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On the Mechanisms of Cytokinesis in Animal Cells

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We present a model that attempts to explain some aspects of cytokinesis in animal cells. We propose two separate phases of cytokinesis. The first is not dependent on the presence of the mitotic apparatus and involves a general activation of cortical contractile elements resulting in the development of a surface tension. In the second phase the asters of the mitotic apparatus interact and modulate the activities of the tension generating elements in the cortex to produce gradients of surface tension with the highest values being at the equator. Tension generating elements are assumed to be free to move in the plane of the cortex so that they will consequently move up the gradient of tension and accumulate as an equatorial belt of oriented elements i.e. the contractile ring. The model was simulated on a computer and is capable of reproducing some of the wide variety of cleavage configurations that are observed.

Introduction

Cytokinesis is the process of cytoplasmic cleavage which, together with mitosis, makes up the act of cell division. These two processes have been extensively studied since it was first realized that all organisms are cellular, yet neither is completely understood at the present time. Cleavage occurs in animal cells by the formation of a furrow which typically appears as an annular constriction at the equator of the cell. The position of the furrow is almost always such that the mitotic spindle is bisected after anaphase has occurred, thus ensuring the partitioning of the two sets of chromosomes into the daughter cells. An understanding of the process of cytokinesis would have to offer explanations as to the nature, shape and position of the furrow and the timing of furrowing relative to the mitotic activity of the cell. Many theories have been put forward to explain the various aspects

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of cytokinesis; indeed it has been said that all the currently favoured ideas were suggested before 1904 (Rappaport, 1971). While there may well be some truth in this statement, there has been a wealth of observational and experimental data accumulated since that time, and it may be worth trying to re-evaluate and extend some of these ideas. One way of assessing the consequences of a particular theory is to incorporate its basic tenets into a computer model. If computer simulations of the model are able to reproduce accurately the wide variety of cleavage patterns seen, then it is likely that the theory is self consistent and is able, in principle, to explain the observed phenomena. We have used this technique to develop an extension of a theory that was originally proposed by Wolpert (1960, 1966). Before embarking on a detailed discussion of the theory it will be necessary to outline briefly the current state of knowledge on cytokinesis. The literature on this subject is vast but there are a couple of excellent recent reviews which cover the field in some depth (Rappaport, 1971; Arnold, 1976). We shall summarize some of the relevant observations and the conclusions that have become generally accepted.

The Nature and Location of the Force Generating Machinery

The internal cytoplasm of a cell may be mechanically stirred (Rappaport, 1969*a*), partially removed (Rappaport, 1969*b*), or displaced by an oil drop (Hiramoto, 1964) without affecting furrowing activity, so it is now generally accepted that the force generating elements that mediate cytokinesis reside in a cortical region adjacent to the plasma membrane. Recent ultrastructural studies have revealed a circumferential band of microfilaments associated with the leading edge of the furrow (Arnold, 1976) which is generally referred to as the contractile ring. The contractile ring is not a permanent cell organelle, as it is absent during interphase and appears only at the onset of furrowing. Furthermore the volume of the microfilament bundle decreases as furrowing progresses (Schroeder, 1972) implying that it may be disassembling during this phase of cleavage. It is tempting to think of this bundle as a purse string which gathers in the equator of a cell (Arnold, 1969), but this is probably a misleading notion, as, in the case of unilateral furrowing the cleavage furrow is not always a complete ring. In cells which have an eccentrically positioned spindle the furrow first appears at the point on the cell surface nearest the centre of the mitotic apparatus (MA) and then progresses in each direction until it joins up at the far side of the cell (Arnold, 1976). It is difficult to see how an incomplete band of filaments could sustain a tension to produce a furrow in these cases given the two dimensional fluid nature of membranes.

Actin and myosin (Schroeder, 1973; Fujiwara & Pollard, 1976) and other muscle associated proteins (Nunally, D'Angelo & Craig, 1980) have been shown to be present in the contractile ring and it has been demonstrated that furrowing requires ATP (Landau, Marsland & Zimmerman, 1955) and is blocked by anti-myosin (Mabuchi & Okuno, 1977). It therefore seems very probable that the force generating elements are fibrilar proteins similar to those present in muscle. The origin of the filaments of the contractile ring is not known. They could be assembled *de novo* or they could be recruited from adjacent regions of cortex. Cortical contractile proteins are not unique to the contractile ring; most cells are covered with microvilli which have cores containing bundles of actin filaments (Burgess & Schroeder, 1977), also random networks of filaments have been seen in the cortices of cells (Franke *et al.*, 1976). It is likely that these filaments can be mechanically active since cortical contractions at regions not associated with a contractile ring can be illicited by wounding (Bluemink, 1972; Gingell, 1970), intracellular injections of calcium (Baker & Warner, 1972) or surface application of calcium ionophores (Schroeder & Strickland, 1974). Thus the contractile ring seems to be a special case in which the normally disordered filaments of the cortex have become organized into a parallel bundle.

Relationship Between the Mitotic Apparatus and the Cleavage Furrow

The general observation that the cleavage furrow bisects the MA suggests a causative link between the two. This has been confirmed by mechanically repositioning the MA within the cell by centrifugation (Conklin, 1917) or micromanipulation (Hiramoto, 1956). If this displacement is done before the furrow is fully established the furrow is seen to form in the correct position relative to the MA even though this may be in a completely different position from that which it would have normally occupied in an unperturbed cell. If the formation of the mitotic apparatus is blocked by colchicine, no furrow is formed (Beams & Evans, 1940). However, if the spindle is disrupted just prior to the onset of furrowing (Hiramoto, 1968), or even if the MA is completely removed from the cell (Hiramoto, 1956), a normal furrow will form and progress to completion. Furrowing, therefore, is not dependent upon the continued presence of the MA, but rather, it seems as if the MA sets up the initial conditions that are necessary for a furrow to form, and that once initiated, furrowing then proceeds autonomously.

In an elegant experiment Rappaport (1961) has demonstrated that the apposition of two asters can be a sufficient condition for the initiation of

furrowing even if the two asters belong to different MAs. He has also shown that in a given cell type there is a maximum separation of asters and a maximum distance of the asters from the cell cortex beyond which furrowing will not be induced (Rappaport, 1969*c*). In cases of polyspermy where several asters appear in a fertilized egg, multiple furrows have been seen (Sugiyama, 1951), with furrows forming between all pairs of asters. Thus it appears that asters are probably the active agents of the MA which induce furrowing and that all asters are equivalent i.e., they do not exist as complementary pairs. The nature of the interaction between the asters and the furrowing mechanism is completely unknown at the present time. It is reasonable to suppose, however, that microtubules or their associated proteins are involved because of the colchicine sensitivity of the interaction.

Many cells exhibit a progressive separation of their poles during anaphase-B (Inoue & Ritter, 1975). This will have the consequence of altering the geometric relationship between the asters and the cell cortex during cleavage. It is unlikely, however, that this phenomenon provides any of the driving forces for the shape changes that occur during cleavage, since furrowing can proceed in the absence of an intact MA.

Changes in the Mechanical Properties of Cells Associated with Cleavage

It is a common observation that cells tend to round up prior to division. The act of rounding up can perform external mechanical work (Danielli, 1952) and so presumably is an energy consuming process. Measurements have been made of cortical stiffness (Mitchison & Swann, 1955; Wolpert, 1966) and internal hydrostatic pressure (Hiramoto, 1963*a*), both increase prior to cleavage and start to fall off at the onset of furrowing. These changes in the mechanical properties of a cell are not an inevitable consequence of furrowing, since cleavage can be blocked by destruction of the MA, yet cyclic changes in surface properties carry on with the same period as the original cell cycle (Swann & Mitchison, 1953). Recent work has shown that similar cyclic changes can also occur in enucleate cell fragments (Hara, Tydeman & Kirschner, 1980). These observations suggest that there is a general increase in the surface tension of a cell prior to cleavage that is probably mediated by the cortical contractile apparatus and furthermore, that this process is independent of the presence of an intact MA. Thus there seem to be two separable aspects to cytokinesis. The first is a general rise in the activity of the contractile apparatus in the cortex, and the second is the interaction of the bipolar MA with the cortex that specifies the position of the cleavage furrow (Schroeder, 1981). These two activities are coordinated in the normal cell cycle to produce cleavage.

When a cell divides there is generally no change in total cyctoplasmic volume (Hiramoto, 1968), however, there will be an increase of about 26% in the total surface area if both mother and daughters are spherical. This implies that new plasma membrane has to be supplied during cleavage. This could be recruited from pre-existing microvilli (Erickson & Trinkaus, 1976) or be supplied from the endomembrane system. We will not consider this aspect of cleavage any further as we will assume that it plays no active part in furrow formation.

Summary of the Key Aspects of Cytokinesis

Contractile elements are contained within the cortical regions of cells and may become activated under certain circumstances to affect changes in cortical tension. Cytokinesis is preceded by a general activation of these cortical elements which is independent of the presence of the MA. The asters of the MA interact with the cortex in an as yet unknown way to produce a circumferential band of contractile elements at the equator. The subsequent contraction of this band results in the formation of a cleavage furrow.

Geometrical Considerations of the Interaction of the Asters with the Cortex

In order to produce an equatorial constriction in a cell by means of surface forces, there has to be a difference in surface tension between the equatorial and polar regions (Hiramoto, 1963*b*). This may be achieved by either increasing the tension at the equator or reducing it at the poles; both possibilities have been postulated for cleavage (Rappaport, 1971). Because of the demonstrated role of asters in the establishment of a cleavage furrow it is likely that the tension variation in the cell surface is produced by an interaction of the asters with the cortical contractile machinery. Asters are generally spherically symmetric structures. They seem to lack an intrinsic polarity since cleavage furrows can appear on both sides of an aster in certain circumstances (Rappaport, 1961). Therefore it would seem to be reasonable to assume that the stimulating activity of an aster should be spherically symmetric. If one makes two further assumptions; (a) that the cortical stimulating activity from the two asters combine in a simple additive way and (b) that the magnitude of the stimulus varies as an inverse power law of the distance of the centre from the aster to the cortex, then the combined stimulus activity from the two asters is always less at the equator than at the poles. This is so for an initially spherical cell no matter what separation the poles may have (Figs 1 and 2). Thus, this rather

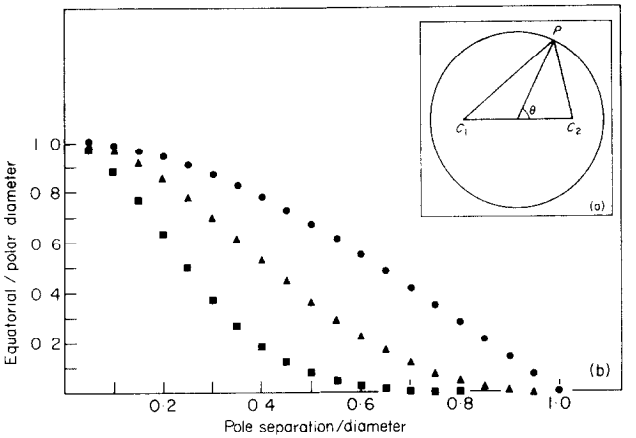

FIG. 1. If the stimulus from an aster is spherically symmetrical and obeys an inverse power law then the scalar sum of the stimulus from both asters is always greater at the poles of a spherical cell than at the equator. This can be seen from simple geometric arguments: Figure 1(a) shows a cell with a radius r having two astral centres at C_1 and C_2 separated by a distance $2d$. For any point P on the surface of the cell the distances to the astral centres will be:

$$C_1P^2 = d^2 + 2rd\cos\theta + r^2$$

$$C_2P^2 = d^2 - 2rd\cos\theta + r^2$$

where θ is the angle the radius from point P makes with the spindle axis. If one assumes a stimulus that obeys an inverse square law and has a unity constant of proportionality then the combined stimulus from the two asters at point P will be:

$$\frac{1}{d^2 + 2rd\cos\theta + r^2} + \frac{1}{d^2 - 2rd\cos\theta + r^2}$$

this will have a maximum value when $\theta = 0$ i.e., at the poles and a minimum value when $\theta = \pi/2$ i.e., at the equator. The ratio of stimulus at the equator and the poles is plotted for different astral separations and power laws in Fig. 1(b). In all cases, the stimulus is lowest at the equator. ●●● = inverse power; ▲▲▲ = inverse square power; ■■■ = inverse fourth power.

simple-minded view always predicts a lower level of stimulation at the equator than at the cortical regions in closest proximity to the asters. These notions are consistent with observations that have been made on cleaving sea urchin eggs, where it has been found that the density of astral microtobules in the cortex is greater at the poles than at the equator (Asnes & Schroeder, 1979). One is therefore led to conclude that an astrally mediated relaxation of a previously established uniform tension is the most plausible mechanism for setting-up the required differential of tension between equator and poles; a notion that was first put forward by Wolpert (1960). This theory is attractive in that it is based on and neatly encompasses two important aspects of cleavage, i.e. the initial activation of the cortical

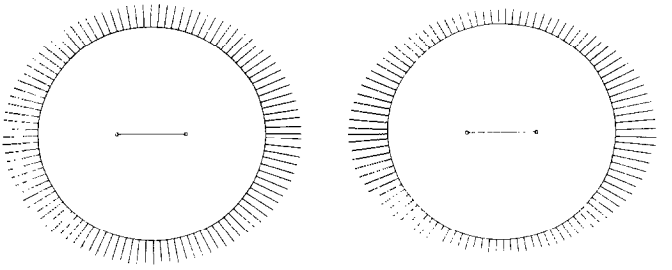

FIG. 2. Computer plots of stimulus distribution on the surface of a spherical cell. The lengths of the lines drawn from the cell envelope represent the magnitude of the stimulus at each point. The points represent the centres of the asters; Figure 2(a) with an inverse square power law and Fig. 2(b) with an inverse fourth power law. Note that even with a high power law the region of lowest tension at the equator is rather broad. (All computer plots are compressed vertically by 6.5% because of the characteristics of the plotting system used.)

tension-producing elements and the interaction of the asters with the cortex. As it stands, however, it is not a sufficient explanation of cytokinesis for reasons which we outline below.

Computer Simulations

It has been shown analytically that differential surface tension can produce equatorial furrowing (Greenspan, 1977). We have explored the properties of this type of model by means of computer simulations. The basic assumptions that we made in setting up these simulations were:

(a) An initially uniform isotropic tension in the cell cortex is modulated by a stimulus emanating from the asters.

(b) The stimulus from the asters induces a relaxation of cortical tension proportional to the magnitude of the stimulus.

(c) The stimulus from each aster is spherically symmetric and obeys and inverse power law.

(d) The net value of the stimulus at each point of the cell surface is the scalar sum of the stimulus from each aster.

(e) Cell volume remains constant during cleavage.

(f) All cell movements associated with furrowing are viscosity limited. (This is undoubtably an over-simplification as cytoplasm is known to be viscoelastic; however, the incorporation of elastic terms in the computer simulations made no appreciable difference to the shape of the cleavage forms, so they were not used)

(g) The internal hydrostatic pressure resulting from the cortical surface tension is uniform throughout the cytoplasm.

In order to simplify computations so that programs would run in a reasonable time only rotationally symmetric cleavage configurations were simulated. In this way it was necessary to consider only one medial section of a cell as all medial sections are identical. The outline of the medial section was approximated by 100 points connected by straight line segments. At each point both the radius of curvature of the cell envelope in the two principal axes and the surface tension were determined. The resultant inward-directed pressure due to surface tension was compared to the internal hydrostatic pressure of the cell (Fig. 3). At the points where

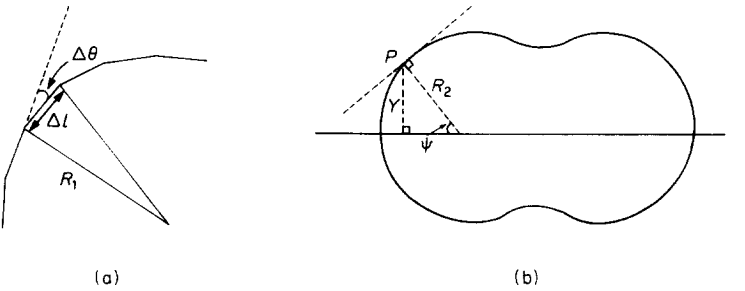

FIG. 3. The pressure P due to an isotropic surface tension force T acting in a spherical surface with a radius of curvature R is given by: $P = 2T/R$ this can be generalized to any curved surface which has orthogonal principal radii of curvature R_1 and R_2 and to an anisotropic surface tension force if the direction of anisotropy corresponds to one of the principal axes. If the tensions along the two principal axes are T_1 and T_2 then the pressure will now be given by: $P = T_1/R_1 + T_2/R_2$. For a point to be in equilibrium on the surface the surface tension forces at that point must be balanced by hydrostatic pressure. Restricting simulations to rotationally symmetric figures simplifies calculations of curvature. Only one medial section has to be considered, the two principal axes being in the plane of the section and orthogonal to it. The radius of curvature in the plane of the section is given by $R_1 = d\theta/dl$ this is approximated by $R_1 = \Delta\theta/\Delta l$ (Fig. 3(a)). The orthogonal radius of curvature is given by $R_2 = Y/\sin\psi$ where Y is the y coordinate of the point and ψ the angle of intersection of the normal at the point of the axis of rotation (Fig. 3(b)).

the stimulation from the asters is weakest (i.e., in the equatorial regions) the pressure due to surface tension is greater than the hydrostatic pressure (which will tend to be the integrated mean of all the surface generated pressures). Points in this region will therefore be subject to a net inward-directed force. Similarly, points in the polar regions will be subject to a net outward directed force, because of the excess of hydrostatic pressure over surface tension generated pressure. Each point was then displaced a small amount along the direction of the force imbalance, the length of the displacement being made proportional to the magnitude of the force imbalance. Thus the velocity of the point was proportional to the force acting

on it, as would be the case for viscosity limited movements. The displacement of the surface points was made sufficiently small that the shape changes appeared as essentially continuous movements. Cell volume was held constant throughout the simulations by applying global inward or outward directed forces on the elements after each iteration to correct for any deviations from the initial volume. Movements of the poles that occur at anaphase-B were included in the program to approximate those observed in cleaving cells (Hiramoto, 1958). The results of the computations were either displayed on a computer graphics screen or plotted.

A plot of an astral relaxation model of the type described above is shown in Fig. 4. The asters in this simulation had to be positioned unnaturally far

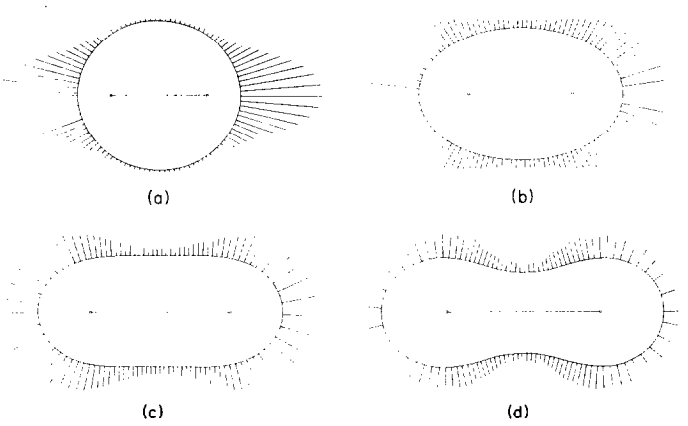

FIG. 4. Computer plots of the astral relaxation model using an inverse fourth power law. This version of the model does not allow for any movement of the tension generating elements, apart from stretching and shrinkage of the surface, so the surface tension forces are isotropic at all points. The cleavage furrow is a saddle surface as the radius of curvature of one of the principal axes is negative. As curvature in this direction increases i.e., as the furrow sharpens, the pressure generated by surface tension forces is reduced until an equilibrium condition is reached, Fig. 4(d), when the furrow will progress no further. The aster separation has to be unnaturally large for a cleavage furrow to form at all.

apart to get a cleavage furrow to appear at all; even so the furrow was shallow and would not progress to completion. The reason for this behavior is simply that although surface tension is greater at the equator than at the poles, it is isotropic at all points (assumption (a)). The geoemetric form of a furrow is a saddle surface in which one of the radii of the principal axes of curvature is positive and the other negative. Thus if these are similar in magnitude, there can be no inward directed force due to surface tension and hence no further furrowing i.e. cleavage progresses to an equilibrium

point between concave curvatures that will pull in and convex curvatures that push out. In order for a furrow to progress it has to be very shallow and bears little resemblance to a furrow in a cleaving cell (Hiramoto, 1958).

Sharper furrows may be obtained if anisotropic surface tensions are specified in assumption (a), with tension being highest in the circumferential direction. In the case of the sea urchin egg it has been calculated, on the basis of measurements of internal hydrostatic pressure and surface shape, that there is a factor of two difference in tension in the two principal directions at the furrow region (Hiramoto, 1968). The circumferential organization of filaments around the contractile ring also strongly implies that there will be a high degree of anisotropy in the force generated by these elements. This then raises the questions of the origins of the tension anisotropy and the genesis of the contractile ring. We would like to suggest the following extension to the astral relaxation theory which we think answers these questions and which can explain many of the variations of cleavage that are seen.

Consequences of the Mobility of Contractile Elements

We have assumed up to now that the cortical contractile apparatus consists of a random network of linear elements evenly distributed throughout the cortex. There has also been the implicit assumption that they are fixed and do not move during cleavage. Elements may however maintain their cortical location and yet also have a high degree of lateral mobility. We would like to propose, therefore, that this is the case. We now explore the properties of the astral relaxation model with this feature incorporated as assumption (h):

(h) Elements are free to move in the plane of the cortex.

Asters interact with the cortex as before, resulting in the highest levels of cortical tension in the equatorial regions. Elements which are between the poles and the equator experience a laterally directed force towards the

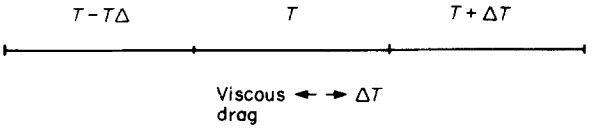

FIG. 5. A network of interconnected tension generating elements producing a uniform tension on a spherical surface will be in equilibrium with surface tension forces being counteracted by internal hydrostatic pressure. If a gradient of tension is set up in such a network the individual elements will no longer be in equilibrium and each element will encounter a force of ΔT (in the case of a uniform gradient) going up the gradient. This will result in a movement of the elements up the gradient such that the lateral force on each is countered by viscous drag.

equator because of the gradient in cortical tension from pole to equator (Fig. 5). They therefore move toward the equator with a viscosity limited velocity. This migration of elements has two initial consequences: (1) there is a pile-up of contractile elements in the equatorial regions giving rise to yet higher surface tension in this region (Greenspan, 1978), (2) elements are likely to become preferentially oriented in an equatorial direction because of the geometric distortions that they incur when they contract uni-directionally and move toward the equator (Fig. 6). This results in the

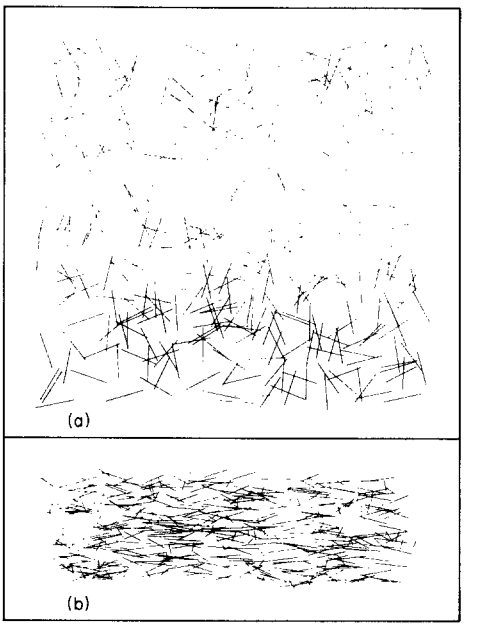

FIG. 6. Tension elements are initially distributed as a uniform layer of randomly oriented elements in the cortex. A gradient of tension is set up in the cortex by the interaction of the asters with the contractile apparatus. This will result in the tension elements migrating towards the equator which is the region of highest tension. In so doing they will suffer geometric distortions which will probably result in a preferential alignment of the elements in a circumferential direction. This will result in the surface tension forces becoming anisotropic at the equator. This figure shows how a 4:1 compression of a randomly oriented network of elements results in their partial orientation.

development of an anisotropic surface tension. Figure 2(b) shows the initial configuration of a simulation of this version of the model and Fig. 7 its subsequent progression. The initially uniform distribution of elements changes such that there is a band of accumulated elements in the equatorial region. Accompanying this process is a gradual flattening and isotropic

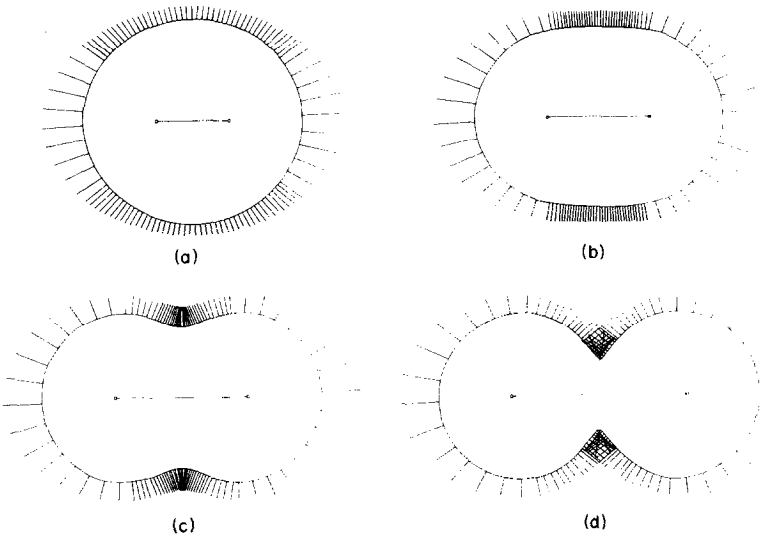

FIG. 7. Sequence of computer plots of a cleavage simulation using an inverse fourth power stimulus function. The length of the projecting spikes is a measure of the combined stimulus from the two asters and the density of the spikes is a measure of the density of the contractile elements. The initial stimulus distribution for this simulation is shown in Fig. 2(b). (a) and (b) show the subsequent redistribution of tension elements to form a broad equatorial belt. The equatorial region changes from a spherical to a cylindrical shape (b). The furrow when it first appears is rather broad (c) but rapidly sharpens as it progresses (d).

shrinkage of a broad equatorial region of the cell (Fig. 7(a), (b)). Similar shape changes have been seen in cleaving sea urchin eggs (Hiramoto, 1958). These initial movements result in the equatorial regions taking on a transiently cylindrical shape (Fig. 7(b)). At this point tension elements in these regions that are not oriented parallel to the equator will experience a component of their internally generated tension tending to orient them in an equatorial direction Fig. 8(a). Thus the initial partial orientation of the contractile elements brought about by the geometric distortions incurred as a result of their redistributions, is augmented by a further ordering caused by the tendency of the elements to move to a circumferential orientation on the transiently cylindrical equatorial surface. When a furrow eventually appears it is initially rather broad (Fig. 7(c)) reflecting the rather broad underlying band of circumferentially oriented tension elements lying on the equator at this point. However, tension elements which are situated at the sides of the furrow will experience a component of their internal tension driving them down to the bottom of the furrow (Fig. 8(b)) where they will be in lateral equilibrium (Greenspan, 1978). The furrow therefore

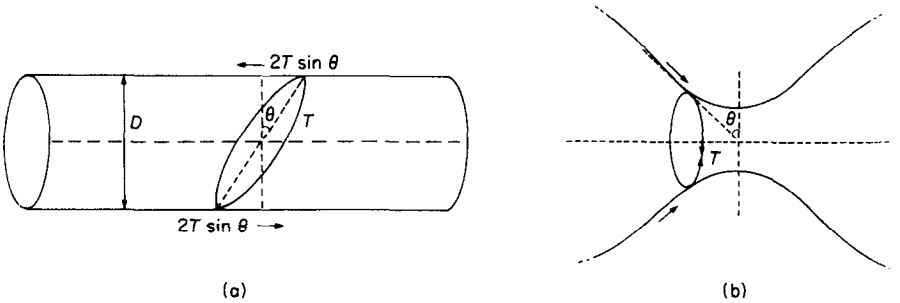

FIG. 8. Tension elements that lie in a non-circumferential direction on a cylindrical surface will experience a component of their internally generated tension tending to drive them to a circumferential orientation. This can best be visualized by considering a ring of elements wrapped around a cylinder at an angle θ (Fig. 8(a)). If the elements are free to move in the surface of the cylinder diameter D and are generating a tension T, then a component $2T \sin \theta$ of their tension will act along the axis of the cylinder to produce a turning moment of $2DT \sin \theta$ on the ring of elements. This will go to zero when $\theta = 0$ i.e., when the ring is in a circumferential orientation and hence in lateral equilibrium. When a furrow starts to form, circumferential rings of elements at each side of the furrow will no longer be in lateral equilibrium but will experience a force driving them to the base of the furrow (Fig. 8(b)). If the angle of the furrow to the equatorial plane is θ then the lateral force on a ring of elements having a tension T will be $2T \cos \theta$.

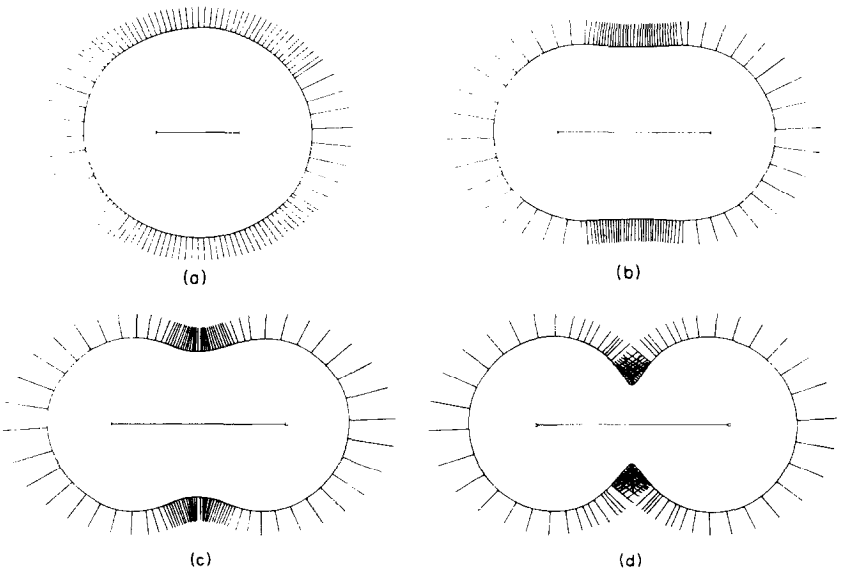

FIG. 9. Similar sequence to that shown in Fig. 7 but using an inverse square stimulus power function. The initial conditions are shown in Fig. 2(a).

rapidly sharpens and narrows (Fig. 7(d)). Thus, as a further consequence of the lateral mobility of tension elements, a furrow once initiated, will tend to deepen and become self-sharpening. In this way an initially rather broad stimulus pattern (Fig. 2) will give rise to a narrow contractile ring. With a suitable choice of parameters computer simulations of this model can give good approximations to real cleavage shapes (Fig. 10). The main parameters that are relevant to cytokinesis are the lateral and vertical mobilities of the tension elements; to get the closest approximation to real cleavage figures the ratio of these mobilities was set to about 4:1. The model is rather insensitive to the power law of the relaxing stimulus of the asters (compare Fig. 7 with Fig. 9). The important point is that the stimuli from the asters are spherically symmetric and additive. The other parameters in the computer model were specific to the method of computation and were used to maintain stability at all times.

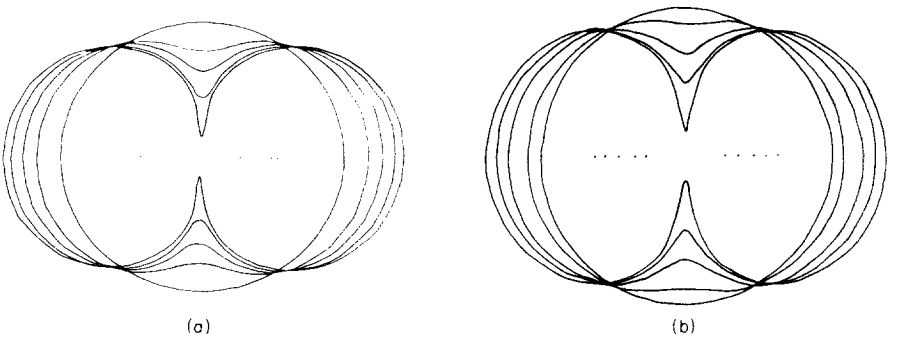

FIG. 10. Comparison of a sequence of computer simulations (a) with measurements made on a cleaving sea urchin egg (b) data described in Hiramoto, 1958. In order to get the best match the astral stimulus was removed in the computer simulations at the time the equatorial regions attained a cylindrical shape.

Summary of Model

The basic tenets of the model that has been developed are:

(1) That cleavage is a consequence of differential cortical tension.

(2) Cortical tension is generated by an initially even distribution of randomly oriented linear tension producing elements that are free to move in the plane of the cortex.

(3) These elements are brought into a state of uniform activation.

(4) The presence of the asters modulates this activation by reducing the tension of elements in their proximity.

The inevitable consequences of these tenets are:

(1) The relaxing influence of the asters will result in the establishment of gradients of cortical tension, the region of highest tension being at the equator.

(2) Tension elements will move up the gradient towards the equator.

(3) In so doing they probably will become preferentially oriented circumferentially.

(4) The surface tension forces will become anisotropic as a consequence of this orientation.

(5) The cell will start to change shape, initially flattening in the equatorial regions. This will result in a further circumferential orientation of the equatorial elements.

(6) A furrow will appear initially as a broad band but this will rapidly sharpen because of the tendency of the contractile elements to "fall into" the furrow.

We will now go on to discuss the ability of this model to explain some of the different forms of cleavage that are observed.

Variations in Cleavage Configurations

(A) CLEAVAGE OF ELONGATED CELLS

It has been demonstrated that a cell may be artificially elongated and yet still produce a normal cleavage furrow that bisects the MA (Rappaport, 1960). This observation has been interpreted as suggesting that the stimulus is equatorial rather than polar as the poles were a long way from the cleavage furrow in these experiments. However the stimulus in the model we have described is assumed to emanate from the asters and so it will only be strongest at the poles of cells that are initially spherical. An elongated cell was simulated on the computer by setting up an initially ellipsoidal cell envelope. Reasonable looking cleavage figures were obtained (Fig. 11). The simulations made the interesting prediction that polar caps of accumulated contractile elements could be produced under these conditions.

(B) ASYMMETRICAL CLEAVAGES

Most cleavages are not completely symmetric, the asymmetries range from barely perceptible to the extreme example of polar body formation. In all these cases, however, the general rule that the MA is bisected by the cleavage furrow is followed, implying that the MA is displaced along the axis of the cell prior to cleavage. This situation is easily set up in the

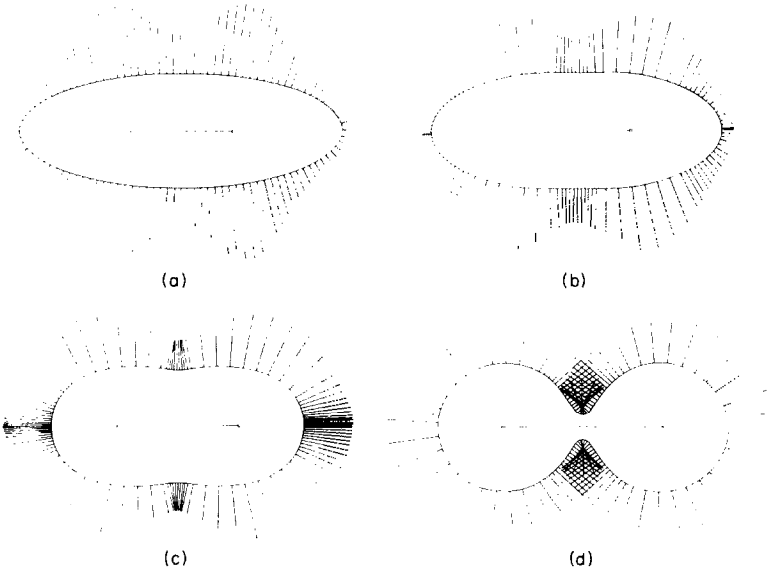

FIG. 11. An initially ellipsoidal cell will cleave normally even though the poles are not the regions of maximum stimulation. The simulations made the prediction that there will be a transient polar accumulation of contractile elements in these situations.

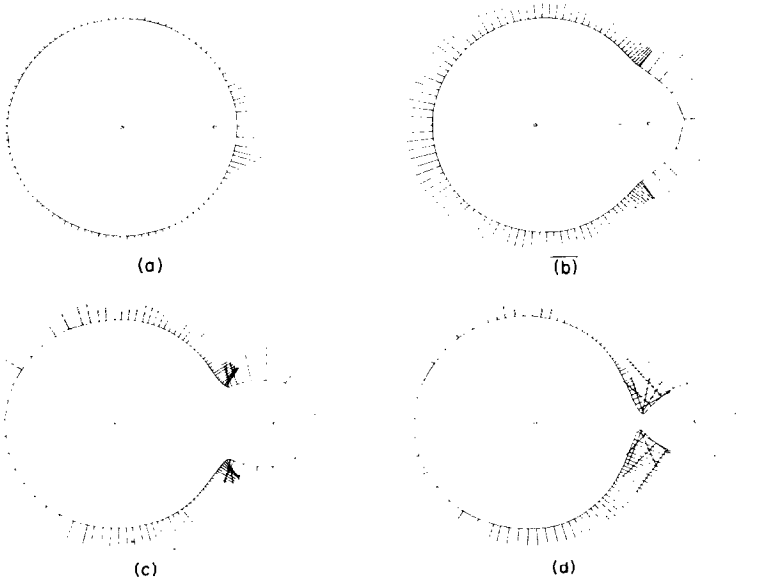

FIG. 12. If the spindle is displaced along its axis towards one side of the cell the cleavage will be assymmetric resulting in the formation of unequally sized daughters.

computer and the resultant cleavages are asymmetric (Fig. 12). Extremely asymmetric cleavages are difficult to simulate but this is probably because of the limited resolution (100 points describing the envelope shape) of the computer models.

(C) CAPPING

Simulations of a cell which has only a single eccentrically positioned aster generally result in the contractile elements being redistributed into a polar cap with no cleavage furrow formed (Fig. 13). Accumulations of

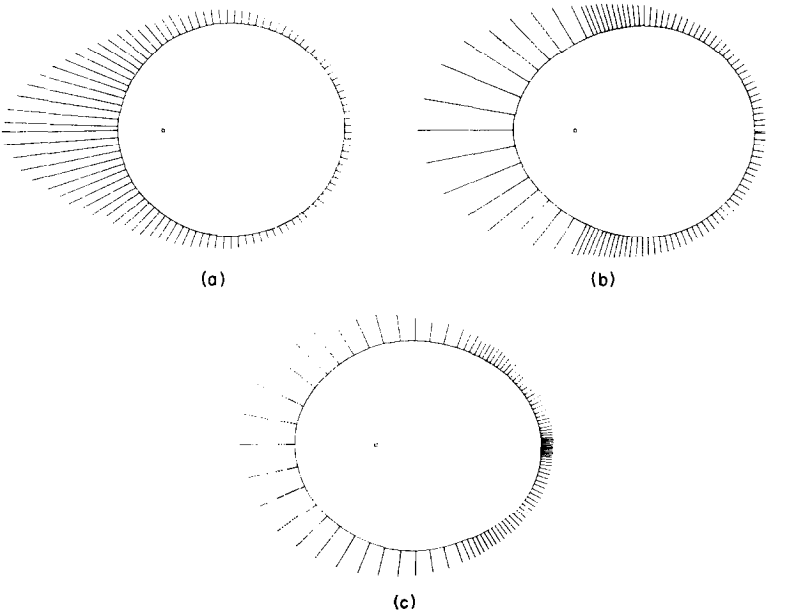

FIG. 13. If a cell contains only one eccentrically positioned aster, generally no cleavage occurs, but the tension elements will be redistributed into a polar cap.

actin have been visualized in cells that have been induced to cap by cross linking surface receptor (Toh & Hard, 1977). This suggests a possible interpretation of the capping phenomenon (Bourguignon & Singer, 1977) in which perhaps some cytoplasmic component may act as an aster when a cell is stimulated and direct the redistribution of cortical contractile elements. The contractile elements may be associated with trans membrane proteins (Koch & Smith, 1978) and sweep them along to the pole.

(D) SPINDLE REMOVAL PRIOR TO CLEAVAGE

We have outlined above the experimental evidence leading to the conclusion that the MA is necessary only for the initiation of cleavage i.e., it can be removed just prior to the appearance of the cleavage furrow yet furrowing will proceed normally. These experiments were simulated on the computer by simply turning off the stimulus from the asters prior to furrowing. Normal looking cleavage figures could still be produced (Fig. 14) as long as the stimulus was turned off after the redistribution of the

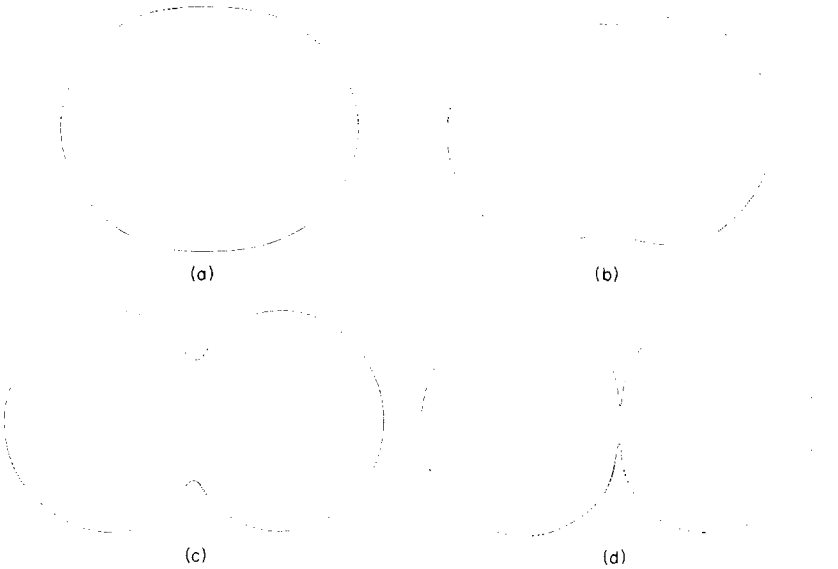

FIG. 14. The stimulus from the asters is only necessary to induce the initial redistribution of tension elements. Once this has occurred it can be removed. Yet cleavage will proceed normally. In this simulation the stimulus was removed when the equator started to flatten.

contractile elements had taken place. The stimulus is necessary, therefore, only to induce the rearrangement of contractile elements, once this has been accomplished furrowing will proceed in the absence of any stimulus. Indeed the continued presence of the stimulus may act against furrowing. In cases where the poles do not move far apart at anaphase, the advancing furrow will approach the poles, thus experiencing an increase in stimulation, which would tend to relax the tension elements. It is therefore a prediction of the model that at least in some cases (e.g., cleavage of sea urchin eggs, Fig. 10) the stimulus has to be transient and start to decay as furrowing commences.

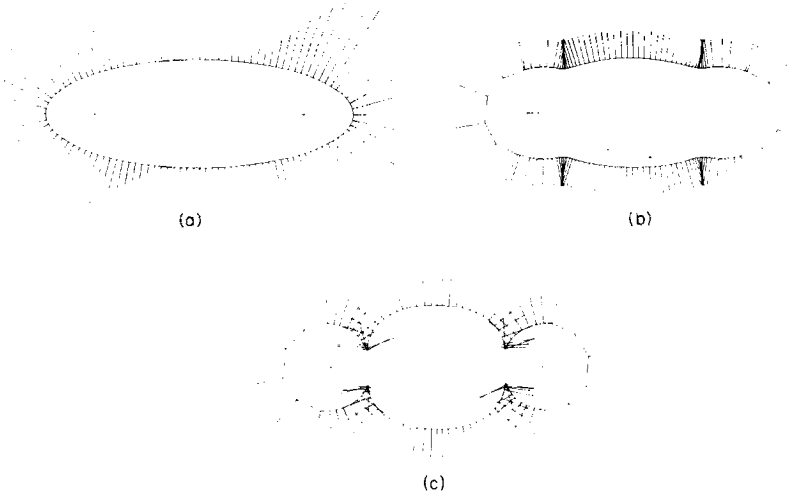

FIG. 15. It is possible to get two cleavage furrows formed if the two asters are widely separated in an elongated cell. Such a situation may occur during the maturation divisions of nematode spermatocytes (Wolf *et al.*, 1978; Ward *et al.*, 1981) where two cleavage furrows are seen to form.

(E) MONO-ASTRAL FURROWING

If a situation is simulated in which asters are widely separated in an elongated cell it is possible to get two cleavage furrows formed (Fig. 15). This situation is interesting as it implies that it is possible in certain circumstances to get a cleavage furrow from a single aster. Observations that may relate to this prediction have been made on the maturation of nematode spermatocytes (Wolf, Hirsh & McIntosh, 1978). Prior to the second meiotic division the spermatocyte elongates and chromosomes along with other cell organelles cluster at each pole. Two cleavage furrows are then seen to form resulting in the centre piece of cytoplasm being sloughed off as a cytoplast (Delawault, 1952). There are, however, suggestions that these cleavages may not be mediated by contractile elements (Ward, Argon & Nelson, 1981).

(F) ECCENTRICALLY POSITIONED SPINDLES

(*a*) *Polar lobe formation*

Although computer simulations have only been done for rotationally symmetric figures, it may be interesting to try to extrapolate some of the results to cases of cleavage with eccentrically positioned spindles. It has

been demonstrated in the previous example that when the asters are sufficiently far apart, two separate cleavage furrows may form. When this occurs the orientation of the furrow is orthogonal to the line joining the aster to the nearest point on the cell surface. If a MA with widely spaced asters is displaced to an eccentric position in a spherical cell the cleavage furrows will tilt towards each other (Fig. 16(a)) until a point is reached

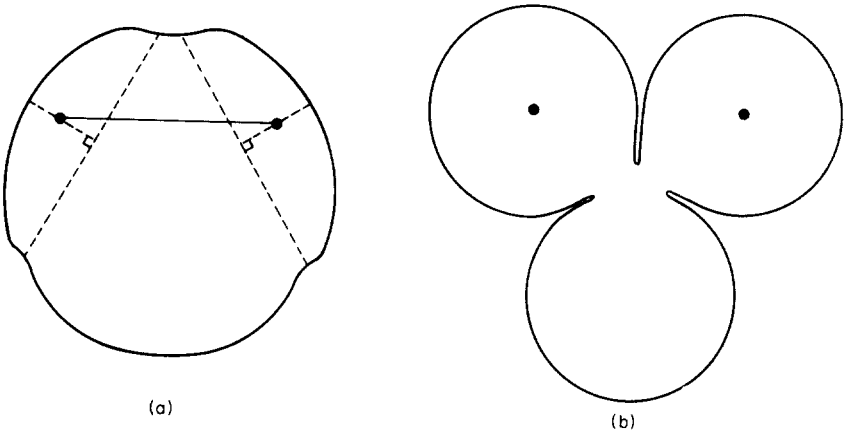

FIG. 16. Computer simulations have only been carried out using rotationally symmetric cleavage figures. It may be possible, however, to extrapolate these results to cases where the MA is eccentrically positioned. If the asters were sufficiently far apart in this situation two furrows would form. The orientation of each furrow would be normal to a line joining the aster and the nearest point on the adjacent cortex (Fig. 16(a)). Thus if the MA was in a sufficiently eccentric position the furrows would touch at the top. When the furrows progress in this configuration the resultant cleavage figure would look like Fig. 16(b). This type of cleavage pattern is seen in polar lobe formation.

when they will touch. If the cleavage furrows are allowed to progress in this configuration a bridge of cytoplasm will form beneath the dividing cells (Fig. 16(b)). Polar lobe formation is a phenomenon which is observed in the development of some invertebrates (Conrad, 1973). The early cleavages have a form closely resembling the shapes shown in Fig. 16(b), with a lobe forming under two dividing cells (Fig. 8 in Schroeder, 1975). A band of microfilaments has been observed at the neck of the lobe and it has been suggested that the lobe is formed by a contractile ring analogous to that seen in cytokinesis (Conrad, 1973). We would therefore like to suggest that polar lobe formation is simply another aspect of cytokinesis. It will occur when the MA is eccentrically positioned and the asters are sufficiently far apart such that their influences do not overlap and do not reach the lower part of the cell.

(b) Cleavages in early amphibian eggs

The conditions that have been outlined above as being necessary for polar lobe formation, given the tenets of the model, may be present in any large cell with an eccentrically positioned MA, yet not all such cells form polar lobes when they cleave. Amphibian eggs, for example, cleave unilaterally with no sign of lobes. The question then arises of why this is so, or more specifically in terms of the model; how does the stimulus from the asters influence the remote regions of cortex to the same extent as the adjacent cortex in order to produce the redistribution of tension elements necessary for cleavage.

Cortical waves have been proposed as the mediators of several forms of cell motility (Hewitt, 1979; Durham, 1974). Time-lapse cine studies of cleaving amphibian eggs have revealed waves of cortical contraction which are initiated at the animal pole and then propagate to the vegetal pole (Hara, 1971). The cleavage furrow always first appears at the site of origin of the contractile wave and follows at a fixed distance behind it. Waves of cortical stiffness have also seen to propagate in the same manner (Sawai & Yoneda, 1974) and presumably these are another manifestation of the same phenomenon. The point of origin of the wave is not always exactly at the animal pole, yet the point of initiation of furrowing is always at the point of initiation of the wave (Hara, 1971). Together these observations suggest a causal relationship between the contractile wave and the cleavage furrow. We have not, up to this point, considered the initiation of cortical contractile activity in detail; the simulations have assumed that all points on the cortex become activated simultaneously. This may be reasonable for small cells but the observations on early amphibian cleavages suggest that this assumption may not always be valid. The model can, however, be naturally extended to accommodate a wave-like initiation of cortical activity and in so doing resolve the paradox of highly unilateral cleavages.

Cortical contractions are presumably mediated by the activation of cortical tension generating elements. These we assume, as before, are linear, randomly oriented and are free to move in the plane of the cortex. A propagating wave of tension has a region of increasing tension at its leading edge and a region of decreasing tension at its trailing edge (Fig. 17). We will assume for simplicity that these regions of tension gradient are both linear and have the same slope.

An element in the leading edge of the wave will experience a force tending to drive it up the gradient and so it will move in a direction opposite to that of wave propagation. Conversely, an element in the trailing edge will tend to move in the same direction as wave propagation. Therefore,

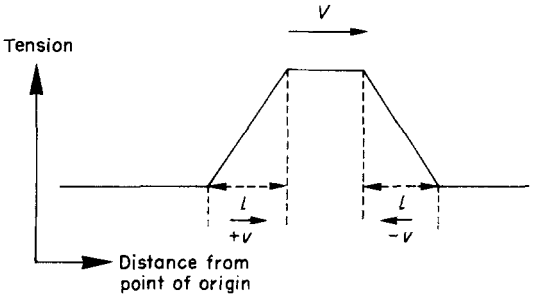

FIG. 17. A propagating wave of tension moving through an array of tension elements will cause elements to be displaced along the direction of the wave. If a wave of tension with linear leading and trailing wavefronts of unit length has a velocity V and is propagating in the cortex, then elements in the leading wavefront will be in a gradient of tension and will move up the gradient (i.e., in the opposite direction to wave propagation) with a constant, viscosity-limited velocity. Let this velocity be $-v$. Similarly, elements in the trailing wavefront will also be in a tension gradient but this time the gradient is reversed so they will move with a velocity v (i.e., in the same direction as wave propagation). Therefore an element spends a time of: $1/(V+v)$ under the influence of the leading wavefront and $1/(V-v)$ under the influence of the trailing wavefront. Consequently the net displacement of the element (D) will be given by: $D = v/(V-v) - v/(V+v)$. This will be positive for $V > v$ i.e., in the same direction as wave propagation. Thus the displacement of elements is predominantly determined by the action of the trailing wavefront.

the velocity of an element relative to the wave is greater in the leading edge than in the trailing edge. This will result in the element spending a longer time under the influence of the trailing gradient than the leading gradient (Fig. 17). Thus an element will suffer a net displacement in the direction of the trailing edge of the wave after it has passed. The initiation event and point of initiation of the contractile wave we will assume are independent of the presence of the MA. However, the asters of the MA interact with the activated cortex as before to initiate two centres of relaxation on the adjacent cortex. These propagate away from the centres of initiation as two fronts of relaxation which collide (Fig. 18). The net displacement that tension elements will suffer if their mobility is comparable to the contractile wave velocity, will be predominantly in the direction of the trailing edge of the wave. When the trailing wave fronts collide, elements will be deposited and will accumulate along the locus of the paths of collision (Fig. 18). Thus the consequence of a propagating cortical contractile wave, with a trailing wavefront geometry that has been determined by the position of the asters, will be the progressive deposition of accumulated contractile elements in an equatorial belt. This we assume by analogy with the rotationally symmetric cleavage simulations, is a sufficient condition for cleavage to proceed.

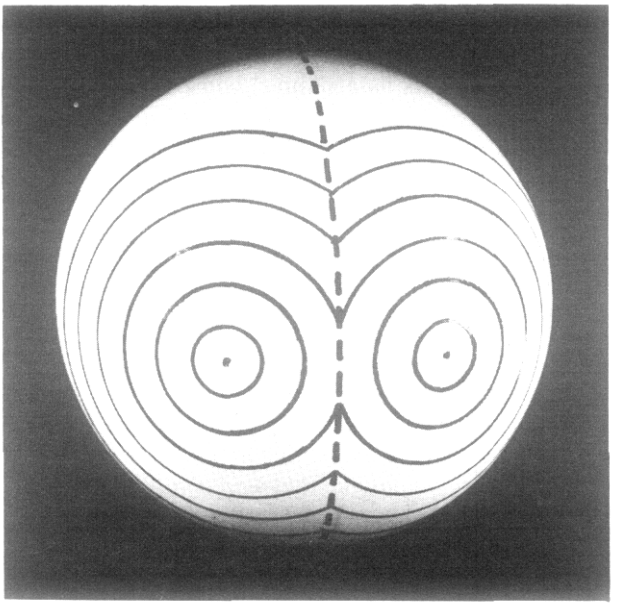

FIG. 18. Tension elements will tend to be displaced by the passage of a trailing wavefront of tension. The displacement will be in the direction of wave propagation. Thus two colliding trailing wavefronts will leave an accumulation of tension elements along the locus of the points of collision. If asters initiate the trailing wavefronts of a cortical contractile wave, two centres of relaxation will be initiated on the cortical regions adjacent to the asters. Wavefronts will propagate from these points and collide. The locus of collisions will be a closed loop around the cell. Tension elements will accumulate on this locus to form a contractile ring.

If unilateral furrowing does take place in the manner that has been suggested then two puzzling features of this mode of cleavage can be simply explained. The first, that has already been mentioned, is the apparent ability of a partially formed contractile ring in a unilaterally cleaving cell, to maintain a tension yet having no obvious attachment points at the free ends. One of the main tenets of the model we have presented is that the contractile ring is formed by the redistribution of tension elements and is not assembled *de novo*, thus at the advancing tip of a furrow in a unilaterally cleaving cell there will be no discontinuity in tension elements but rather a region where they will be migrating and accumulating (Fig. 19). Thus the tension in the furrow will be dispersed at the advancing tip of the contractile ring and be taken up in the adjacent isotropic regions of the cortex. The second puzzling feature of extreme unilateral cleavage is the non-trivial problem of how the advancing tips of the furrows always

exactly join up at the far side of the cell even if they have been deviated en route by mechanical disturbances to the cell surface (Sawai, Kubota & Kojima, 1969). This follows as a natural consequence of the notion that the path of the extending band of accumulated tension elements is the locus of intersection of two circular wavefronts. Such a locus always has to be a closed loop.

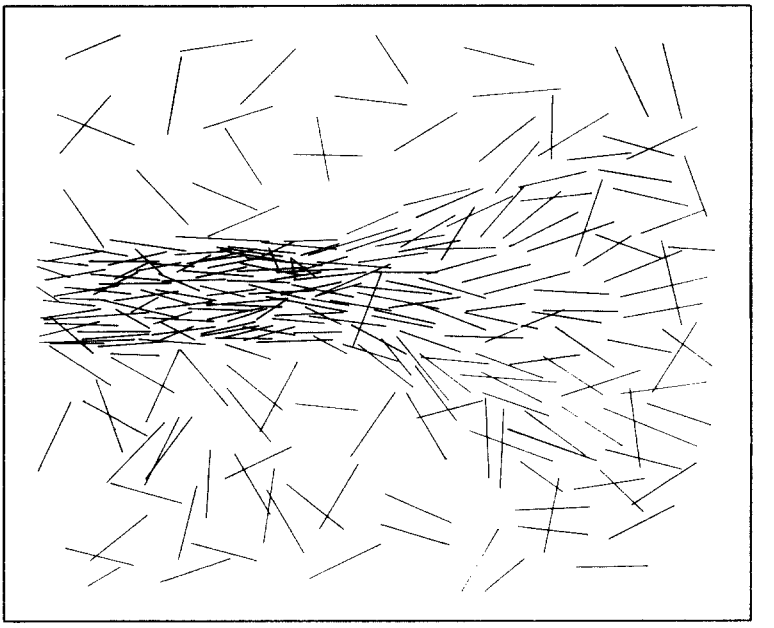

FIG. 19. The contractile elements of cleavage furrow are formed by the redistribution of an initially uniform, randomly oriented network of cortical elements. In the case of unilateral cleavage where the redistribution is attained by the propagation and collision of two cortical wavefronts the contractile ring will be initiated at a point nearest the MA and will propagate around the cell. There will be no discontinuity of tension elements at the advancing tip of the contractile ring so the tension generated by the accumulated and oriented elements in the ring is fanned out at the ends and taken up by the surrounding randomly oriented elements.

This view of unilateral cleavage suggests that surface contraction waves that have been seen on amphibian eggs may be a device to allow large cells with eccentrically placed MAs to cleave normally. An eccentrically placed MA in a cell which did not have the capacity to propagate waves of cortical contraction may result in the formation of a polar lobe at cleavage if the asters are sufficiently far apart.

Summary

There are three main aspects to the model which we have presented.

(A) CORTICAL TENSION GENERATING ELEMENTS

These are assumed to be linear and initially uniformly distributed with random orientations. Tension elements maintain their cortical location yet are free to move in the plane of the cortex. They are assumed to interact with each other locally so that tension may be propagated through the network. The first stage of cytokinesis is a generalized activation of these tension elements resulting in an increase in surface tension and cortical stiffness. The initial activation is independent of the presence of the MA

(B) RELAXING INFLUENCE OF ASTERS

The asters of the MA interact with the activated cortex in such a way as to reduce the tension in the adjacent elements. The magnitude of this effect is assumed to be proportional to some simple function of the distance of the element from the aster. The effects of the two asters are assumed to combine by scalar addition. Our simulations have shown that, in at least some configurations, the stimulus has to be transient, since a continuous stimulus would act to inhibit the later stages of furrow progression. The transient nature of the stimulus may result from the brief life time of the asters.

(C) REDISTRIBUTION OF TENSION ELEMENTS

When two asters interact with an activated cortex the locally-relaxing influence of the asters will give rise to gradients of cortical surface tension, the region of highest tension being at the equator. Tension elements will tend to move up this gradient because of their free mobility in the cortex. In so doing they become partially oriented circumferentially because of the geometric distortions that occur when they migrate and accumulate as a broad equatorial belt. This results in the surface tension increasing and becoming anisotropic at the equator such that the direction of maximum tension is circumferential. The cell will now start to change shape as a result of the non-uniform surface tension. Initially the equatorial regions will flatten to become cylindrical. This shape change results in the tension elements at the equator becoming aligned circumferentially which is their minimum energy configuration. The cylindrical shape is transient and further contraction of the tension elements leads to the appearance of an

initially broad furrow. This rapidly sharpens as tensions elements fall into the base of the furrow.

BIOCHEMICAL NATURE OF ASTER/CORTEX INTERACTIONS

In developing this model we have deliberately avoided making any assumptions about the biochemical processes that must be taking place during cytokinesis. This is because the essentially mechanistic arguments that we have used give no clue as to their nature. The model is however, consistent with what little is known on this topic. The stimulus from the asters has been shown to be colchicine sensitive and non-diffusible (Rappaport, 1971). This strongly implicates the microtubules of the asters or their associated proteins as being the mediators of the stimulus, specifically it may be the free ends of the microtubules which are the active agents (Bray, Thomas & Shaw, 1978). Calcium ions activate cortical contractions (Franke *et al.*, 1976; Bluemink, 1972) and may be the effectors of the initial cortical activation (Cande, 1980). There is evidence that cortical actin may be freely convertible from G to F forms and this may also occur on cortical activation (Spudich & Spudich, 1979). The cortical location of tension elements mediating cytokinesis may be the consequence of the attachment of the ends of actin filaments to specific membrane bound complexes such as occurs in microvilli, or there might be some more generalized association of membrane proteins along the length of actin filaments (Koch & Smith, 1978).

GENERALIZATION TO INTERPHASE CELL MOTILITY

The microtubules of an interphase cell may modulate the activity of the cortical tension elements in the same way as has been proposed for the cleaving cell. The main difference is that the microtubules of an interphase cell are generally not organized into two spherically symmetric arrays and therefore cannot set up the appropriate pattern of stimulus on the cortex for cleavage to occur. However, bundles of microtubules approaching the cortex may induce local relaxations in the region of interaction resulting in pseudopodial extensions being driven out by internal pressure. Attachment of the pseudopod to a substrate could consolidate such an extension and provide the basis for cell locomotion (Abercrombie, Heaysman & Pegrum, 1970).

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