

A QUANTITATIVE DESCRIPTION OF PROTOPLASMIC MOVEMENT DURING CLEAVAGE IN THE SEA-URCHIN EGG

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Quantitative information on the protoplasmic movement during cleavage is one of the most important desiderata in the study of cell division. Spek (1918) described the protoplasmic streaming during cleavage and emphasized the importance of the surface tension in the cell division, giving attention to the similarity of this protoplasmic streaming to the streaming within an oil drop dividing as a result of local difference in surface tension. Dan and his collaborators (Dan, Yanagita & Sugiyama, 1937; Dan, Dan & Yanagita, 1938; Dan & Dan, 1940, 1942, 1947; Dan, 1943; Dan & Ono, 1954; Ishizaka, 1958) reported a series of experiments in which the cortical movement during cleavage was followed by measuring the movement of kaolin particles adhering to the cell surface. The results obtained by Spek and by Dan provide qualitative information on the protoplasmic movements during cleavage. However, from their data it is neither possible to make a quantitative analysis of the protoplasmic movement nor to infer the correlation between cortical and endoplasmic movements in detail. In the present work protoplasmic (both cortical and endoplasmic) movement during cleavage of the sea-urchin egg is described in a quantitative fashion, in order to provide basic data for the study of cell division.

The determination of the location of the motive force is an essential point for the study of cell division. Recently, cleavage without mitotic apparatus was reported in sea-urchin eggs by Swann & Mitchison (1953) and by the author (Hiramoto, 1956), which is favourable for the cortical activity theory. The present work is concerned with the protoplasmic movement during cleavage of the egg without mitotic apparatus, and seeks to compare the effect of the presence and absence of the mitotic apparatus on the distribution of stress within the dividing cell.

MATERIAL AND METHODS

As material the eggs of the heart-urchin, *Clypeaster japonicus* were used. Eggs were fertilized in the ordinary way and both the fertilization membranes and the hyaline layers were dissolved in an isotonic solution of urea. The eggs were then washed with fresh sea water and were cultured in a Syracuse dish until the amphiaster stage. Before experimentation, a drop of egg suspension was put on a slide, together with blocks of thin glass plate as supports, and a cover-slip was put over it. Because the

drop was surrounded with the blocks of glass plate and separated from them by an air gap, the eggs were not compressed by the cover-slip and the evaporation of the medium was minimized. When it was necessary to abolish evaporation completely, mineral oil was applied between the cover-slip and the blocks; but this was usually unnecessary. For the measurement of protoplasmic movement eggs were selected in which the spindle lay in the horizontal plane.

Determination of cortical movement. For the determination of cortical movement carbon particles were previously attached to the surface of the egg and the movement of the particles on the circumference of the largest optical section of the egg was traced by photographing them at appropriate time intervals.*

Photographs obtained from the same egg at successive stages of cleavage were projected on a sheet of graph paper;* the contours of the egg and the positions of the surface indicated by the particles in successive stages were traced; the tracings were then superimposed, using the spindle axis and the cleavage plane as reference, in the way described by Ishizaka (1958) (cf. Fig. 1).†

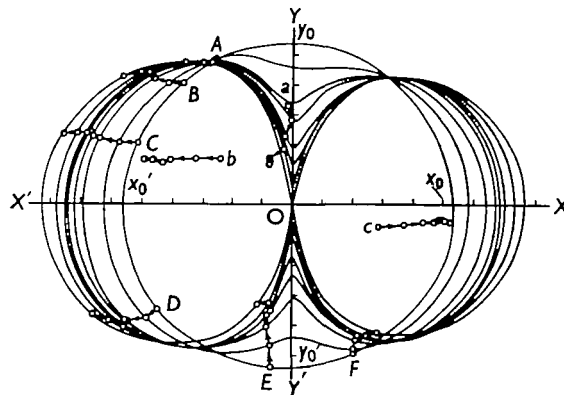


Fig. 1. Movement of particles adhering to the cell surface (*A, B, C, D, E, F*) and movement of endoplasmic granules (*a, b, c*) during cleavage of an egg of *Clypeaster japonicus*. The *x*-axis represents the spindle axis and the *y*-axis the cleavage plane. The smallest divisions on the co-ordinate axes are 10μ .

Since only a few particles are found on the circumference of an egg at one time and their points of adherence to the cell surface are determined by chance, movement of only particular portions of the cortex is revealed by a single experiment. In consequence, data obtained from individual eggs are not directly comparable one with another because of individual differences as to (1) the size of the egg, (2) the stages of cleavage photographed, and (3) the positions on the cortex of the particles whose movements were traced.

* Distortion of the image by the optical system was almost negligible, since the distortion due to the objective lens was well compensated by a 'Periplan' eye-piece and that due to the projection lens could be eliminated by using an iris diaphragm.

† The spindle axis is represented by the *x*-axis, and the cleavage plane in the largest optical section is represented by the *y*-axis.

As shown in Fig. 1, *Clypeaster* eggs are not perfectly spherical even before the initiation of furrowing, and cleavage is not perfectly symmetrical with respect both to the spindle axis (x -axis) and to the cleavage plane (y -axis). Thus the circumference of the egg just before cleavage intersects the co-ordinate axes at different distances from the origin (O). Now, these distances are defined to be x_0 , $-x'_0$, y_0 and $-y'_0$ (Fig. 1), x'_0 and y'_0 being negative. In order to convert the movement of a portion of the cortex, as obtained from the tracings of photographs, into normalized movement as for an egg having a radius of unity, x/x_0 (or x/x'_0) and y/y_0 (or y/y'_0) are taken as co-ordinates of the portion (x, y), whose movement was traced, where x_0 (or x'_0) and y_0 (or y'_0) have the same signs as x and y , respectively, i.e. both x/x_0 (or x/x'_0) and y/y_0 (or y/y'_0) are positive. For example, for normalized co-ordinates of point B in Fig. 1 just before cleavage ($-36.8\mu, +40.5\mu$),

$$x = 0.651 (= -36.8 / -56.5) \quad \text{and} \quad y = 0.751 (= 40.5 / 53.9)$$

are taken, since x'_0 and y_0 are -56.5μ and $+53.9\mu$, respectively (cf. Fig. 2).

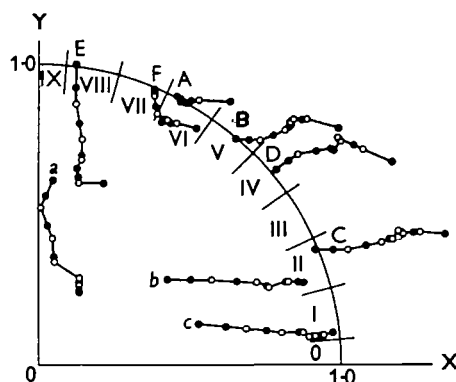


Fig. 2. Normalized movement of the surface particles and of the endoplasmic granules from Fig. 1. White circles indicate normalized positions. Black circles indicate normalized positions at reference stages which are determined by interpolation.

Since the stages of cleavage at which the egg was photographed are different in each egg, it is necessary to standardize the stages of cleavage in order to correlate the results obtained from different eggs. The stage of cleavage is defined by the percentage of the width of the furrow neck to the original egg diameter; e.g. the stage of cleavage is '100%' just before the beginning of furrowing, it is '0%' when the egg has just divided, and intermediate values are allotted to intermediate stages. In the present study, stages '100%', '90%', '70%', '50%', '30%', '15%' and '0%' are taken as reference stages. Positions of the cortical point at the reference stages are determined by interpolation (black circles in Fig. 2).

In order to obtain averaged movement of the cortex, experimental results are divided into ten groups according to topographical positions of the cortical points with reference to the cleavage plane in the following way (cf. Fig. 2). In the normalized graph, if the angle (θ), between the x -axis and the line connecting the origin (O) to the point which is moving, turns out to be larger than 5° and smaller

than 15° at stage '100%', such data are classified as group I. Cases in which θ is larger than 15° and smaller than 25° at stage '100%' are referred to group II. Similarly, group 0 ($5^\circ > \theta$), group III ($35^\circ > \theta > 25^\circ$), group IV ($45^\circ > \theta > 35^\circ$), group V ($55^\circ > \theta > 45^\circ$), group VI ($65^\circ > \theta > 55^\circ$), group VII ($75^\circ > \theta > 65^\circ$), group VIII ($85^\circ > \theta > 75^\circ$) and group IX ($\theta > 85^\circ$) are defined. The x -co-ordinates and the y -co-ordinates of all cortical points belonging to the same group at the same stage are averaged. The results are shown in Table 2 and in Fig. 4 (C_1, C_2, \dots, C_8); in this figure the averaged positions in group 0 and in group IX are not shown, the averaged positions of the pole (C_p) and of the furrow (C_f) being indicated instead.

Determination of endoplasmic movement. In some eggs of *Clypeaster*, several granules within the cytoplasm are clearly distinguishable from other cytoplasmic granules by their larger size (about 1μ in diameter). The number of recognizable granules in the largest optical section of the egg differs in batches, up to ten in some batches and none in others. The movement of endoplasm could be determined using these granules as markers.

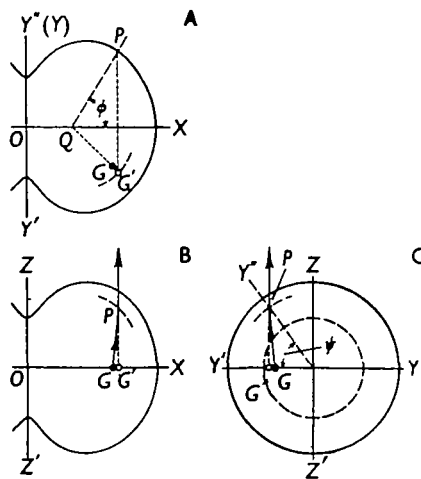


Fig. 3. A diagram indicating the refraction of light at the surface of a dividing sea-urchin egg. The spindle axis of the egg is taken as the x -axis (XX'), and cleavage plane is represented by the Y - Z plane. The optical axis of the microscope is the z -axis (ZZ'). The optical path is indicated by arrows in B and C. A: view from x -direction; the optical axis is perpendicular to the plane of the page; B, view from y -direction; C, view from x -direction. For further explanation, see text.

As is pointed out elsewhere (Hiramoto, 1957), since the light passing through egg protoplasm is refracted at the cell surface, the apparent positions of granules, as seen in the microscope, are not necessarily the true ones. The degree of the optical deviation from the true position can be calculated, if the refractive index of egg protoplasm and the geometrical form of the cell are given. The refractive index of egg protoplasm has been obtained (the first method in Hiramoto, 1957) and was found to be 1.385 using eggs shortly before cleavage.*

* Refractive index of sea water: 1.3383 (25° C.).

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The correction of the position of the granule is made assuming that the refractive index remains unchanged during cleavage and that the egg has an axial symmetry. In Fig. 3 true and apparent positions of a granule in the largest optical section are shown (G and G'). The determination of the exact position of the granule by ray tracing is troublesome in procedure since the geometrical form of the egg is not simple. However, the true position of the granule (G) is approximately obtained by tracing the axial ray, since the maximal deviation of the position thus calculated is only of the order of 1μ and only rays which are within 30° (in sea water) from the optical axis enter the objective lens ($40\times$, N.A.: 0.65).

P is the point where the cell surface is intersected by the line passing through G' parallel with the optical axis (ZZ' in Fig. 3 B, C, which is perpendicular to the page in Fig. 3 A); Q is the point where the x -axis is intersected by the line normal to the cell surface at P . The corrected position of the granule is $[x' - k(x' - q), y' - k'y']$, where q is x -co-ordinate of Q , and x' and y' are respectively the x - and y -co-ordinates of G' . k is a constant which changes in accordance with the angle (ϕ) between the normal PQ and x -axis and k' is another constant which varies with the angle (ψ) between the $X-Y'$ plane (including the x -axis and point P) and $X-Y$ plane (cf. Table 1). Usually a value of $1/30$ can be used as k or k' , the error resulting from this approximation being negligible.

Table 1. k - (or k' -) values for various ϕ - (or ψ -) values

ϕ (or ψ)	90°	80°	70°	60°	50°	40°	30°	20°	10°
k (or k')	0.035	0.034	0.034	0.034	0.034	0.033	0.032	0.031	0.025

Typical movement of endoplasmic granules in the largest optical section of an egg is shown in Fig. 1. The movement of the granules is normalized in the same way as in the analysis of the cortical movement by taking x/x_0 (or x/x'_0) and y/y_0 (or y/y'_0). For example, the normalized position of the granule b (Fig. 1) at the initial spherical stage is (0.428, 0.282) (Fig. 2), as its position is $(-24.2\mu, +15.2\mu)$ and x'_0 and y_0 are -56.5μ and $+53.9\mu$ respectively. The positions of the granule at the reference stages (100, 90, 70, 50, 30, 15 and 0%) are obtained by interpolation (black circles in Fig. 2).

Normalized positions of the granules at stage 100% are indicated by white circles in Fig. 4A. These points are obtained from seventeen selected eggs dividing with considerable regularity. Regular cross-lines at stage 100% (Fig. 4A) indicate different strata in the endoplasm, the distortion of which in Fig. 4B-G shows general movement within the cell.

Colchicine treatment. Cortical and endoplasmic movements during cleavage were similarly obtained in eggs treated with colchicine. The eggs deprived of both the fertilization membranes and the hyaline layers were treated with 3×10^{-3} M or 5×10^{-3} M colchicine shortly before cleavage, and the determination of protoplasmic movement was made after the disappearance of the mitotic apparatus.

RESULTS

I. *Normal egg*

In a diagram such as Fig. 1, in which contours of an egg at successive stages of cleavage are so superimposed that they share the spindle axis and cleavage plane, cortical movement shows the following characteristic features. Roughly speaking, the furrow region of the cortex moves toward the spindle axis parallel with the cleavage plane throughout the cleavage (*E*), the polar region of the cortex moves in the polar direction parallel with the spindle axis (*B*, *C*, *D*), and the borders between these regions scarcely move (*A*, *F*) during cleavage. The presence of such borders which do not move during cleavage was discovered in other species of sea urchin by Ishizaka (1958) who named them 'stationary surface rings'.

Cytoplasmic granules in the region close to the cleavage plane move toward the spindle axis but away from the cleavage plane during cleavage (cf. *a* in Fig. 1); the granules in the region close to the spindle axis move in the polar direction (*b*, *c* in Fig. 1); the granules in the peripheral region of the cell move together with the cortex. Both cortical and endoplasmic movements are symmetrical with respect to both the spindle axis and the cleavage plane. These results are more clearly and quantitatively indicated in the averaged results in Fig. 4.*

Since the movement of every point in the egg can either be computed from Table 2 or can be graphically estimated from Fig. 4, various conditions governing protoplasmic movement during cleavage can be anticipated as follows.

Constancy of the cell volume during cleavage. The total volume of the egg is calculated as the revolution about the spindle axis of the shape of the egg in the largest optical section shown in Fig. 4. The volume of the egg equals $2\pi \int_0^{x_p} y^2 dx$, in which *y* is the *y*-co-ordinate of the cell surface and x_p is the *x*-co-ordinate of the pole (C_p). The values are calculated using Simpson's formula. The result is shown in Table 3. Considering the fact that an error of 0.4μ in the egg diameter would result in a volume error of more than 1%, the constancy of the volume is surprising.

Linear and areal changes of the cell surface during cleavage. Dan and his collaborators (Dan *et al.* 1937; Dan *et al.* 1938; Dan & Dan, 1940; Dan & Ono, 1954) described regional differences in the degree of expansion of the circumference of sea-urchin eggs during cleavage. Similar results, shown in Fig. 5, are obtained by measuring the linear change of the circumference of the egg indicated in Fig. 4. Polar region, subpolar region, subfurrow region and furrow region by the definition of Dan *et al.* correspond to regions between C_p and C_4 , C_4 and C_6 , C_6 and C_8 , and C_8 and C_f in Fig. 4, respectively. The contour along the circumference of the egg is

* As is mentioned elsewhere (Hiramoto, 1957), there is a cortex at the surface of sea-urchin egg which differs in physical properties from the endoplasm. Therefore some discontinuity in the cytoplasmic movement is to be expected at the boundary between the cortex and the endoplasm because of the high rigidity of the cortex. Although this is not indicated in the figure, since the precise determination of displacement within such a narrow space was impossible, it does not seem that greater precision would invalidate the argument in the present paper.

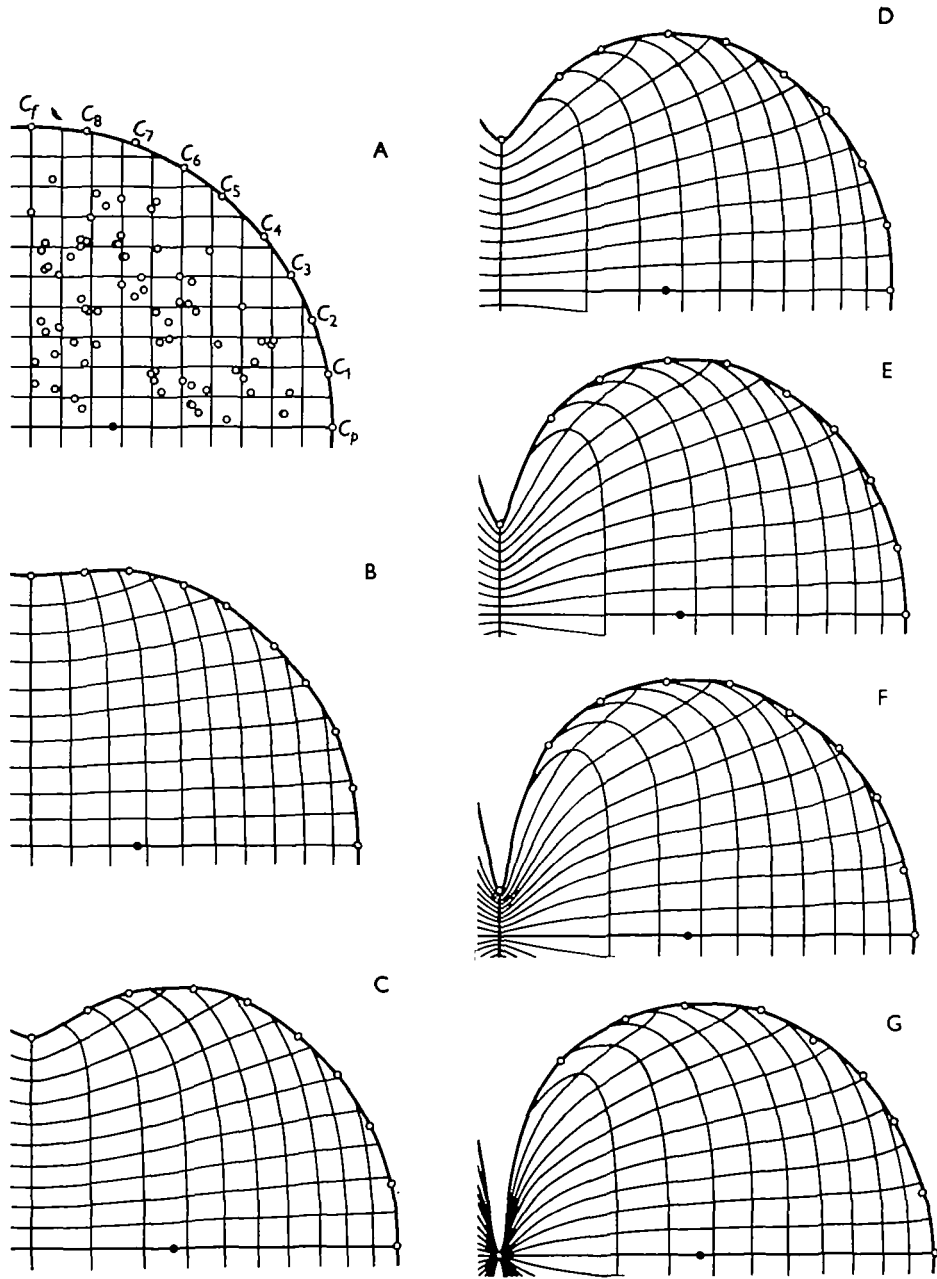


Fig. 4. Diagram indicating protoplasmic movement during cleavage of *Clypeaster* egg. A quarter of the egg is shown. A, stage 100%; B, stage 90%; C, stage 70%; D, stage 50%; E, stage 30%; F, stage 15%; G, stage 0%. Cross-lines within the egg indicate different strata of the endoplasm in the largest optical section of the egg. Movements of white circles on the cell surface in A ($C_p, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_7$) can be followed in the other figures. Black circles indicate positions of the astral centre.

determined by assuming that it consists of series of circular arcs.* It will be seen from Fig. 5 that the general characteristics of the linear changes of the surface along the circumference coincide with those found by Dan *et al.* except for small quantitative differences, some of which may be attributable to the difference in material.

Changes in area of the surface during cleavage are shown in Fig. 7A. The surface area is calculated by assuming that the blastomeres consist of series of zones and

Table 2. *Averaged cortical movement of the sea-urchin egg during cleavage*

Stage	100 %	90 %	70 %	50 %	30 %	15 %	0 %	No. of points averaged
C_p x	1.000	1.080	1.208	1.288	1.344	1.374	1.438	36
y	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
C_1 x	0.984	1.067	1.190	1.274	1.313	1.337	1.398	13
y	0.178	0.189	0.207	0.217	0.221	0.215	0.201	
C_2 x	0.933	1.008	1.120	1.193	1.224	1.249	1.303	17
y	0.356	0.379	0.401	0.421	0.447	0.454	0.440	
C_3 x	0.861	0.912	1.013	1.073	1.103	1.126	1.203	17
y	0.507	0.538	0.572	0.599	0.616	0.620	0.593	
C_4 x	0.771	0.807	0.886	0.938	0.950	0.962	1.037	19
y	0.635	0.663	0.700	0.721	0.739	0.741	0.714	
C_5 x	0.634	0.650	0.719	0.747	0.751	0.767	0.867	10
y	0.766	0.796	0.816	0.828	0.837	0.838	0.813	
C_6 x	0.507	0.505	0.537	0.554	0.555	0.558	0.619	20
y	0.861	0.867	0.860	0.853	0.847	0.843	0.830	
C_7 x	0.344	0.325	0.321	0.332	0.326	0.338	0.417	12
y	0.947	0.915	0.848	0.803	0.788	0.782	0.783	
C_8 x	0.183	0.177	0.185	0.192	0.168	0.165	0.202	15
y	0.985	0.912	0.794	0.714	0.654	0.635	0.648	
C_7 x	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
y	1.000	0.900	0.700	0.500	0.300	0.150	0.000	

Table 3. *Volume of dividing sea-urchin egg*

Stage	100 %	90 %	70 %	50 %	30 %	15 %	0 %
Volume (%)	100.0	100.0	99.9	99.5	99.8	100.6	100.3

segments of spheres following the method of Dan & Ono (1954), although the procedure of calculation is different. In Fig. 6B, the area of curved surface of the zone is $2\pi dy$, in which d is the length of the chord (LN) and y is the distance between the axis of rotation and the mid-point (M) of the arc. The total area is the sum of these areas ($2\pi \Sigma dy$). In the case of the segment of a sphere the surface area can be calculated in the same way. This method of calculation is as simple as that of Motomura (1940), which is less accurate than the present one because his calcula-

* Between length of the arc (l in Fig. 6A), length of the chord (d), height of the bow shape (x), and angle α , the following relations hold:

$$l/d = \alpha / (2 \sin \frac{1}{2} \alpha), \quad x/d = \frac{1}{2} \tan \frac{1}{2} \alpha.$$

Therefore, l is directly obtained using a graph of $\tan \frac{1}{2} \alpha$ plotted against $\alpha / \sin \frac{1}{2} \alpha$.

tion is made under an assumption that the blastomeres consist of series of trapezoids, and is as accurate as that of Dan & Ono (1954), which is somewhat troublesome in procedure because, to obtain the area of the surface, a circle which would circumscribe the curved surface of the cell must be found by successive approximation. Moreover, the present method is applicable with exactitude when the cell surface is a part of a cone (Fig. 6C), and is applicable with approximation unless the curvature

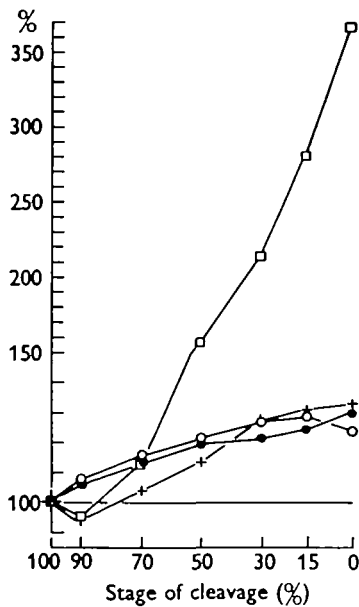


Fig. 5

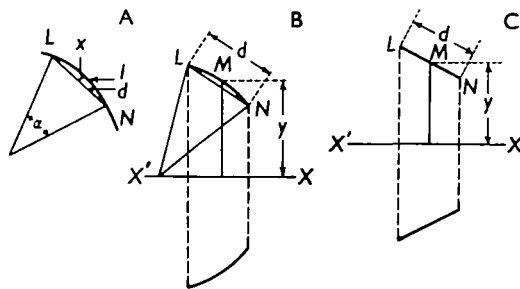


Fig. 6

Fig. 5. Linear elongation or shrinkage of the cell surface during cleavage. Curves are constructed by measuring sections of the circumference between two adjacent white circles shown in Fig. 4. O, length between C_p and C_4 (polar region); ●, length between C_4 and C_6 (subpolar region); +, length between C_6 and C_8 (subfurrow region); □, length between C_8 and C_7 (furrow region).

Fig. 6. Diagram illustrating the methods of calculation of the length and the area of curved surface. A: the length of the arc (LN) is estimated from the relation between $\tan \frac{1}{2}\alpha$ and $\alpha/\sin \frac{1}{2}\alpha$, because $l/d = \alpha/(2 \sin \frac{1}{2}\alpha)$ and $x/d = \frac{1}{2} \tan \frac{1}{2}\alpha$, where l is the length of the arc (LN), d is the length of the chord (LN), and x is the height of the bow shape. B: the area of the surface of a zone of a sphere having the axis (XX') is $2\pi dy$. The largest optical section of the zone is indicated by heavy lines, where d is the length of the chord (LN) and y is the distance of the mid-point (M) of the arc from the axis (XX'). C: the area of the surface of a cone, the cross-section of which is indicated by heavy lines, is also $2\pi dy$, where d is the length of line (LN) and y is the distance of its mid-point (M) from the axis of the cone (XX').

of the cell surface is too large. As is shown in Fig. 7A, the result agrees in general with that of Dan & Ono (1954).

Fig. 7B indicates the areas occupied by each of the four regions as percentages of the total surface area at various stages of cleavage. In the early stage of cleavage (100–70%), furrow and subfurrow surfaces contract, whereas polar and subpolar surfaces expand. In the middle stage (70–15%), the percentage of area occupied

by each region is almost unchanged although total area increases. In the late stage (15-0%), furrow surface expands, polar surface contracts, and subfurrow and subpolar surfaces retain their percentages of the total surface area almost unchanged. Areal change in the late stage mentioned above implies the initiation of a large expansion of the furrow membrane accompanying the contraction of the polar membrane which has been pointed out by Dan *et al.* (Dan *et al.* 1938; Dan & Dan, 1940; Dan, 1954) as a characteristic feature of the post-cleavage period.

General characteristics of endoplasmic movement. Endoplasmic movement indicated in Fig. 4 is qualitatively similar to that reported by Spek (1918). It will be noted in this figure that although the vertical lines within the endoplasm in A are not so much deformed in the central zone of the egg during the course of cleavage, they are much deformed in the peripheral zone.

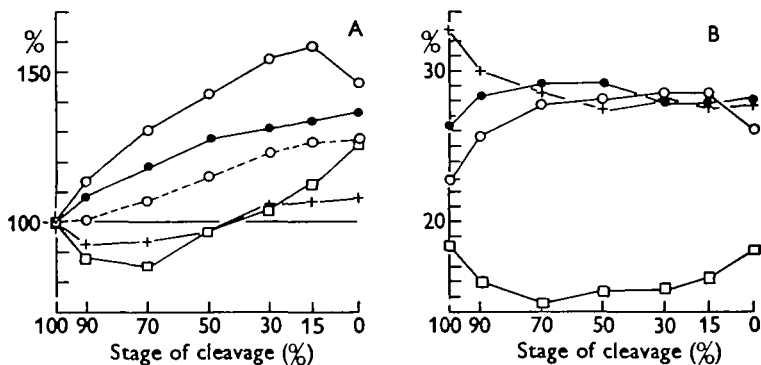


Fig. 7. Areal change of the cell surface during cleavage. Curves are constructed by measuring the surface of dividing egg shown in Fig. 4. A: percentage changes, taking the initial state as 100. Broken line indicates change of the total surface area. B: changes in area of individual regions as percentages of the total surface area. Definition of symbols is the same as in Fig. 5.

This fact is more clearly shown by the movement of endoplasm through the stationary surface ring (Fig. 8). In Fig. 8A, the y -axis represents the plane of the stationary surface ring in the largest optical section of the egg.* This figure illustrates the endoplasmic movement through the stationary surface ring by indicating where endoplasmic elements, constituting the plane of the stationary surface ring at the stage 70%, were at stages 90 and 100% and where they will be at future stages. To avoid overlapping of curves, the scale of the x -axis of Fig. 8A is magnified five times in Fig. 8B. It is clear that in the central part the lines are approximately parallel, while in the peripheral zone they converge rather steeply toward the y -axis as much-deformed parabolas. This fact may suggest the presence of some gelled structures such as the spindle and the asters which impede the free movement of

* As is shown in Fig. 4, every point on the cell surface changes its position in the x - y diagram in a strict sense. Therefore, for convenience, the part of the cell surface which is in position (0.550, 0.831) in Fig. 4A (stage 100%) is taken as 'stationary surface ring' because it is almost motionless. This part, of course, moves during cleavage, e.g. it is in (0.600, 0.855) at stage 70% (Fig. 4C). The y -axis in Fig. 8 is determined in such a manner that it passes the above-mentioned part, namely (0.550, 0.831) at stage 100% and (0.600, 0.855) at stage 70%.

protoplasm in the central zone of the cell, because it is expected that the endoplasmic displacement curves ought to approach perfect parabolas if the endoplasm is composed of a homogeneous liquid whose movement during cleavage is caused solely by the deformation of the cortex. Kamiya (1950) reported a velocity distribution curve of similar shape in the intracapillary flow of the protoplasm of myxomycete plasmodium.

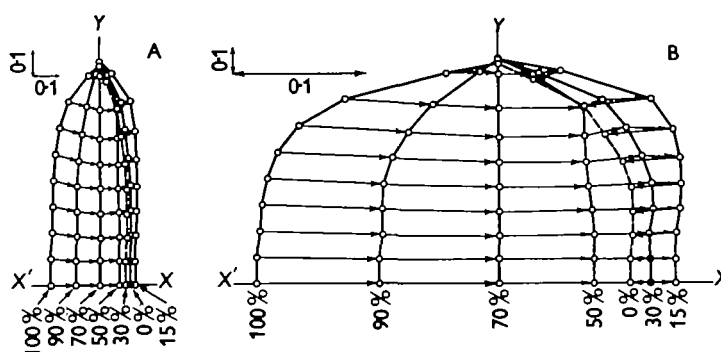


Fig. 8. Displacement of endoplasm within the plane of the stationary surface ring of the dividing sea-urchin egg. Curves are constructed from the data in Fig. 4. Though the x -axis (XX') of the figure is the same as the abscissa of Fig. 4, the y -axis here is taken to indicate the plane of the stationary surface ring in the largest optical section of the egg. Circles connected by heavy lines represent loci of endoplasmic elements which constitute the plane of the stationary surface ring at the stage 70%. The scale of the x -axis in A is magnified five times in B.

Backward movement of endoplasm during the period between stages 15 and 0% (Fig. 8) may be due to the initiation of the expansion of the furrow membrane mentioned above.

Change in shape and length of astral rays during cleavage. Black circles in Fig. 4 indicate the averaged positions of the astral centre. It will be noted that although the absolute position of the astral centre changes during cleavage, its position relative to the surrounding protoplasm is unaffected. In other words, the astral centre moves together with surrounding protoplasm, i.e. no slipping occurs between them.

The changes in shape of the astral rays can be estimated graphically by assuming that the individual parts of the astral rays move together with surrounding protoplasm over their entire lengths. In Fig. 9, ten astral rays designated $A_p, A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8$ and A_f are represented, which are attached to the cortex at points $C_p, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8$ and C_f (Fig. 4), respectively. In this figure, the peripheral parts of the astral rays indicated by broken lines are hardly visible in living material. Although it is unlikely that the astral rays in the peripheral zone of the egg have rigidity, especially in the late stage of cleavage, it is probable that the material of the rays may remain in the positions indicated by broken lines. It will be seen that the astral rays, which are straight at stage 100%, bend during cleavage. The bending of astral rays during cleavage has been observed by many investigators (cf. Dan, 1943).

Fig. 10 indicates the changes in length of these astral rays during cleavage, as obtained from Fig. 9. Astral rays attached to the furrow region of the cortex (A_f , A_8) shorten in the early stage of cleavage. If the furrowing results from the mechanical pull of the astral rays attached to the cortex, the astral rays attached to

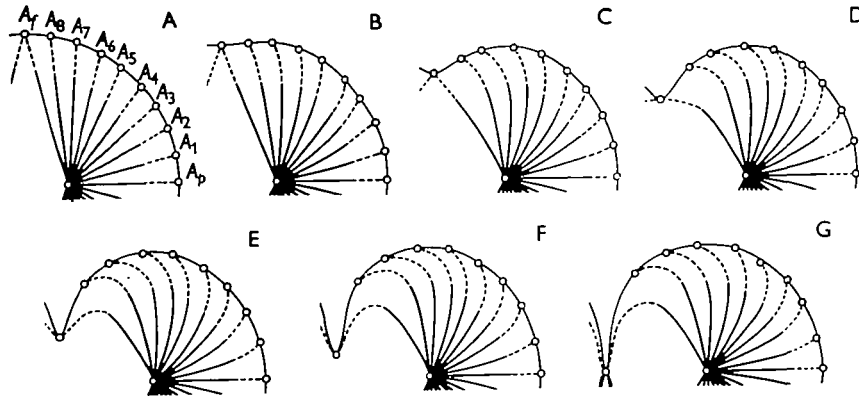


Fig. 9. Changes in shape of the astral rays during cleavage. The shapes of the astral rays are estimated graphically from the protoplasmic movements indicated in Fig. 4. A_p , A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 , A_8 and A_f are hypothetical astral rays attached to the cortex at points C_p , C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 and C_f (Fig. 4), respectively. Peripheral parts of rays indicated by broken lines are hardly seen in living materials.

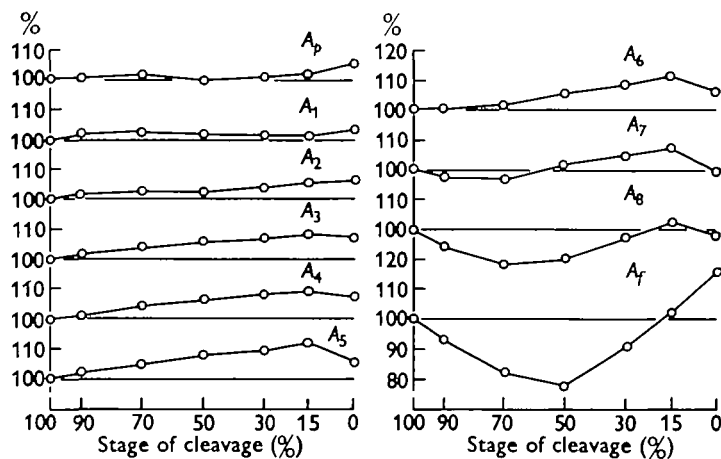


Fig. 10. Changes in length of astral rays during cleavage. A_p , A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 , A_8 and A_f are the hypothetical astral rays indicated in Fig. 9.

the furrow cortex must be stretched. On the other hand, if the furrowing results from the active constriction of the furrow cortex, the astral rays must be shortened by compression. The result shown in Fig. 10 is favourable to the latter interpretation, i.e. the contracting-ring theory is preferable to the radiate-aster theory (Dan, 1943) in this connexion.

Behaviour of an injected oil drop. The normalized endoplasmic movement of Fig. 4 was checked by observing the movement of paraffin oil injected into eggs. The behaviour of the successfully injected drop was roughly the same as that of the endoplasmic granules mentioned above.* In some eggs, however, slight deviation of the movement of the oil drop was observed from that expected from Fig. 4. This deviation may be due to imperfect recovery of the cell from the deformation at the time of micro-injection. Simultaneous observation of the oil drop and of endoplasmic granules in the same position within the same egg did not reveal any difference in the movements of these two kinds of marker.

II. *Egg without mitotic apparatus*

Averaged protoplasmic movement of the colchicine-treated egg is shown in Fig. 11. Although the spindle and the peripheral parts of the asters disappear after treatment with colchicine, the central parts of the asters do not completely disappear; they remain as two bright areas within the cell even when the drug is sufficiently concentrated to inhibit cleavage. Black circles in Fig. 11 indicate the averaged positions of the centre of this bright area. It will be seen that this point moves together with the surrounding protoplasm. The difference in position between the bright spot in colchicine and the astral centre in the normal egg (black circles in Fig. 4) may be due to the rearrangement of endoplasm resulting from disintegration of the mitotic apparatus.

Figs. 12 and 13 indicate linear and areal changes of the cell surface during cleavage of the colchicine-treated egg. The results are similar to those for the normal egg except that the changes are slightly more exaggerated in the colchicine-treated egg than in the normal one.

In contrast to the cortical movement, the endoplasmic movement is much affected by treatment with colchicine. The displacement of endoplasm in the central zone of the cell is much greater in the colchicine-treated egg than in the normal egg. Vertical lines in Fig. 4 appear as curved (convex) toward the pole in Fig. 11. Fig. 14, which corresponds to Fig. 8B in the normal egg, brings out this fact more clearly. The displacement curves of endoplasm through the stationary surface ring† are almost simple parabolas with their axes coinciding with the spindle axis. Since the protoplasmic viscosity decreases after colchicine treatment (Swann & Mitchison, 1953), it seems likely that the impediment to the movement of the endoplasm is absent in the colchicine-treated egg. From these facts and from the argument in the preceding section it is suggested that the displacement curves indicated in Fig. 14 are interpreted as passive flow of endoplasm which has become solated by the treatment with colchicine.

* In some experiments, the injected drop was gradually pushed out through the path of the insertion of the pipette. This may be due to unsuccessful impalement by the micro-pipette, as pointed out by Tyler, Monroy, Kao & Grundfest (1956).

† The position on the cell surface which is at (0.530, 0.850) at stage 100% and is at (0.550, 0.856) at stage 70% in Fig. 11.

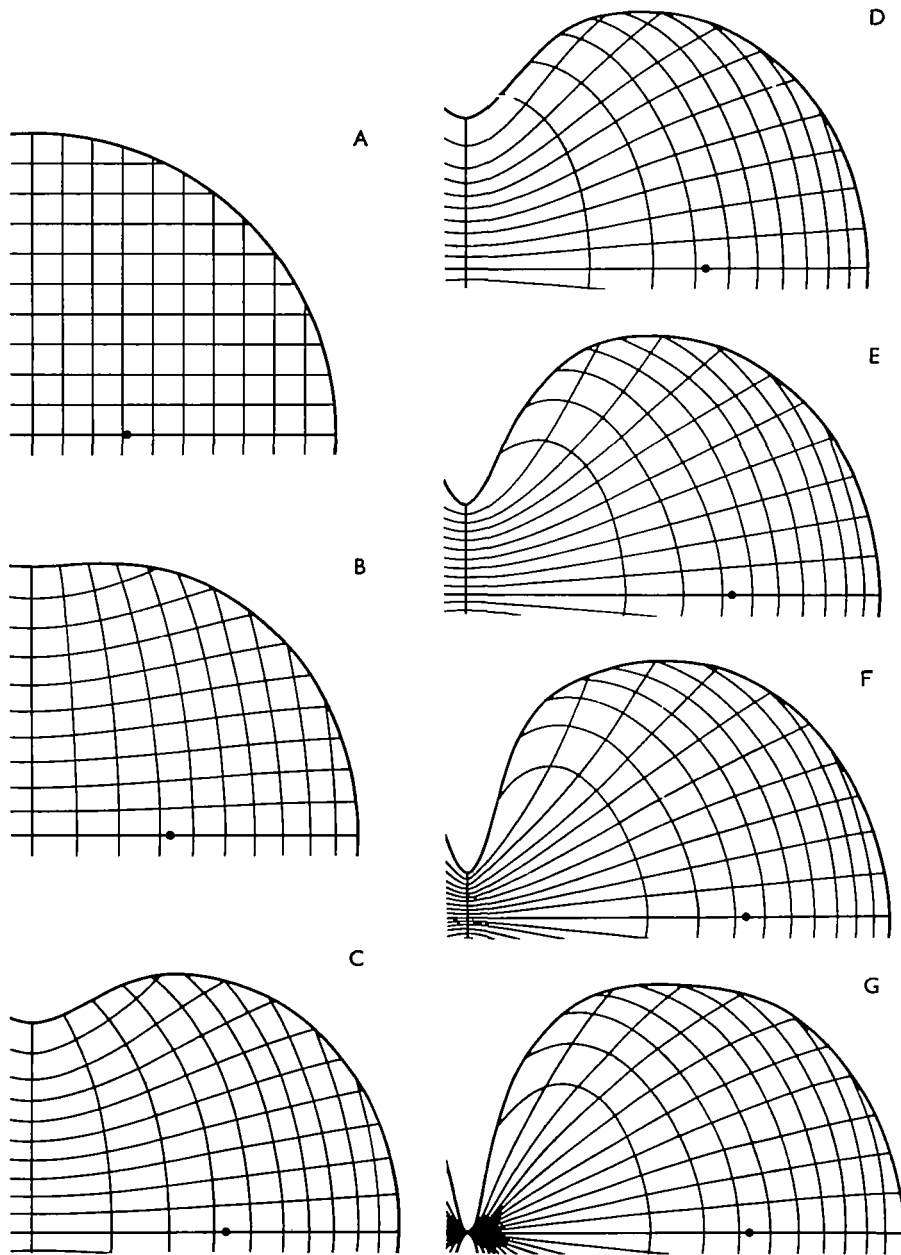


Fig. 11. Diagram indicating the protoplasmic movement during cleavage of the colchicine-treated egg. A, stage 100 %; B, stage 90 %; C, stage 70 %; D, stage 50 %; E, stage 30 %; F, stage 15 %; G, stage 0 %. Cross-lines within the egg indicate different strata of the endoplasm in the largest optical section of the egg. Black circles indicate positions of the remnant of the astral centre.

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An attempt was made to trace the protoplasmic movement during cleavage in eggs from which the spindles had been removed by sucking them into a micropipette (Hiramoto, 1956). However, since the genuine movement of protoplasm

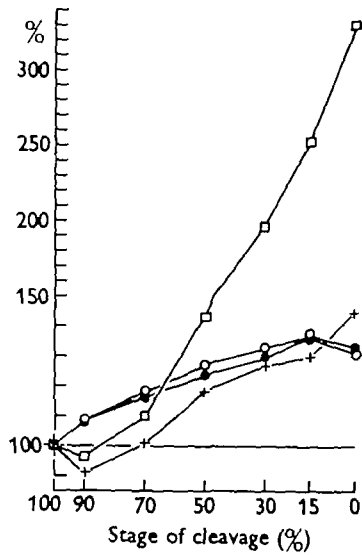


Fig. 12

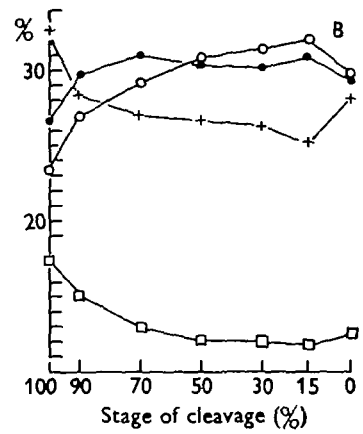
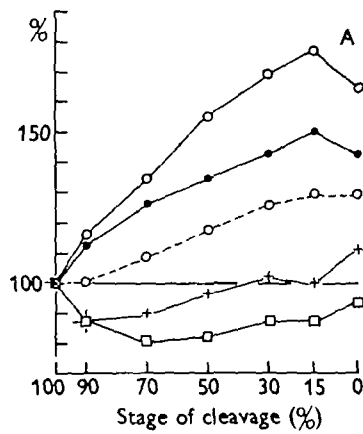


Fig. 13

Fig. 12. Linear elongation or shrinkage of the cell surface during cleavage of the colchicine-treated egg. Curves are constructed from Fig. 11. O, polar region; ●, subpolar region; +, subfurrow region; □, furrow region.

Fig. 13. Areal change of the cell surface during cleavage of the colchicine-treated egg. A: percentage changes taking the initial state as 100. Broken line indicates change in total surface area. B: changes in area of individual regions as percentages of the total surface area. Definition of symbols is the same as in Fig. 12.

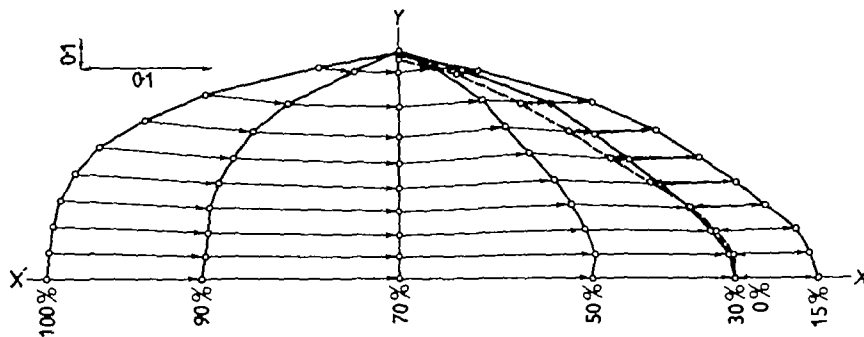


Fig. 14. Displacement of endoplasm through the plane of the stationary surface ring of the dividing colchicine-treated egg. The x-axis (XX') corresponds to the abscissa in Fig. 11. The y-axis indicates the plane of the stationary surface ring. Circles connected by heavy lines indicate the changes in position of endoplasmic elements constituting the plane of the stationary surface ring at the stage 70%. The scale of the abscissa is magnified five times.

by cell division overlaps with the protoplasmic rearrangement resulting from the recovery process from strain set up during the operation, it was impossible to obtain definite information.

DISCUSSION

In this study, cortical movement during cleavage has been described by using particles attached to the surface of the egg as markers. The movement of particles seems to represent the cortical movement of the egg in so far as no slipping occurs between the cortical cytoplasm and particles adhering to the cell surface (cf. Dan & Dan, 1940). Endoplasmic movement has been determined by tracing the movement of granules within the endoplasm. The movement of these granules represents the movement of the protoplasm in which they are suspended, because they move together with an injected oil drop whose movement seems to be entirely passive.*

Whether the motive force of cell division is located in the cortex or in endoplasmic structures such as the spindle and the asters is one of the most important problems concerning the mechanism of cell division. As is mentioned above, the shortening of astral rays attached to the furrow cortex in the early stage of cleavage suggests the active movement of the cortex. According to Dan (1943), the distance between the astral centre and the furrow surface (namely, the length of astral ray) is kept unchanged during early stages of cleavage in *Hemicentrotus* (*Strongylocentrotus*) and *Mespilia* eggs (cf. figs. 6 and 7 in his paper). In eggs of these species the shortening of the astral rays in the furrow region may be too small to be ascertained in early stages.

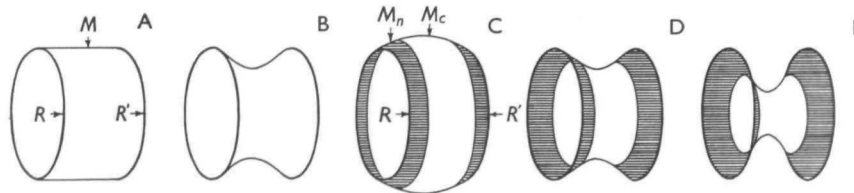


Fig. 15. Supposed changes in the shape of a membrane spanning the space between two immovable rings (a model imitating the behaviour of the furrow and the subfurrow regions of the dividing sea-urchin egg bordered on both sides by its stationary surface rings). A, B: uniformly contracting membrane (M) at rest (A) and in contraction (B). C, D, E: a membrane composed of contractile (M_c) and non-contractile (M_n) parts at rest (C) and in the process of contraction (D, E). R, R' : immovable rings.

The three-dimensional contraction of the furrow surface cannot necessarily be expected from the contracting-ring (band) theory, because contraction of the furrow surface cannot take place freely since the volume of the cell is required to be kept unchanged. Now we shall imagine a model which is composed of two rigid rings of the same size lying in planes perpendicular to their common axis, like two wheels

* Iida (1942) found cytoplasmic granules stainable with neutral red in *Mespilia* and *Clypeaster* eggs which made peculiar movements, but they are different in kind from the granules in the present work.

on a shaft (R and R' in Fig. 15 A) and a membrane connecting these two rings (M). The two wheels represent a pair of stationary surface rings. If this membrane shrinks in area while the distance between the rings is kept unchanged, the membrane must change its shape as shown in Fig. 15 B: the area of the membrane decreases while the contour in the lateral view increases. If the surface bulges out in the lateral view as shown in Fig. 15 C, and if it is further assumed that the contractile region is restricted to the middle part of the membrane (M_c) while the remaining parts are non-contractile (M_n), then the shape of the membrane, when M_c is made to contract, must be identical with the shape in Fig. 15 B. In this case, the contour line of the active region in the lateral view will decrease when the contraction is small (Fig. 15 D), but it will increase when the contraction is large (Fig. 15 E), while its area decreases steadily regardless of the degree of contraction, and the non-contractile membrane (M_n) expands both in length and in area. The situation in dividing eggs is similar to that in the latter case. The behaviour of the surface of the furrow and subfurrow regions of the cortex in the early stages of cleavage can be imitated by the above model.*

'Contraction of the furrow cortex' in the present paper means the development of a contractility of the furrow cortex higher than that of other regions, but the other regions may also change their mechanical properties during cleavage (cf. Mitchison & Swann, 1955).

Increase in surface area in the furrow region during the late stages of cleavage (after stage 70%, Fig. 7 A, B) must be explained by assuming that the contractility of the furrow cortex is greater in the direction parallel to the equator ('latitude') than in the direction along the lines joining pole to pole ('longitude').

Abrupt increase in area of the furrow surface in the late stage of cleavage (after stage 15%) accompanying the contraction of the polar surface has been pointed out in the present experiment. The author believes that the active phase of cell division almost comes to an end in the stage 15%, and subsequent separation of blastomeres can be brought about by their surface forces without further contraction of the furrow surface, in the same manner as the separation of an elongated liquid drop into two.

SUMMARY

1. Protoplasmic movements during cleavage in the eggs of the heart-urchin *Clypeaster japonicus* have been followed by tracing the movements of cytoplasmic granules and of carbon particles adhering to the surface.
2. These movements are quantitatively described in normal eggs and in eggs whose mitotic apparatus has been destroyed by colchicine.
3. The results obtained are qualitatively similar to those obtained by Spek and by Dan and his collaborators.

* An initial shrinkage phase in linear change is observed in the subfurrow region as well as in the furrow region in the present experiment (Fig. 5), while it is observed only in the furrow region in the experiments of Dan and his collaborators. This discrepancy may be due to the difference in the breadth of the contracting band in these two cases.

4. Endoplasmic movement and changes in the length and shape of the astral rays are readily explained by the contracting-ring (band) theory.
5. The location of the motive force of cell division is discussed.

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