SURFACE CHARACTERS OF DIVIDING CELLS

I. STATIONARY SURFACE RINGS

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INTRODUCTION

Though many theories have been put forward on the mechanism of cell division, no general agreement has so far been reached. In spite of the fact that each theory brought up various important aspects of the phenomena, such lack of agreement may partly be caused by the inadequacy of data on the stresses acting on the surface of cleaving cells, particularly concerning their magnitudes and the modes of their changes during the process of cell division.

The stress, in the present paper, is considered in terms of displacement of cell parts with reference to the centre of gravity of the cell.

MATERIALS AND METHODS

The eggs of the sea-urchins *Mespilia globulus* and *Hemicentrotus pulcherrimus* were used. In these eggs the mitotic apparatus can clearly be observed through the cytoplasm. The contour of the cell-body is radially symmetrical about the spindle axis, and the cleavage is equal. Accordingly, by choosing eggs in which two astral centres are in the same optical plane, it is easy to find eggs whose spindle axes lie in a strictly horizontal plane.

Since the egg cell shows neither translational nor rotational movement during its first division, it is justifiable to think that the centre of gravity of the egg is at the mid-point between the two astral centres. Once the centre of gravity of the cell is determined, stresses developed during the division process can be expressed as the displacement of cell parts in relation to this centre. This is allowable, because division can be achieved irrespective of any movement of the cell as a whole.

To visualize displacement of the cortex, fine particles of animal charcoal were applied to egg surfaces which had been denuded of the fertilization membrane and hyaline layer according to the technique of Dan, Yanagita & Sugiyama (1937).

The subsequent procedure was as follows: an egg with a horizontal spindle was chosen; the contours of the largest optical section, the positions of particles attached to the contour and those of the astral centres were sketched by a camera lucida at each succeeding stage of division. After completion of the division process such series of sketches were superimposed with reference to the centre of gravity and the spindle axis. In this way, the loci of the particles were obtained.

RESULTS

In Figs. 1 and 2, such superpositions of sketches obtained respectively for *Mespilia globulus* and *Hemicentrotus pulcherrimus* are shown.

On the whole, particles on the furrow surfaces are pulled in, more or less converging to the point of the last attachment of the blastomeres, while particles on the polar surfaces are pushed away in a slightly diverging fashion. But the most surprising thing is that, between the two zones, there are four definite points (in two-dimensional figures) at which all contour sketches intersect. Evidently these points are symmetrical with respect to the spindle axis as well as to the cleavage plane. Therefore, extending this statement to the three-dimensional condition by rotating the sketch around the spindle axis, the four crossing points can be integrated into two rings which are held in common by the egg surfaces at each successive stage of division.

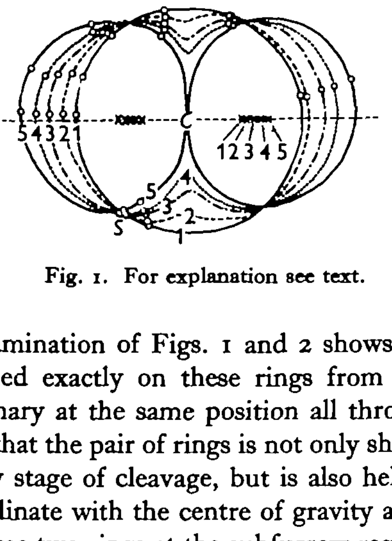

Fig. 1. For explanation see text.

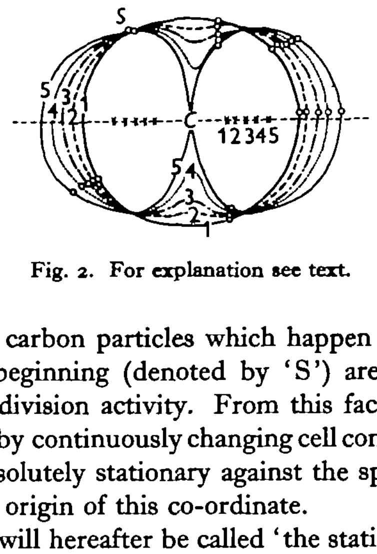

Fig. 2. For explanation see text.

Examination of Figs. 1 and 2 shows that carbon particles which happen to be attached exactly on these rings from the beginning (denoted by 'S') are held stationary at the same position all through division activity. From this fact it is clear that the pair of rings is not only shared by continuously changing cell contours of any stage of cleavage, but is also held absolutely stationary against the spindle co-ordinate with the centre of gravity as the origin of this co-ordinate.

These two rings at the subfurrow region* will hereafter be called 'the stationary surface rings'.

At any rate, as long as the rings remain in the same position, it is beyond any dispute that stresses are balanced at these points (or along these circles, more strictly speaking) at any moment of cleavage, in spite of lack of concrete data on how many components the stresses can be divided into or in what direction and with what magnitude each component is working.

Another fact which should be pointed out in connexion with the figures is the fact that before cleavage the astral centres are situated about 3/8 radius away from the centre of the egg, whereas after the division is complete they are pushed farther away from each other and now lie outside the rings. This means that the astral centres pass through the stationary surface rings during the polar elongation of the cell.

* Furrow, subfurrow, and polar region are defined after Dan *et al.* (1937).

DISCUSSION

As stated above, it has been found that there is a pair of stationary surface rings in the subfurrow region of the dividing sea-urchin egg.

The polar expansion theory of cell division which was proposed by Mitchison (1952) can anticipate the sliding of the whole surface toward the furrow side. But if there are two immobile rings on each side of the furrow region, it may become very difficult to visualize how the movement of the polar region alone can let the furrow surface cut into the cell body.

A reverse situation is encountered by the constricting ring theory of the furrow region advocated by Marsland & Landau (1954). In other words, with the stationary ring interposed, the furrow region and the polar region are more or less clearly separated and it will be very difficult to correlate the behaviour of the two regions at successive stages such as observed by Dan *et al.* (1937).

On the other hand, there seems, at least superficially, to be more possibility of reconciling the present facts with Dan's theory (1943). In the first place, when the convex surface of the furrow region changes towards concavity as it cuts into the cell-body, with its two sides bordered by the stationary rings, the only possible way for this to happen is for the furrow surface first to shrink to flatness and then to reverse its curvature. This corresponds exactly to what Dan calls the initial shrinkage of the furrow region.

In the second place, it is even possible to explain the surface ring by Dan's theory. Dan attributes the main feature of division activity to the pushing away of the two asters by an autonomous elongation of the spindle. He considers that the expansion of the polar surface is due to fountain-like bending of the astral rays as the asters are being pushed toward the polar surface, while the shrinkage of the furrow is due to the pull by the median crossing rays of these same asters as they are departing from the median plane. In other words, simple movement of the asters can give paradoxically opposite effects to the polar and furrow regions, the former being carried away from the median plane while the latter is shrinking toward the median plane. If so, there should be a dividing line between the two which must be in equilibrium between the two opposing forces.

As a corollary, it might be said that the cortical activity theory may have to discover different causes for the polar and the furrow behaviour as the result of the presence of the stationary rings, while the internal activity theory has the advantage of explaining the behaviour of both these regions in terms of a single common cause.

SUMMARY

1. The surface movements during division have been studied by marking the naked surface of the sea-urchin egg with charcoal particles.

2. The contours of the largest optical section, the positions of the particles thereon and the positions of the astral centres are recorded in a series of camera lucida drawings.

3. The drawings are then superimposed, the centre of gravity and spindle axis being used for reference.

4. It is thereby shown that there are two surface rings which remain in the same positions throughout the whole process of division.

5. It is concluded that these rings indicate regions where stresses remain balanced during division.

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