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

progressive decrease in agglutination, namely, from about 5 hours to 24 hours. This decrease is associated with a decrease in agglutinin. In the third phase there was continued decrease in agglutination, due to liberation of anti-agglutinin.

When eggs were overripe at the time of shedding, corresponding portions of the first or even second phase did not occur. These phases took place prior to shedding.

Overripening gave rise to the same kind and the same intensity of changes whether within or without the body.

B. When freshly shed sperm from different freshly collected males were aged at 20° C. and tested at each age under strictly comparable conditions, by either the same egg water or by freshly shed ripe eggs, agglutination was widely different. There were 3 phases. In the first there was practically no change, *i. e.,* from 0 to about 3 hours. In the second, agglutination *increased* progressively and markedly, from the third to the twenty-fourth hour. The average increase was 120%. In the third phase there was a rapid and progressive decrease in agglutination.

When the freshly shed sperm were overripe, or were precociously overripened by high temperature, corresponding portions of the first or second phase were not evidenced.

C. When the freshly shed eggs and sperm were not overripe, and the same germ cells tested at successive ages, under strictly comparable conditions, there occurred the same cyclical *increase,* then decrease in agglutination. The increase, however, was greater than when eggs alone or sperm alone were aged.

When the freshly shed eggs or sperm or both were overripe changes like those above mentioned occurred, namely, absence of corresponding portions of the first or second phases in the agglutination cycle.

D. Eggs undergo with age a cyclical change in agglutinin. Sperm undergo a corresponding cyclical change. The two cycles are not synchronous. The agglutination at any time depends upon the physiologic condition of the eggs and of the sperm at that time, and is the sum of the effects produced by the eggs and the sperm.

The agglutination phenomenon is conditioned by 4 major factors, viz., (1) degree of overripeness of eggs and of sperm when shed. (2) time or age. (3) condition of ageing, temperature, H ion concentration, etc. (4) different rates of change in ageing eggs, and sperm.

E. Sperm are not a fixed entity reacting in the same way with same intensity to a given dose of agglutinin. Sperm is a varying

entity either secreting, with age, varying amounts of a substance or substances which activate agglutination, or, sperm undergo a cyclical change or changes as a result of which they become increasingly reactive to a given dose of agglutinin.

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The Factors that Modify Agglutination of Ageing Sperm.

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The previous studies demonstrated that ageing "dry" or concentrated sperm gave rise to a marked increase, then decrease in agglutination, provided, however, the sperm were not overripe when shed. In the present study, the sperm were overripened in a standard 1% suspension at 22° C. and tested at each age by samples of the same egg water. There was the same, but quicker, cyclical change. The first phase began in 5 to 20 minutes after the initial test. The second phase ended in 15 to 125 minutes. The third phase ended in 260 minutes. The increase in agglutination was from 77 to 328%.

The greater the overripeness of the dry sperm when shed, the earlier the maximum values, and the sooner was the cycle ended. When sperm was not overripe when shed, subsequent ageing gave rise to very little or no increased phase, followed by a decreasing phase.

The experiments were so devised as to eliminate such factors as a change in agglutinin, change in jelly content, change in temperature, or H ion concentration of the medium.

To determine whether the increase in agglutination was due to a substance or substances liberated by the sperm, increasing concentrations of sperm were tested with the same egg water solutions. The concentrations of sperm ranged from 1% to 25%. More concentrated suspensions could not be used, on account of the difficulty in distinguishing the agglutinated clusters in the thick medium. Sufficient time was allowed for the substance if present to be liberated. There was no change in agglutination values with the marked increase in concentration of sperm whether ripe or overripe.

The CO_2 liberated by the eggs and the active sperm plays a large rôle in decreasing the activity of the sperm, in clumping the sperm into aggregations, but plays no rôle in increasing the agglutination of ageing sperm.