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Determinations of the pH of Developing Fundulus Eggs.

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By the use of a micro-pipette, solutions of Clark and Lubs' indicator dyes were injected into the subchorionic space, the pericardial cavity, and the yolk of developing Fundulus eggs, 4 to 6 days after fertilization, and the colorimetric pH values of the contents were determined. A few injections were also made into the brain vesicles. Single injections were made in most of the eggs. In several cases an injection was made into the subchorionic space, then the pipette was carried into the pericardial cavity, and another injection made in order to detect simultaneously any possible difference in the pH of different spaces in the same egg. The color tints of the indicators were always determined at the horizon of the egg in order to eliminate interference from the yellowish color of the yolk.

The color of the subchorionic space indicated a pH identical with that of sea-water. The critical indicators used were phenol red and cresol red, but the indicators above and below the ranges of these two were also injected. If the developing eggs were placed in distilled water for 18 hours and then injected, the pH of the subchorionic space was found to be that of the surrounding medium, *viz.*, 5.6 ± 1. Controls in sea-water exhibited a subchorionic pH of sea-water. There was no leakage around the pipette at the point of puncture, because the outward diffusion of the injected dye occurred only through the membrane as a whole, and not around the pipette at the site of the puncture.

In his experiments on the permeability of developing Fundulus eggs to acid, Loeb[1] stressed the importance of placing the eggs in distilled water for 24 hours previous to the experiment in order to rid the membrane of adhering electrolytes. He found that more consistent results were obtained in the experiments performed on material so treated but, as pointed out above, such treatment changes the pH of the subchorionic space, a possibility he did not suggest.

The pH of the pericardial cavity was also found to approach that of sea-water. However, the pericardial cavity differed from the subchorionic space in that its pH did not change in eggs immersed in distilled water, nor did the dye diffuse out of the embryo. The

tissue at the immediate site of puncture became yellow almost immediately when the phenol red or cresol red was injected, which was probably due to the acidity of injury, the importance of which has been stressed by Needham and Needham[2] and by Chambers and Pollock[3] in pH determinations on living tissues. There was, however, no outward leakage of the dye at the site of puncture. The few injections into the brain vesicles indicated that it had the same pH as that of the pericardial cavity. The pH of the yolk differed from that of the pericardial cavity and brain vesicles, as it was distinctly acid to cresol red and phenol red.

By the use of a micro hydrogen electrode Bodine[4] found the mean average internal pH value of the developing Fundulus egg to be 6.39. He did not, however, discuss the possibility of variations of pH between the different structures and cavities within the developing egg. His average probably represents some one constant sample which he always obtained by his technique, possibly yolk mixed with some subchorionic fluid which would be obtained in his procedure. In a differentiating organism it would seem preferable to study the pH of the several differentiated structures, rather than to attempt the average which would necessitate maceration with a resulting acidity of injury.

Since the subchorionic space changes so readily in pH with changes in pH of the environment but without change within the embryo, it is suggested that it is not at the chorionic membrane that the acid-salt antagonism takes place, as Loeb thought. Experiments have already been conducted on a large number of developing embryos from which the egg membrane has been removed, and it has been found that the cellular wall of the embryo and not the chorionic membrane is the site of the acid-salt antagonism.

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[1] Loeb, J., *J. Gen. Physiol.*, 1922-23, v, 231.

[2] Needham, J., and Needham, D. M., *Proc. Roy. Soc. London*, Series B, xcix, 173.

[3] Chambers, R., and Pollock, H., *J. Gen. Physiol.*, 1926-27, x, 739.

[4] Bodine, J. H., *J. Gen. Physiol.*, 1926-27, x, 533.