SURFACE CHARACTERS OF DIVIDING CELLS

II. ISOTROPY AND UNIFORMITY OF SURFACE MEMBRANE

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INTRODUCTION

One outstanding characteristic of the surface movement of dividing sea-urchin eggs is the presence of a pair of so-called stationary surface rings (Ishizaka, 1958). These stationary zones are symmetrically placed on both sides of the cleavage plane, girdling the two incipient blastomeres. As can be seen in Fig. 1, the circular zones not only retain their respective radii unchanged \(s_1, s_2, s_3\), but they also maintain a constant distance from the cleavage plane throughout the furrowing process, notwithstanding the fact that the furrow is steadily sinking and the distal (polar) ends of the cell are continuously moving away from each other. Although the polar surface and the equatorial (furrow) surface must be exerting forces upon each other during division, as long as the circular zones remain absolutely stationary no work is being performed between the two regions of the surface. This means that whatever force is...
accelerating the furrow, it must work through the interior of the cell and not through the surface as Mitchison (1952) and Wolpert (1960) contend.

In the present study of the surface movements during furrow formation of the spermatocytes of the grasshopper, *Acrida lata*, a marking technique is adopted which is the same as that used for sea-urchin eggs (Ishizaka, 1958). In comparing the results obtained in the two forms, consideration is extended to isotropy and uniformity of the surface layer of the cell.

**METHODS**

The material was prepared in the following way. After cutting off the tip of the testicular follicle with a sharp knife, the spermatocytes suspended in body fluid are put on a coverglass on which fine charcoal particles have been sprinkled beforehand.

![Surface movement of the dividing grasshopper spermatocyte](image)

If the spermatocytes shrink by being dried during the preparation or as a result of the physiological condition of the grasshopper blood in dry weather, the preparation is breathed on to dilute the medium until the cells round up. Then the coverglass with the cells is inverted over a depression slide and sealed with liquid petrolatum. The bottom of the depression has been wetted with saliva to prevent clouding by condensation of vapour. In such a preparation it is possible to control the tonicity of the medium by warming or cooling the slide (not the coverglass), which makes the vapour condense either upon the hanging drop or upon the bottom of the depression as desired. As long as the tonicity is maintained within a certain range, furrow formation is completed between 12 and 15 min. at 30° C. with no sign of abnormality.

Finally, carbon particles firmly attached to the cell surface (not showing Brownian motion) are selected as markers and a series of camera-lucida drawings is made of (1) the cell contours of the largest optical section, (2) the positions of the markers thereon, and (3) the positions of the spindle poles. The displacements of markers are
analysed by superimposing the drawings with reference to two co-ordinates, one passing through the spindle axis and the other coinciding with the plane of furrow, as illustrated in Fig. 1 for sea-urchin egg and in Fig. 2 for the *Acrida* spermatocyte.

**RESULTS**

Data for *Acrida lata* are shown in Fig. 2. Three features must be pointed out concerning this figure.

(A) The contours of the *Acrida* spermatocytes, unlike those of sea-urchin eggs, can be fitted to segments of a circle within 3% of deviation as long as the tonicity of the medium is so maintained as to keep the cells spherical.

(B) As in sea-urchin eggs, superimposed contours of a dividing spermatocyte intersect at four points (designated as S), which indicates the presence of a pair of special circular zones, each on an incipient daughter cell. Needless to say, these circular zones run parallel to each other and are placed symmetrically on both sides of the furrow plane.

(C) However, there is a definite difference between sea-urchin egg and spermatocyte in the behaviour of a marker in the vicinity of the intersection points. A particle S on the upper right-hand side of Fig. 2 shows an undeniable sliding movement toward the pole as the furrow advances, in contrast to absolute immobility of markers on sea-urchin eggs.

(D) The horizontal shift of a marker around the intersections in *Acrida*, just mentioned, brings up the following aspect particularly clearly. In Fig. 2, a marker lying practically on the furrow pursues a nearly vertical course with a slight sidewise shift only at the last stage of division. The loci of the next marker show a greater tendency for sidewise sliding. By other data not illustrated here, it is evident that the nearer a marker to the intersection point, the more horizontal are its loci. If the particle S were situated slightly more to the right, its loci would have been perfectly horizontal.

A mirror-image situation is found on the polar side. On the whole, loci of particles on the polar side diverge radially. However, as the position of a particle is shifted toward the intersection point, the loci become more horizontal. For instance, in Fig. 2, the loci of the lower marker lie on a line which passes through the centre of the cell, which means they are radial, while those of the higher marker are entirely off centre, which means they deviate from the radial towards a horizontal direction.

**CALCULATIONS**

Since the cell contours can be fitted to spheres (result A), the quantitative characteristics of the dividing cell surface can easily be analysed by a procedure shown in Fig. 3. A perpendicular is drawn from a marker m to the spindle axis, the length of which is designated as R and the distance between the foot of the perpendicular and the furrow plane is expressed by z. Then (z, R) is a cylindrical co-ordinate for points on a cell circumference, including m, and running parallel to the furrow plane.

To begin with, the movements of markers in the direction of the spindle axis will be examined. In Fig. 4 the horizontal components (z) of the movement of a marker,
$m$, are plotted against the changing lengths of the cell as measured by the pole ($z_p$). In such plots $z_p$ serves as a scale for the advancement of division. Therefore, if a marker, $m$, happens to be attached to the pole of the cell, since $z$ becomes equal to $z_p$, the plots will be a straight line inclining by $45^\circ$ (the uppermost line of Fig. 4). If a marker is at the centre of the furrow, the plots coincide with the abscissae as $z$ remains always 0.

Fig. 3. Geometrical relation. $C_0$, Origin of the co-ordinates at the centre of the cell; $z$, a co-ordinate along the spindle axis; $R$, radial co-ordinate perpendicular to $z$-axis; $r$, radius of curvature of the cell contour. $P(0, R_f)$, the deepest part of the furrow; $P(z_p, 0)$, pole of the cell; $C_r(z_c, 0)$, centre of curvature; $m(z, R)$, arbitrary marker.

Fig. 4. Displacement of marker along the spindle axis. Ordinate: $z$, distance between a marker and the furrow plane. Abscissa: $z_p$, distance between the cell pole and the furrow plane. $r(0)$, Radius of the spherical stage before division; $r'$, radius of incipient daughter cell.
Plots of markers for intermediate positions are also shown in Fig. 4. It is rather surprising not only that all the plots fall on straight lines but also that their extrapolations converge to the origin of the graph. Expressed otherwise, this means that the displacement of any part of the surface parallel to the spindle axis is strictly proportional to the change of cell length \( z_p \) all through division.

Denoting the stage before the beginning of furrow formation by an indicator, \( (o) \), the above relation is represented by a simple formula, \( z/z_p = z(o)/z_p(o) \).

Since the contours of a dividing Acrida spermatocyte can be approximated to segments of spheres (result A), by denoting the radii of such spheres as \( r \) and the radius of the initial spherical stage as \( r(o) \), the above formula can be converted into

\[
2\pi rz / 2\pi rz_p = 2\pi r(o) z(o) / 2\pi r(o) z_p(o).
\]

In this form, the denominator is the surface area of the incipient daughter cell (see also Dan & Ono, 1954), while the numerator is the surface area of the part of the cell delimited between the furrow plane and the cell circumference including \( m \), parallel to the former. This means that in terms of area expansion, too, the degree of expansion of any part of the cell surface is strictly proportional to the total expansion of the entire cell surface throughout the division process: in other words, the cell surface behaves uniformly in all its parts. This is not the case with sea-urchin eggs (Dan & Ono, 1954).

As the next step, the movement of markers perpendicular to the spindle axis will be considered. The change of ratio in the radius of the circumference including \( m \) is represented as \( R/R(o) \) and this is plotted against change in the radius of the furrow itself, \( R_f / R(o) \), the latter again serving as a scale for deepening of the furrow. The results are shown in Fig. 5.

Changing values of \( R \) (the radius of the circumference involving \( m \)) are calculated on three bases.

(i) Uniform expansion of the surface area (equation (1)).

(ii) The cell contour as a segment of a sphere (result A),

\[
R^2 = r^2 - (z - z_p)^2 \quad \text{or} \quad R = [r^2 - (z - (z_p - r))^2]^{1/2}.
\]

(iii) Volume constancy during furrowing (Hiramoto, 1958). The volume of a segment of a sphere is calculated by

\[
2\pi(r - z_p/3) z_p^2 = 4\pi r(o)^3 / 3.
\]

Observed data fall approximately on the theoretical curves obtained by calculation. Especially noteworthy is the fact that the calculated curve for the intersection point, \( z(o) = r(o)/(z_p - 1) \), remains consistently on the level of 1. This means that at the intersection point the radius remains unchanged throughout the division process just as it does in the case of the stationary surface rings of sea-urchin eggs.

Therefore, the only difference found between the surface ring of the sea-urchin egg and that of the Acrida spermatocyte is that the latter fails to remain absolutely stationary, but slides toward the pole as the furrow deepens. This behaviour of the Acrida surface ring is a natural consequence of a uniform expansion of the cell surface during division which carries the ring away from the furrow plane.

In order to examine this difference more closely the tracing of the movement of
markers is extended until after the completion of division in the two forms. In the grasshopper spermatocytes no further movement takes place after division, while in sea-urchin eggs a marker attached to the stationary ring, in spite of the fact that it has remained at the same spot during division, is now pushed toward the pole parallel to the spindle axis (s in Fig. 1). Therefore, the ultimate position of the markers turns out to be very similar in the two forms if the tracing is extended till after the completion of division.

![Diagram](https://via.placeholder.com/150)

**Fig. 5.** Displacement of marker perpendicular to the spindle axis. Abscissa: ratio of the decreasing radius of advancing furrow to that of the initial diameter of the cell. Ordinate: ratio of $R$ of a marker to the initial $R(o)$. Solid lines: calculated curves with observed markers. Broken lines: calculated curves without markers.

**DISCUSSION**

Judging from the fact that spermatocyte, which is flattened by a force applied from outside, can round up again in a few minutes when the force is removed, the rheological relaxation time of the spermatocyte surface may be of the order of about 1 min. On the other hand, the duration of the division process in this cell is as long as 12-15 min. so that the speed of advance of the furrow (inversely related to the duration of division) will be too slow to induce a viscous drag.
In cleaving sea-urchin eggs, by contrast, not only have viscosity and elasticity effects been detected by many investigators (Marsland, 1938, 1939; Mitchison & Swann, 1954, 1955; Wolpert, 1960; Hiramoto, 1963a, b), but the speed of advance of the furrow is much faster than in the grasshopper spermatocytes (about 5 min. for summer species). On the basis of these observations it can be anticipated that transient visco-elastic forces are more effective in cleaving sea-urchin eggs than in dividing spermatocytes.

Therefore, if it is assumed that it is the visco-elastic effect by which the stationary surface ring of the sea-urchin egg is held fixed in position during cleavage, it is not surprising to find that such a ring will gradually be pushed towards the poles to an equilibrium position similar to that reached in the spermatocyte after the transient stress is removed on completion of cleavage.

In the grasshopper spermatocyte, as was pointed out earlier, the surface area expands uniformly during division and the surface curvature is also uniform, conforming to a segment of a sphere. Therefore stress in such a surface must also be isotropic, like surface tension. For the surface under isotropic and uniform tension to expand, mechanical work must be supplied by other parts of the cell, and it is very likely that the equatorial furrow cortex and/or the endoplasm will provide the external force.

The point which the present author wishes to emphasize is that the cell surface of both sea-urchin egg and Acrida spermatocyte must be isotropic and uniform in the equilibrium state. Surprisingly enough, after the completion of division even the contour of the furrow region becomes spherical as in the other regions, although the furrow surface has been subjected to extremely anisotropic strain during division. Hiramoto (1963a, b) regarded the surface stress in the sea-urchin egg as isotropically and uniformly elastic, with a Poisson ratio of 0.5. Yoneda (1964) recently measured the surface stress of the sea-urchin egg, allowing ample time for equilibrium to be reached, and he in fact concluded that the surface stress was uniform and isotropic, like surface tension.

Therefore, whether the circular zone remains stationary or slides during division is related to a difference in the reaction of such surfaces to division forces imposed on them by the tip of the furrow or by the endoplasm. While the spermatocyte surface accommodates itself immediately relative to the slowly imposed stress, there is a considerable lag in the response of the surface of the sea-urchin egg, owing to a rather high visco-elastic resistance. If so, the difference will be lessened as the time of observation is deferred closer to the relaxation time of the system and the difference will no longer be detected in the final equilibrium state.

**SUMMARY**

1. Surface movement of the dividing spermatocyte of the grasshopper, *Acrida lata*, was followed by a marking method.
2. Throughout the division process of the spermatocytes, incipient daughter cells maintain spherical contours.
3. By direct observation of markers and calculation using the condition given in item 2, the following points are established.
(a) As in the sea-urchin egg, there are a pair of circular zones on a grasshopper spermatocyte surface which retain their respective radii unchanged while the cell undergoes a division.

(b) In the grasshopper spermatocyte, unlike the sea-urchin egg, the surfaces of these circular zones do change their positions and move towards the poles during division.

(c) As a spherical cell goes through a constricted form to become two daughter cells, not only is the radius of curvature of the surface everywhere uniform (item 2), but both axial length and surface area increase uniformly everywhere except in the region of the furrow.

4. From the findings of item 3 it is inferred that the prevailing surface stress is uniform and isotropic, like surface tension, and that the force causing division must be derived from some other parts of the cell such as the furrow cortex or the endoplasm.

5. Basically, the nature of the surface of sea-urchin eggs is similar to that of the spermatocyte. That the circular zones of the former are stationary while those of the latter move steadily during cleavage is tentatively explained in terms of the speed of advance of the furrow in relation to the relaxation time of the cortex.

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REFERENCES


