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THE TOXICITY OF ATMOSPHERIC OXYGEN FOR THE EGGS OF THE SEA-URCHIN (*STRONGYLOCENTROTUS PURPURATUS*) AFTER THE PROCESS OF MEMBRANE FORMATION.

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In a former paper I have stated that the process of artificial membrane formation will produce two kinds of effects in the egg of the sea-urchin, namely: it will cause the phenomena of nuclear division to begin, and second, it will cause the egg to disintegrate within a limited number of hours, unless it is treated previously or subsequently with hypertonic sea-water (or in some other way, as we shall see later). The process of nuclear and cell division may go on in such eggs until they reach the six- or eight-cell stage; as a rule, however, the eggs only form the first spindle and then the process of disintegration begins. Since the eggs in which no membrane formation has been produced may live for a week or more it seems as if the chemical process connected with the nuclear and cell division might be responsible for the process of disintegration. I showed ten years ago that the process of nuclear and cell division in the egg of the sea-urchin (and probably generally) depends upon the presence of free oxygen, and stops as soon as the oxygen is replaced by hydrogen[1] or as soon as the oxidations are stopped by potassium cyanide. It is therefore obvious that the processes underlying

[1] Loeb: Pflüger's Archiv., Vol. 62, p. 249, 1895.

the development of the egg, especially underlying nuclear and cell division, are processes of oxidation. Later I have pointed out the possibility that these oxidative processes are identical or connected with the synthesis of protoplasmic material into nuclear compounds.[1] Since the process of membrane formation in the egg of the sea-urchin starts these oxidative processes it occurred to me to try whether it was not possible to prevent the premature death of such eggs by preventing oxidations. One method used to accomplish this consisted in submitting them to sea-water through which a current of hydrogen was carried and from which in this way the air was removed; the second method consisted in stopping oxidations in the eggs by adding KCN to the sea-water. It was found that in such cases the death of the eggs with a membrane could indeed be prevented. A few illustrations of these experiments may be quoted. The hydrogen was prepared from zinc and sulphuric acid and was washed carefully in two bottles with a saturated solution of Na_2CO_3, in a bottle with a solution of potassium permanganate and a flask with sea-water. The latter was made slightly alkaline and colored red by a few drops of phenolphthalein in order to indicate whether or not the hydrogen was really free from acid. Only such experiments were used in which no noticeable change of color occurred in this sea-water during the experiment.

In the eggs of one female the process of membrane formation was produced by submitting them for one and a half to two minutes to a mixture of 50 c.c. sea-water and 2.9 c.c. n/10 butyric acid. The eggs were ten minutes later distributed into two lots. One was put into a flask with sea-water through which pure (medicinal) oxygen was driven; the other was put into a flask with sea-water from which the air had been previously removed and through which a weak current of hydrogen continued to bubble. After twenty-three hours the eggs which had been left in contact with air or in the oxygen apparatus were disintegrated beyond recognition. When the eggs were taken after twenty-three hours from the hydrogen apparatus they had the same normal appearance which they had when first put into the apparatus. No nuclear division could occur, of course, on account of

[1] This Series, Vol. 2, p. 147, and Vol. III, p. 1, 1905.

the lack of oxygen and it seemed therefore as if the lack of oxidations preserved the life of these eggs. But in order to convince myself that they were alive not only in appearance but in reality I put them after they came from the current of hydrogen into hypertonic sea-water (50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ n NaCl). Portions of the eggs were taken out from this solution after thirty, forty-three, fifty-five, sixty-five, and eighty minutes. A number of the eggs taken out after forty-three and fifty-five minutes and a few of those taken out after sixty-five minutes developed into perfect plutei.

In another experiment the eggs of a female were submitted to the process of membrane formation and distributed in three sets of flasks, one in contact with air, a second through which a current of oxygen was carried, and a third through which a current of hydrogen was carried. Eggs were taken out at various intervals varying from one to ten hours. The eggs that had been exposed to air or pure oxygen all began to show the spindle after the proper time and began to disintegrate in from four to six hours. Those taken after ten hours from the hydrogen current appeared normal and could be caused to develop. The experiments were repeated a number of times with the result mentioned here, but the monotony of the experiments was broken by the additional observation that the eggs which had been exposed after the membrane formation to a current of hydrogen for a sufficiently long time could undergo a perfectly normal development without any further treatment with hypertonic sea-water. The number of eggs which developed under such conditions was very small indeed, never more than 1 per cent.—while the treatment with hypertonic sea-water caused 50 to 100 per cent. of the same eggs to develop—but the control experiments were such as to exclude all possible error. I tried to ascertain the minimal time during which the eggs with a membrane had to remain in the current of hydrogen in order to undergo such a development and found that the minimum was 160 minutes at 15° C.

I then tried whether the same results could be obtained by the addition of KCN to sea-water. The process of membrane formation was caused by butyric acid and the eggs were five minutes later put into 50 c.c. of sea-water to which 1 c.c. of a presumably 1 per cent. solution of KCN was added. Some eggs were taken from this solution after fifteen, thirty, forty-five, sixty-five and eighty-five minutes. In those eggs which had been exposed to the KCN forty-five and sixty-five minutes a number of eggs developed. This development did not begin until about fourteen hours after the eggs had been taken from the KCN sea-water and only those eggs developed which were put into a shallow watch-glass, while the eggs kept in a finger bowl where they were covered with a thicker layer of water perished through KCN poisoning. Those eggs which did develop developed very slowly indeed. In another experiment 1, 2, 4 and 8 c.c. of a 1/10 per cent. KCN solution was added to 50 c.c. of sea-water, and the eggs in which a membrane had been produced artificially were left in this sea-water from one to twenty-three hours. A small percentage of good larvae was obtained after an exposure of from three to seven hours to 50 c.c. sea-water + 2 or 4 c.c. 1/10 per cent. KCN solution. The segmentation did not begin until about fifteen hours after the eggs were taken out of the poisoned sea-water. It is therefore obvious that the eggs remain for a considerable time under the influence of the KCN. Other experiments gave similar results.

It must be remembered that while the eggs were deprived of oxygen, or under the influence of KCN, catalytic processes or chemical reactions other than oxidations were not excluded and in all probability continued in the eggs.

These experiments support the idea that the premature death of the eggs after membrane formation is connected with the processes of development started by the membrane formation, and since these processes are oxidative in character it is comprehensible that an exclusion of oxygen or of the oxidative processes by KCN prevents the disintegration after the process of membrane formation.

But how was it possible that a prolonged exclusion of oxygen could act, although in a much less degree, like the treatment of the eggs with the hypertonic sea-water? Three years ago E. P. Lyon[1] published an excellent paper on the artificial parthenogenesis of the eggs of sea-urchins at Naples in which he

[1] E. P. Lyon: Am. Journal of Physiology, Vol. 9, p. 308, 1903.

stated that in four experiments he had observed that unfertilized eggs of a sea-urchin when put for twenty-four hours into a weak solution of KCN in sea-water could afterwards develop. The number of eggs which developed was always very small and it seems that the method was not always reliable. At that time the relation of the process of membrane formation to artificial parthenogenesis was not known, and Lyon used eggs without membranes. But his experiments seem to prove that lack of oxygen if continued long enough at a sufficiently high temperature can induce the process of development even without a membrane formation. Lyon suggests an idea which I had expressed in connection with experiments on the transformation of negative into positive heliotropism in copepods, namely, that hypertonic sea-water might act by causing a lack of oxygen in the cells submitted to it. We shall see in the next paper that this conclusion is not correct, although it is a fact that a condition of lack of oxygen if prolonged sufficiently may act like hypertonic seawater.

We shall find the explanation for this fact in the next paper. In my experiments on heliotropism I found also that by lowering the temperature the same effects could be produced as with lack of oxygen. I tried therefore whether eggs after the membrane formation would live longer if kept at a lower temperature. This is undoubtedly the case, and, moreover, eggs which were kept after the membrane formation at a temperature below 10° C. as a rule went through a series of segmentations, while those at a higher temperature did not.