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On the isolation of oöcytase, the fertilizing and cytolyzing substance in mammalian blood-sera. (Preliminary report.)

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It has been observed by Loeb that the eggs of sea-urchins (*Strongylocentrotus purpuratus*) may be induced to form fertilization-membranes by immersing them in mammalian blood-sera which have been rendered isotonic with sea-water by the addition of NaCl. Occasionally, if the serum be especially potent, simple immersion of the eggs in the serum suffices to bring about this result, but as a rule previous treatment of the eggs with a sensitizing agent ($SrCl_2$ or $CaCl_2$) is required. Prolonged action of the serum upon sensitized eggs results in the cytolysis of the eggs. Very potent sera usually cause agglutination of the eggs as well, especially of sensitized eggs.

I have isolated from ox-serum a fraction which is extremely potent in fertilizing, cytolyzing, and agglutinating sea-urchin eggs.

To 860 c.c. of fresh, whipped, and centrifugalized ox-serum, which had been rendered isotonic with sea-water by the addition of $2\frac{1}{2}M$ NaCl solution, I added 400 c.c. of 7 per cent. $BaCl_2$. A thick cloud was produced. This mixture after standing for an hour in a warm place until its temperature rose to 37° C. was centrifugalized. The entire precipitate, consisting of $BaCO_3$, $BaSO_4$, and the barium compound of the fertilizing agent and, possibly, of other substances, settled in the form of a cake at the bottom of the centrifuge-tubes. This precipitate was thoroughly drained and then suspended several times in 2 per cent. $BaCl_2$ and re-centrifuged in order to free it from adherent serum. It was then stirred up for an hour in 100 c.c. of $N/10$ HCl and the insoluble residue (probably barium sulphate) was centrifuged out. To the clear fluid thus obtained were added 10 c.c. of 10 per cent. Na_2SO_4 in order to free it from barium. This mixture was allowed to stand for some hours at 50° and then centrifuged. The clear

yellowish fluid thus obtained yielded no precipitate or opalescence upon the further addition of Na_2SO_4 and was therefore free from barium. To this fluid were added 4 volumes of acetone. A light flocculent precipitate was formed at once which settled readily. This was collected upon a hardened filter, washed in alcohol and ether, and dried for 3½ days over H_2SO_4 at 36° C.

The substance thus obtained does not dissolve in sea-water. It dissolves readily in *N*/10 HCl and remains in solution upon neutralization. To this solution sufficient *2½M* NaCl was added to render it isotonic with sea-water and the solution (0.5 per cent.) thus obtained was diluted to 1, ½, . . . , etc., by the addition of sea water. On adding sea water, the mixture becomes very opalescent but the substance is not precipitated.

Eggs of *Strongylocentrotus purpuratus* which have not undergone previous sensitization are fertilized and agglutinated by solutions of this substance in dilutions of from 1 part in 200 to 1 part in 800. Eggs which have been sensitized by immersion for 4 minutes in *M*/2 $CaCl_2$ are fertilized and agglutinated by dilutions of from 1 part in 200 to 1 part in 1,600. Eggs which have been sensitized by immersion for 4 minutes in *M*/2 $SrCl_2$ are agglutinated by dilutions of from 1 part in 200 to 1 part in 25,000. The sensitizing action of $SrCl_2$ and $CaCl_2$ is clearly seen to reside in their power to precipitate the fertilizing agent upon the egg.

Since this substance is thermostable, withstanding 18 hours' exposure to a temperature of 50° without destruction, and there is some reason for suspecting that it is not present as such in circulating blood but is discharged from white corpuscles in shed blood, it would appear to present many analogies to the cytases or cell-liquefying substances found by Metchnikoff in white corpuscles. Accordingly, I propose that it be called "Oöcytase."