# THE RELATION BETWEEN CLEAVAGE AND TOTAL ACTIVATION IN ARTIFICIALLY ACTIVATED EGGS OF *URECHIS*

ALBERT TYLER

(*From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California and the William G. Kerckhoff Marine Laboratory, Corona del Mar, California*)

It is generally assumed in most work on artificial parthenogenesis that cleavage and development result when the initial response of the egg to the artificial agent most closely resembles its response to the sperm. The percentage of eggs that respond in this fashion varies, of course, with the length of exposure to the artificial agent, presumably reaching a maximum for the exposure producing the highest percentage of activation.[1]

It would follow then that the cleavage-activation relation should be such that as the percentage of activation increases, the percentage of cleavage increases; that is, that the percentage of cleavage is directly proportional to the percentage of activation. Although this relation is practically always tacitly assumed in parthenogenesis experiments, detailed data on this point are lacking. If, however, exposures giving higher percentages of activation do not produce increasing percentages of eggs whose response is most nearly like that induced by the sperm, or if such eggs were not the ones which cleave and develop, an entirely different cleavage-activation relation might be expected. The determination of this relation is important, then, in an analysis of the factors which determine whether or not an artificially activated egg will cleave.

The variation of the percentage of activation with the length of exposure to the artificial agent is in itself a highly interesting fact, since it is not manifested in insemination of a normal batch of eggs with normal sperm. This variation may be attributed to variability in the amount of treatment necessary to activate a given egg, or, less likely perhaps, to a variation in the time at which the change produced by the activating agent reaches a given egg. Whatever its source, the way in which the percentage of activation varies with the length of exposure is useful in helping to elucidate the mechanism by which the artificial agent activates the egg.

[1] Any egg in which initial developmental changes have taken place will be termed "activated" in this paper, regardless of maturation or cleavage.

In the parthenogenesis experiments on *Urechis* eggs a unique relation between cleavage and activation was found, such that as the percentage of activation increases, the percentage of cleavage decreases. The variation of percentage activation with length of exposure was found to give a particular type of distribution curve in certain of the experiments. These results together with their interpretation are presented in detail in this report.

## Material and Method

The eggs used in these experiments were those of the echiuroid, *Urechis caupo*, described by Fisher and MacGinitie (1928). The changes undergone by the egg upon normal fertilization, and upon artificial activation, and the method used in activating the eggs were described in detail in a previous publication (Tyler, 1931). Briefly, it was found that dilutions of sea water ranging from 80 per cent to distilled water were effective in activating the *Urechis* eggs.[2] In order to treat the eggs, a batch was transferred with as little sea water as possible to a Stender dish containing a large volume of the hypotonic solution. Samples were then removed after various intervals of time to Syracuse dishes containing normal sea water. All the usual precautions in regard to contamination by sperm or foreign matter, hypertonicity, etc., were taken.

Table I

*Unfertilized Eggs Treated with Distilled Water, Temperature 21.8° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.05 | . 57.0 | 32.4 |
| 0.08 | 95.0 | 10.0 |
| 0.17 | 99.6 | 0.6 |
| 0.25 | 100.0 | 0.0 |
| 0.33 | 100.0 | 0.05 |
| 0.42 | 100.0 | 0.1 |
| 0.50 | 100.0 | 0.0 |
| 0.67 | 100.0 | 0.0 |
| 0.83 | 100.0 | 0.0 |
| 1.00 | 100.0 | 1.5 |
| 1.50 | 100.0 | 0.0 |
| 2.00 | 100.0 | 0.0 |
| 3.00 | 100.0 | 0.0 |
| 4.00 | 100.0 | 0.0 |
| 5.00 | 99.0 | 0.0 |

[2] Eighty per cent sea water, for example, is made up of eight parts sea water and two parts distilled water. The sea water used was always taken at the same height of tide.

The percentages of cleavage and of activation were based on counts of at least three hundred eggs; frequently, especially for very low or very high percentages of activation, a much larger number were counted.

It was shown that two types of activated eggs appear as a result of the treatment. One type is characterized by initial changes which are indistinguishable from those induced by the sperm. In this type the breakdown of the germinal vesicle, the rounding out of the indentation, the elevation of the membrane, and the extrusion of polar bodies occur in very much the same manner as when the egg is fertilized by a sperm. The time relations for these various changes, allowing for the time of exposure, compare very closely with the time schedule of the same events in the fertilized egg. However, in spite of the remarkable similarity in behaviour of this type of artificially activated egg to that of the fertilized egg, none of the eggs divide.[3]

The other type of artificially activated egg departs widely in its behaviour from that of the normal fertilized egg. The only visible

TABLE II

*Unfertilized Eggs Treated with 20 Per Cent Sea Water, Temperature 22.0° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.08 | 25.0 | 69.2 |
| 0.17 | 58.1 | 58.7 |
| 0.25 | 92.0 | 31.4 |
| 0.33 | 98.8 | 17.3 |
| 0.50 | 100.0 | 3.1 |
| 0.67 | 100.0 | 1.0 |
| 0.83 | 100.0 | 4.2 |
| 1.00 | 100.0 | 0.5 |
| 1.17 | 100.0 | 0.1 |
| 1.33 | 99.5 | 2.2 |
| 1.50 | 100.0 | 0.1 |
| 1.67 | 100.0 | 0.0 |
| 1.83 | 100.0 | 0.0 |
| 2.00 | 100.0 | 0.0 |
| 2.50 | 100.0 | 0.0 |
| 3.00 | 100.0 | 0.0 |
| 3.50 | 100.0 | 0.0 |
| 4.00 | 100.0 | 0.0 |
| 5.00 | 100.0 | 0.0 |
| 7.00 | 100.0 | 0.1 |
| 10.00 | 100.0 | 0.3 |
| 15.00 | 100.0 | 0.6 |
| 20.00 | 100.0 | 1.5 |
| 40.00 | 100.0 | 0.0 |

[3] In only three cases were eggs with two polar bodies seen to divide. The three eggs proceeded only as far as the two-cell stage.

change that occurs in this type of egg within the first three-quarters of an hour after treatment is the dissolution of the germinal vesicle. The egg remains indented, no membrane elevation occurs, and no polar bodies are extruded. After that time the eggs begin to round up, and lift off membranes, but no polar bodies appear. Practically all of the eggs of this type divide, the time of first division varying from one hour and twenty minutes to about three hours. The eggs which cleave and develop are thus the ones which show a poor initial response to the treatment. In what follows, then, the percentage of cleavage is practically identical with the percentage of "poorly activated eggs," and the data on percentage of cleavage and of activation will also show the relation between the percentage of "imperfectly" and of "perfectly" activated eggs for various strengths of hypotonic solutions.

## The Variation of Percentage of Activation and of Cleavage with Length of Exposure for Various Dilutions of Sea Water

### *Treatment with Distilled Water*

The action of distilled water is extremely rapid in causing activation of the eggs. After 3 seconds' treatment, 57 per cent of the eggs

Table III

*Unfertilized Eggs Treated with 30 Per Cent Sea Water, Temperature 22.1° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.17 | 3.8 | 37.5 |
| 0.33 | 59.8 | 13.8 |
| 0.50 | 99.7 | 1.4 |
| 0.67 | 99.6 | 0.9 |
| 0.83 | 100.0 | 0.4 |
| 1.00 | 100.0 | 0.6 |
| 1.33 | 100.0 | 0.4 |
| 1.67 | 98.1 | 3.1 |
| 2.00 | 99.2 | 0.7 |
| 2.50 | 99.0 | 0.5 |
| 3.00 | 99.3 | 0.8 |
| 3.50 | 98.5 | 1.6 |
| 4.00 | 83.6 | 2.3 |
| 4.50 | 86.1 | 3.2 |
| 5.00 | 81.8 | 11.1 |
| 6.00 | 86.7 | 1.0 |
| 8.00 | 100.0 | 0.0 |
| 10.00 | 100.0 | 0.1 |
| 15.00 | 100.0 | 0.0 |
| 20.00 | 100.0 | 0.0 |
| 40.00 | 100.0 | 0.0 |

become activated, and after 15 seconds all of the eggs are activated. The results of one series of exposures are given in Table I. Another series run in the same manner gave quite similar results. The percentage of the activated eggs that cleave (column three in the table) is seen to drop very rapidly as the percentage of activation increases. Thus, when 100 per cent activation is obtained, there is practically no cleavage.

Cytolysis sets in after 2 minutes' exposure and reaches 90 per cent at 5 minutes' treatment. The activated eggs in that range are somewhat abnormal in appearance, having a relatively wide membrane and forming blisters over the surface so that polar bodies are often indistinguishable.

*Treatment with Twenty Per Cent Sea Water*

The action of 20 per cent sea water is less rapid than that of distilled water. The results of one series are given in Table II.

TABLE IV

*Unfertilized Eggs Treated with 40 Per Cent Sea Water, Temperature 22.0° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.17 | 0.0 | 0.0 |
| 0.33 | 52.1 | 31.4 |
| 0.50 | 94.0 | 13.5 |
| 0.67 | 97.8 | 2.3 |
| 0.83 | 99.7 | 0.9 |
| 1.00 | 99.9 | 0.1 |
| 1.33 | 100.0 | 0.1 |
| 1.67 | 100.0 | 0.2 |
| 2.00 | 99.6 | 0.0 |
| 2.50 | 99.0 | 2.4 |
| 3.00 | 98.8 | 1.1 |
| 3.50 | 98.3 | 1.6 |
| 4.00 | 97.5 | 3.2 |
| 5.00 | 85.3 | 6.4 |
| 7.00 | 88.0 | 0.9 |
| 10.00 | 99.9 | 0.4 |
| 15.00 | 100.0 | 0.0 |
| 20.00 | 100.0 | 0.0 |
| 40.00 | 100.0 | 0.0 |

Fifteen seconds longer treatment is required to give 100 per cent activation than for the distilled water. The rise in percentage of activation with time of exposure is again seen to be accompanied by a drop in cleavage. No exceptions are seen in the first part of the table and the ones occurring in the latter part are of small magnitude. Cytolysis sets in after 4 minutes' exposure and reaches 70 per cent after 15 minutes and 90 per cent after 20 minutes. Increasing numbers of abnormal eggs of the type described above are found in that range. Two other series of experiments were run, and closely similar results obtained.

TABLE V

*Unfertilized Eggs Treated with 45 Per Cent Sea Water, Temperature 21.0° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs | Volume in $\mu^3 \times 10^{-5}$ |
|---|---|---|---|
| *min.* | *per cent* | *per cent* | |
| 0.17 | 0.2 | 0.0 | |
| 0.33 | 11.5 | 50.0 | |
| 0.50 | 39.0 | 29.2 | |
| 0.67 | 81.3 | 10.6 | |
| 0.83 | 92.9 | 4.8 | |
| 1.00 | 98.6 | 1.4 | 8.12 |
| 1.33 | 100.0 | 0.2 | |
| 1.67 | 100.0 | 0.0 | |
| 2.00 | 99.5 | 0.0 | 8.90 |
| 2.50 | 98.3 | 0.3 | |
| 3.00 | 96.4 | 0.8 | 9.51 |
| 3.50 | 87.3 | 2.2 | |
| 4.00 | 65.7 | 4.0 | 10.10 |
| 5.00 | 34.4 | 20.5 | 10.45 |
| 6.00 | 21.2 | 26.2 | 10.92 |
| 8.00 | 7.9 | 22.2 | 11.49 |
| 10.00 | 6.9 | 50.0 | 11.92 |
| 15.00 | 0.8 | 43.0 | 12.95 |
| 20.00 | 1.3 | 51.6 | 13.51 |
| 40.00 | 3.3 | 8.3 | 13.96 |

*Treatment with Thirty Per Cent Sea Water*

With 30 per cent sea water the percentage of activation rises less rapidly than with 20 per cent. The results again show that as the percentage of activation increases, the percentage of cleavage decreases. Table III gives the results of one series. The percentage of activation shows a slight drop after about one and one-half minutes' exposure which becomes quite marked at 4 to 6 minutes' exposure. But as the activation drops, the cleavage is seen to rise, so that at 5 minutes' exposure, where the activation has dropped to 82 per cent, the cleavage has risen to 11 per cent. The activation then rises again to 100 per cent and the cleavage drops to zero.

Cytolysis sets in after 6 minutes' treatment and reaches 50 per cent after 40 minutes. The abnormal eggs referred to above again appear in this range of exposures.

Four other series of experiments were run, at temperatures ranging from sixteen to twenty-three degrees, and very similar results obtained.

The inverse relation between cleavage and activation was evident in each series. If every case in which an increase (or decrease) in activation accompanied by an increase (or decrease) in cleavage to the extent of at least one per cent is considered an exception, then out of a total of eighty-one dishes there are seven exceptions.

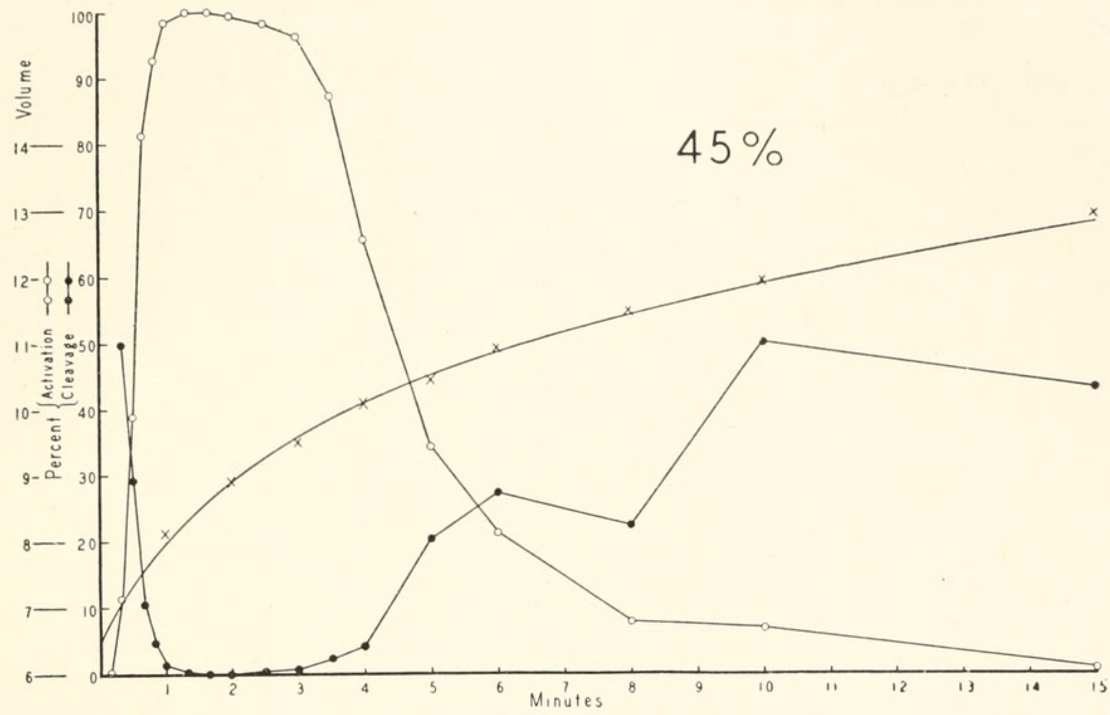


FIG. 1. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 45 per cent sea water. Data of Table V.

*Treatment with Forty Per Cent Sea Water*

The percentage of activation for eggs treated with 40 per cent sea water rises less rapidly than for eggs treated with any of the preceding dilutions. Table IV gives the results of one series of exposures. The activation is seen to rise rapidly to 100 per cent, drop more slowly to 85 per cent and return again to 100 per cent. The percentage of cleavage of the activated eggs decreases as the activation increases, and increases as the activation drops. The inverse relation between cleavage and activation is thus again clearly shown, only one exception occurring in the table, namely at the three minute exposure, where a drop in activation is followed by a drop in cleavage greater than one per cent.

At the 8 minutes' exposure there is 4 per cent of cytolysis, which increases to about 30 per cent for the 40 minutes' treatment. The abnormal eggs again occur in this range.

Three other series were run with 40 per cent sea water, totaling forty-seven dishes. Out of these a total of four exceptions of magnitude greater than one per cent were obtained.

TABLE VI

*Unfertilized Eggs Treated with 50 Per Cent Sea Water, Temperature 21.5° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs | Volume in $\mu^3 \times 10^{-5}$ |
|---|---|---|---|
| *min.* | *per cent* | *per cent* | |
| 0.17 | 0.1 | 100.0 | |
| 0.33 | 2.0 | 80.1 | |
| 0.50 | 43.2 | 40.6 | |
| 0.67 | 91.0 | 21.6 | |
| 0.83 | 96.7 | 10.6 | |
| 1.00 | 99.2 | 5.3 | 7.83 |
| 1.33 | 99.8 | 0.7 | |
| 1.67 | 100.0 | 0.4 | |
| 2.00 | 100.0 | 0.4 | 8.72 |
| 2.50 | 100.0 | 1.2 | |
| 3.00 | 90.8 | 26.9 | 9.29 |
| 3.50 | 93.4 | 21.9 | |
| 4.00 | 86.0 | 39.2 | 9.72 |
| 4.50 | 68.3 | 63.2 | |
| 5.00 | 33.9 | 76.2 | 10.10 |
| 6.00 | 34.9 | 80.0 | 10.46 |
| 8.00 | 18.8 | 94.7 | 11.08 |
| 10.00 | 7.1 | 83.3 | 11.42 |
| 15.00 | 0.5 | 40.0 | 12.20 |
| 20.00 | 0.4 | 79.0 | 12.72 |
| 40.00 | 1.5 | 61.3 | 12.85 |

*Treatment with Forty-five Per Cent Sea Water*

The results obtained with 45 per cent sea water differ in two respects from those obtained with the preceding dilutions of sea water. These are, first, that the percentage of activation returns practically to zero after its initial rise to 100 per cent, and second, that very little cytolysis sets in.

In Table V the results of one series of experiments are presented. The rate of increase in activation is slower than with the preceding dilutions. After 8 minutes' exposure a few of the eggs become cytolysed and the amount of cytolysis reaches 5 per cent after 40 minutes.

The inverse relation between cleavage and activation is quite evident in the table and is illustrated graphically in Fig. 1. The "exceptions" generally occur in the dishes showing low percentages of activation. Similar results were obtained in three other series of experiments run with 45 per cent sea water. Out of a total of seventy-three dishes examined, sixteen exceptions were found, all of them in dishes showing less than 8 per cent activation.

The increase in activation occurs much more rapidly than the decrease. This may readily be seen in the graph (Fig. 1), where the percentage of activation plotted against time gives a skew curve. The probable interpretation of this result will be presented later. But in connection with the activation-time curve, it is of interest to

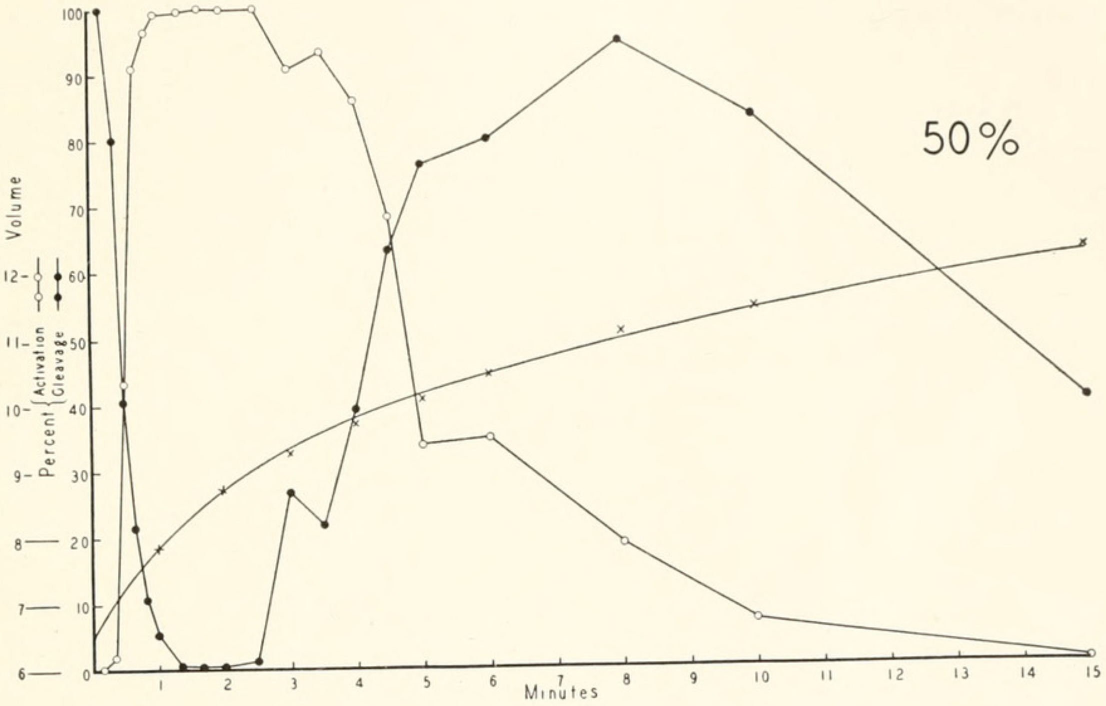


FIG. 2. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 50 per cent sea water. Data of Table VI.

present here the curve showing the increase in volume with length of exposure to the 45 per cent sea water. The data from which the curve was drawn are given in Table V. Each point represents the average of the volumes of three eggs. The measurements of the diameters were made with a Filar ocular micrometer. With this micrometer measurements accurate to 0.1 per cent may be obtained. However, the variations in volume, for the data presented here and below, ranged as high as 5 per cent. This is probably due to the rapid change in volume that is taking place as the measurements are made and to the variability of the eggs. The volume measurements are being repeated on a larger scale and by means of a cinematograph in order to obtain accurate data for an analysis of the swelling process itself. But even the relatively rough data presented here will be shown to be useful in an analysis of the activation-time curves obtained in these experiments.

The swelling curve of Fig. 1 shows that the eggs continue to increase in volume even after the percentage of activation begins to drop. The curve itself is of the exponential type, the slope continually decreasing. In other words, the increase in volume occurs less rapidly as the time of exposure increases.

Table VII

*Unfertilized Eggs Treated with 55 Per Cent Sea Water, Temperature 21.2° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs | Volume in $\mu^3 \times 10^{-5}$ |
|---|---|---|---|
| *min.* | *per cent* | *per cent* | |
| 0.17 | 0.4 | 100.0 | |
| 0.33 | 0.9 | 100.0 | |
| 0.50 | 10.0 | 53.3 | |
| 0.67 | 38.4 | 37.4 | |
| 0.83 | 70.3 | 29.4 | |
| 1.00 | 89.5 | 17.6 | 7.74 |
| 1.33 | 96.2 | 9.6 | |
| 1.67 | 98.4 | 2.0 | |
| 2.00 | 99.9 | 1.0 | 8.73 |
| 2.50 | 97.8 | 1.6 | |
| 3.00 | 93.3 | 5.6 | 9.32 |
| 3.50 | 88.8 | 7.1 | |
| 4.00 | 85.6 | 12.4 | 9.57 |
| 5.00 | 66.4 | 21.3 | 9.82 |
| 6.00 | 64.1 | 21.0 | 10.11 |
| 8.00 | 47.3 | 16.6 | 10.49 |
| 10.00 | 13.9 | 15.4 | 10.89 |
| 12.00 | 1.7 | 44.2 | |
| 15.00 | 1.1 | 70.6 | 11.41 |
| 20.00 | 0.0 | 0.0 | 11.63 |
| 40.00 | 2.2 | 90.9 | 11.84 |

### *Treatment with Fifty Per Cent Sea Water*

The results obtained with 50 per cent sea water are quite similar to those obtained with 45 per cent sea water, except that the increase in activation occurs more slowly and practically no cytolysis occurs in any of the dishes. The increase in volume of the eggs in 50 per cent sea water is also somewhat slower than for those exposed to 45 per cent sea water, and the equilibrium volume attained is, of course, smaller.

Table VI contains the results of one series of experiments and a set of volume measurements (means of three eggs) obtained at different times. The data are presented graphically in Fig. 2. The results again bear out the inverse relation between cleavage and activation. It is interesting to note that the irregular rise in the activation curve at the three and one-half minutes' exposure is accompanied by a drop in cleavage. The exceptions occur only in the last four points of the graph, where the percentage of activation is low. A total of eighty-two dishes in five series of experiments gave sixteen exceptions all of the same type illustrated here.

The activation shows a drop to practically zero after its initial rise

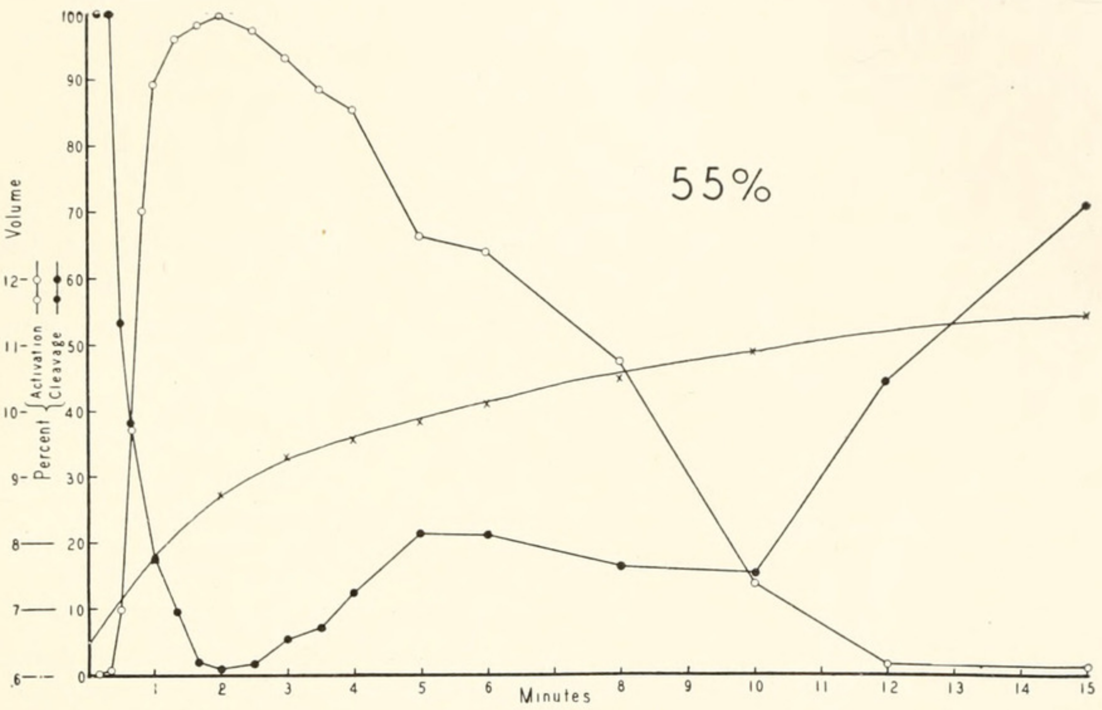


FIG. 3. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 55 per cent sea water. Data of Table VII.

to 100 per cent, as in the preceding case. The rise in activation is again seen to occur more rapidly than the subsequent decrease, giving the skew activation-time curve shown in Fig. 2. The volume curve for the eggs in the 50 per cent sea water is of the same type as obtained with the preceding dilution.

*Treatment with Fifty-five Per Cent Sea Water*

The results of one series of experiments with 55 per cent sea water and the volume data for the same dilution are presented in Table VII and Fig. 3. The data again show a decrease in cleavage as the activation increases and an increase in cleavage as the activation decreases. Four series of experiments totaling seventy-four dishes gave twelve exceptions—chiefly at low percentages of activation.

The activation-time curve is of the same shape as that obtained in the preceding case, but it is shifted slightly to the right, so that the time required for the maximum percentage of activation and for the return to zero per cent activation is longer than with 50 per cent sea water.

The swelling curve (Fig. 3) shows that the volume continues to increase after the percentage of activation has reached a maximum. It is also of the exponential type in which the rate of increase in volume decreases with time.

### *Treatment with Sixty Per Cent Sea Water*

The results obtained with 60 per cent sea water again differ from the preceding only in the time relations of activation and cleavage and the volume curve. The data is given in Table VIII, and graphically represented in Fig. 4.

TABLE VIII

*Unfertilized Eggs Treated with 60 Per Cent Sea Water, Temperature 22.3° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs | Volume in $\mu^3 \times 10^{-5}$ |
|---|---|---|---|
| *min.* | *per cent* | *per cent* | |
| 0.17 | 0.1 | 0.0 | |
| 0.33 | 1.0 | 0.0 | |
| 0.50 | 1.0 | 50.0 | |
| 0.67 | 13.4 | 33.3 | |
| 0.83 | 30.3 | 25.8 | |
| 1.00 | 61.3 | 25.9 | 7.88 |
| 1.33 | 73.1 | 13.7 | |
| 1.67 | 88.7 | 7.7 | |
| 2.00 | 99.4 | 0.4 | 8.68 |
| 2.50 | 100.0 | 0.1 | |
| 3.00 | 100.0 | 0.2 | 9.16 |
| 3.50 | 97.6 | 2.9 | |
| 4.00 | 88.2 | 9.3 | 9.45 |
| 5.00 | 85.7 | 5.5 | 9.69 |
| 6.00 | 75.0 | 30.6 | 10.01 |
| 7.00 | 42.4 | 46.5 | |
| 8.00 | 16.0 | 61.5 | 10.16 |
| 10.00 | 14.7 | 81.2 | 10.49 |
| 12.00 | 9.5 | 37.1 | |
| 15.00 | 5.1 | 72.3 | 10.94 |
| 20.00 | 0.0 | 0.0 | 11.10 |
| 40.00 | 0.0 | 0.0 | 11.11 |

The cleavage-activation relation shows up quite clearly. Out of eighty-nine dishes in five series of experiments, fourteen relatively unimportant exceptions were obtained.

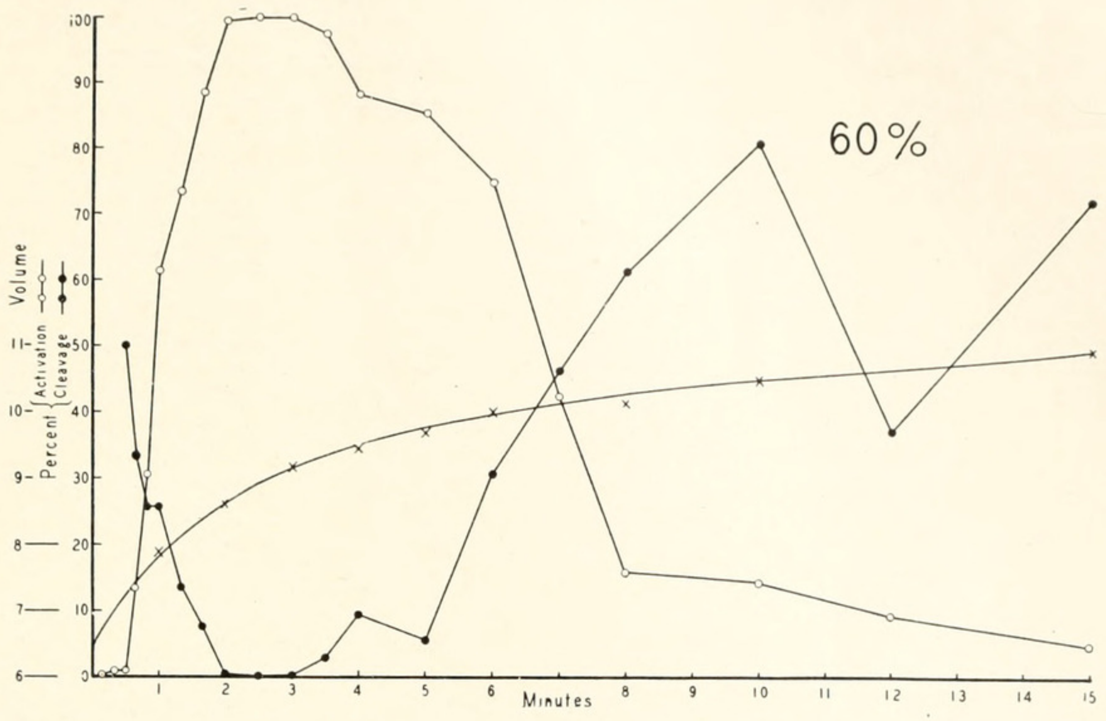


FIG. 4. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 60 per cent sea water. Data of Table VIII.

The activation-time curve shows a slight shift to the right when compared with the preceding ones, but its asymmetry is still quite evident.

The swelling curve is of the same type as in the preceding cases but approaches a lower equilibrium volume.

### *Treatment with Sixty-five Per Cent Sea Water*

Sixty-five per cent sea water gives results which differ from the preceding in the same direction as the results obtained with the 60 per cent sea water differ from those obtained with 55 per cent. One series of experiments and a set of mean volumes are shown in Table IX and Fig. 5.

The inverse relation between cleavage and activation is evident in spite of certain relatively large irregularities. From three series of experiments seven relatively large and eight minor exceptions were obtained out of a total of fifty-nine dishes.

## Table IX

*Unfertilized Eggs Treated with 65 Per Cent Sea Water, Temperature 22.0° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs | Volume in $\mu^3 \times 10^{-5}$ |
|---|---|---|---|
| *min.* | *per cent* | *per cent* | |
| 0.17 | 0.0 | 0.0 | |
| 0.33 | 0.2 | 0.0 | |
| 0.50 | 0.0 | 0.0 | |
| 0.67 | 0.7 | 40.0 | |
| 0.83 | 6.4 | 36.3 | |
| 1.00 | 13.6 | 11.8 | 7.61 |
| 1.33 | 77.8 | 20.8 | |
| 1.67 | 87.6 | 13.4 | |
| 2.00 | 92.0 | 10.2 | 8.32 |
| 2.50 | 96.5 | 6.1 | |
| 3.00 | 98.6 | 2.1 | 8.73 |
| 3.50 | 92.5 | 6.7 | |
| 4.00 | 71.9 | 22.5 | 9.25 |
| 5.00 | 49.0 | 56.7 | 9.56 |
| 6.00 | 21.8 | 46.9 | 9.49 |
| 7.00 | 20.3 | 51.0 | |
| 8.00 | 13.8 | 42.4 | 9.88 |
| 10.00 | 13.7 | 34.9 | 10.15 |
| 12.00 | 6.0 | 40.9 | |
| 15.00 | 3.9 | 15.3 | 10.50 |
| 20.00 | 0.2 | 100.0 | 10.55 |
| 40.00 | 0.0 | 0.0 | 10.55 |

The activation-time curve (Fig. 5) is asymmetrical as in the preceding cases, but it shows a slight shift to the right.

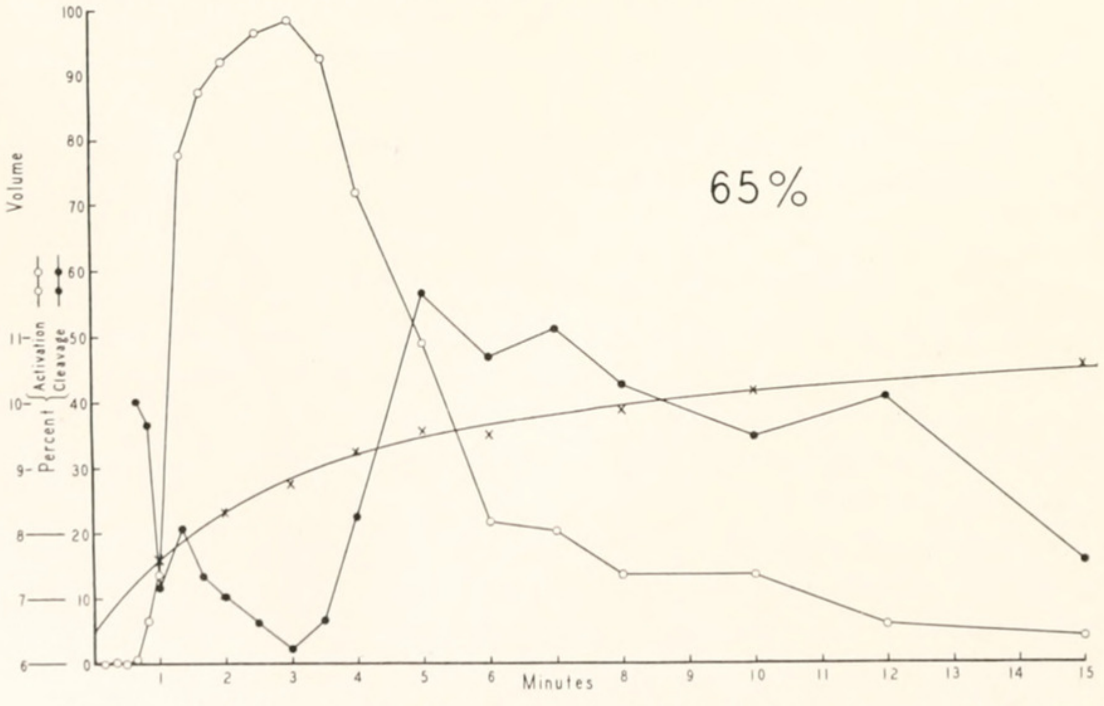


Fig. 5. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 65 per cent sea water. Data of Table IX.

The volume increase is slower than for the eggs in more dilute sea water, and approaches a lower asymptotic value.

### *Treatment with Seventy Per Cent Sea Water*

The results of one series run with 70 per cent sea water are tabulated in Table X. Figure 6 shows that trend of the data graphically.

TABLE X

*Unfertilized Eggs Treated with 70 Per Cent Sea Water, Temperature 21.2° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.17 | 0.4 | 57.1 |
| 0.33 | 0.8 | 50.0 |
| 0.50 | 2.5 | 55.2 |
| 0.67 | 2.4 | 29.8 |
| 0.83 | 4.1 | 30.2 |
| 1.00 | 4.6 | 29.5 |
| 1.33 | 6.4 | 20.3 |
| 1.67 | 20.4 | 19.5 |
| 2.00 | 46.1 | 10.2 |
| 2.50 | 96.2 | 9.1 |
| 3.00 | 100.0 | 2.3 |
| 3.50 | 97.5 | 10.9 |
| 4.00 | 69.2 | 19.7 |
| 4.50 | 51.6 | 35.7 |
| 5.00 | 31.6 | 55.5 |
| 6.00 | 16.7 | 65.2 |
| 8.00 | 7.4 | 82.9 |
| 10.00 | 13.3 | 76.3 |
| 15.00 | 12.7 | 85.8 |
| 20.00 | 4.6 | 83.3 |
| 40.00 | 0.3 | 66.7 |

No serious divergence from the cleavage-activation relation is evident. Two series of experiments totaling thirty-seven dishes gave seven minor variations.

The activation-time curve (Fig. 6) is again decidedly asymmetrical. It is displaced to the right, so that the return to zero per cent activation requires a longer exposure than in the preceding cases.

The volume data are not presented for this or for the succeeding dilutions of sea water. The volume increase proceeds more slowly, of course, and reaches a smaller equilibrium volume with increasing concentrations of sea water.

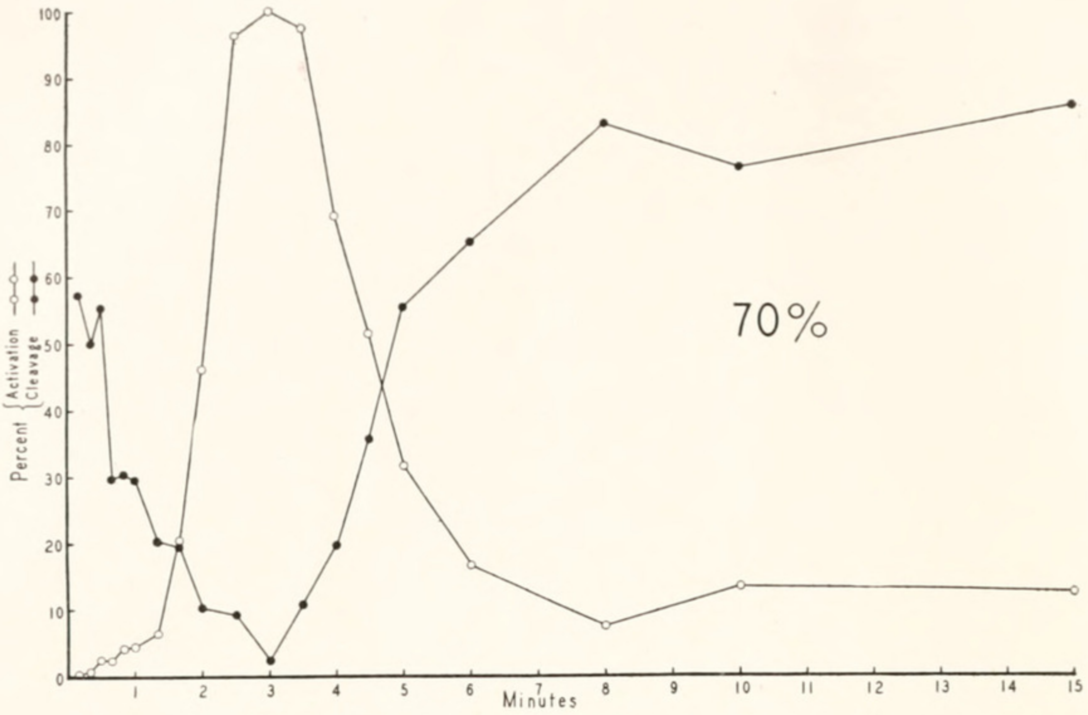


FIG. 6. Variation of percentage activation (open circles) and percentage cleavage (solid circles) with time of exposure to 70 per cent sea water. Data of Table X.

*Treatment with Seventy-five Per Cent Sea Water*

Table XI and Fig. 7 contain the results of one series of experiments with 75 per cent sea water.

TABLE XI

*Unfertilized Eggs Treated with 75 Per Cent Sea Water, Temperature 20.8° C.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.17 | 0.0 | 0.0 |
| 0.33 | 0.0 | 0.0 |
| 0.50 | 0.0 | 0.0 |
| 0.67 | 0.0 | 0.0 |
| 0.83 | 0.0 | 0.0 |
| 1.00 | 0.0 | 0.0 |
| 1.33 | 3.3 | 71.4 |
| 1.67 | 8.2 | 50.0 |
| 2.00 | 33.9 | 45.9 |
| 2.50 | 83.2 | 49.5 |
| 3.00 | 86.2 | 32.3 |
| 3.50 | 90.0 | 28.4 |
| 4.00 | 79.3 | 28.6 |
| 5.00 | 47.8 | 59.2 |
| 6.00 | 30.2 | 65.0 |
| 8.00 | 7.4 | 61.3 |
| 10.00 | 1.2 | 66.7 |
| 15.00 | 0.1 | 40.0 |
| 20.00 | 0.0 | 0.0 |

As before, the percentage of cleavage varies inversely with the percentage of activation, although the difference between the maximum of activation and the corresponding minimum of cleavage is not as great as in the cases listed above. Two series of experiments totaling thirty-one dishes gave four exceptions.

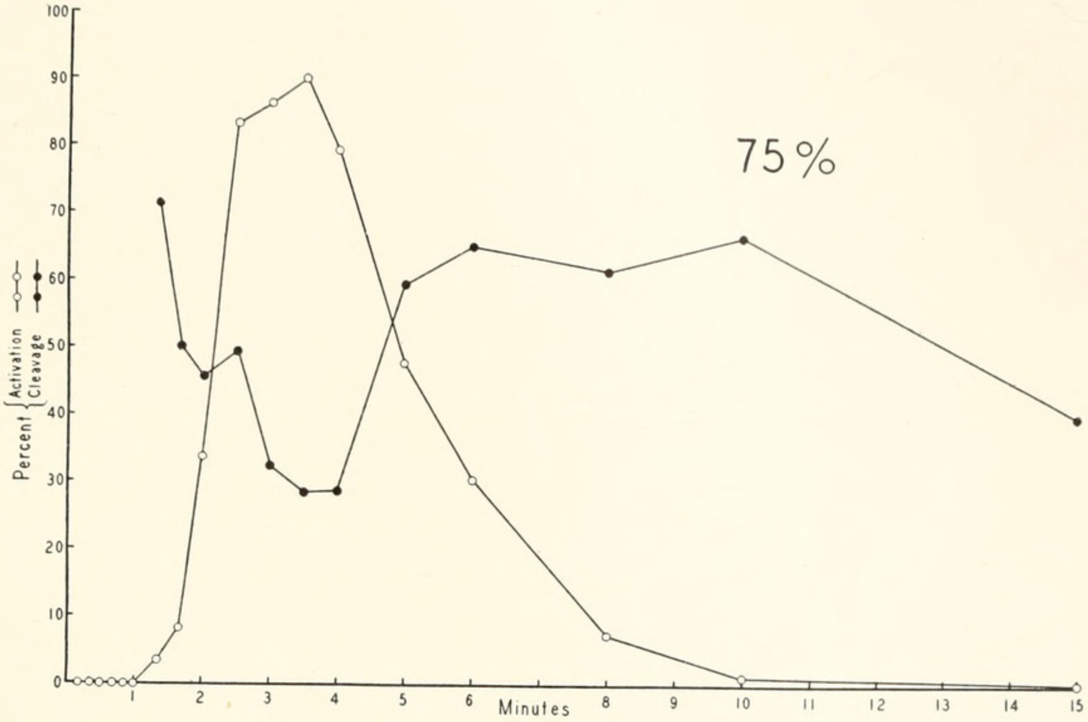


FIG. 7. Variation of percentage activation (open circles) and percentage cleavage (solid circles) with time of exposure to 75 per cent sea water. Data of Table XI.

For the activation-time curve (Fig. 7), the time to reach a maximum is longer than in the preceding case, but the drop to zero per cent occurs sooner. However, the maximum value reached is only 90 per cent activation as compared with 100 per cent in the previous cases. The curve itself is still asymmetrical.

*Treatment with Eighty Per Cent Sea Water*

Eighty per cent sea water generally fails to give more than one to two per cent activation. In one series of experiments, however, an exceptionally high percentage of activation was obtained. The results are given in Table XII and Fig. 8.

It is readily seen from the data that practically every increase (or decrease) in activation is accompanied by a decrease (or increase) in cleavage, bearing out the inverse relation between cleavage and activation.

The activation-time curve reaches its maximum as quickly as for the 75 per cent sea water, but that is undoubtedly due to the higher temperature at which this series was run. The activation curve does not return to zero, but maintains a relatively high percentage of activation and a correspondingly high percentage of cleavage.

Table XII

*Unfertilized Eggs Treated with 80 Per Cent Sea Water, Temperature 22.5° F.*

| Length of Exposure | Activation | Cleavage of Activated Eggs |
|---|---|---|
| *min.* | *per cent* | *per cent* |
| 0.17 | 0.0 | 0.0 |
| 0.33 | 0.0 | 0.0 |
| 0.50 | 0.0 | 0.0 |
| 0.67 | 0.0 | 0.0 |
| 0.83 | 0.2 | 100.0 |
| 1.00 | 1.4 | 42.8 |
| 1.33 | 6.6 | 31.2 |
| 1.67 | 14.5 | 30.8 |
| 2.00 | 58.2 | 26.8 |
| 2.50 | 94.1 | 21.5 |
| 3.00 | 95.7 | 17.6 |
| 3.50 | 98.8 | 3.5 |
| 4.00 | 98.7 | 8.3 |
| 5.00 | 81.6 | 57.8 |
| 6.00 | 92.5 | 37.1 |
| 8.00 | 80.7 | 48.6 |
| 10.00 | 65.5 | 59.1 |
| 15.00 | 64.1 | 38.2 |
| 20.00 | 62.5 | 48.8 |
| 40.00 | 47.6 | 64.2 |

## The Variation of Percentage of Activation with Volume for Various Dilutions of Sea Water

When the percentage of activation is plotted against the mean volume attained by the eggs at different lengths of exposure, a curve is obtained which is much more symmetrical than the activation-time curve. Figure 9 shows five curves of that type, for 45, 50, 55, 60 and 65 per cent sea water. The percentages of activation were plotted in each case against the volumes attained at corresponding times of exposures, the volumes being taken from the smooth curves.

The activation-volume curves of Fig. 9 approach in shape the normal distribution curve. The individual curves have the same abscissa but the ordinates are raised successively for each dilution of sea water. It can readily be seen that even with the same coördinates the curves would not coincide; but their divergence is no greater than would be expected when one considers the statistical nature of the activation values and the errors involved in the volume measurements. Moreover, there are probably injury factors operative in the lower concentrations of sea water that are not present in the higher concentrations, as indicated by the cytolysis obtained in 45 per cent sea water.

## Fertilization of "Over-Exposed" Eggs

The activation-time curves for concentrations of sea water above 40 per cent are seen to rise to a maximum of about 100 per cent activation and then drop off to zero.

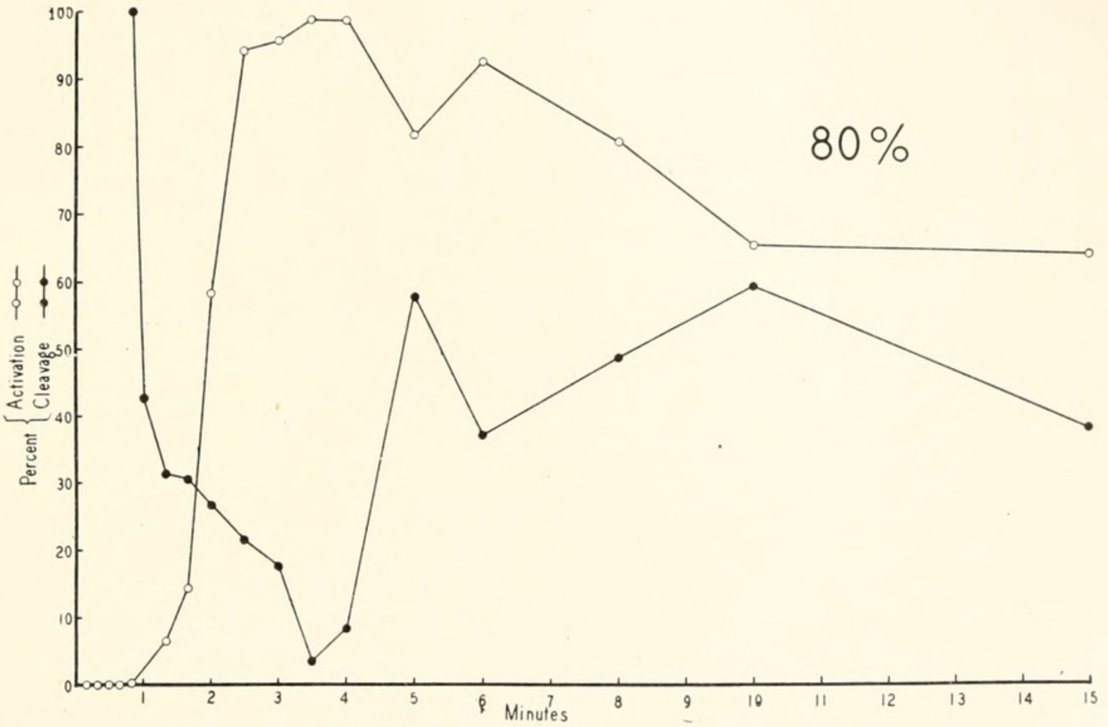


Fig. 8. Variation of percentage activation (open circles) and percentage cleavage (solid circles) with time of exposure to 70 per cent sea water. Data of Table XII.

The eggs which do not respond before the "optimum exposure" is reached may be termed "under-exposed" unactivated eggs, and those which do not respond upon longer exposures may be termed "over-exposed" unactivated eggs.

The failure of the "over-exposed" unactivated eggs to respond to the treatment might presumably be due to an injury effect, or other change produced in the eggs. The "over-exposed" unactivated eggs as well as the under-exposed unactivated eggs were therefore inseminated with fresh sperm in order to determine whether they would become fertilized and produce normal embryos. The results obtained with 45 to 65 per cent sea water are given in Table XIII. The third column in the table gives the total percentages of activation obtained with the lengths of exposure listed in column two. The fourth column gives the percentage of the unactivated eggs that become fertilized upon addition of sperm, and the fifth column, the percentage of the fertilized eggs that produce normal larvæ.

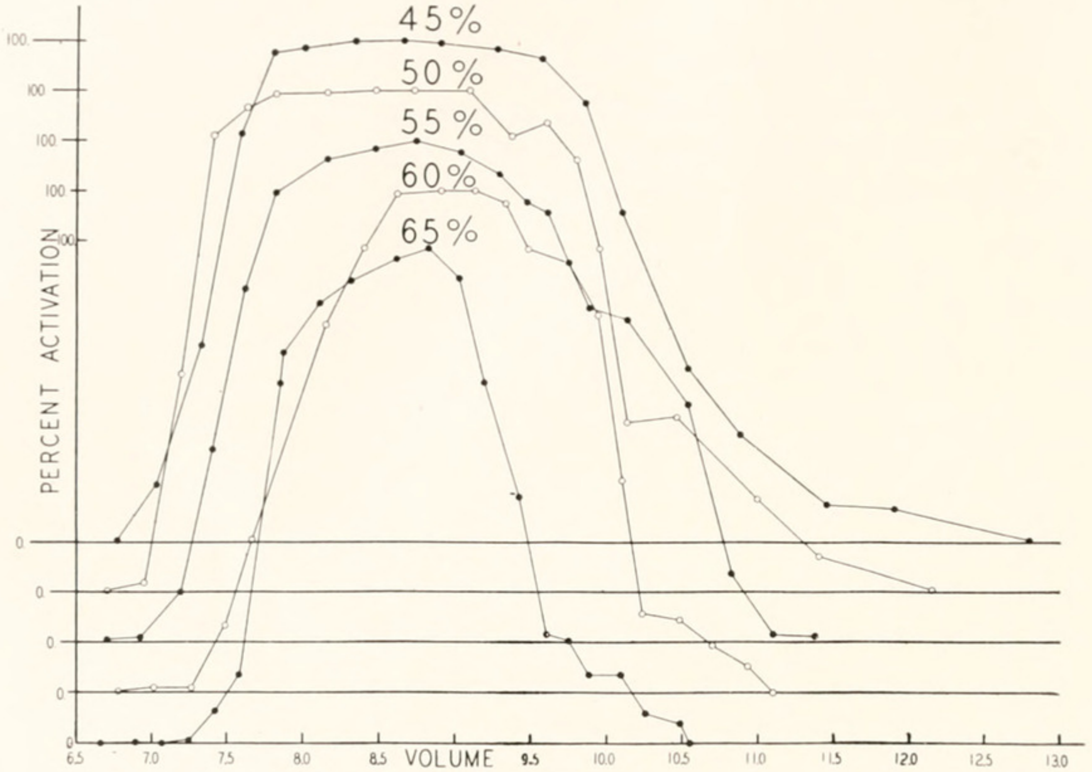


Fig. 9. Variation of percentage of activation with mean volume of eggs attained at corresponding times of exposure to 65, 60, 55, 50, and 45 per cent sea water. Ordinates raised successively for each dilution of sea water. Data from Tables V to IX; volumes taken from the smooth curves of Figs. 1 to 5.

The unactivated eggs were transferred to a separate dish and inseminated at about 2 to 3 hours after treatment. Control eggs (listed in the table as 0.0 minutes' exposure) were inseminated at the same time.

The "under-exposed" unactivated eggs are not given for the 55 and the 60 per cent sea water. In the other three cases the "under-exposed" unactivated eggs show practically 100 per cent fertilization and a high percentage of normal embryos. The "over-exposed" eggs show a high percentage of fertilization in every case, comparing quite favorably with that given by the control eggs. The percentage of normal embryos obtained varies considerably, but is quite as good as that obtained from the controls, except for the 45 per cent sea water. However, in the latter case a relatively large percentage of the eggs were polyspermic.

TABLE XIII

*Insemination of "Under-Exposed" and "Over-Exposed" Unactivated Eggs*

| Concentration of Sea Water | Length of Exposure | Activation | Fertilization | Normal Embryos |
|---|---|---|---|---|
| *per cent* | *min.* | *per cent* | *per cent* | *per cent* |
| | 0.0 | — | 95 | 40 |
| | 0.17 | 0.0 | 99 | 50 |
| | 1.50 | 100.0 | — | — |
| 45 | 10.00 | 5.7 | 70 | 20 |
| | 15.00 | 0.0 | 60 | 2 |
| | 20.00 | 0.3 | 60 | 5 |
| | 40.00 | 0.5 | 60 | 5 |
| | 0.0 | — | 98 | 25 |
| 50 | 0.17 | 0.1 | 100 | 60 |
| | 2.00 | 100.0 | — | — |
| | 15.00 | 0.5 | 80 | 70 |
| | 0.0 | — | 65 | 70 |
| 55 | 2.00 | 100.0 | — | — |
| | 20.00 | 0.0 | 50 | 65 |
| | 0.0 | — | 100 | 100 |
| | 2.75 | 100.0 | — | — |
| 60 | 7.00 | 42.4 | 100 | 100 |
| | 10.00 | 14.7 | 100 | 95 |
| | 20.00 | 0.0 | 100 | 100 |
| | 0.0 | — | 100 | 65 |
| | 0.17 | 0.0 | 100 | 100 |
| 65 | 3.00 | 99.0 | — | — |
| | 7.00 | 28.1 | 100 | 40 |
| | 10.00 | 18.3 | 90 | 75 |
| | 20.00 | 0.0 | 99 | 50 |

The results show that the "over-exposed" unactivated eggs are still capable of becoming fertilized, even though a shorter exposure would have resulted in every egg becoming activated upon return to normal sea water.

## DISCUSSION

### 1. *Variation of Rate of Increase in Activation with Dilution of Sea-Water*

It is evident from the results presented above that the factors causing activation are brought into action more quickly, the lower the concentration of the sea water used for the treatment. In the dilute sea water the egg swells due to intake of water. The volume increase also occurs more quickly, the lower the concentration of the sea water in which the eggs are allowed to swell. This parallel behaviour suggests that volume increase in the dilute sea water may be used as a basis for an interpretation of the results presented above; but this is not meant to imply that water-intake alone is responsible for the activation of the egg.[4]

### 2. *Activation-Time Curves*

For concentrations of sea water ranging from 45 to 75 per cent the percentage of activation was seen to rise rapidly to a maximum and then fall off more slowly. In terms of volume change this means that when the egg is in a definite volume range it will become activated upon return to normal sea water, but before or after passing through that volume range the egg does not become activated upon return to normal sea water. This volume range is evidently well below the equilibrium volume, since the eggs continue to swell after the time of exposure giving the maximum activation. The reason why a range of volumes rather than one definite volume is specified will be indicated below. There is considerable variability in the time at which different eggs pass through the same volume range when a given batch is exposed to a given dilution of sea water. Thus some of the eggs will have reached the volume range from which return to normal sea water results in activation before the others have entered that range. Correspondingly, some of the eggs will have passed through that volume range while the others are still in it. Let us term the volume range resulting in activation the "optimum volume range." The percentage of eggs passing through a given volume range at a given time will depend on the kind of variability shown by the eggs. If the variability of this material is expressed by the normal distribution curve, then we would expect the variability in the percentage of eggs passing through the "optimum volume range" to be expressed by that type of curve only if the increase in volume were a linear function of the time of exposure. But the volume increase is a logarithmic function of time, the rate of swelling continually decreasing with time of exposure. The eggs therefore enter the "optimum volume range" more rapidly than they leave it. Thus the variation in the percentage of eggs passing through the "optimum volume range" with time of exposure should be expressed by a skew distribution curve with its mode displaced to the left. In other words, the variation of percentage activation with time of exposure should give a skew curve, since the percentage of eggs passing through the "optimum volume range" is by definition identical with the percentage of activation. This is in fact the type of curve that is

[4] The change in hydrogen ion concentration, for example, might be an important factor. It ranged from pH 8.2 for the sea water to pH 7.1 for the distilled water used.

obtained when percentage of activation is plotted against length of exposure (Figs. 1 to 8).

The reason for assuming a range of volumes rather than one definite optimum volume results from the following consideration. The maximum of the activation-time curve is at 100 per cent activation. This means that all of the eggs must be in such a condition after a certain time of exposure that removal to normal sea water at that time results in every egg becoming activated. But the volume measurements show that the eggs vary in the time of exposure at which a given volume is reached. Therefore, if we adhere to the volume interpretation we must assume that a range of volumes, at least as great as the variation in volume of the individual eggs, is effective in causing activation upon return to ordinary sea water. The time of exposure at which all of the eggs are in that "optimum volume range" then results in 100 per cent activation.

On this basis the more rapid swelling obtained with progressively lower concentrations of sea water should cause a shifting of the activation-time curve to the left proportional to the increase in rate of swelling and likewise a shortening of the time range of activation. The results presented above show that this is in general true. But with extreme dilutions of sea water (40 per cent to distilled water) the drop to zero per cent activation does not occur. This is probably due to a secondary effect as indicated by the fact that there is a tendency for the activation to drop (see Tables II, III, IV), but as cytolysis sets in a second rise in activation (of an abnormal type) takes place.

One should also expect, according to the volume interpretation, that the concentration of sea water in which the equilibrium volume of the eggs is within the "optimum volume range" should give an activation-time curve that does not drop. This is presumably approached by the 80 per cent sea water (Table XII and Fig. 8).

### 3. *Activation-Volume Curves*

If the variation of the percentage of activation with time of exposure is correlated with the variation in volume of the eggs attained at corresponding times of exposure, then the percentage of activation plotted against mean volume should give a normal distribution curve, which should be identical for the various dilutions of sea water. The results show that this is roughly true. The curves obtained with various dilutions of sea water (Fig. 9) are quite symmetrical when compared with the activation-time curves. The probable reasons for the failure of the various curves to be exactly identical have been given above.

The expectation of a normal distribution curve for percentage activation plotted against mean volume is based on the assumption that the variation in volume of the eggs, at each time of exposure considered, is expressed by the normal probability curve. This is the type of variation that is generally assumed for biological material in the absence of further information. To obtain such information in this case it would be necessary to measure the volumes of a large number of eggs at various times of exposure. This has not been done on a large enough scale and accurately enough to determine whether the chance law holds for the volumes at every exposure used, but the measurements obtained on untreated eggs indicate that their variation in volume is of that type.

### 4. "*Over-Exposed*" *Unactivated Eggs*

It has been shown that the over-exposed unactivated eggs obtained with solutions ranging from 45 per cent to 65 per cent sea water can still be fertilized and may produce normal embryos. This may be taken to mean that the eggs have not been irreversibly affected by treatment with these dilutions of sea water. Consonant with this fact is the observation previously reported, that no visible changes aside from the swelling are seen to occur in the treated eggs while in the dilute sea water. It is also in accord with the result that the time for the initial stages (*e.g.* polar body extrusion) of the artificially activated eggs is comparable with that of the fertilized eggs only if allowance is made for the time of treatment.

It is evident then that no developmental changes occur in the egg while in the hypotonic solution, but that activation is initiated by the return to normal sea water after a definite time of exposure (or after a certain amount of water has been taken in). The question may therefore be raised as to why a longer exposure fails to evoke a response in the egg upon return to normal sea water when a shorter one does. If the egg were found to be injured by the longer exposure this question might be more readily answered. But the data presented here show that this is not so. The question bears directly on the mechanics of activation. With the data available we can only answer by restating the result in the following terms—that a definite change (enabling the egg to become activated upon return to normal sea water) *is* produced in the egg by the intake of an amount of water within a certain range, but that the change is reversed when more water is taken in. In other words, by the difference in behaviour upon return to normal sea water, an egg in the optimum exposure range must be intrinsically different from an egg in the earlier or later ranges, and by the similarity in behaviour upon return to normal sea water, an egg in the earlier range of exposures must be intrinsically the same (neglecting the manifest difference in volume) as an egg in the later range; hence the change produced must be reversed.

The return to original condition of eggs that have been allowed to swell in dilute sea water has also been noted in eggs of *Nereis* (Just, 1930) and eggs of *Arbacia* (McCutcheon and Lucké, 1926). But in neither of these cases is it stated whether activation is obtained at shorter exposures.

The ability of eggs that have been "over-exposed" to butyric acid to become fertilized has been noted by Moore (1916) for *Arbacia*, Just (1919) for *Echinarachnius*, and Lillie (1921) for *Strongylocentrotus*. But in these cases the cleavage and development were stated to be abnormal.

5. *Cleavage-Activation Relation*

The inverse relation between the percentage of cleavage and the total percentage of activation may now be interpreted in a similar way provided we introduce a "sub-optimum volume range" on both sides of the "optimum volume range." The justification of this arises from a consideration of the results reported in a previous publication (Tyler, 1931). It was shown that the activated eggs that extrude both polar bodies practically never divide, even though the response of that type of egg to the treatment is outwardly indistinguishable from the response of the egg to the sperm. Only the eggs that produce no polar bodies were the ones to cleave, but such eggs were shown to respond in a relatively very slow and abnormal fashion to the treatment in respect to the breakdown of the germinal vesicle, rounding out of indentation, and membrane elevation. Such is the type of result one would expect from a "sub-optimum" treatment. In terms of volume change this "sub-optimum" exposure would be obtained in a "sub-optimum volume range." Practically no eggs of that type are obtained at the time of exposure giving 100 per cent activation, but they occur in increasing numbers to either side of that exposure time. Since, at the time of exposure giving 100 per cent activation, all of the eggs are assumed to be in the "optimum volume range," the "sub-optimum volume range" must occur on each side of the former.[5]

Thus, when a batch of eggs is treated with dilute sea water, the eggs will pass through a "sub-optimum volume range" both before and after entering the "optimum volume range." At relatively short

[5] The "sub-optimum volume range" must evidently be shorter than the range of variability of the volumes of the eggs, since 100 per cent cleavage (with 100 per cent activation) is never obtained for any given exposure.

times of exposure, then, one would expect most of the activated eggs to be within the "sub-optimum volume range," and so give a high percentage of cleavage (of the activated eggs). But with longer exposures as the total activation increases one would expect more and more of the eggs to enter the "optimum volume range" and so give a low percentage of cleavage. The results would then be reversed upon passing through the second "sub-optimum volume range" with longer exposures.

This leads to a relation between percentage of cleavage and percentage total activation that is identical with that described in the text.

This interpretation can be tested in a much better fashion by following the volume changes of individual eggs in various dilutions of sea water and noting their behaviour when removed to normal sea water after having been allowed to swell to various volumes. Such experiments are now in progress.

The results reported here have an important bearing on what is generally termed the "optimum treatment" in parthenogenesis experiments. It has generally been assumed that the treatment producing the highest percentage of activation (similar to that produced by the sperm), and of cleavage and development is the optimum treatment. But in *Urechis* it has been shown that the treatment that is optimum for activation is not so for cleavage and development. Thus, if one wishes to produce the most parthenogenetic development, the length of exposure used is different from that which would be chosen if one wished to produce the highest percentage of eggs whose initial response to the treatment was most similar to that induced by the sperm. It is preferable, I think, to term the latter the optimum treatment, for the reasons stated above. The failure of eggs receiving the optimum treatment to divide is probably connected with insufficient chromatin (since all such eggs extrude two polar bodies and are left with the haploid number of chromosomes). It should be possible then to produce cleavage in such eggs by suppressing the polar divisions. This is somewhat difficult to accomplish without initiating other changes in the eggs, but the results obtained thus far indicate that suppression of the polar divisions of the "optimally" stimulated eggs results in cleavage.

The inverse relation between percentage of cleavage and percentage of activation appears then to depend on the fact that only the "poorly activated eggs" which extrude no polar bodies are the ones to divide. Thus the extent to which this relation is general for eggs of various forms will probably depend on whether or not the eggs that extrude both polar bodies divide. In eggs of the sea urchin type, where the polar bodies are extruded in the ovary and where cleavage is apparently possible with the haploid number of chromosomes, we might not expect this relation to hold.

In eggs of *Thalassema neptuni*, which, from the descriptions are very similar to the *Urechis* eggs, artificial activation by means of isotonic solutions has been reported by Hobson (1928). The variations of percentage of activation and of percentage of cleavage [6] are presented for several short series of exposures, but Hobson thinks that the results show an increase in cleavage with increase in activation. However, he notes (pp. 73 and 74) that the maximum of cleavage often fails to coincide with the maximum of activation, when both composition of medium and length of exposure are varied.

## Summary

1. The rate of increase in percentage activation of *Urechis* eggs with hypotonic sea water is shown to decrease as the concentration of sea water used is increased from distilled water to 80 per cent sea water.

2. The rate of increase in volume also decreases with increased concentration of sea water.

3. For dilutions of sea water ranging from 75 per cent to 45 per cent, the activation passes through a maximum (usually 100 per cent) and then returns to zero per cent with longer exposures. For lower concentrations of sea water the return to zero per cent is not obtained, but a high percentage of activation is maintained. With 80 per cent sea water the return to zero per cent activation also does not occur.

4. The activation-time curves for 75 per cent to 45 per cent sea water are of the form of skew distribution curves, rising rapidly to 100 per cent activation and falling more slowly to zero per cent.

5. The activation-volume curves are presented for 65 per cent to 45 per cent sea water and are of the form of a normal probability curve. They are roughly identical for the various dilutions of sea water.

6. Practically every series of experiments shows an inverse relation between the percentage of total activation and percentage of cleavage (of the activated eggs); so that as the percentage of activation increases with time of exposure, the percentage of cleavage decreases, and when

[6] Hobson's total activation does not include cleavage. It is not stated in the paper whether the percentage of cleavage is that of all the eggs or of the activated eggs, though it seems to be the former. When the data of his tables is recalculated on this basis, there are thirteen cases in which an increase (or decrease) in activation is accompanied by an increase (or decrease) in cleavage and six cases in which the inverse relation holds.

the percentage of activation decreases with exposure the percentage of cleavage increases.

7. The over-exposed unactivated eggs are still capable of fertilization and of producing normal embryos in spite of the fact that a shorter exposure would have resulted in their becoming activated upon return to normal sea water.

8. The variation in rate of activation with concentration of sea water, the type of activation-time curves, the activation-volume curves, and the fertilization of over-exposed eggs are shown to be interpretable on the basis of volume change occurring in the dilute sea water, a definite volume range being optimum for activation. The cleavage-activation relation is shown to be the outcome of the previously reported result that only the "poorly activated" eggs divide, and its interpretation, based also on the exposures producing such eggs, involves the assumption of a "sub-optimum volume range" on both sides of the optimum.

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