THE MECHANICAL PROPERTIES OF THE CELL SURFACE

III. THE SEA-URCHIN EGG FROM FERTILIZATION TO CLEAVAGE

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INTRODUCTION

In earlier papers (Swann, 1952; Mitchison, 1952) we put forward a new conception of the mechanism of cell division—the expanding membrane theory. This postulated an active expansion of the cell membrane or cortex as the immediate cause of cleavage; it implied that the cell membrane must be sufficiently rigid to expand in area without buckling, and that there could be little or no internal pressure in the cell during cleavage. We produced evidence in support of this theory from optical changes (Mitchison & Swann, 1952), from the effect of colchicine (Swann & Mitchison, 1953), and from micro-dissection (Mitchison, 1953). It was clearly important, however, to test a mechanical theory of this kind by measuring the mechanical properties of the cell membrane during cleavage. We therefore developed a method of measuring the properties of the cell membrane with an instrument we have called the 'cell elastimeter', which is described in the first paper of this series (Mitchison & Swann, 1954*a*). The second paper of the series (Mitchison & Swann, 1954*b*) described the application of this method to the unfertilized sea-urchin egg, and showed amongst other things that the cell membrane behaved as a relatively rigid structure of appreciable thickness. In the present paper we shall describe measurements of the stiffness and, indirectly, of the internal pressure of the sea-urchin egg from fertilization to the second interphase. We shall also briefly discuss these measurements in relation to other work, and to our own theory of the mechanism of cleavage. A full consideration of all the points involved will be published elsewhere.

STIFFNESS CHANGES DURING THE FIRST INTERPHASE AND DIVISION

The measurements of stiffness changes in fertilized sea-urchin eggs were made with the cell elastimeter using the experimental technique and methods of calculation described in the first and second paper of this series. The fertilization membranes were removed by passing the eggs through fine-mesh bolting silk about 2 min. after fertilization, and the eggs were measured both in ordinary sea water and in calcium-free artificial sea water, which removes the hyaline layer.

The results from a series of measurements on *Psammechinus miliaris* are given in Table 1. The eggs are grouped by stages of development and the stiffness is the

average value for each group. This stiffness is given in dynes/cm.$^2/\mu$ deformation corrected for the standard condition of a 100μ diameter egg and a 50μ diameter pipette.

A difficulty arises in the case of the eggs which were measured when they were in the process of cleaving. Strictly speaking, neither the model experiments nor the correction process described in the first paper are applicable to the irregular shape of cleaving eggs. We have, however, treated eggs at this stage as though they were spheres of the same diameter as eggs before cleavage (105μ). This is somewhat arbitrary, but no other possible treatment seems to be any better. The effect is to make the corrected stiffness values for eggs in the later stages of cleavage somewhat smaller than the true values. If, on the other hand, these eggs had been treated as spheres of the same diameter as the blastomeres in second interphase (83μ), the stiffness values would have come out greater than the true values and would have been 1·52 times the values given in Table 1.

Table 1. *Stiffness of the membrane during development*, Psammechinus miliaris

(Corrected stiffness in dynes/cm.$^2/\mu$ deflexion for 100μ diameter egg and 50μ diameter pipette)

Stage	Approximate times after fertilization (min.)	Ordinary sea water			Calcium-free sea water		
		Corrected stiffness	Young's modulus × 10^4 dynes/cm.^{2}	No. of eggs	Corrected stiffness	Young's modulus × 10^4 dynes/cm.^{2}	No. of eggs
Unfertilized	—	8·2	0·91	50	9·3	1·03	10
Early sperm aster	3–15	5·2	0·58	8	4·2	0·47	9
Late sperm aster	16–30	8·4	0·93	12	4·5	0·50	13
Streak	31–40	10·3	1·14	9	3·4	0·38	11
Prophase, metaphase, early anaphase	41–53	12·6	1·40	12	5·4	0·60	20
Mid anaphase	54–55	33·9	3·75	2	20·1	2·24	5
Late anaphase, early cleavage	56–58	61·3	6·81	9	57·0	6·32	8
Mid-cleavage, late cleavage	59–60	39·4	4·36	6	49·0	5·44	5
Interphase	61–	17·7	1·96	6	12·5	1·39	8

Note. (1) Average diameter of uncleaved eggs, 105μ, average diameter of blastomeres of cleaved eggs, 83μ. (2) Pipette diameter, 50μ. (3) The same batch of unfertilized eggs which gave the stiffness of 9·3 in calcium-free sea water, gave a stiffness of 9·5 in ordinary sea water.

The figures for Young's modulus are derived from the corrected stiffness values by the method described in the first paper. It should be emphasized that they are only approximate, and that they assume a membrane thickness of 1·6μ and no internal pressure in the egg.

The stiffness values from Table 1 shown are in graphical form in Fig. 1, where the stiffness for each stage is plotted against the central time for that stage. A dotted line (whose maximum height is uncertain) is put in immediately after fertilization. This is derived from the results given in the next section (p. 738).

The results in ordinary sea water show a sudden rise in stiffness at fertilization followed by a fall, during the early sperm aster stage, to the lowest value reached

during development. The stiffness then rises slowly until metaphase, after which it rises rapidly to reach a maximum during late anaphase and the early stages of cleavage. During the later stages of cleavage (when the furrow is cutting through the egg) the stiffness falls again and reaches a value in the second interphase which is more or less constant but is about twice as high as in the first interphase. The stiffness alters in all about twelvefold, the lowest value being in the early sperm aster stage, and the highest value in late anaphase (a change in Young's modulus from $0{\cdot}58 \times 10^4$ to $6{\cdot}81 \times 10^4$ dynes/cm.^{2}).

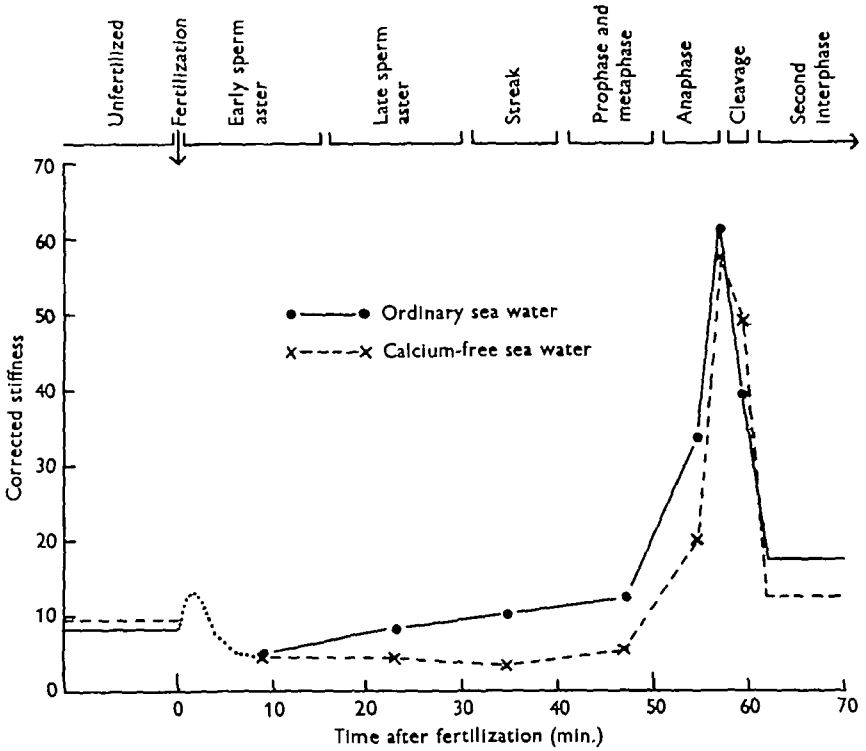

Fig. 1. Stiffness changes during first interphase and division. Eggs of *Psammechinus miliaris*. Corrected stiffness is in dynes/cm.$^2/\mu$ deformation for 100μ diameter egg and 50μ diameter pipette.

The results in calcium-free sea water show the stiffness changes after the removal of the hyaline layer which appears on the outside of fertilized eggs during the sperm aster stage. There is no significant difference between the values in ordinary and in calcium-free sea water when the hyaline layer is not present, i.e. during the early sperm aster stage and in the unfertilized egg. As the hyaline layer develops, eggs in normal sea water become progressively stiffer than naked eggs, the greatest absolute difference (13·8 units) occurring in mid-anaphase. In the later stages of cleavage the naked eggs give a higher stiffness value, but this is almost certainly due to the errors inherent in averaging on the rapidly falling curve. The naked eggs thus show the same general changes in stiffness as the eggs in ordinary sea water, except for the

absence of the slow rise up to metaphase. One can assume therefore that this slow rise is due to the development of the hyaline layer, which acts as an additional elastic membrane on the surface of the cell.

Although we have only presented one set of results on the fertilized eggs of *P. miliaris*, we have made a large number of similar measurements on the eggs of this species and of *P. microtuberculatus* and *Paracentrotus lividus*. These have all shown changes in stiffness similar to those illustrated in Fig. 1.

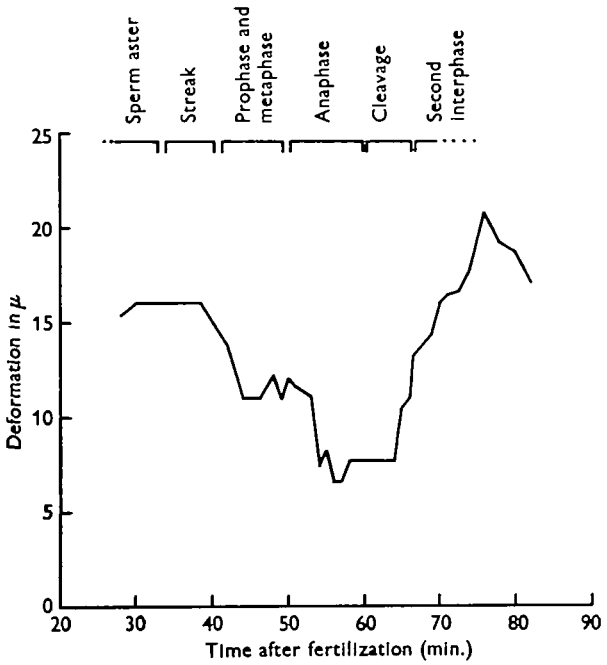

Fig. 2. Changes of deformation with constant pressure during first interphase and division. Egg of *Psammechinus miliaris*.

It might be expected, particularly at cleavage, that there would be variations in stiffness between different regions of the cell surface. We have looked repeatedly for such variations but have never found them at any stage, and there seems no doubt that the poles and the furrow regions of the dividing egg (at any rate during the early stages of cleavage) have similar mechanical properties at any given time. This is in agreement with the findings of Selman & Waddington (1955) on the very much larger eggs of amphibians. It is still possible, however, that the membrane in the depths of the furrow during late cleavage may have different mechanical properties, since this region cannot be reached with the elastimeter pipette.

An alternative method of investigating the changes in the membrane, is to keep an egg on the pipette with a constant pressure and follow the changes in deformation over a period of time. An example of such an experiment (a 'constant pressure run') is shown in Fig. 2, for a fertilized egg of *Psammechinus miliaris* in calcium-free sea

water with a 41 μ pipette and a pressure of 6 dynes/cm.^{2}. The deformation here behaves in the opposite way to stiffness, and it can be seen that there is a marked drop in the deformation during anaphase and the early part of cleavage. The advantage of this method is that it is easy to get the exact time relations of the changes in the membrane. For instance, this particular example shows a characteristic feature of other constant pressure runs, namely that the reduction in deformation (corresponding to the increase in stiffness) starts during prophase and reaches its minimum value in the middle of anaphase, *before* the start of cleavage. These constant pressure runs, however, suffer from the serious disadvantage that they do not give quantitative values for stiffness, and for this reason we have not used them to any great extent.

STIFFNESS CHANGES AT FERTILIZATION

It is impossible to make direct measurements of stiffness during the fertilization of normal eggs because of the fertilization membrane. As soon as the eggs are fertilized the stiffness rises to a high value, and the effect of the cell membrane cannot be separated from that of the fertilization membrane which is much stiffer than the interphase egg. If the fertilization membranes are stripped off with bolting silk, measurements cannot be made until at least 3 min. after fertilization, since the membranes must be fully elevated before stripping, and this takes about 2 min. The only way round this difficulty is to use eggs where the elevation of the fertilization membrane has been inhibited by previous treatment with trypsin. Runnström, Monné & Broman (1943), who discovered this effect, believe that it is due to the action of trypsin in digesting away the vitelline membrane, which acts as the precursor of the fertilization membrane. Whether or not there is a morphologically separable vitelline membrane, it is certainly true that trypsin causes a large and irreversible drop in the membrane stiffness of unfertilized eggs (down to $\frac{1}{4}$–$\frac{1}{8}$ of normal eggs). This effect will be described in more detail in a subsequent paper on the effect of chemical agents on the unfertilized egg. After fertilization, trypsin-treated eggs develop and cleave normally.

Experiments were done with the eggs of *P. microtuberculatus* which had been treated, when unfertilized, with a solution of 0·1 % by weight of trypsin in sea water for 5 min. These eggs were fertilized on the elastimeter slide, and stiffness measurements made as soon as possible. All the eggs showed a sharp rise in stiffness, which reached a peak of 4–5 times the value of the unfertilized trypsin-treated eggs within the first 3 min. after fertilization. In the next 5 min. the stiffness fell back gradually to a value slightly below that for normal eggs at the sperm aster stage. Fig. 3 shows the results from four typical eggs, together with the corrected stiffness values for trypsin-treated unfertilized eggs, for normal unfertilized eggs, and for normal fertilized eggs at the sperm aster stage. The precise position of the height and timing of the peak value for stiffness is uncertain, since it is impossible to make instantaneous measurements at fertilization. The peak, therefore, may be higher and come nearer to the moment of fertilization than the graphs indicate. These results are incorporated in Fig. 1 as the dotted line in the first part of the curve.

It should be mentioned that constant pressure runs on trypsin-treated eggs give substantially similar results, with a drop in the deformation soon after fertilization.

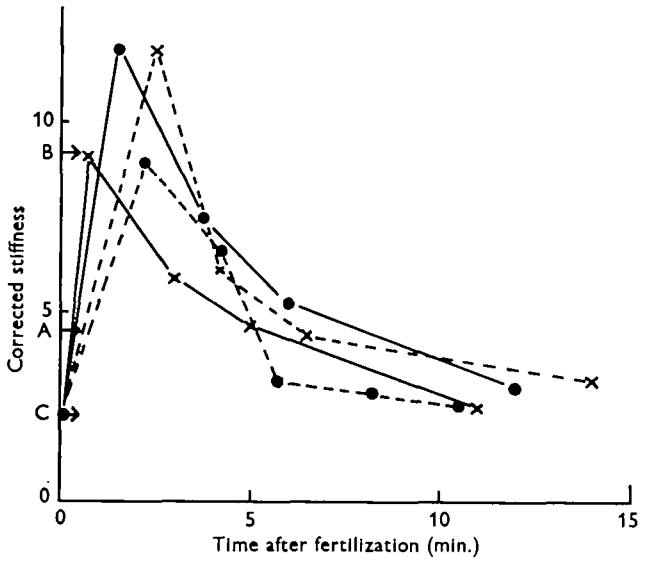

Fig. 3. Stiffness changes at fertilization. Four eggs of *Psammechinus microtuberculatus*. A, stiffness of normal unfertilized eggs; B, stiffness of unfertilized eggs treated with trypsin; C, stiffness of normal fertilized eggs at sperm aster stage. Corrected stiffness is in dynes/cm.$^2/\mu$ deformation for 100μ diameter egg and 50μ diameter pipette.

MEASUREMENTS ON SWOLLEN AND SHRUNKEN EGGS

For a correct interpretation of stiffness values, some knowledge of the internal pressure of the egg is needed. This problem is discussed in the second paper of the series, on the unfertilized egg (Mitchison & Swann, 1954*b*), and the reader should refer to this paper for a fuller description of the methods which can be used to give an indication of internal pressure. Only a summary of the methods will be given below.

It should be possible to determine whether or not an egg has an internal pressure (and, therefore, a resting tension in the membrane) by measuring the stiffness when the egg has been shrunk in a hypertonic solution. If there is pressure initially, the stiffness should fall, while if there is no pressure the stiffness should remain more or less constant. In either case, if the egg is swollen in a hypotonic solution the stiffness should rise.

Stiffness measurements on swollen or shrunken eggs must be made at a stage when the normal stiffness is known fairly accurately. It is only possible, therefore, to use two stages in the fertilized egg: the sperm aster stage, where the stiffness remains relatively constant for a long time; and the late anaphase or the 'wall-sided' stage of early cleavage, where the equatorial region of the egg is straight-sided and the exact stage of the egg is accurately known. Stiffness measurements were accordingly made

on these two stages after the eggs (*P. microtuberculatus*) had been immersed for periods of 2–10 min. in the following media:

		Approx. molarity
'Full hypotonic'	50 ml. Ca-free sea water + 20 ml. dist. water	0·39
'Half hypotonic'	50 ml. Ca-free sea-water + 10 ml. dist. water	0·45
Normal Ca-free sea water		0·54
'Quarter hypertonic'	50 ml. Ca-free sea water + 5 ml. 2M-NaCl	0·67
'Half hypertonic'	50 ml. Ca-free sea water + 10 ml. 2M-NaCl	0·78
'Full hypertonic'	50 ml. Ca-free sea water + 20 ml. 2M-NaCl	0·96

The half and full hypertonic media were not used with the dividing eggs since they inhibited cleavage.

The results for late anaphase and early cleavage eggs are given in the first half of Table 2 and Fig. 4. It can be seen that there was a rise in stiffness, both in the hypotonic and the hypertonic media. Owing, however, to the rapid changes in stiffness that take place at this stage, there was a wide scatter in the measurements (see the last column of Table 2). A t test ($P=0{\cdot}05$) shows that, although the difference between the stiffness in full hypotonic and in normal sea water is significant, the other differences are not. We can conclude, therefore, that the cleavage eggs were behaving as though they had no internal pressure in ordinary sea-water; they showed a rise in stiffness when swollen, but no significant change in stiffness when shrunk.

The results for the eggs at the sperm aster stage are given in the second half of Table 2 and Fig. 5, and all the differences are significant ($P=0{\cdot}05$) except those between eggs in half hypertonic or half hypotonic and eggs in normal sea water. Although these results show a fall in stiffness in hypertonic media (as would be expected if there was an internal pressure in the normal egg at this stage), they also show a fall in hypotonic media. This experiment has been repeated twice, and in each case has given results similar to those in Table 2. The fall in stiffness in hypotonic media is an anomalous result if the egg is behaving like a simple elastic walled ball, since, whatever the normal pressure, the stiffness should increase when the egg is swollen. These results are discussed later (p. 744), and a possible explanation for them is given.

A direct method, of quite a different sort, was used to set an upper limit for the possible initial stretch of the membrane. The existence of an internal pressure implies, of course, that there is such an initial stretch. Eggs of *P. microtuberculatus*, at the sperm aster stage (15 min. after fertilization) and at late anaphase, were placed in hypertonic solutions of different strengths and were examined under the microscope after about 3 min. in order to find the smallest degree of hypertonicity which caused a visible wrinkling of the membrane. These solutions were found to be 50 ml. sea water + 5 ml. 2M-NaCl for the sperm aster eggs, and 50 ml. sea water + 15 ml. 2M-NaCl for the late anaphase eggs. Eggs from the same female were also photographed in these solutions and subsequently measured, together with controls in normal sea water. Figures are given in Table 3 for the mean diameters (mean of major and minor axes) of fifty eggs, for the standard deviation of the mean diameters and for the average ellipticity (major axis/minor axis).

Table 2. *Stiffness of swollen and shrunken eggs*, Psammechinus microtuberculatus

Stage	Medium	Molarity	No. of eggs	Average diameter (μ)	Corrected stiffness (dynes/cm.^{2}/μ deflexion for 100μ egg and 50μ pipette)	
					Mean	Standard deviation
Late anaphase and early cleavage	Full hypotonic	0·39	37	119	56·0	29·9
	Half hypotonic	0·45	19	115	36·5	22·5
	Normal	0·54	15	110	34·4	16·6
	Quarter hypertonic	0·67	18	105	45·9	19·8
Sperm Aster	Full hypotonic	0·39	16	119	2·04	0·76
	Half hypotonic	0·45	14	115	3·67	0·94
	Normal	0·54	5	110	5·04	0·89
	Half hypertonic	0·78	13	102	4·41	1·69
	Full hypertonic	0·96	15	96	2·41	0·36

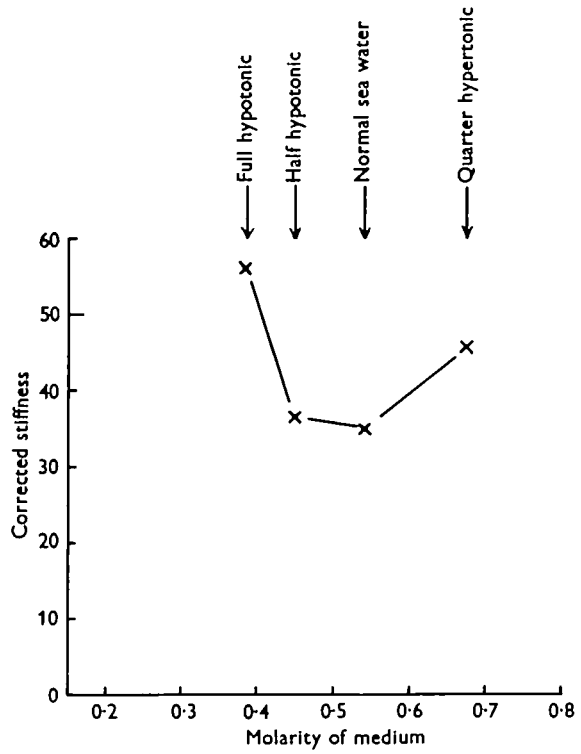

Fig. 4. Stiffness of swollen and shrunken eggs during later anaphase and wall-sided stages. Eggs of *Psammechinus microtuberculatus*. Corrected stiffness is in dynes/cm.^{2}/μ deformation for 100μ diameter egg and 50μ diameter pipette.

These results show that wrinkling first occurred when there had been a 3·5% linear shrinkage with the sperm aster eggs and a 9·0% shrinkage with the anaphase eggs. Since wrinkling cannot take place when there is an internal pressure, the membrane cannot normally be stretched by more than 3·7% (linear) from the resting state in the case of sperm aster eggs, and 9·7% in the case of anaphase eggs.

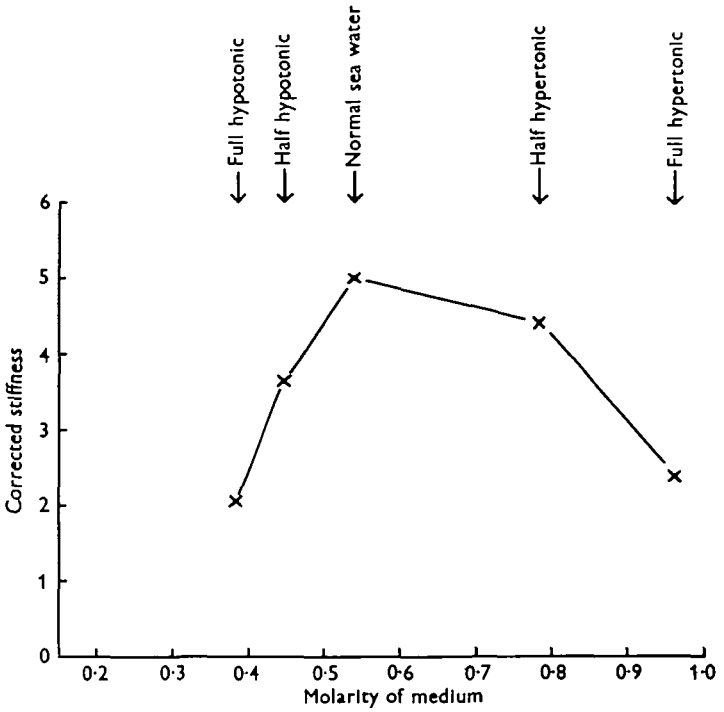

Fig. 5. Stiffness of swollen and shrunken eggs during the sperm aster stage. Eggs of *Psammechinus microtuberculatus*. Corrected stiffness is in dynes/cm.^{2}/μ deformation for 100 μ diameter egg and 50 μ diameter pipette.

Table 3. *Wrinkling of fertilized eggs*, Psammechinus microtuberculatus

Stage	Medium	Average diameter (μ)	Standard deviation (μ)	Average ellipticity
Sperm aster	Normal sea water	113	5·5	1·025
Sperm aster	50 ml. sea water + 5 ml. 2M-NaCl	109	6·9	1·027
Late anaphase	50 ml. sea water + 15 ml. 2M-NaCl	103	11·3	1·038

By combining these figures with the stiffness values from Table 1 and with the data given in fig. 16 of the first paper, we can now set an upper limit for the possible internal pressure in normal eggs. During the early sperm aster stage, the maximum possible internal pressure is 19 dynes/cm.^{2}, which would reduce the value for Young's modulus from $0{\cdot}58 \times 10^4$ to $0{\cdot}48 \times 10^4$ dynes/cm.^{2}. During late anaphase, the maximum possible pressure is 500 dynes/cm.^{2}, and this would reduce the value

for Young's modulus from $6{\cdot}81 \times 10^4$ to $4{\cdot}35 \times 10^4$ dynes/cm.^{2}. It must be emphasized that these pressure values are only upper limits and do not imply that there is necessarily any pressure at all. It may also be noted that there are at least two reasons for supposing that these experiments may overestimate the degree of shrinkage before wrinkling, and therefore the internal pressure. In the first place, the earliest signs of wrinkling are very difficult to detect under the microscope, and may occur with even smaller amounts of shrinkage than those found above. Secondly, it is probable that the initial effect of reducing the volume of a slightly elliptical body like an egg will be to increase the ellipticity rather than to produce wrinkling. Such an increase was in fact found in these experiments, as can be seen from Table 1.

DISCUSSION

I. *The experimental results*

The elastimeter measurements have shown that there are large changes in the stiffness of fertilized eggs during the division cycle. We have assumed that these represent changes in the elastic (Young's) modulus of the membrane, but there are three other factors which could also affect the measured stiffness. These are changes in the membrane thickness, changes in the internal pressure, and the existence of internal cytoplasmic rigidity, e.g. asters. Each of these factors will be discussed below.

It has been assumed, for the purpose of calculating Young's modulus, that the cortical thickness stays constant at about $1{\cdot}6\mu$. Changes in thickness would, of course, produce changes in the measured stiffness, but the thickness would have to increase about eightfold, i.e. from 1·6 to 12μ, to account for the twelvefold increase of stiffness between the sperm aster and the anaphase egg (see first paper, figs. 11 and 12). In fact there is no evidence at all of such a change, and Mitchison (1956) concludes that the membrane is roughly $1{\cdot}5\mu$ thick throughout the first interphase and division. The only evidence that conflicts with this is the observation by Chambers (1938*a*), that an oil drop in a cleaving *Lytechinus* egg is indented about 5μ ahead of the advancing furrow. This large figure for the membrane thickness may be due to different conditions in another species of sea urchin (Chambers finds that the experiment does not work with *Arbacia*), or it may simply be an overestimate caused by the difficulty of making accurate optical observations in the narrow furrow neck.

The presence of the asters (sperm aster and amphiaster) within the egg may also affect the elastimeter readings. From microdissection experiments asters are known to behave as discrete solid bodies, so that, unless care is taken, a deformation of the egg designed to measure the mechanical properties of the membrane may in fact be measuring the properties of the asters. This seemed to us a serious objection to Cole's (1932) method of measuring membrane properties by means of large compressions, and we designed the elastimeter to get over this difficulty as far as possible. The maximum deformation used with the elastimeter (a hemispherical bulge) only involves about a 5% increase in the surface area of the membrane, and it is very unlikely that an aster could affect the readings unless it completely filled the whole

cell and was attached to the membrane. Despite the arguments of Dan (1943), it seems that the asters are *not* attached to the surface since they can be moved about both by microdissection needles and by centrifuging (Harvey, 1935). It is also apparent in our own time-lapse films that the granules in the surface of a cleaving egg move independently of the asters. A further piece of evidence which suggests that the asters are not involved in the stiffness measurements is that the lowest stiffness values are reached during the sperm aster stage when there is an aster filling most of the cell. The stiffness only begins to rise markedly in metaphase when the sperm aster has disappeared and the amphiasters are so small that they could not affect the elastimeter readings. This is the exact reverse of what would be expected if the asters were responsible for the stiffness. Admittedly, the highest stiffness values are reached in late anaphase when the amphiasters are at their maximum size, but a similar rise in stiffness is shown at the equivalent time in eggs where the asters are suppressed by the action of colchicine (Swann & Mitchison, 1953). The peak stiffness of eggs in colchicine is not as high as in normal cleaving eggs, but it seems likely that colchicine weakens the membrane as well as destroying the asters. In conclusion, we believe that the asters are not affecting the stiffness measurements, though we cannot produce definite evidence at all stages. Ideally, we should like to make completely separate measurements on the membrane and the asters in a normal cleaving egg, but it would be difficult to do so.

The third factor which may affect the stiffness measurements, namely internal pressure, raises the most difficult problem of all. We have shown in the first and second papers that for any given stiffness value there is a series of solutions for Young's modulus and internal pressure varying from no internal pressure and a relatively high modulus to a high internal pressure and a relatively low modulus. The measurements on the wrinkling point set an upper limit to the possible internal pressure, and, in the case of the sperm aster stage, show both that the maximum possible pressure is very low (19 dynes/cm.^{2} or 1/50,000 of an atmosphere) and that there is only a small difference between the possible limits of the modulus ($0{\cdot}58 - 0{\cdot}48 \times 10^4$ dynes/cm.^{2}). In the case of the late anaphase eggs, however, although the limits of the modulus are still fairly close ($6{\cdot}81 - 4{\cdot}35 \times 10^4$), the maximum possible pressure is relatively large (500 dynes/cm.^{2} or 1/2000 of an atmosphere) because of the much higher absolute value of the modulus. This is not very informative if we wish to know the pressure changes at cleavage (which may be important in deciding between certain theories of cell division) since, as should be emphasized again, the method only sets an upper limit. The only other information about the internal pressure comes from the stiffness changes in hypo- and hypertonic media. With the anaphase and early cleavage eggs, the results seem to show that there is no internal pressure since there is no significant fall in the stiffness in hypertonic solution. Exactly comparable results were found for the unfertilized egg, as described in the second paper of the series. We have, however, to take account of the anomalous result with sperm aster eggs, which showed a fall in stiffness when swollen in hypotonic media. The most likely explanation of this result appears to be that the membrane itself is becoming hydrated and therefore weaker in hypotonic

media, and that this weakness more than compensates for the increased stiffness caused by the stretching. Another possibility is that the weak membrane at this stage of development reaches a 'yield point', analogous to that described in the first paper, after only a small degree of stretch. In view of this effect, however, we do not feel justified in drawing any conclusions about the fall in stiffness of the shrunken sperm aster eggs in hypertonic media, which would otherwise indicate a positive pressure in the normal egg. Although these anomalous effects only occur when the membrane has the very low stiffness values characteristic of the sperm aster stage, they inevitably throw some doubt on the validity of the other stiffness measurements made in hypo- and hypertonic media on unfertilized and cleaving eggs.

Even if the maximum possible pressure were present within the eggs, it is clear that the Young's modulus of the membrane would still follow a curve very similar in shape to that given in Fig. 1. The peak value in late anaphase and early cleavage would be reduced by 36%, and the trough at the sperm aster stage would be reduced by 17%. We cannot say definitely what would happen to the values at other stages, but it seems very likely that they would be scaled down proportionately.

In conclusion, it is evident from the wrinkling experiments, that the maximum possible pressure at the sperm aster stage is very low, but it is difficult to be certain of the pressure in the cleaving egg. Wrinkling point experiments set a relatively high upper limit for pressure at cleavage, though stiffness measurements on shrunken eggs suggest that there is in fact no pressure at this stage. But the anomalous results of comparable experiments on the sperm aster stage throw some doubt on all the stiffness measurements on shrunken eggs.

The only means of settling this problem finally would be to make direct measurements of internal pressure, but the values are so low in absolute terms that this would be very difficult. Measurements have, in fact, been made of the internal pressure of other eggs by inserting into them a micropipette connected to a manometer, but they have mostly given very high and, we suspect, quite misleading values. We have found from our own experience that there are two sources of error in such measurements which are very difficult to overcome; the tendency of the cytoplasm to clog the pipette, and the inadequacy of the seal between the pipette and the cell surface.

II. *Earlier work on the mechanical properties of fertilized egg membranes*

One of the earliest attempts at measuring changes in the mechanical properties of the cell surface during the division cycle was that of Vlès (1926), who observed the degree of flattening of the sea-urchin egg under gravity, and calculated from this the surface tension using a 'sessile drop' formula. He found a decrease after fertilization and a sharp rise at about metaphase, followed by a fall during and after cleavage. His absolute values lie between 10 and 20 dynes/cm., and are 100 or more times greater than the values found by Cole (1932) and others. However, McCutcheon, Lucké & Hartline (1931), and we ourselves, have been unable to detect any flattening under gravity, while Harvey & Fankhauser (1933) have pointed out that

the formula used by Vlès to calculate surface tensions is in any case wrong. It seems fairly certain, therefore, that Vlès's results are erroneous.

Harvey & Fankhauser (1933) applied the same method as Vlès to salamander eggs, though using another formula. They found a fall in tension at fertilization, with a slight rise before the first cleavage. But they mention that these results may be vitiated by changes in cytoplasmic gelation, and in the density of different regions of the egg. In any case, as Harvey & Danielli (1938) point out, no sessile drop formula is strictly applicable to a cell with an elastic membrane.

Cole & Michaelis (1932) applied Cole's (1932) gold-beam method to fertilized *Arbacia* eggs, but somewhat surprisingly found no changes during the mitotic cycle. There are, however, two reasons why these results cannot be regarded as conclusive. First, the experiments were done, as the authors point out, on unsatisfactory eggs at the end of the season. Secondly, this method of measurement involves large compressions of the eggs and is probably inaccurate when they contain asters.

The remaining attempts to measure the mechanical properties of the surface have involved centrifugation. Harvey (1933) measured the ease with which *Arbacia* eggs could be broken in two by centrifuging, and found that the strength of the surface declined within a few seconds of fertilization, continued to decline for some minutes, and then later rose again. This method, however, relies on first stratifying the cytoplasm, and is therefore sensitive to variations in cytoplasmic gelation as well as the strength of the surface. There is also an additional complication in *Arbacia* due to the increase at fertilization of the proportion of the dense pigment granules which are caught in the cortex. This should diminish the apparent ease of splitting. Somewhat similar experiments have been carried out by Heikens (1947) using *Limnaea* eggs. He found that 'tension at the surface', expressed as the ratio of breadth to length in centrifuged eggs, was low during polar body extension, and shortly before cleavage, but rose during and after cleavage. Here again the results are inevitably confused by the state of the cytoplasm and the ease with which it can be stratified.

A different technique was employed by Brown (1934), who measured the centrifugal force necessary to dislodge the pigment granules from the cortex of *Arbacia* eggs, and move them to one side of the cell. He expressed his results in terms of relative units of viscosity, though pointing out that this is not strictly appropriate. He found a small peak of viscosity in the early stages of mitosis, and a sharp rise just before cleavage.

Brown's results have been criticized by Wilson (1951), on the grounds that many of the pigment granules in *Arbacia* are not contained in the cortex, and that the method really measures a combination of cortical and cytoplasmic rigidity. Wilson himself has attempted to get over this difficulty by observing the cortical granules themselves in the eggs of *Chaetopterus*, rather than noting the appearance or otherwise of granules at the centrifugal pole of the egg. There were no very striking changes in cortical rigidity, though there was a slight fall after fertilization. Rigidity appeared to be constant before and after cleavage.

Marsland (1951) used the same criterion as Wilson for observing the changes in cortical rigidity in *Arbacia* eggs, though in some earlier papers he had used Brown's criterion. He found a tenfold increase in the centrifugal force required to displace the cortical granules in a cleaving egg as compared with an unfertilized egg. This is in agreement with the increase in rigidity at cleavage which we find with the elastimeter, but is in direct opposition to Wilson's results. The difference may be due to the material used, though it seems unlikely that there could be such a fundamental difference in the mechanical properties of two eggs which are fairly similar in size, appearance, and general behaviour.

It is not altogether clear what property is measured by the force necessary to shift cortical granules. It is likely to be related to rigidity, but, as Wilson points out, it is more akin to shear strength. It may perhaps bear some relation to the yield point we have noticed in our own experiments. In any case, it must be emphasized that the measurements will be sensitive to changes in size and density of the granules themselves, and it is perhaps wiser to suspend judgement on the results until we have a better idea of what mechanical property is being measured.

A short but interesting series of experiments was carried out by Danielli (1952), who compressed dividing sea-urchin eggs beneath a fragment of cover-slip, and found that shortly before cleavage they exerted sufficient force to raise it. He has interpreted this as a rise in membrane tension, but it should be pointed out that such a conclusion does not necessarily follow since the rise in Young's modulus which we have found, would produce the same result without any rise in tension or internal pressure. For example, a punctured tennis ball, compressed by a weight, will raise the weight if the Young's modulus of the rubber is increased by moderate heating. Since there is a hole in the ball, there can be no increase in the internal pressure. It should be mentioned at this point that the decrease in the deformation at cleavage during the constant pressure runs (p. 737) is analogous to Danielli's experiment.

It is evident that these experiments cannot be regarded as wholly satisfactory, since none of them make it possible to distinguish between tension and rigidity in the membrane. Nevertheless, there does seem to be a certain consensus of opinion that, as we have found, the strength of the cell surface declines during the first interphase, and increases at cleavage.

III. *Relevance to theories of cleavage*

A full discussion of the relevance of these results to the various theories of the mechanism of cleavage would take too much space for the present paper. Nevertheless, it will be apparent without going into details that the main result of this work, the rise in Young's modulus over most if not all of the cell surface at the time of cleavage, is not what would be expected on the basis of some of the theories.

For instance, theories that ascribe the active role in cleavage to the mitotic figure, and a merely passive one to the cell surface (e.g. Gray, 1924; Dan, 1943), would not be expected to lead to such striking changes in Young's modulus as we have found

nor should they lead to changes beginning before the visible onset of cleavage. Still less would the surface be expected to become about ten times more rigid at the very moment when it has to be passively extended. Moreover, cleavage by this means should lead to a tension in the surface and the evidence, such as it is, points to there being no tension at this time. We believe also that our earlier work on cleavage without asters in the presence of colchicine (Swann & Mitchison, 1953) and on the timing of optical changes in the cell surface (Mitchison & Swann, 1952) provide strong evidence against this type of theory.

Somewhat similar arguments tell against the group of theories that suppose cleavage to be brought about by a contractile ring in the cell surface in the region of the furrow (e.g. Chambers, 1938*b*; Lewis, 1951; Marsland, 1951). There should, for instance, be evidence of different mechanical properties between the equatorial and polar regions of an egg that is cleaving, or about to cleave, but we have found no such evidence, nor have Selman & Waddington (1955) using our method on the very much larger amphibian egg. As before, it is highly improbable that the *whole* surface should get ten times stiffer at the very time when all but the equatorial ring has to be passively extended. The tension in the surface should also rise sharply, and while we have not been able to dispose of this possibility altogether, the evidence is certainly against it. Finally, there is our earlier work (Mitchison, 1952, 1953; Mitchison & Swann, 1952) on surface movements, optical changes and micro-dissection, which we believe also tells against any form of contractile ring theory.

The last group of theories depends on the expansion of the cell surface, either by growth (e.g. Schechtman, 1937; Selman & Waddington, 1955) or by molecular reorientation (Mitchison, 1952; Swann, 1952). Our present findings are not inconsistent with either of these two types of mechanism. Neither scheme should lead to an increase of tension in the surface. And while there is perhaps no obvious reason why growth should be associated with changes in rigidity, it might be argued that since growth is the result of an increased tendency to molecular aggregation, it should perhaps lead to an increased Young's modulus (compare, for example, the growth of the mitotic figure, or the growth of a crystal from the mother liquor). In the case of the 'expanding membrane' theory of cleavage, an increase in rigidity is very much to be expected, since the whole surface has to increase in area and force the furrow inwards.

Further discussion on the growth and expansion theories involves a detailed consideration of a number of points, and for this reason we propose to leave it to a later paper. It may be noted, however, that even on our own theory of expansion, there must be growth at some stage if the cell cortex is not to get progressively thinner. We are therefore inclined to consider the possibility of combining the two points of view, expansion by growth, and expansion by molecular reorientation, into a single theory. In any particular type of cell the contributions of the two mechanisms to cleavage would no doubt vary. In the case of the sea-urchin egg we believe that the evidence from the cell elastimeter, from surface movements and from optical changes all point to expansion by molecular reorientation over most of the egg

surface being the more important process. In the very much larger amphibian egg, on the other hand, cleavage may be largely brought about by growth in the furrow.

SUMMARY

1. Measurements with the cell elastimeter on the stiffness of the cell membrane of fertilized sea-urchin eggs show the following general features. There is a sudden rise at fertilization, followed by a fall during the early sperm aster stage to the lowest value reached during development (a Young's modulus of about $0{\cdot}58 \times 10^4$ dynes/cm.^{2}). The stiffness rises slowly until metaphase, after which it rises rapidly to reach a maximum during late anaphase and early cleavage ($6{\cdot}81 \times 10^4$ dynes/cm.^{2}). During the later stages of cleavage the stiffness falls again and reaches a value in the second interphase which is about twice as high as in the first interphase. Measurements on naked eggs in calcium-free sea water indicate that the slow rise in metaphase is due to the development of the hyaline layer.

2. Measurements on swollen and shrunken eggs at cleavage indicate that there is no internal pressure in the eggs at this stage, but similar experiments with eggs at the sperm aster stage yield anomalous results. Observations on the wrinkling point in shrunken eggs show that the *maximum possible* internal pressure is 19 dynes/cm.^{2} for sperm aster eggs and 500 dynes/cm.^{2} for cleaving eggs.

3. The bearing of these results on various theories of the mechanism of cleavage is briefly discussed. The rise in Young's modulus of the whole cell surface at cleavage argues against theories depending on the action of the spindle and asters, and against theories proposing a contractile ring in the surface. The rise is, however, what might be expected on the basis of the expanding membrane theory.

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ADDENDUM

Since completing this work, we have read an interesting paper by Vlès (1933) on the mechanical deformation of sea-urchin eggs. Vlès used an apparatus which was similar in principle to our cell elastimeter, but he was concerned only with very great deformations when the egg was either largely or completely sucked up into the pipette. Using an approximate method, he arrived at a value of about 10^3 dynes/cm.^{2} for the Young's modulus of the cell surface of unfertilized eggs. This is about one-tenth of the value we have found, but, as Vlès himself pointed out, his method only gave an estimate of the order of magnitude of the modulus. He also made a series of measurements on the return of the deformed egg to its original shape, and some observations on fertilized eggs which had been deformed within their fertilization membranes.

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