

pendent of the daily output of this product, *i. e.*, of the amount of work these cells do.

Further investigation of this phenomenon is already under way.

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Agglutination of "Normal" Germ Cells.

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It is widely believed that freshly shed germ cells are relatively constant. In previous studies^{1, 2, 3, 4} an extremely wide variation in many characters and characteristics was noted. In the present study agglutination of sperm by egg water, which agglutination lends itself to fairly exact quantitative determination, was studied under very precise experimental conditions.

The sea urchin (*Arbacia punctulata*) was used. These were freshly collected, opened immediately upon arrival at the laboratory, the "best" egg lots selected and immediately tested for the duration of agglutination. Duplicate tests and tests of aliquot divisions of the eggs gave a difference of 0 to 4 seconds, averaging 1 second or 4.5%. This was considered the experimental error.

Thirty series of 3 to 4 females each were studied. When eggs from each female in a series were tested separately, under strictly comparable conditions (including the same sperm suspension), the agglutination time varied very extensively, namely, from 2 to 55 seconds, or 9 to 1300%, with an average of 12 seconds or 142%.

Eggs that gave high agglutination values with sperm from one individual gave consistently high, though not the same values, with sperm from other individuals. Similarly eggs that gave intermediate or low values with one male, gave intermediate or low values with other males.

This wide variation and increase in agglutination time parallels corresponding wide changes in size, color, shape of eggs, loss of jelly, membrane formation, cleavage, etc.^{1, 2, 3, 4} The degree of change in any one of these traits measures the extent of overripening of the eggs prior to shedding.

¹ Goldforb, A. J., *Proc. Nat. Acad. Sci.*, 1917, iii.

² Goldforb, A. J., *Carnegie Inst. Publication* 251, 1917.

³ Goldforb, A. J., *Biol. Bull.*, 1918, xxxiv.

⁴ Goldforb, A. J., *Biol. Bull.*, 1918, xxxv.

It was also noted that severe storms that delay natural shedding, also higher temperature of the sea water, give rise to overripening of the eggs within the body of the sea urchin, with all the symptoms above enumerated.

Not only were the eggs of different individuals in widely different stages of overripeness, even when freshly shed, but sperm from different males freshly collected, immediately tested, in the same concentration of sperm suspension with the same egg water, gave widely different agglutination values, namely, 11 to 3300%. This wide range in agglutinability of different sperms is interpreted to indicate corresponding differences in overripeness prior to shedding.

Sperm which gave high agglutination values with one female, gave high, though not the same, values with other females, and *vice versa*, sperm which gave intermediate or low values with one female gave correspondingly intermediate or low values with other females.

This extremely large variation in freshly shed germ cells may in very small part be due to genetic differences. But the major differences appear to be due to corresponding degrees of overripeness prior to shedding.

"Normal", *i. e.*, chronologically freshly shed germ cells may be physiologically fresh eggs, or they may be in nearly all stages of physiological deterioration or overripeness. As the subsequent behavior of the fertilized eggs depends upon the physiologic condition of the germ cells at the time of fertilization, it is therefore necessary, in experimental work, to determine by the agglutination or other tests their exact physiologic condition prior to experimentation.

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Agglutination Changes in Ageing Germ Cells.

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A. When freshly shed eggs of *Arbacia* were physiologically ripe (not overripe) and then aged in sea water at 20° C. there occurred a profound series of changes in agglutination. Three phases may be readily distinguished. In the first phase there was a progressive and marked *increase* in agglutination, reaching a maximum of about 200% in 3 to 5 hours. This increase is due to a corresponding increase in liberation of agglutinin. In the second phase there was a