

## TENSION AT THE SURFACE OF THE DIVIDING SEA-URCHIN EGG

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Several theories have been put forward to explain the mechanics of cleavage of animal cells. According to the expanding membrane theory of Mitchison and Swann (Mitchison, 1952; Mitchison & Swann, 1955; Swann & Mitchison, 1958), the cleaving furrow is passively pushed in as a result of 'active' expansion of poles; while the constricting ring theory (Marsland, 1950, 1956*a, b*) anticipates that the furrow region has independent power to contract. A third alternative is 'astral relaxation theory' of Wolpert (1960), who advocates that cleavage is initiated by relaxation of the membrane in the polar region which allows the furrow to contract.

In order to scrutinize these theories it would be most important to collect information about the mechanical properties of the cell surface during cleavage. In fact, measurements have already been made by several workers, with a view to obtaining some quantitative data on the physical properties of the cell surface (Mitchison & Swann, 1955; Wolpert, 1963, 1966; Hiramoto, 1967, 1968).

The aim of the present paper is to give a fuller account of the time-course of the change in the tension at the surface of the sea-urchin eggs at the time of cleavage. Preliminary notes on this experiment have been presented elsewhere (Dan, 1963).

### MATERIALS

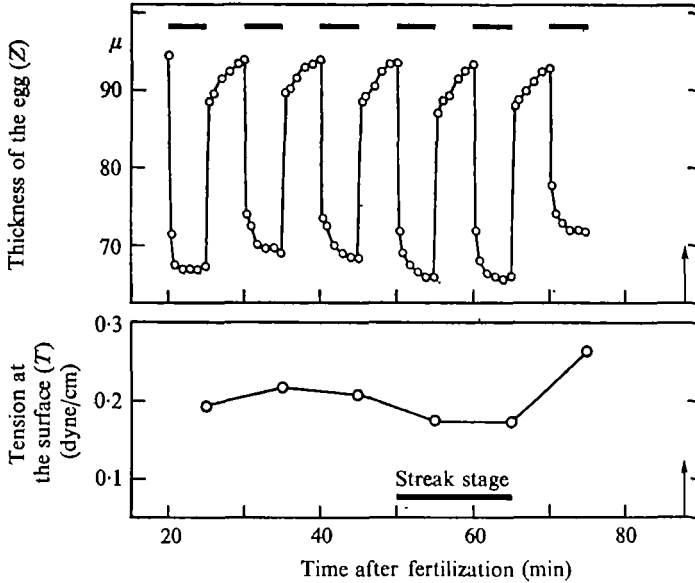
Fertilized eggs of the sea-urchins *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus* and *Temnopleurus hardwickii* were used. Eggs were deprived of the fertilization membrane and the hyaline layer by treatment with 1 M urea following fertilization, and were kept thereafter in sea water.

### METHODS

Compression method developed by Cole (Cole, 1932; Cole & Michaelis, 1932) was employed to measure the mechanical property of the cell surface. Details of the experimental arrangement are similar to those used for unfertilized eggs (Yoneda, 1964) and a brief account of the principal features of the experimental arrangement will be given here.

A pair of small pieces of coverglass are attached to the ends of two glass beams, one on each beam. One of the beams is very flexible and acts as a bending balance for measuring the force of compression. The compliance of the balance has been

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Text-fig. 1. Upper-curve: thickness  $Z$  of a fertilized egg of *Hemicentrotus pulcherrimus* compressed under the force of  $1.4 \times 10^{-3}$  dyne for 5 min with 5 min recovery in six repetitions (marked by horizontal lines); arrow indicates the appearance of the cleavage furrow.  $14^\circ\text{C}$ . Lower curve: tension at the surface calculated from data shown in the upper curve.

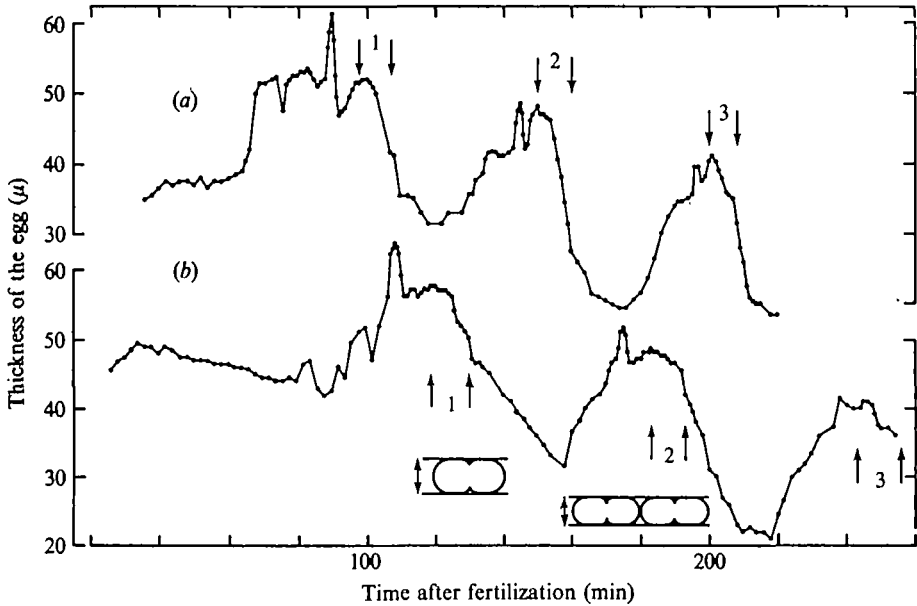
determined beforehand ( $10^4 \sim 10^5 \mu\text{m}/\text{dyne}$ ). Each beam is mounted on a micro-manipulator in such a way that the surfaces of the coverglass are horizontal and accurately apposed to each other. These are submerged in sea water filling a trough mounted on the stage of a horizontal microscope. One fertilized egg at a time is compressed between the pair of coverglasses. The force of compression is equal to the compliance of the bending balance multiplied by the yielding of the beam, the latter being read by an ocular micrometer. Observations are made with a  $\times 20$  objective of long working distance (Nikon) and a condenser of long focal length. The camera used for photography is an Olympus PM 6.

#### RESULTS

The upper curve in Text-fig. 1 illustrates the time-course of the change in thickness ( $Z$ ) of the egg when subjected to a constant force of  $1.4 \times 10^{-3}$  dyne for 5 min in six repetitions during the period from fertilization to the first cleavage. The time required for equilibrium is 3–5 min, being comparable to that for unfertilized eggs (Yoneda, 1964; Hiramoto, 1963). The equilibrium thickness,  $Z$ , divided by the initial diameter of the egg,  $Z_0$ , will be denoted by relative thickness,  $z$ , which serves as a semiquantitative estimate of the tension at the surface, i.e. higher  $z$  means higher tension at the surface. The absolute value of the tension  $T$  for spherical eggs can be calculated by the equation

$$F = \pi Z_0 T (-ds/dz), \quad (1)$$

where  $F$  is the force of the compression and  $s$  is the ratio of the surface area under compression to the initial surface area (relative surface area). Values of  $-ds/dz$  have

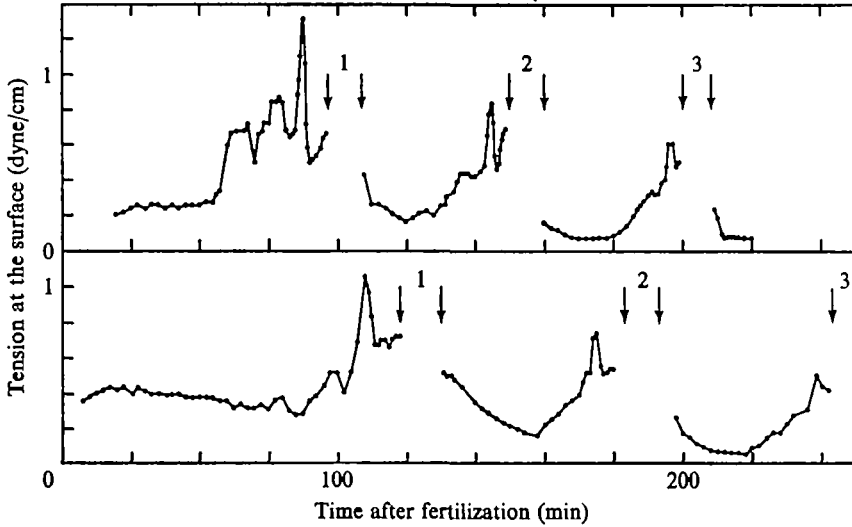


Text-fig. 2. Thickness ( $Z$ ) of eggs under lateral compression. Pairs of arrows indicate the start and termination of the first, second and third cleavage in *Hemicentrotus pulcherrimus*. Two examples are shown: (a)  $13^{\circ}\text{C}$ ,  $Z_0 = 98\ \mu\text{m}$ . (b)  $12^{\circ}\text{C}$ ,  $Z_0 = 93.5\ \mu\text{m}$ . Force of compression ( $F$ ) is  $9.8 \times 10^{-3}$  dyne.

been determined experimentally elsewhere (Yoneda, 1964, 1972), so that the tension  $T$  can be calculated if  $F$ ,  $Z_0$  and  $\alpha$  are known.

The lower curve in Text-fig. 1 is the tension thus calculated. Up to the streak stage, since the tension changes rather slowly, intermittent compressions with 10 min intervals are applicable, assuring a good physiological condition for the egg. At the time of cleavage, however, since the change is so rapid, continuous compression is applied. Text-fig. 2 illustrates the change in the thickness of continuously compressed eggs under the constant force of  $9.8 \times 10^{-3}$  dyne throughout the three division cycles. In such a condition, since the diaster develops always in a direction parallel to the plane of compression, as expected from Hertwig's classical rule (Hertwig, 1884), the compressing force is automatically directed laterally to the spindle. Therefore the resulting blastomeres lie invariably side by side without overlapping one another. The compression along such a direction will hereafter be called 'lateral compression'. Cyclic change in the thickness correlating closely with the division cycle will be noted, which means a similar pattern of change in the tension at the surface (Text-fig. 3). Hiramoto (1963) earlier reported such a change up to the second cleavage.

One point which deserves mention in connexion with Text-figs. 2 and 3 is a sharp peak in the tension which occurs several minutes prior to the onset of cleavage, lasting only 4 min at  $12\text{--}15^{\circ}\text{C}$ . The peaks were observed for all the eggs so far studied, although sometimes less marked at the second and the third cleavages, owing probably to incomplete synchrony of division among blastomeres. The temporal relation between the peak in the tension and the appearance of the cleavage

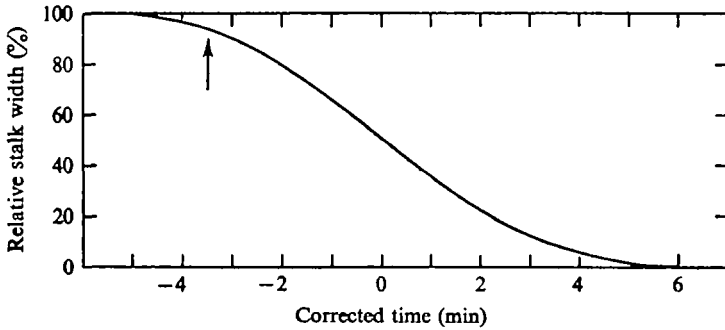


Text-fig. 3. Tension at the surface of fertilized eggs calculated from data shown in Text-fig. 2. Gaps in the curves corresponding to the time of cleavages are due to inadequacy of equation (1) to describe the cell in dumb-bell shape.

Table 1. Intervals between the peaks of tension and the appearance of the furrows in the first cleavage

<i>Hemicentrotus</i>			<i>Pseudocentrotus</i>		
Temperature (°C)	Intervals (min)	Force of compression ( $\times 10^{-3}$ dyne)	Temperature (°C)	Intervals (min)	Force of compression ( $\times 10^{-3}$ dyne)
12	10.5	9.8	18.5	5	4.7
12	8	9.8	19	4	4.7
13	8	9.8	20	4	4.7
13	6.5	1.4	20	6	4.7
13.5	8	1.4			
14	8.5	1.4	Mean 19	5	
14	8	1.4			
14.5	7	3.3			
Mean 13	8				

furrow is fairly close (Table 1), which suggests that this change is closely related to some unidentified event preceding cleavage. Wolpert's data (1966) on the 'stiffness' of the eggs of a Scandinavian sea-urchin show a similar situation. In the upper curve of his Fig. 5 one aberrant point, which he ignored, corresponding to a measurement 6 min before the start of cleavage, stays about twice as high as adjacent points preceding and succeeding it by 1 min. Time-lapse cinemicrographs (1 frame/5 sec) of Sirakami (1968) show a kind of pulsating motion of the egg of *Astriclypeus manni* occurring 4 min before the cleavage at 30 °C. First, the egg abruptly assumes an oblate shape with the short axis probably coinciding with the axis of cleavage, then elongates to a prolate, the maximum change in the diameter being about  $\pm 3-5\%$ . Such a cycle of deformation is repeated twice or more, although progressively decaying. It takes some 45 sec for one cycle of pulsation. Considering the possible

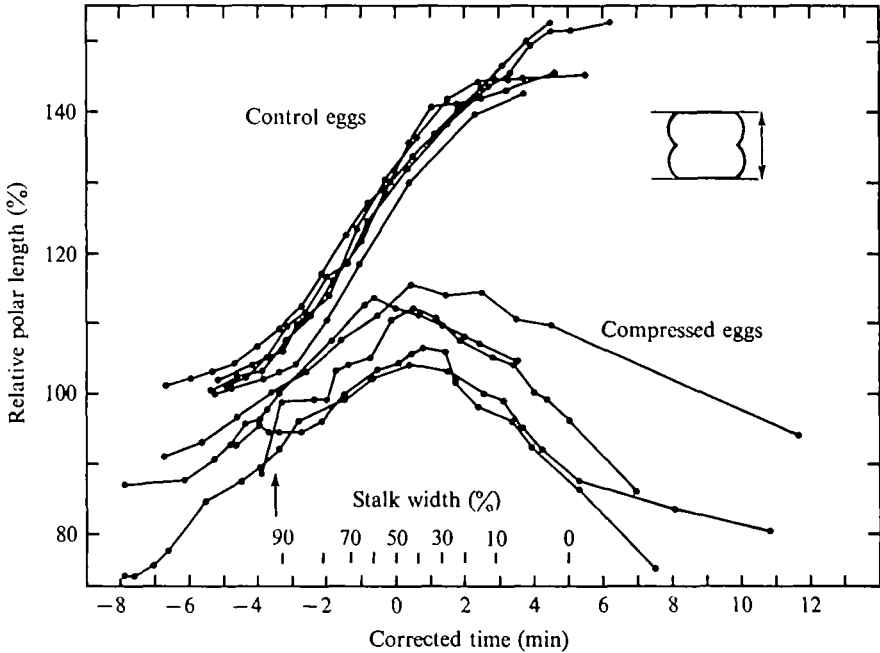


Text-fig. 4. Normalized time-course of reduction in the stalk width of dividing eggs of *Pseudocentrotus depressus*. The arrow indicates the appearance of the furrow.

temperature dependence of the speed of cleavage process, we may imagine that the pulsating motion (4 min before cleavage at 30 °C) and the sharp rise and fall in the tension (8 min before cleavage at 13 °C, or 5 min at 19 °C) are closely related to a common event preparing for a cleavage.

Technical simplicity is a merit of lateral compression, which is advantageous for studying long-term change in the rigidity of the cell as a whole. On the other hand, the disadvantage is that the radial symmetry of the egg is lost in the laterally compressed egg during cleavage. Photomicrographs taken from one direction alone are not enough to describe the three-dimensional form of the cleaving egg, and consequently to calculate the tension at the surface. All that can be said is that the apparent thickness ( $z$ ) decreases gradually and steadily throughout the cleavage stage to a minimum in the second interphase.

A more crucial experiment would be to flatten the egg along the axis of cleavage, or to measure the compressibility of the egg along axial direction. This will be termed 'axial compression'. One egg in the late diaster stage is held between the parallel compressing plates, and is kept in position for about 1 min until the egg lightly sticks to the surface of both glass plates. Then orientation of the egg is adjusted, by slight horizontal movement of the plate, until the mitotic apparatus of the egg is brought perpendicular to the compressing plates. After that, force of a definite magnitude is constantly applied along the direction of the cleavage axis. Even under the load, all the eggs so far studied cleaved with slight retardation. Photomicrographs taken every 30 sec during the cleavage are employed for measuring (1) the length of the egg along the cleavage axis (polar length) and (2) the depth of the furrow (stalk width). These parameters will hereafter be expressed as percentage of the initial diameter of the egg in order to eliminate the variation of the size of the individual egg (93–97  $\mu\text{m}$  in *Pseudocentrotus* egg, the average being 95  $\mu\text{m}$ ). When the stalk width is plotted against time, it is found that the rate of reduction of the stalk is linear within the range of 80 to 30% of the initial diameter both in control eggs and in compressed eggs. The average speed of furrowing is 15%/min for the control egg at around 15 °C (Text-fig. 4). Now the mid-level of the curve (the stalk width of 50%) will be taken as the origin of the time-scale and the scale is so adjusted that the inclination of the curve becomes a standard value of 15% per minute, so as to

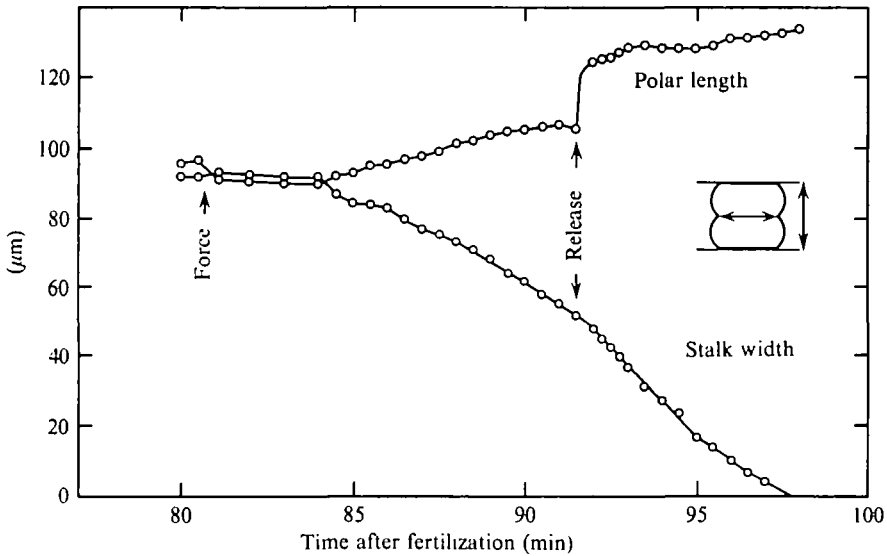


Text-fig. 5. Change in polar length with the normalized time in eggs of *Pseudocentrotus* under axial compression. Force of compression ( $F$ ) is  $7.5 \times 10^{-8}$  dyne. Arrow indicates the appearance of the furrow.

eliminate the differences among individual cleavage times. The corrected time can be taken as the reference of the course by which cleavage proceeds.

Text-fig. 5 shows the polar elongation of the control (upper curves) and the compressed eggs (lower curves). The cleavage indentation is perceived at the stage corresponding to the stalk width of about 92% of the initial diameter, as indicated by an arrow, being about 3.5 min (corrected time) before the 50% furrowing. The polar length of the control egg increases monotonically, until it eventually acquires twice the diameter of one blastomere (theoretically 158%). On the other hand, the egg under compression elongates until the stage in which the stalk reduces to 50% of the initial diameter, after which the polar length decreases rather rapidly, suggesting progressive weakening of the cell membrane. Since the cleaving egg under axial compression remains axially symmetric in shape, photographic records provide sufficient data for detailed study of the cell shape, on the basis of which it is possible to calculate the absolute value of the tension at the surface in the consecutive stages of cleavage.

Results reported so far relate to the mechanical property of the cleaving egg as a whole without focussing attention on any particular part of the cell surface. The last experiment is an attempt to estimate qualitatively the rigidity of the furrow region. The egg is allowed to cleave under axial compression until the furrow deepens to about 50% of the initial diameter, when the force of compression is quickly released. Resulting change in the polar length and the stalk width are shown in Text-fig. 6. Whereas the polar length tends to quickly regain the control value on release of the force, no such 'elastic recoil' is detected for the stalk width, the only response being



Text-fig. 6. Changes in polar length and stalk width of an egg of *Pseudocentrotus* when the force of compression ( $7.5 \times 10^{-8}$  dyne) is suddenly released in the middle of cleavage.  $15.5^\circ\text{C}$ .

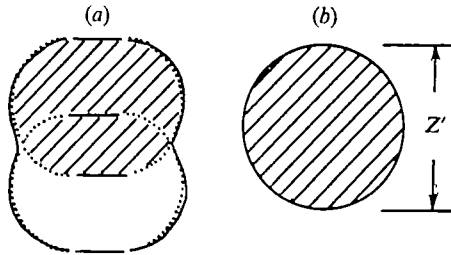
the simple speeding up of the furrowing. This fact strongly suggests that the furrow region is distinctly stiffer than the polar or subpolar surface, or speaking more exactly, the instantaneous elastic coefficient of the furrow region is very high. A similar statement was made by Wolpert (1966) using the cell-elastimeter, and by Hiramoto (1965) who injected a large oil drop.

#### CALCULATION OF THE TENSION AT THE POLAR SURFACE IN AXIAL COMPRESSION

An estimation will now be made for the absolute value of the tension at the polar surface, assuming that the mitotic apparatus exerts no appreciable amount of force against the cell surface.

Compressing unfertilized eggs, Yoneda (1964) found that the shape of the contour of the compressed eggs is fitted fairly well with the theoretical contour, which has been drawn on the basis of the assumption that the tension at the surface of the compressed egg is uniform. This is also the case for fertilized eggs before the onset of cleavage (Yoneda, 1972). Theoretical contours calculated with an IBM 7090 computer in 50 steps of compression (from  $z = 0.969$  down to  $z = 0.422$ ) were drawn, each on a separate sheet, on such a scale that the volume of each theoretical egg is equal to that of a sphere of 64  $\mu\text{m}$  in diameter. Since 50 values of  $-ds/dz$  are tabulated for various degrees of compression, it is simple to find values of  $-ds/dz$  by fitting the theoretical contours with photomicrographs properly enlarged. If the magnification of the image of the best fit is  $M$  times, the initial diameter  $Z_0$  should be  $64 \mu\text{m}/M$ . Accordingly, the tension  $T$  is calculated by equation (1), if  $F$  and  $M$  are known.

In order to apply the above procedure to cleaving eggs a modification is necessary



Text-fig. 7. (a) The contour of the dividing egg under compression (solid line) reproduced from the fifth photograph on Plate 1, and a pair of theoretical contours fitted to it (dotted line). (b) Sphere with volume equal to the hatched portion in (a) having diameter  $Z'$ .

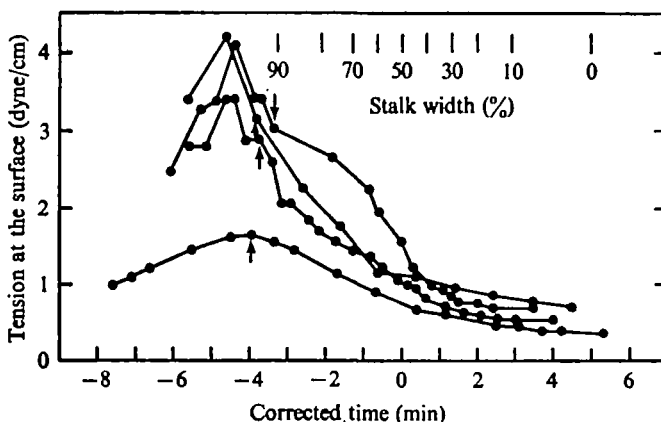
Table 2. Calculation of the tension at the polar surface of the dividing egg shown in Plate 1

Time after fertilization (min)	39.5	40	41	41.5	42	43	44	45	49
Stalk width ( $\mu\text{m}$ )	95.5	93	88	82	77	61	39	—	—
Tension = $F/\pi Z'(-ds/dx)$ (dyne/cm)	1.75	1.75	1.30	1.15	0.90	0.49	0.37	0.31	0.08

since the postulate of uniformity in tension is no more valid, owing to the presence of a stiff furrow region. At present the curve-fitting is done separately for each incipient blastomere under axial compression. Fortunately it is possible, for any stage of cleavage, to find a single-fit contour covering polar, subpolar and sub-furrow surface of each incipient blastomere (Plate 1), and thus to find their  $-ds/dx$  values. Text-fig. 7(a) shows one example which is reproduced from the fifth photograph of Plate 1. Here the image of the compressed egg magnified by 776 times (M) and IBM data no. 10 matches satisfactorily. The volume of the fitted portion (hatched) should be equal to that of a sphere with the diameter of  $64 \text{ mm}/776 = 82.5 \mu\text{m}$  ( $Z'$  in Text-fig. 7b). Since the tension of the fitted contour will be the same as that when the sphere with the diameter  $Z'$  is compressed to this degree, it can be easily calculated. It is justifiable to neglect the presence of the lower surface overlapping the opposite blastomere since it is separated from the main portion of the blastomere by a plane perpendicular to the axis of symmetry. At any rate, fitting of the major portion of the curved contour is the main importance. As is evident from Text-fig. 7, the two contours deviate from each other slightly at the furrow region. As mentioned before, since this portion is mechanically different from the rest of the cell surface, the force of constriction will be dealt with separately.

Results of the application of this procedure for each stage of the *Temnopleurus* egg shown in Plate 1 are collected in Table 2 and another set of data for four eggs of *Pseudocentrotus* is graphically shown in Text-fig. 8. It should be stressed that although the egg continues to elongate to the stage of 50% stalk width, the calculated tension reaches a maximum at the start of cleavage, when the first sign of deviation from the sphericity is perceived preceding the appearance of the furrow (arrows) by 1 min at  $15^\circ\text{C}$ . The tension steadily decreases all through the process of cleavage down to less than 0.5 dyne/cm. In contrast to such a marked decrease in the tension, the increase in the surface area is theoretically only 26% ( $\sqrt{2} = 1.26$ ). In other words the mechanical energy stored in the surface (= surface area  $\times$  tension) decreases during





Text-fig. 8. Tension at the polar surface of four eggs of *Pseudocentrotus depressus* at the time of cleavage. Arrows indicate the appearance of the cleavage furrow. Note that the maximum tension is reached at the start of the cleavage.

cleavage. This would be a quantitative expression of 'relaxation' of the cell surface proposed by Wolpert (1960).

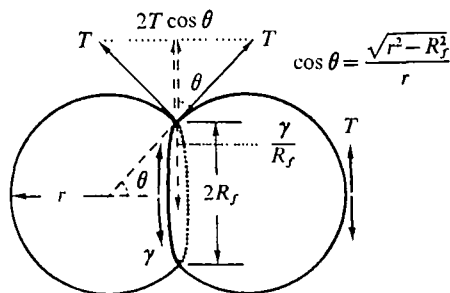
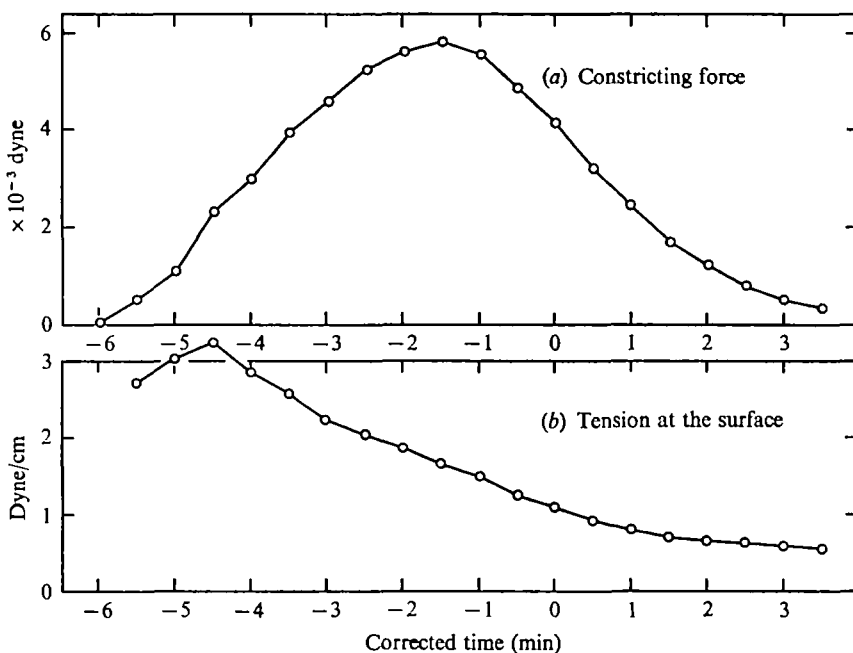
The coincidence of the contour of the egg with the theoretical contour assures the uniformity of the tension at the surface of the cell except the region of furrow. Going back from this conclusion, the contour of a dividing cell without compression should be a segment of a sphere, which is not the case. Ishizaka (1966) explained this in terms of transient viscous drag of the cell surface and rapid change in the cell shape during cleavage. It is highly possible that the extra stretching of the surface caused by compression causes equilibrium to be reached faster.

#### DISCUSSION

Mitchison & Swann (1955) measured the stiffness of the egg surface by the cell-elastimeter. The stiffness rises slowly until metaphase, and rapidly thereafter to reach a maximum during the late anaphase and early cleavage. During the later stage of cleavage the stiffness falls and reaches a minimum level in the second interphase. The overall pattern of the change in the stiffness is in good accordance with the change in the tension reported in the present paper.

The steady decrease in the tension at the polar surface during cleavage is incompatible with the constricting ring theory of Marsland, since passive stretching due to furrow constriction would naturally result in either no change or an increase in the polar tension. Although the present data in the main favours cortical relaxation theory of Wolpert (1960), the data do not still exclude the possibility of active furrow constriction. Therefore it would be appropriate to estimate the force of constriction at the furrow region, according to Ishizaka's theoretical approach (cf. Dan, 1963). Assuming that the incipient blastomere is a part of a sphere with diameter  $r$ , and considering the balance of force between the polar tension and the force of constriction ( $\gamma$ ) we have

$$\frac{\gamma}{R_f} = \frac{2\sqrt{(r^2 - R_f^2)}}{r} T \quad (2)$$

Text-fig. 9. Balance of forces between the polar tension ( $T$ ) and the constricting force ( $\gamma$ ).Text-fig. 10. (a) Constricting force  $\gamma$  calculated on the basis of data shown in Text-figs. 4 and 8. *Pseudocentrotus pulcherrimus*. (b) Tension at the polar surface normalized by averaging four sets of data in Text-fig. 8.

in which  $2R_f$  is the stalk width (Text-fig. 9). Assuming the constancy of the cell volume during cleavage,  $R_f$ ,  $r$  and  $Z_0$  are related as

$$2\pi(r - Z_p/3) Z_p^3 = \pi Z_0^3/6, \quad (3)$$

where  $Z_p = r + \sqrt{(r^2 - R_f^2)}$  (Ishizaka, 1966). Substituting equation (3) into equation (2), it is possible to calculate the minimum necessary force of constriction  $\gamma$  if  $R_f$ ,  $Z_0$  and  $T$  are known. The four curves of the tension at the polar surface in Text-fig. 8 were averaged for  $T$  (the lower curve in Text-fig. 10). The value of  $R_f$  was read from Text-fig. 4, and  $Z_0$  was taken as  $95 \mu\text{m}$ . Results of the calculation are shown in Text-fig. 10, which indicates that the constricting force reached the maximum, amounting to  $6 \times 10^{-3}$  dyne, about 3 min after the maximum of the tension at the

polar surface. If the equatorial ring which generates the constricting force is assumed to be  $6.5 \mu\text{m}$  wide (see later), the force of  $6 \times 10^{-3}$  dyne is equivalent to the tension of  $9 \text{ dyne/cm}$  ( $= 6 \times 10^{-3} \text{ dyne}/6.5 \mu\text{m}$ ), being definitely higher than the maximum tension developed at the polar surface ( $3 \text{ dyne/cm}$ , see Text-fig. 10*b*). This would suggest that the furrowing is not merely a passive result owing to the polar relaxation but is due, in addition, to an autonomous generation of a constricting force at the equatorial ring.

The force of constriction exerted by the furrow of echinoderm eggs was directly measured by Rappaport (1967). According to him, the force amounted to  $2.00 \times 10^{-3} \pm 0.43 \times 10^{-3}$  dyne for eggs of *Pseudocentrotus depressus*, with which the present result is in substantial agreement.

Recently, several workers have succeeded in demonstrating a bundle of microfilaments which might provide a structural basis for the contractile ring at the furrow region (Goodenough, Ito & Revel, 1968; Schroeder, 1968, 1970; Szollosi, 1968, 1970; Tilney & Marsland, 1969; Arnold, 1969; Bluemink, 1970). If it is assumed that the force of constriction is due to force of contraction of microfilaments, the value of  $2-6 \times 10^{-3}$  dyne divided by the total number of filaments would give the force exerted by a single microfilament. In the sea-urchin egg the bundle of microfilaments assumes the form of a circular girdle measuring  $0.1 \mu\text{m}$  in thickness (Tilney & Marsland, 1969). Tilney and Marsland's photograph indicates that the girdle is about  $6.5 \mu\text{m}$  wide. If the separation between microfilaments is assumed to be  $100 \sim 150 \text{ \AA}$  (cf. Schroeder 1970), we have  $10^{-8}$  dyne for the force exerted by a single microfilament.

Finally, a reference should be made on a new method developed by Hiramoto (1968). He determined the intracellular pressure  $P$  from the relation between the deformation and the force when the egg was deformed by a minute truncate rod applied to the polar surface of the egg and calculated the tension  $T$  using a modified form of the Laplace formula  $P = T/R_1 + T/R_2$  ( $R_1$  and  $R_2$  are radii of principal curvature of the egg surface), giving the result that the maximum tension was of the order of  $1 \text{ dyne/cm}$  for eggs of *Temnopleurus toreumaticus*. The maximum is reached in the middle of cleavage, which sharply disagrees with the present result. No satisfactory explanation has yet been found with respect to the discrepancy between results of Hiramoto and present authors.

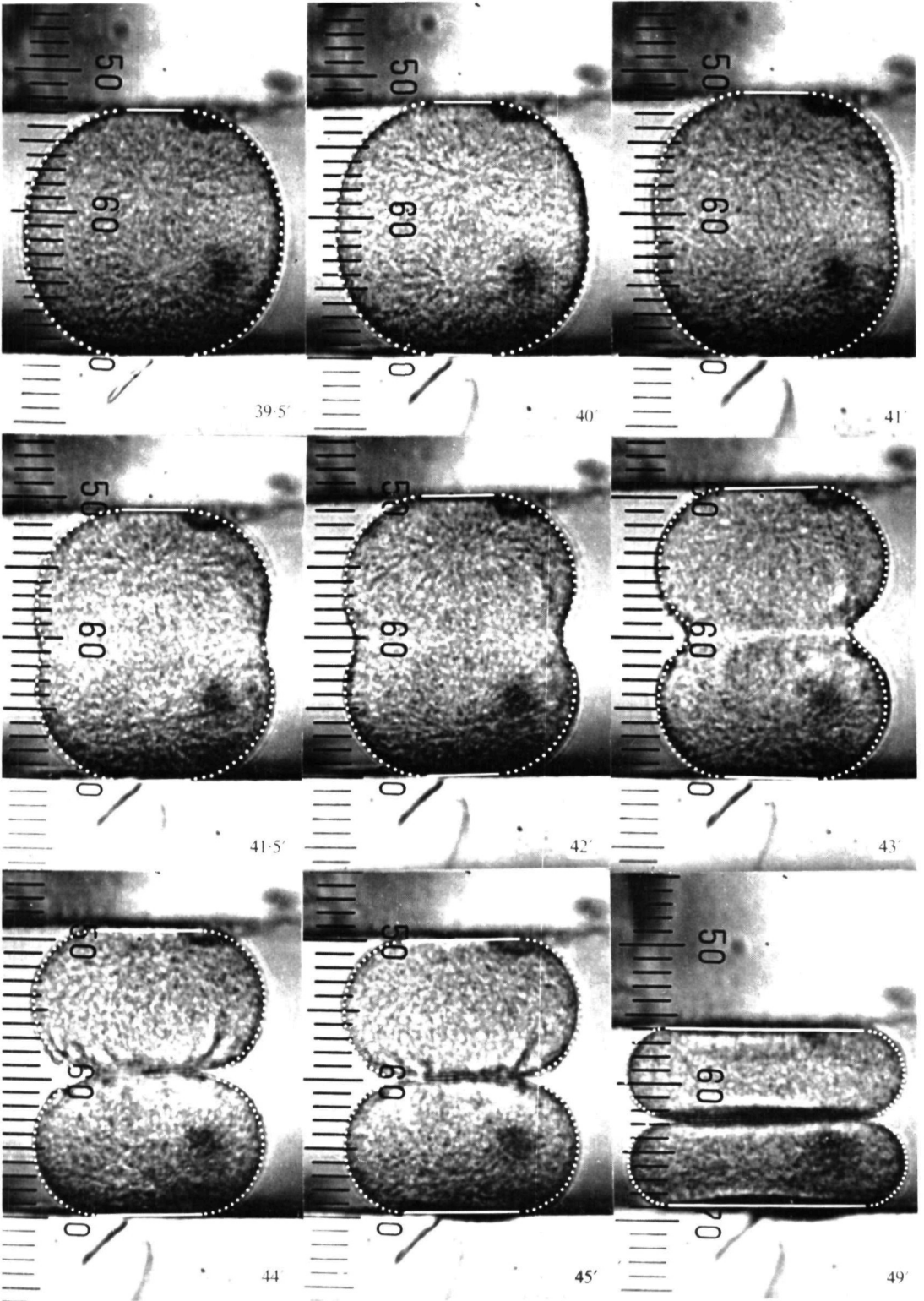
#### SUMMARY

1. Cyclic changes in tension correlating with the division cycle were demonstrated by compression method of Cole.
2. A sharp peak in the tension was found 8 min before the onset of cleavage at  $13^\circ\text{C}$ .
3. A computer method indicated that the tension reached a maximum at the start of cleavage, after which it steadily decreased throughout the course of cleavage.
4. A rough estimation revealed that the force of constriction at the equatorial ring amounted to  $6 \times 10^{-3}$  dyne, which compares well with Rappaport's result (1967).

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EXPLANATION OF PLATE

Photomicrographs of a dividing egg of *Temnopleurus hardwickii* under axial compression at 27.5 °C. The theoretical contours shown in dotted lines are superimposed. Numerals: the time after fertilization in minutes. One division of the micrometer scale: 5.1  $\mu\text{m}$ . Force of compression:  $2.7 \times 10^{-8}$  dyne. Initial diameter of the egg: 96.5  $\mu\text{m}$ .