A MICRO INJECTION STUDY ON THE PERMEABILITY OF THE STARFISH EGG.

By ROBERT CHAMBERS.

(From the Eli Lilly Research Division, Marine Biological Laboratory, Woods Hole.)

(Received for publication, September 27, 1922.)

It is well known that selective permeability, or semipermeability, is one of the essential characteristics of the living cell. So far, however, there is no evidence as to whether the semipermeability of protoplasm is a property of its entire mass or of its surface only.

Apparently the only means by which the action of substances on the interior alone of protoplasm may be studied is by injection. Animal cells can be injected by using the very fine glass pipettes and the mercury injection method which Barber devised for bacteriological work. I have used this method. The pipettes, both as regards their size and the ease of making, leave nothing to be desired. The method, however, is not only very difficult, but is unsatisfactory, owing to the fact that the pressure required for injection depends upon the expansion of mercury by heat, and this cannot be instantly controlled. Kite[1] tried it, but substituted for most purposes the far cruder method of blowing into his pipettes through a rubber tube. This operation necessitates larger pipettes than can be properly used for cell injection. The erroneous conclusions arrived at by Kite were due not only to the difficulty of the procedure, but mainly to the extraordinary ability of protoplasm to form films over torn surfaces. Pushing a pipette, especially a comparatively large one, into an egg cell frequently causes the surface of the cell to become invaginated and thus forms a deep pocket. The tip of the pipette, even if it should finally break through the surface, is apt to be separated from the protoplasm of the interior by the formation of a new surface film continuous with the original surface of the cell. Kite apparently did not guard against this contingency, and his experi-

[1] Kite, G. L., *Biol. Bull.*, 1913, xxv, 1.

mental results indicate that his solutions never actually entered the protoplasm of the cell. The injected fluid simply seems to have filled the bottom of a pocket and then flowed down the side of the pipette to the exterior. When Kite says that he found no difference in permeability, whether a substance be applied to the surface of a cell or to any spot in its interior, he was perfectly correct. The spot in the interior was an infolding from the surface of the cell. At best, he was only comparing the permeability of the original surface of the cell with that of a newly formed surface film which surrounded the tip of his pipette.

I have recently succeeded[2] in devising a simple but efficient piece of apparatus with which one can accurately and easily control the injection of fluids through a micro pipette having an aperture of less than 1 micron in diameter. The pipette, when properly made, tapers rapidly to a tip with a sharp cutting edge. The apparatus is so constructed that the pipette can be quickly changed. By keeping in mind the ease with which protoplasmic surface films are formed, one can, with this method, readily and accurately inject fluids directly into the interior of the protoplasm of a cell.

This summer Jacobs kindly set at my disposal a manuscript which the reader will find in this number of this Journal, in which are described the interesting results that were obtained by immersing neutral red vitally stained starfish eggs in ammonium chloride, and in sodium bicarbonate solutions. Jacobs found that a 1/2 M NH_4Cl solution, which is sufficiently acid to redden neutral red, will cause the neutral red within the eggs to turn yellow, indicating the entrance into the eggs of NH_3 and not of HCl. He also found that neutral red stained eggs will turn a deeper red when immersed in an alkaline solution of 1/2 M $NaHCO_3$ charged with CO_2, indicating the entrance into the eggs of CO_2 and not of NaOH.

Jacobs' results confirm those of Loeb,[3] Bethe,[4] Warburg,[5] Harvey,[6]

[2] Chambers, R., *Anat. Rec.*, 1922, xxiv, 1.

[3] Loeb, J., *Biochem. Z.*, 1909, xv, 254.

[4] Bethe, A., *Arch., ges. Physiol.*, 1909, cxxvii, 261.

[5] Warburg, O., *Z. Physiol. Chem.*, 1910, lxvi, 305.

[6] Harvey, E. N., *J. Exp. Zool.*, 1911, x, 507; *Internat. Z. physik. Chem. u. Biol.*, 1914, i, 463.

and Crozier[7] that weak acids and bases freely penetrate living cells, whereas strong acids and bases do not. This is presumably because of their solubility in the organic solvents (lipoids) of the protoplasmic surface layer.

I have injected the solutions which Jacobs used into starfish eggs vitally stained with neutral red, and obtained decided and consistent results, which show that HCl and NaOH will permeate protoplasm freely as long as there is no protoplasmic film to serve as a barrier. The semipermeability of protoplasm, in all probability, depends upon the surface film having properties different from those of the continuous internal protoplasm.

EXPERIMENTS.

Mature starfish (*Asterias forbesii*) eggs were vitally stained with neutral red. They were then placed in a hanging drop in Barber's moist chamber, and those eggs selected which showed a neutral tint of an orange-red hue. All the experimental work was done under a Leitz $\frac{1}{7a}$ oil immersion objective and ocular 15. This objective gives a remarkably long working distance together with a sharp definition that allows of the use of high powered oculars.

Treatment with 1/2 M NH_4Cl.

Eggs were placed in a hanging drop of 1/2 M NH_4Cl in which, as Jacobs has shown, their vitality remains unimpaired for a period of 10 to 15 minutes. With a microdissection needle deep cuts were made in the eggs. The cut surfaces were immediately bounded by surface films continuous with the surface of the egg, and no injurious effect of the surrounding medium was noticeable. During this time, the neutral red within the egg, gradually turned yellow.

This experiment indicates that the NH_4Cl does not prevent the formation of films over the cut surfaces of the egg, and also that the solution will not, within the time limits of the experiment, penetrate those films.

The interior of stained eggs was made to flow out in a drop of 1/2 M NH_4Cl by the following means. The egg was torn at one spot on its surface and then caught on the other side and pulled to the edge of the drop. In every case the rapidly outflowing interior turned rose-red upon coming into contact with the surrounding solution and cytolyzed into a frothy semisolid mass. The change from an orange color to a red, with an accompanying cytolysis, extended from the out-

[7] Crozier, W. J., *J. Biol. Chem.*, 1916, xxiv, 255.

flowing area into the egg itself, and spread to the original cortex until the entire egg was cytolyzed.

This experiment shows that if the egg be torn in such a way as to cause its interior to flow out rapidly, no surface film forms. The NH_4Cl at once penetrates the protoplasm which undergoes the characteristic color change and cytolysis.

Stained eggs were placed in a hanging drop of sea water and 1/2 M NH_4Cl injected under the egg membrane. This is fairly easy to do in the unfertilized egg but more so in the fertilized egg in which the membrane has already lifted off as the so called fertilization membrane. In the unfertilized egg, the injected solution at first bulges the membrane giving rise to a localized blister, and then usually spreads quickly over the egg, lifting the membrane from its entire surface. The permeability of the egg to the NH_4Cl is not affected by this treatment. This demonstrates that it is not the egg membrane which protects the egg from the NH_4Cl, but the actual surface film of the protoplasm lying under the membrane.

Stained eggs in a hanging drop of sea water were punctured with a glass pipette having an aperture of 1 micron in diameter, and a minute quantity of 1/2 M NH_4Cl injected directly into the interior of the egg. The injected area immediately changed from an orange to a rose-red color, and then underwent cytolysis. The color change and accompanying cytolysis spread from the injected area. In some cases this spread was arrested by the formation of a surface film which converted the injected and disintegrated area into a vacuole. In other cases the cytolysis spread till it reached the cortex which disintegrated from within outward.

This experiment demonstrates that 1/2 M NH_4Cl, which causes an alkaline color change within eggs immersed in it, will, when injected into the interior of the eggs, produce the acid color change and accompanying cytolysis which characterizes the presence of HCl.

Treatment with 1/2 M $NaHCO_3$ + CO_2.

Stained eggs were cut and torn in a hanging drop of the $NaHCO_3$ solution. In contrast to the reaction in the presence of NH_4Cl there was no tendency for the formation of surface films over their cut surfaces. The protoplasm simply flowed out and was dispersed in the solution, the color changing meanwhile from red to yellow.

Injection of $NaHCO_3$ beneath the egg membrane of eggs in sea water had no other effect than that produced upon eggs by immersing them in the solution; *viz.*, deepening of the red color in the egg owing to the selective penetration of CO_2.

Stained eggs were injected with the $NaHCO_3$ solution. The injected area immediately turned yellow, and cytolysis with liquefaction took place. No surface film formed about the cytolyzing area, and the yellow color spread throughout.

CONCLUSION.

The experiments with the NH_4Cl are similar to, and corroborate micro injection experiments performed in connection with some work on mustard gas in which the writer[8] collaborated. Eggs immersed in sea water containing decomposed mustard gas, at a certain low concentration are not affected. If, however, the solution be injected, the egg quickly cytolyzes owing to the free HCl present.

A similar impermeability of the protoplasmic surface film to certain substances was also encountered in injection work on *Amœba*.[9] *Amœbæ* immersed in an aqueous solution of eosin will not take the stain till after death. On the other hand, the eosin, when injected into the *Amœba*, quickly permeates the protoplasm, to be arrested only at the surface.

The semipermeability of a living cell appears primarily to be a function of its surface film. It is immaterial whether this film be that of the original cortex of the cell, a film newly formed over a cut surface, or a film that surrounds an artificially induced vacuole within the cell. As long as such a surface film exists neither the acid group of the NH_4Cl nor the alkaline group of the $NaHCO_3$ can, within certain concentration limits, penetrate the protoplasm. These solutions, if injected beneath the surface film, however, will produce their characteristic effects upon the protoplasm.

[8] Lillie, R. S., Clowes, G. H. A., and Chambers, R., *J. Pharmacol. and Exp. Therap.*, 1919–20, xiv, 75.

[9] Chambers, R., *Proc. Soc. Exp. Biol. and Med.*, 1920–21, xviii, 66.