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Mechanical behavior of closed lamellar membranes as a possible common mechanism for the establishment of developmental shapes

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ABSTRACT **The mechanical behavior of a closed membrane composed of two layers in contact is described as it is obtained by finding the minimum of the membrane bending energy at constant membrane area, constant difference between the areas of the two layers, and constant enclosed volume. It is shown that the membrane bending energy is a discontinuous function of the volume (v) and difference between the layer areas (Δa) defined relative to the volume and area difference of a sphere with the same membrane area, respectively. However, for different classes of shapes it is possible to obtain regions in the $v/\Delta a$ diagram within which the shapes change continuously with v and Δa. These regions are shown for the egg, dumbbell and cup shape classes, respectively. The results of the shape analysis are used in the discussion of cell polarity, cytokinesis and gastrulation. Cell polarity is related to the decrease in symmetry during the transition from the radially symmetrical spherical shape to the asymmetrical shapes of the egg class. It is proposed that symmetrical cytokinesis occurs within the dumbbell class and that asymmetrical cytokinesis occurs within the egg class. Gastrulation is described as shape transformations within the class of cup shapes.**

KEY WORDS: *lamellar membranes, developmental shapes, cell polarity, cytokinesis, gastrulation*

Introduction

The development of a multicellular organism from a single cell to an adult involves shape transformations that take place at different stages in the formation of the final body. Especially in early development there are many processes that are characterized by changes in either the shape of individual cells or the shape of cell aggregates such as epithelial sheets. To understand the mechanisms underlying such shape transformations an obvious approach is to study the molecular origin of the forces involved, and the relationship between these forces and the respective deformations. However, it has been observed in the case of epithelial invagination that the shape change can be the result of different mechanisms including, for instance, cell adhesion properties, microfilament-mediated changes in cell shape, or cell growth and division (Ettensohn, 1985). Thus, different mechanisms can result in the same kind of shape transformation. It can also be noted that shapes observed at different levels of body organization can be quite similar. For instance, the cup shapes of red blood cells are like the shapes of embryos at the gastrula stage. The question therefore arises whether there are some more general and common properties of these systems which govern the establishment of their shapes. In this communication we attempt to demonstrate that some general principles for shape formation may emerge from the general behavior of lamellar membranes with closed surfaces, and suggest that the same principles may also govern the behavior of more complex systems such as cellular sheets.

Cell membranes are lamellar structures. Their basic structure is that of a bilayer composed of two phospholipid monolayers in close contact at their hydrophobic sides. Cell membranes in general may comprise additional lamellar structures in the form of the membrane-associated cytoskeleton and the glycocalyx. Cellular sheets can also be considered as lamellar structures that include the cell membranes on opposite faces of the sheet, as well as additional layers including cytoskeletal structures organized parallel to the membrane and extracellular matrix. Thus, the lamellarity of these systems can be quite complex.

The essential relevant property of a lamellar structure is that an asymmetrical alteration of the composition (or of any other property) of constituent layers may generate lateral stresses within these layers which consequently give rise to a bending deformation of the whole system. Several possible deformations exist depending on the interaction between the

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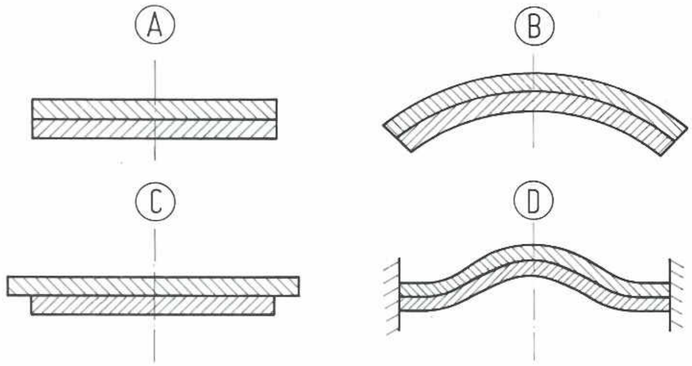

Fig. 1. Schematic representation of possible bending deformations arising as the result of an increase in the area of the upper layer of a circular section of a two-layered membrane. (A) *Initial state.* **(B)** *Deformation of a membrane with connected layers.* **(C)** *Response of a membrane with unconnected layers.* **(D)** *Deformation of a membrane with unconnected layers but subjected to lateral constraints.*

layers and the external constraints applied to them. This is exemplified in Fig. 1 for the case of a flat circular piece of a bilayered system (Fig. 1A). The two constituent layers can be either connected or unconnected. If connected, an asymmetrical increase in the area of the upper layer will cause the system to curve upward in order to reach the state with the minimum value of elastic energy (Fig. 1B). If unconnected, the upper plane is able to slide upon the lower plane and there is no need for bending (Fig. 1C). If, however, the system of unconnected layers is laterally constrained, it will bend (Fig. 1D). Such constraints are encountered when the membrane forms a closed surface.

In the present analysis we shall describe the properties of a system consisting of two closed and unconnected layers which are in close contact with each other and are laterally homogeneous. We propose that knowledge and understanding of the general properties of such a system regarding its possible shapes represents the basis for understanding the behavior of more involved systems such as finite but constrained lamellar systems, laterally inhomogeneous membrane systems, or lamellar systems composed of more than two layers. Thus, in the first part of this work the relevant properties of closed, lamellar, unconnected bilayers are described. In the second part, the relevance of these properties in developmental biology is discussed. Three examples are considered: cell polarity, cell division, and gastrulation.

Determination of shapes of membranes forming closed surfaces

The system investigated is a membrane forming a closed surface with the area A enclosing volume V which is in general smaller than the volume of the sphere with the same surface area ($V_{sph}= A^{3/2}/6\pi^{1/2}$). An object with the relative volume $v<1$, where $v = V/V_{sph}$, can assume an infinite number of different shapes. We presume that the interior of this object has no structure. The shape of such an object is governed by the membrane energy, *i.e.* the internal energy of the membrane and the interaction of this membrane with its surroundings. Here we restrict ourselves to the simple case of an unsupported membrane, *i.e.*, one for which the surrounding phase is also structureless. It is anticipated that of all possible shapes at a given value of v the described object attains the shape which corresponds to its minimum membrane energy.

The problem to be solved consists of two parts. First we must find the expression of the membrane energy, which involves the essential structural features of the membrane. The second part involves the mathematical procedures which yield the shape with the minimum energy.

The membrane energy is expressed in terms of macroscopic elastic coefficients and the corresponding energy terms. As the basic unit we consider a phospholipid monolayer which is an unavoidable constituent of any plasma membrane. Its elastic properties can be described in terms of the isotropic stretching coefficient and the bending coefficient. Such a description follows from the assumption that a monolayer behaves as a two-dimensional liquid on the plane of the membrane and as an elastic body in bending. Depending on the shapes of phospholipid molecules constituting a monolayer it may have a tendency to bend spontaneously, which means that a monolayer in general exhibits spontaneous curvature (Helfrich, 1973). Similar elastic properties can be ascribed to unicellular sheets. Provided that cells can change their positions on the plane of the sheet, this sheet also behaves like a two-dimensional liquid. Due to the mechanical properties of cells it is anticipated that energy is needed in order to bend the sheet, and because cells are usually polar, as is the case with the epithelial cells, it is expected that the bending energy will be minimal at a certain spontaneous curvature.

On the basis of the described elastic properties of monolayers it is possible then to derive the elastic energy of lamellar membranes forming closed surfaces. In biological systems we are frequently involved with unconnected lamellar structures where the constituent layers are in contact but can slide against one another. The simplest example is the phospholipid bilayer. In the bilayer the two constituent monolayers are in contact because of strong hydrophobic forces between their hydrocarbon chains, but the chains of one layer do not appreciatively affect the lateral movement of the chains of another layer. Consequently, the two monolayers respond to lateral mechanical stresses independently of each other. The two monolayers of a closed membrane such as we have in phospholipid vesicles or in cell membranes nevertheless affect each other's elastic state because of the geometrical constraint they impose on each other by being in contact. We have previously shown (Svetina *et al.*, 1985) that the elastic behavior of a bilayer forming a closed surface is characterized by three coefficients. The isotropic stretching coefficient is the sum of the corresponding coefficients of the constituent monolayers. The bending coefficient is the sum of the corresponding bending coefficients of the monolayers. The relative stretching coefficient, which characterizes the change in energy due to relative stretching of the two layers at a constant membrane area, is given by the stretching coefficients of the two layers and the distance between them. Spontaneous curvature vanishes in the case of a symmetric phospholipid membrane, *i.e.* a bilayer composed of two structurally equivalent monolayers. For any membrane composed of two unequal layers the spontaneous curvature has in general a non-

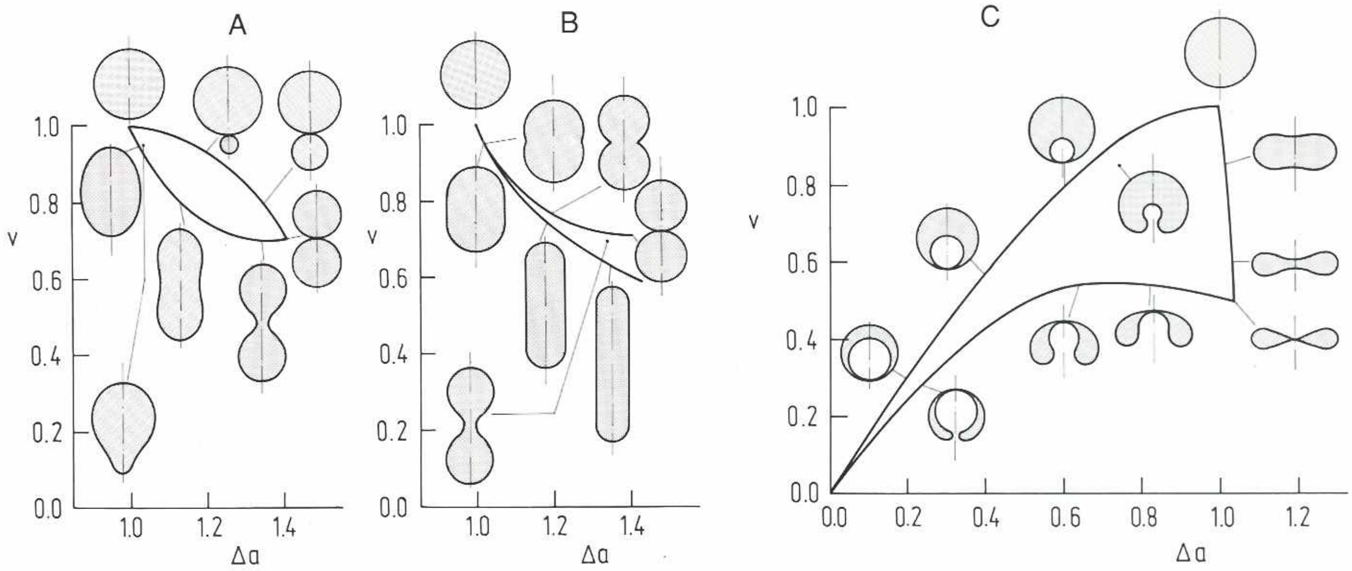

Fig. 2. Regions in the v/Δa diagram where there are: *the egg* **(A)**, *dumbbell* **(B)** *and cup* **(C)** *classes of cell shapes. The characteristic shapes at class boundaries are shown as is the characteristic member shape for each class.*

zero value. It is important to emphasize that the same contributions to the elastic energy obtained in the case of phospholipid membranes can be used to characterize the behavior of any membrane that can be considered to be composed of two sliding layers in contact. For instance, a possible view of the plasma membrane of red blood cell is that its phospholipid part constitutes one layer and the spectrin skeleton attached to its cytoplasmic side the other one. These two layers are in contact because the skeleton is attached to integral membrane proteins. However, they can also respond independently to lateral mechanical stresses because the integral proteins are mobile within the plane of the membrane. Unicellular sheets can also be considered as two-layered structures with the described mechanical properties. For example, the cell layer can be considered as one layer of the structure and an associated extracellular matrix as another layer.

In many instances biological membranes are in fact multilamellar structures. Red blood cell membrane for instance can be considered to be a trilayer formed from the two monolayers of the phospholipid part of the membrane and the cytoskeleton. However, the elastic energy of a closed multilayer can be expressed by the same energy terms as a bilayer except that the meaning of parameters is not as obvious (Svetina *et al.*, 1988). Thus, the consequences of the membrane lamellarity on the shape of the enclosed object can be derived from the analysis of a two-layered membrane. Therefore, we shall in the forthcoming discussions restrict ourselves to the description of the properties of a closed bilayered system. In fact, the essential properties of the system can be revealed by simply taking into consideration the membrane bending energy with the spontaneous curvature zero. Technically, the shape of the object is determined by finding the minimum of the membrane bending energy at constant object volume, constant membrane area and constant difference between the areas of the two layers ($\Delta A = A_2 - A_1$ where A_2 is the area of the outer and A_1 the area of the inner layer, respectively). Below we present some previously derived (Svetina and Zeks, 1989) properties of the described basic system and the recipes for generalizing the results to treat more complex cases.

The result of minimization of the bending energy does not depend on the value of the bending coefficient. The shapes obtained depend only on the values of two geometrical parameters: the relative volume v (defined above) and the relative difference between the areas of the two membrane layers. The latter quantity is defined as $\Delta a = \Delta A/\Delta A_{sph}$, where $\Delta A_{sph} = 4\delta(\pi A)^{1/2}$ is the layer area difference for a sphere with the area A, δ being the distance between the two layers. The analysis (which was performed for axisymmetric shapes) shows (Svetina and Zeks, 1989) that the bending energy is in general a discontinuous function of v and Δa. However, there are regions in the v/Δa diagram within which shapes can be changed continuously by continuously changing v and Δa. Thus there are different classes of shapes where a given class comprises all shapes of the same symmetry characteristics that can be obtained in such a continuous manner. Classes may overlap, that is, at given values of v and Δa different shapes with different energies can exist. Some of these shapes are not stable. Classes have well-defined boundaries. Some of them are lines representing the v/Δa dependence of the limiting shapes. These limiting shapes are in general composites of sections of spheres and cylinders where only two radii are possible. Other class boundaries mark a transition between shapes with different levels of symmetry. Namely, the treated system exhibits symmetry-breaking behavior: by continuously changing v and Δa in some classes it is possible to reach a point where the symmetry changes. The line connecting these points represents a boundary of a class of lower symmetry. The shapes with higher symmetry become unstable at this line. We name a given class by a characteristic shape belonging to it. In Fig. 2 regions are shown in the v/Δa diagram where the egg (A), dumbbell (B) and cup (C) classes are.

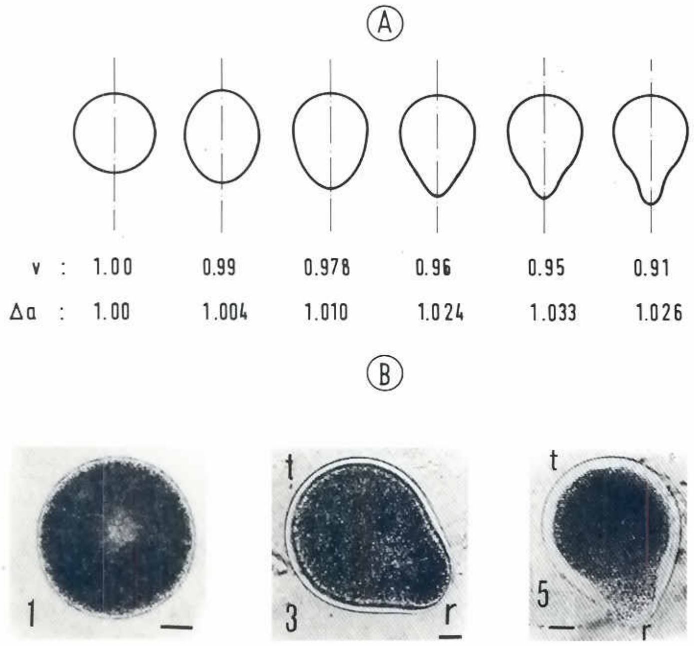

Fig. 3. Illustration of a possible origin of cell polarity. (A) *An example of a possible series of shapes in the course of shape transformations from a sphere to a given egg shape.* **(B)** *A set of shapes of zygote of* P. fastigiata *obtained by Brawley and Robinson (1985) at 2 h, 19 h and 20 h after fertilization (their Figs. 1, 3 and 5, respectively).*

The limiting shape of the egg class at higher Δa values is two linked spheres with their radii depending on v. The low Δa limit is the symmetry breaking line where the shapes attain mirror symmetry with respect to the equatorial plane. The limiting shape of the dumbbell class at lower Δa values is a cylinder with two spherical caps and at higher Δa values a squeezed number eight. The limits of the cup class are at low Δa values two linked spheres of which the smaller one is invaginated, and at high Δa values the symmetry breaking line. The bottom line is defined in such a way that below it are the shapes with the membrane in contact.

The membrane bending energy corresponds to each shape of a given class of shapes. The inclusion of the non-zero value of the spontaneous curvature does not affect the cell shape at given v and Δa values for a given class but does change its energy. The same is true for inclusion of the stretching and relative stretching energy terms.

Cell polarity, cytokinesis and gastrulation

We assume that the described properties of lamellar membranes may underlie some shape transformations that occur during development at the level of single cells or in cell aggregates forming closed cellular sheets. This postulate is supported by the observation that the cell volume, as well as the relative areas of the membrane layers, are regulated by different cellular transport or metabolic processes. Similarly, the inner and outer surface areas and the volumes surrounded by closed cellular sheets are controlled by different mechanisms. Thus the values of the relevant system parameters, *i.e.* the relative volume v and the relative layer area difference Δa in these systems can be under strict regulative control, and so also would be the consequent shapes.

The properties described in the previous section were derived for a laterally homogeneous closed membrane and are therefore not directly applicable for describing the complexity of real biological systems. However, we attempted in the following examples to show that knowledge of the behavior of the basic system described here may help to depict the essential features of some observed phenomena. The examples – cell polarity, cytokinesis and gastrulation – were chosen on the assumption that these processes are influenced by the fact that the respective systems involve closed envelopes.

A possible origin of cell polarity

Many types of cells are polar, *i.e.* the concentrations of their constituents are either on the increase or decrease in a given direction, and their structural features vary accordingly. The physico-chemical basis for a nonhomogeneous distribution of cell components is not yet established. A possibility is that cell constituents form a Turing structure (Turing, 1952), where a specific set of chemical reactions and proper values of the diffusion coefficients of some of the reactants allow the system to attain a stable state far from thermodynamic equilibrium. Recently we proposed (Svetina and Zeks, 1990) that cell polarity may alternatively arise as a consequence of the symmetry-breaking properties of layered membranes described in a previous section. In certain ranges of v and Δa values, the shape of a cell with the minimum membrane energy involves polar asymmetry, and such shapes can arise in a continuous manner from shapes involving equatorial mirror symmetry. They can also arise directly from spherical shapes exhibiting radial symmetry (Fig. 3A). In Fig. 3B the shapes observed (Brawley and Robinson, 1985) in the developing fucoid zygote of *P. fastigiata* are shown for comparison. It has been suggested (Svetina and Zeks,

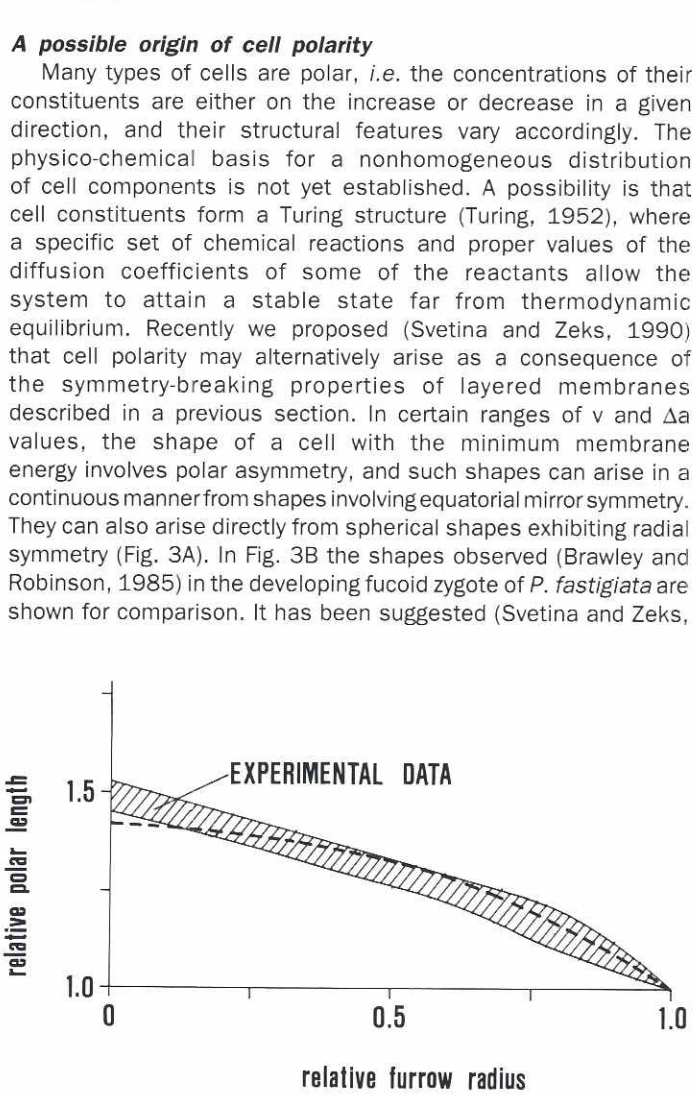

Fig. 4. The change in the relative polar length (defined relative to the polar length of a sphere) with the analogously defined relative furrow radius (broken line), as predicted if shape changes at cytokinesis were following the upper Δa limiting shapes of the dumbbell class *(see Fig. 2B). Comparison is made to the experimental data on the dividing sea urchin egg of Hiramoto (1958) and Yoneda and Dan (1972) as compiled recently by Zinemanas and Nir (1990).*

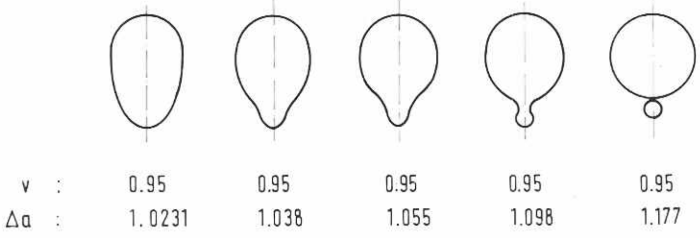

Fig. 5. A possible set of shape changes leading eventually to an asymmetrical cell division. *The shape changes due to the increase of Δa are shown for relative volume v= 0.95.*

1990) that shape asymmetry may cause an asymmetric distribution of membrane components the energies of which depend on the membrane curvature. For example, protein-lipid interaction, which depends on the structure of the part of the protein in contact with phospholipid molecules, may be affected by membrane curvature (Svetina *et al.*, 1990).

Cytokinesis

Cytokinesis is the final act of cell division where a cell divides its cytoplasm into two parts. It is well established that the physical mechanism of cytokinesis is located in the cell surface (Rappaport, 1986). It is possible to explain the observed shape changes from an initially spherical cell to two also spherical daughter cells by invoking different membrane properties, one of which is membrane bending energy. The latter was taken into consideration in the theory of Verhas (1988). He obtained a reasonable time course of cell shapes by assuming a suitable time dependence of the membrane spontaneous curvature. On the basis of the present discussion it is possible to identify the shapes obtained by Verhas as the shapes belonging to the dumbbell class. The shapes as observed in cleavage furrow-forming cell divisions, for example in the sea urchin, are actually very similar to the limiting shapes of this class at higher Δa values (Fig. 2B) which can also be seen by comparing calculated and measured dependence of the polar distance on the furrow radius (Fig. 4). These limiting shapes involve an edge at the equator and therefore an infinite bending energy would be needed for their realization. In view of this it can be postulated that the force generating elements that mediate cytokinesis and reside in a cortical region adjacent to the plasma membrane, such as actin and myosin, are involved primarily in necessary adjustments of the v and Δa values. The accompanying accumulation of cortical contractile elements at the cleavage furrow regions (White and Borisy, 1983) could be the consequence of their lateral redistribution with respect to varying membrane curvatures which may also give rise to the proper lowering of the membrane free energy (Svetina *et al.*, 1990).

The described possibility for cytokinesis using the dumbbell class of shapes is expected to give rise to two equivalent daughter cells because the dumbbells involve the equatorial symmetry plane. In some instances such as a polar body formation, the cell division is asymmetrical: the sizes of the two daughter cells are noticeably different. Such division could occur by using the egg class of shapes as a basis for shape transformations during the cytokinesis. The limiting shape in this class is a large and a small vesicle (Fig. 2A). The radii of these vesicles depend on the relative volume at which this limiting shape is reached (Fig. 5). In view of our discussion on polarity it is plausible to expect that the properties of the two daughter cells are different so that division is asymmetrical not only with respect to the sizes of the daughter cells but also with respect to their composition. An example of direct evidence for the difference between the membranes of the two daughter cells in asymmetrical cell division is an unequal distribution of the fluorescent label at the main cell and the polar body in the fertilized mouse egg (Wolf and Ziomek, 1983).

Gastrulation

Gastrulation is a developmental process involving the rearrangement of cellular sheets. A simple classical example of gastrulation is the sea urchin embryo where the cells in blastula are arranged to form a hollow sphere only one cell layer thick. In the course of the gastrulation process this cellular sheet invaginates. We can conclude from the shapes observed that the gastrulation proceeds with the shape changes within the cup class of shapes (Fig. 2C). In Fig. 6A are shown some consecutive shapes of the cup class obtained by decreasing the volume and varying Δa, which resembles shapes observed (Gustafson and Wolpert, 1967) in the gastrulation of the sea urchin (Fig. 6B).

Discussion

The present work represents a theoretical approach to relate the mechanical properties of closed membranes or unicellular sheets and the shapes of the enclosed bodies. The existence of such a relationship has been postulated for some time (Lewis, 1947; Gierer, 1977; Odell *et al.*, 1981). The par-

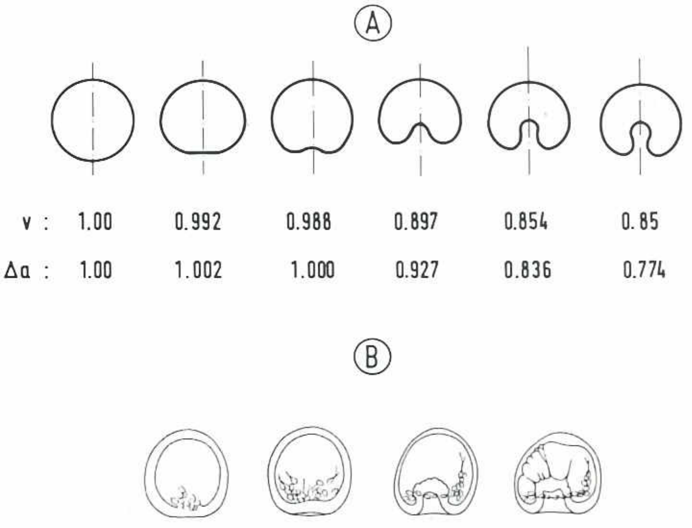

Fig. 6. An example of a possible series of shapes in the course of shape transformations from a sphere to a cup shape simulating the shape changes in the process of gastrulation (A). *For comparison, the shapes of developing larva of* Psammechinus miliaris *are shown* **(B)** *as presented in Fig. 1 of Gustafson and Wolpert (1967).*

ticular idea developed here is that the lamellar character of the membrane structure is important for understanding these processes. The hypothesis of coupled layers previously gave rise to a qualitative description of shape transformations of red blood cells resulting from an asymmetric modification of their membrane (Sheetz and Singer, 1974), and it was also proposed that this approach could be useful for describing the shape behavior of more complex systems. More recently we developed a corresponding theory (Svetina and Zeks, 1989). Here we have used examples to illustrate how this theory might be used to provide a rational mechanism for shape transformations in complex systems.

The theory presented confirms on the one hand earlier concepts about how membrane bending causes evaginations or invaginations. In addition, it emphasizes the importance of the existence of different classes of shapes. An object can easily change its shape within a given class but can be transformed into another class only by overcoming a certain energy barrier. The examples presented here indicate that different classes can be used to explain how cells and closed cellular sheets undergo different shape transformations under different conditions. Cell polarity seems to be a natural consequence of a cell exhibiting an asymmetrical shape such as a shape of any member of the egg class. Cytokinesis may involve either the dumbbell class or the egg class depending on the symmetry of the cell division. Gastrulation seems to involve the cup shapes. It is of interest to note that the three examples treated here have a common initial shape, a sphere. The sphere is the one shape in the system that belongs to all classes and is therefore a suitable shape at which to make the decision about the further destiny of the system.

A significant characteristic of lamellar membranes is their symmetry-breaking behavior as exemplified here by the case of cell polarity. Lowering its symmetry makes a given system more ordered. Therefore it can be concluded that the membrane is the site where the spatial organization of biological systems could be established, at least at some initial stages of development.

The present analysis could also be useful in relating the molecular processes underlying shape transformations during development to the macroscopic behavior of the systems. In many instances it is possible to relate a macroscopic phenomenon such as cytokinesis to a given molecular property such as the structural impairment of a mutated protein or an interaction of a specific modifier with the molecular structures involved. Our results suggest that there are properties of the system intermediate to the molecular and macroscopic levels which make it possible to dissect the problem of connecting molecular and macroscopic events into tractable parts. An important finding is that there are purely geometrical aspects of the problem which arise as the consequence of the fact that membranes are composed of layers. The relevant geometrical properties are relative volume, v, and relative difference between the areas of the two layers, Δa. The search for the mechanisms underlying shape transformations can thus be directed to the search for the mechanisms responsible for the changes in the cell volume, the mean membrane area and the difference between the areas of the two relevant membrane layers, the properties of the system on which v and Δa depend.

It should be noted that the shape transformations represent macroscopic mechanical movements and therefore the mechanisms of shape changes as understood here can be classified as chemo-mechanical and osmo-mechanical transduction mechanisms. It is the chemical processes at the membrane which cause differential changes in the areas of the membrane layers. The alterations in the cell osmotic properties cause changes in v.

The present treatment of shape transformations in developing processes was restricted to shape transformations which are obtained by homogeneous closed lamellar membranes. It is clear from the present discussion and other work that even this idealized system is already quite complex with respect to its shape behavior. We believe that the properties revealed may also help understand more complex situations involving nonhomogeneous membranes. For instance, membrane can become mechanically nonhomogeneous because of the effect of membrane curvature on the lateral distribution of membrane components. This affects the shape. However, the shape obtained still belongs to the same class. The division of the system into classes of shapes remains the same. The complexity of the system can of course increase due to other causes, such as the possibility of phase separation of membrane components or the active participation of cytoskeletal components.

Acknowledgments

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