ON 'THE STATIONARY SURFACE RING' IN HEART-SHAPED CLEAVAGE

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In the foregoing paper, Ishizaka (1958) succeeded in demonstrating the presence of a pair of ring-zones in the cortex of sea-urchin eggs which remain absolutely stationary with reference to the co-ordinates outside the egg through successive changes of the form of dividing ova. The purpose of the present paper is to see whether or not a similar situation exists in the case of cells cleaving in a heart shape; i.e. in cells in which the cleavage furrow of one side appears earlier than that of the opposite side.

As examples of heart-shaped cleavage, the eggs of the sand dollar, *Astriclypeus manni*, and those of the medusa, *Spirocodon saltatrix*, were used. In these eggs, the cleavage furrow appears first at the animal pole; hence the cell takes a heart shape at some moment of the cleavage. The primary cause of the heart-shaped cleavage is considered by the author to be the eccentric position taken by the cleavage spindle, which lies closer to the animal pole (Dan & Dan, 1947*a*, *b*). When *Astriclypeus* eggs are observed along the egg axis, however, since the contour of the largest optical section corresponds to the equatorial surface of the egg between the two poles, the cleavage pattern becomes seemingly symmetrical with no sign of the heart shape. When the eggs are observed perpendicularly to the egg axis, the difference in the phase of furrow formation at the opposite poles becomes maximal. Observation from intermediate angles gives various degrees of asymmetry. Besides this, there is a fairly wide range of variation in the eccentricity of the cleavage spindle among different batches of *Astriclypeus* eggs, so that by combining the selection of females and angles of observation, the eccentricity can be continuously varied within a considerable range.

In *Spirocodon* eggs, because of an exceeding degree of eccentricity of the spindle, the incipient blastomeres flatten out considerably at the middle of the division process and all come to lie on the flat side, thus showing only the broad side to the observer. In this case the animal and vegetal poles are clearly in view.

The method employed was practically the same as that adopted by Ishizaka; that is, to draw a series of camera lucida sketches in which changing cell contours, changing positions of the astral centres and of the carbon markings on the egg surface were recorded. These cell contours were then superimposed.

Some technical difficulty not encountered in regular cleavage was found in the case of heart-shaped cleavage. In Ishizaka's case, since the ova used cleaved equally

and symmetrically around the spindle axis, superposition of the spindle axes and the mid-points of the two centres of the sketches did not leave any room for ambiguity concerning the manner of superposition. Consequently the existence of four intersections on the contour is unequivocal. But in the case of the heart-shaped cleavage, as the result of the eccentric position of the mitotic figure, the asters are supposed to rotate around their own centres which, in turn, makes the spindle bend toward the vegetal pole (Dan & Dan 1947*a*, *b*). Consequently it is impossible to resort to a purely objective method of superposition such as that adopted by Ishizaka. For the present case, therefore, assuming that stationary regions may exist at least around the animal pole, the drawings of the eggs were superposed accordingly. In this sense, the following is a simple trial even anticipating an error just to see whether or not such a stationary zone can be established for heart-shaped cleavage.
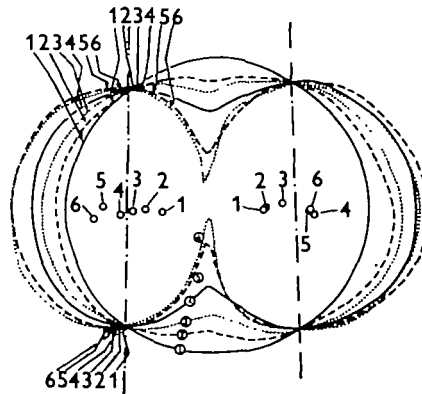

Fig. 1. Superposition of the contour drawings of cleaving egg of *Astriclypeus manni*, a sand dollar, seen along the egg axis. Numbers on the cell periphery are successive positions of adhering kaolin markings and numbers at the centre are those of the astral centres. The contours intersect at four points. Lines connecting two of the points on each incipient blastomere run practically parallel. Loci of the astral centres make roughly two straight lines (eccentricity, 0·5; angle, 4·0°).

The results obtained are illustrated in the figures. As shown in Fig. 1, when the eggs are looked at along the egg axis, that is, when the furrows of the opposite sides appear in a symmetrical fashion, the presence of a pair of stationary surface rings is confirmed beyond any doubt. As was stated before, these skectches were superimposed in such a way as to obtain four stationary intersections. In consequence of such superposition the loci of the astral centres approximate to straight lines. Furthermore, the two planes determined by the stationary rings run almost parallel to each other (in Fig. 1 the two planes deviate from parallel by only 4°).

In the eggs viewed perpendicularly to the egg axis (Fig. 3), if the animal region contours are so superimposed as to give two maximally clear-cut intersection points, the vegetal intersection points vary over a wider range than those encountered in the case of regular cleavage. Yet it is still possible to say that they fall on a relatively narrow portion of the contour. Considering also the case shown in Fig. 2, it is permissible to say that a pair of stationary surface rings occur also for heart-shaped

cleavage with the reservation that they become somewhat blurred in width toward the vegetal side. Tentatively allowing this conclusion, two other features become evident. As the disparity in the phase of furrowing at the animal and vegetal poles increases: (1) the loci of the two astral centres slant upwards and away from each other; (2) the angle included between the two stationary rings becomes more obtuse.
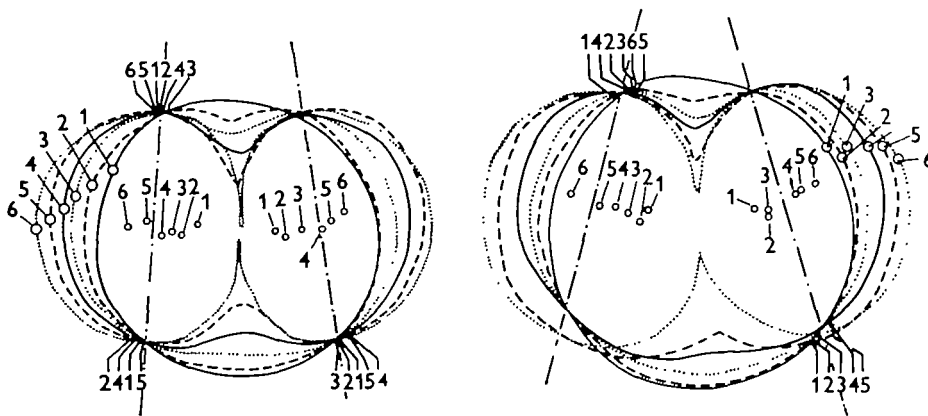

Fig. 2   Fig. 3

Fig. 2. Superimposed contour sketches of dividing *Astriclypeus* eggs, seen obliquely to the egg axis. Note asymmetry in the phase of furrowing on the opposite sides. Numbering as in Fig. 1. Lines connecting two intersection points on either side of the furrow converge and the loci of the astral centres tend to slant up at the ends (eccenticity, 0·47; angle, 13·7°).

Fig. 3. Cleaving *Astriclypeus* egg with an extremely eccentric spindle, seen perpendicularly to the egg axis. Note that the inclination of the planes of the stationary surface rings and the slanting of the astral loci are more emphasized than in the previous figures. Note also that the vegetal intersection points shift around during the course of cleavage (eccentricity, 0·43; angle, 31·0°).

Concerning the V-shape of the astral loci, no explanation is available at present. But it may be worth noting that this resembles the bending of the spindle which normally occurs in heart-shaped cleavage.

In order to state in more quantitative terms the proposition contained in the second point, the eccentricity must be expressed more accurately. In the following, the eccentricity is expressed as a ratio, taking the cell diameter as denominator and the distance from the spindle to the nearest cell periphery, the animal pole, as numerator; in other words, when the spindle is central in position, the ratio is 0·5 and the value decreases as the spindle becomes more eccentric. The measurement of eccentricity is not so easy as it may sound because the position of the mitotic apparatus tends to shift toward the centre of the cell as the asters grow larger. Therefore, the effort was made to take the final position of the spindle before the cell departed from the spherical condition.

In connexion with Fig. 1, it was mentioned that the two lines connecting the intersection points run parallel to each other. In this case the apparent position of

the spindle is central (the eccentricity value is 0·5). The egg shown in Fig. 2 has the value of 0·47 and the angle found between the two lines is 13·7°. In the case of Fig. 3, the corresponding values are respectively 0·43 and 23·0–31·0°.
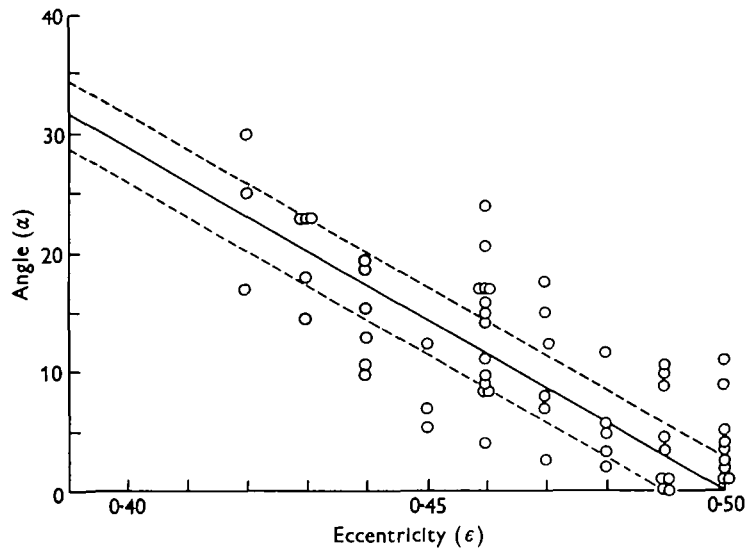

Fig. 4. The data of Table 1 plotted and a straight line fitted to them. The character of the straight line is defined in the text.

Table 1. *Relation between spindle eccentricity and inclination between stationary surface rings*

Eccentricity	Angle in degrees									Average
0·5	4·0	2·5	3·5	11·5	1·0	2·0	5·0	1·0	9·0	4·9
0·49	9·0	10·5	0	10·0	4·5	1·0	3·5	1·0	0	4·9
0·48	3·5	11·5	2·0	5·5	5·0	—	—	—	—	5·5
0·47	12·5	17·5	8·0	2·5	12·5	7·0	15·0	12·5	—	10·9
0·46	20·5	11·0	4·0	17·0	16·0	15·0	17·0	24·0	9·0	—
	14·5	8·5	8·5	9·0	8·5	—	—	—	—	13·0
0·45	5·5	12·5	7·0	—	—	—	—	—	—	8·3
0·44	13·0	10·5	10·0	19·5	19·0	15·5	—	—	—	14·6
0·43	18·0	14·5	23·0	23·0	23·0	—	—	—	—	20·7
0·42	25·0	17·0	30·0	—	—	—	—	—	—	24·0

All the data concerning the angles and the eccentricity are given in Table 1 and plotted in Fig. 4. The plots fall within an inclining belt zone, the two borders of which are almost parallel. The author interprets this as chiefly being due to variation in the eccentricity measurements rather than to error in the measurements of the angles because, in the latter case, the belt would be broader toward the higher values of the angles in a fan shape. On the other hand, it is not surprising to find that the eccentricity varies, as it is not a stable thing itself, and a great deal of chance may be involved in determining the exact stage at which the cell departs from the spherical condition.

If the plots are grouped by 0·01 unit of the eccentricity, the straight line shown in Fig. 4 is obtained, which can be defined as

$$\alpha = \lambda \; (0\cdot 50 - \epsilon),$$

α is angle between the two stationary rings; ϵ is eccentricity of the spindle. λ was found to be 285 by the least square; the mean square being

$$\overline{\Delta^2} = \{\overline{\alpha - \lambda\,(0\cdot 50 - \epsilon)}\}^2 = 5\cdot 3^2,$$
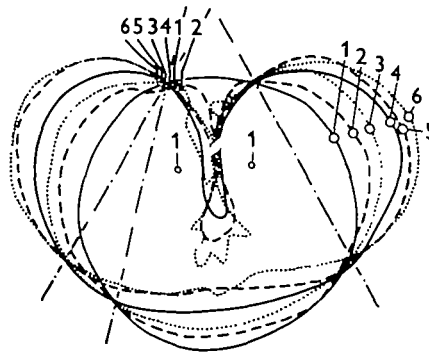
which is shown as broken lines in Fig. 4.

Fig. 5. Superposition of contour sketches of cleaving egg of the medusa, *Spirocodon saltatrix*, seen perpendicularly to the egg axis. In spite of indefiniteness of vegetal intersection points, it is clear that the stationary surface rings lean toward each other embracing a very obtuse angle.
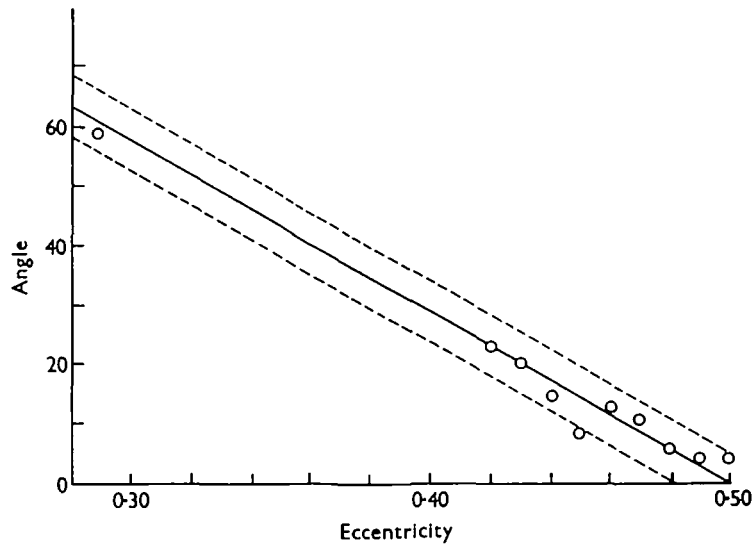

Fig. 6. Exact fit of the *Spirocodon* points on the *Astriclypeus* line. Extreme left point is average value of six *Spirocodon* cases. The group of points on the right are averages of *Astriclypeus* data as grouped by 0·01 unit of eccentricity.

Finally an attempt was made to extend the scheme to include the eggs of the medusan, *Spirocodon saltatrix*. The characteristic cleavage of medusae is considered by the author to be only an extreme case of heart-shaped division. Because of technical difficulties only a few data are available, but from these the following conclusions can be drawn. (1) The stationary lines become still more obscure. (2) It is, nevertheless, clear that the two lines incline toward each other, embracing a very obtuse angle between them. (3) Considering Fig. 3, together with Fig. 5, which illustrates medusan cleavage, the fact that as the cleavage process advances, the vegetal intersection points seem to be shifted toward higher levels suggests this as the cause of the indefiniteness of the vegetal intersection points. In the six observed cases of medusan cleavage, the average of the eccentricity is found to be 0·29 and that of the angle, 58·6°. These data are shown in Fig. 6 to fall on the same straight line as the averages for *Astriclypeus* eggs. This indicates that the same basic mechanism is involved in the cleavage of the two forms.

DISCUSSION

As was the case with regular cleavage (Ishizaka, 1958), the presence of a pair of stationary surface rings is extremely difficult to reconcile with existing theories of cell division, and the nature of these rings remains utterly unknown. However, the fact of a linear correlation between the obtuseness of the angle formed between the two planes of the stationary rings and the eccentricity of the spindle is beyond any doubt. In the author's previous analysis (Dan & Dan, 1947a, b), the conclusion was drawn that in the division of a cell whose spindle is eccentric in position, the two asters rotate in such a direction that their animal pole sides converge and their vegetal sides diverge. This is further manifested by the bending of the spindle so that it becomes convex toward the vegetal pole. The conversion of the two stationary rings toward the animal pole seen here must certainly be caused by the rotation of the internal asters. The same astral rotation may also have an indirect connexion with the V-shaped slanting of the astral loci.

SUMMARY

1. The eggs of the sand dollar, *Astriclypeus manni*, and the medusa, *Spirocodon saltatrix*, were used for the reason that they cleave in heart shape, the cleavage furrow appearing earlier at the animal than at the vegetal pole.

2. By the superposition of drawings showing contours and astral centres as well as the positions of carbon markers on the cell surface, the presence of a pair of stationary circular zones of the cortex can be demonstrated. These remain absolutely stationary through successive stages of cleavage, as was shown to be true of regularly cleaving sea-urchin eggs.

3. The two planes determined by this pair of stationary surface rings tilt toward each other on the animal pole side in linear proportion to the eccentricity of the mitotic spindle within the cell, and the loci of the astral centres tend to slant toward the animal pole.

4. The above phenomena can be explained by the previously proposed theory for heart-shaped cleavage; i.e. the primary cause of heart-shaped cleavage is the eccentric position of the spindle, which in turn causes the rotation of the asters and the bending of the spindle.

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