and fifty-three and two hundred and eighteen minutes. The temperature was 15° C. One lot was left in contact with air, while the other lot was in the hypertonic solution connected with a hydrogen generator and therefore deprived of oxygen. When the eggs were transferred to normal sea-water and examined soon afterwards it was found that those which had been in the hypertonic sea-water in the presence of air had completely disintegrated. It was impossible to find a whole egg; only tiny fragments were found. The eggs, however, that had been in the absence of oxygen in the same hypertonic sea-water for the same length of time appeared perfectly normal and could not be distinguished from eggs which had been left in normal sea-water. Some of these eggs showed later a slight tendency to segment, but no egg went beyond the two-cell stage. When they were fertilized the following morning with sperm many of them developed normally.

The following experiment will show that unfertilized eggs in which the membrane formation was caused by butyric acid can resist the effect of hypertonic sea-water considerably longer in an atmosphere of hydrogen than in air. Such eggs were distributed into several flasks with hypertonic sea-water, some of which were in contact with air, while others were in an atmosphere of hydrogen. The temperature was 15° C. They remained in the hypertonic sea-water one hundred and forty-two minutes. The eggs that had been in the hypertonic sea-water in contact with air segmented and developed abnormally and none reached the pluteus stage; the majority died in the blastula stage. Those that had been in the same solution in an atmosphere of hydrogen developed normally and a large percentage reached the pluteus stage after they had been exposed about thirty minutes more to hypertonic sea-water in an atmosphere of air.

All these experiments show that the toxic effects of sea-water which is rendered hypertonic through the addition of NaCl, $CaCl_2$ or cane sugar, as well as the toxic effects of hypertonic solutions of NaCl and $CaCl_2$, upon fertilized and unfertilized eggs of the sea-urchin can be prevented for some time, at least, by lack of oxygen. This fact harmonizes with the idea that these hypertonic solutions accelerate or otherwise modify the phenomena of oxidation (and possibly other catalytic processes) and that their destructive effect, as far as described in this paper, is due to this influence. This lends further support to the idea expressed in the preceding paper that the hypertonic sea-water causes artificial parthenogenesis by accelerating in the resting egg the process of oxidation, which is identical or intimately connected with the synthesis of nuclein from protoplasmic material. The discussion of the way by which the various salts or hypertonic sea-water modify phenomena of oxidation shall be left to a later paper.