

## CHAPTER 1

# Introduction

### 1.1. Modeling Biological Development

A *mathematical model* in natural science is a systematization of data such that alteration of experimental conditions is reflected ideally by variation of a small number of model parameters. It goes without saying that models are rarely developed in this way. Rather, they are constructed by simplification of hypothetical underlying mechanisms, and their ultimate major utility lies in the physical, chemical, and other mechanisms that they suggest, which are then susceptible to experimental verification. A mathematical model has the advantage of being able to draw upon concepts and techniques from nominally alien disciplines, and the consequent danger that those properties that contribute to the uniqueness of the discipline at hand may be understated due to insufficient universality.

In this course, we address ourselves primarily to the development of multicellular animals from an initial single cell egg. In bygone years, the self-inconsistent concept of *preformation* was in vogue, in which each egg contained in miniature a copy of the adult to which it would eventually give rise. It was not terribly difficult to imagine ways in which this copy could be enlarged and developed—and it was not terribly interesting. We are now reasonably certain that the information used by the developing organism is initially present in a spatially unstructured form, and the problem then is to explain *epigenesis*, the development of the highly complex, highly reproducible spatial structure of the animal from this initial structureless mass. This should not be understood in its most extreme form—there *is* spatial structure in the initial egg, and it *does* play a significant role in some of the ensuing development, but the total information that it yields is small on the scale of that required to define the process of development. Choice of the level of a mathematical model is of crucial importance. Should it describe all animals simultaneously, animal type entering in some fashion as a variable parameter? This is not absurd during early development, and so we will do something like that. Should it instead be “content” with describing in a uniform way all changes in form that can occur in a developing animal? This is the aim of catastrophe theory, which we will examine in the next chapter. Should we attempt at all to describe the development of a single egg, or should we use a stochastic description of an ensemble of similar eggs? The stochastic approach will indeed be a useful tool.

On a more detailed level: Should an effort be made to at least distinguish between the biochemical properties that serve as markers for cell type—the problem of differentiation and pattern formation—and the spatial delineation of tissues formed

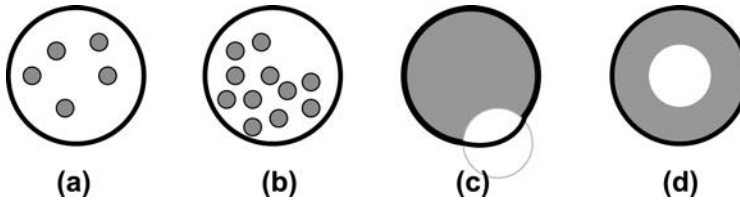


FIGURE 1.1. Yolk distribution in the egg: (a) isolecithal, (b) mesolecithal, (c) telolecithal, (d) centrolecithal.

from cells and extracellular material—the problem of change of form or morphogenesis? We will certainly see to what extent this is feasible. If we focus upon cells as units of structure, to what extent do we have to peer inwards to underlying biochemistry to have control over their overt properties, and to what extent can we deal in some continuum quasi-hydrodynamic way with cell populations, perhaps with an external biochemical field? Can we use the mechanical properties of cells as some sort of intermediate connection, mimicking cellular interaction by quasi-molecular interaction?

This list of questions and associated model types can be enlarged without difficulty, and becomes epistemological with even less difficulty. We will therefore adopt the more prudent course of following the traditional stages of developmental biology, of trying to organize as much phenomenology as possible at each stage, but of not struggling unduly when particular properties of specialized classes of organisms have to be pursued. A fairly broad swathe of mathematical techniques will perforce enter, and very few will exit.

## 1.2. Early Stages: A Brief Survey

The earlier, presumably more primitive stages of development show great similarities among diverse organisms, consistent of course with the gross physical form of the egg. In particular, the presence of yolk retards cleavage, producing larger cells and a general slowness of change. Eggs are thus usefully characterized according to their distribution of yolk: *isolecithal*, with little uniformly distributed yolk, as in echinoderms, on amphioxii, and mammals (Figure 1.1(a)); *mesolecithal*, with a heavier accumulation of yolk on one side, as in amphibia (Figure 1.1(b)); *telolecithal*, with only a small area bare of heavy yolk, as in birds (Figure 1.1(c)); *centrolecithal*, with yolk excluded only from the center and some channels, as in insects, and some coelenterates (Figure 1.1(d)).

The yolky side is the vegetal, the other the animal side or pole, a distinction arising on separation of egg from ovary, and valid in isolecithal eggs as well. In isolecithal eggs, cleavage is uniform straight through, or holoblastic, also true in mesolecithal eggs, but resulting in nonuniform cell size. In telelecithal eggs, cleavage is very incomplete, or meroblastic, with a cleavage area confined to a blastodisc. In centrolecithal eggs, it is generally superficial, separating nuclei but not cells proper.

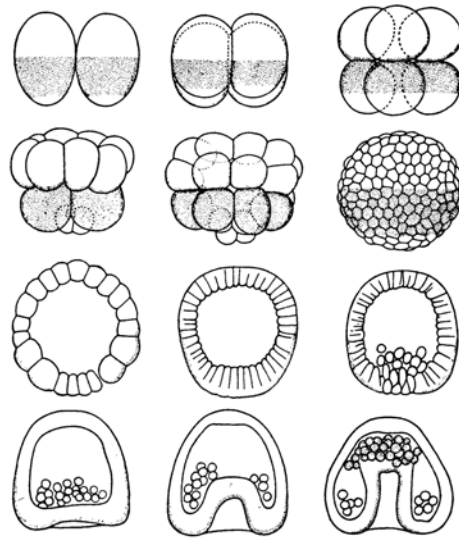


FIGURE 1.2. Cleavage and gastrulation of the sea urchin *Paracentrotus lividus*. After Boveri, 1901; drawn by Ina Mette.

The first commonly named stage of development results in a hollow ball, or *blastula*, after a number of cell cleavages. There may or may not be a discernible solid ball of cells, or *morula*, as an intermediate stage. There follows a complex pattern forming step of *gastrulation*, involving an invagination to produce the *endodermal* layer, the outside corresponding to *ectoderm* and the joining region to *mesoderm*. These three germ layers give rise ultimately to alimentary organs and lungs; skin and nervous system; and a support system of bone, muscle, and vascular elements. The sequences of cleavage, blastulation, and gastrulation occur in the development of diverse organisms—the sea urchin, an invertebrate echinoderm; amphioxus, a protochordate, the entering wedge to vertebrates; the frog, a vertebrate amphibian; the chick, a vertebrate bird; and the mouse, a vertebrate mammal. The form of these events, however, varies widely. We cannot attempt here detailed descriptions, and the reader is urged to consult basic texts such as [1]. We show in Figure 1.2 cleavage and gastrulation of the sea urchin.

### 1.3. An Example: Formation of the Blastocoel

We shall start by trying to model the very earliest morphogenetic change, that of blastulation, or more pointedly of *blastocoel* (the cavity in the blastula) formation; see Figure 1.3. It will turn out that several important concepts and techniques arise even at this level. But what precisely is the problem? It is to describe and at least empirically explain why a cleaving egg abandons the form of a morula or ball of cells and forms a cavity or blastocoel, a switch that occurs almost at once and gradually in sea urchins, but much later and suddenly in mammals. In fact, let us concentrate upon *isolecithal* eggs for the moment to avoid the additional parameters of yolk distribution.

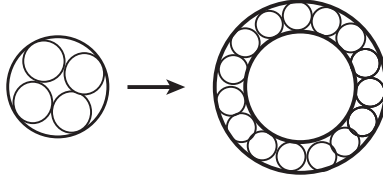


FIGURE 1.3. Formation of the blastocoel.

**1.3.1. Local Behavior.** Two mechanisms have been suggested for blastocoel formation, both relying on the firm attachment of cells to the inner egg surface coat or *hyaline layer*. In Dan's theory [54], the intercellular fluid accumulates large macromolecules, hence takes up more water osmotically and forces the cells attached to the hyaline layer outward.

According to Gustavson and Wolpert [89], cavity formation in the sea urchin is rather the result of hyaline layer adhesion exceeding intercellular adhesion, so that as the cells get smaller during cleavage they remain in a one-cell layer, a more or less fixed total cell volume, thus increasing the hyaline layer surface area and expanding the whole egg, with additional fluid entering passively. (The egg linear expansion need not be large—a shell from  $R = 4$  to  $R = 5$  radius contains roughly the same cell volume as a ball  $R = 4$ .) This adhesive imbalance would presumably not occur until much later in mammalian eggs.

The Gustavson-Wolpert mechanism takes advantage of cell properties that we shall meet frequently. Let us therefore investigate it in further detail. How shall we describe our system? At this stage, the major question is whether there is or is not a cell at a given point in space, so that it is reasonable to introduce a *cell density field*

$$v_{\mathbf{x}} = \begin{cases} 0 & \text{if there is no cell at } \mathbf{x}, \\ 1 & \text{if there is a cell at } \mathbf{x}. \end{cases}$$

We have the option of regarding  $\mathbf{x}$  as continuous, as representing cell-sized boxes that may or may not be filled by cells, as mean cell occupation in a larger volume, or as something in between. We will adopt the second option but will feel free to switch as convenient; the difference is inconsequential only when there are many cells in each structural element of the organism.

Now what determines the configuration that the cells achieve in the course of time? We will start with the assumption of *quasi-static equilibrium*: a system with dynamical degrees of freedom  $\{q_\alpha\}$  is described by some energy function  $E(\{q_\alpha\}, t)$ . This gives rise to generalized forces

$$F_\alpha = -\frac{\partial E}{\partial q_\alpha},$$

which change the dynamical variables. The change may be Newtonian,  $\ddot{q}_\alpha = F_\alpha/m_\alpha$ , hydrodynamic drag  $\dot{q}_\alpha = F_\alpha/\gamma_\alpha$ , or simply unknown. If we suppose that the time scale of the dynamics is fast compared with the explicit externally controlled time

dependence of the function  $E$ , the system will always have time to equilibrate,

$$\frac{\partial E}{\partial q_\alpha} = 0,$$

and remain motionless on the large time scale, no matter what the dynamics. This is quasi-static equilibrium. Of course, we also require that the equilibrium be stable—that a fluctuation not dynamically introduce growing changes. With typical dynamics, this requires

$$\sum dF_\alpha dq_\alpha \leq 0$$

at equilibrium, or

$$\sum \left( \frac{\partial^2 E}{\partial q_\alpha \partial q_\beta} \right) dq_\alpha dq_\beta \geq 0.$$

This means that  $E$  is not just stationary, but must be at least at a *local minimum*.

What are the components of the system energy in the present case? To start with, there is of course the hyaline layer surface adhesion energy

$$E_{\text{bd}} = -\gamma \sum_{x \in \text{bd}} v_x.$$

(bd  $\equiv$  boundary): the more cells at the boundary, the closer to a minimum is  $E$ . Next, there is the cell-cell interaction adhesive energy

$$E_{\text{int}} = -\frac{1}{2} J \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} v_x v_y,$$

where  $\langle \mathbf{x}, \mathbf{y} \rangle$  indicates that the cells at  $\mathbf{x}$  and  $\mathbf{y}$  are nearest neighbors, in contact, and  $J$  is the mean interaction energy (a factor of  $\frac{1}{2}$  occurs here because each neighboring pair occurs twice in double summation). Then there is the restriction that the cell number be fixed:

$$\sum_{\mathbf{x}} v_x = N,$$

which, on insertion with a Lagrange parameter  $\lambda$ , masquerades as an energy contribution

$$E_N = \lambda \left( \sum_{\mathbf{x}} v_x - N \right).$$

Finally, there is the effect of the all-pervading biological component—*fluctuation*—which enters at all levels of macroscopic description. Its simplest manifestation is via the *autonomous mechanical motion* of cells, so that external forces yield only a most likely dynamics, with a considerable spread. The same effect is of course produced by the fluctuation of coupling parameters, such as  $\gamma$  and  $J$ , due to variability of surface contact. And, on a different level, observation of a sample of several nominally identical organisms yields a spread of biological parameters not necessarily derivable from that of a single representative. Such unavoidable fluctuations are at the heart of traditional thermodynamics, where the unavailable energy is accounted for by inclusion of a negative entropy. This takes the form

$$E_T = T \sum_{\mathbf{x}} (\rho_{\mathbf{x}} \ln \rho_{\mathbf{x}} + (1 - \rho_{\mathbf{x}}) \ln(1 - \rho_{\mathbf{x}})),$$

where  $T$  is an effective temperature or fluctuation level, and

$$\rho_{\mathbf{x}} \equiv \langle v_{\mathbf{x}} \rangle,$$

the average cell density, is no longer necessarily 0 or 1. In the absence of compelling energetic reasons, this gives rise to a nondescript soup of half cell, half not-cell. For many purposes, it is sufficient to Taylor expand  $E_T$  about its minimum at  $\rho_{\mathbf{x}} = \frac{1}{2}$ , yielding

$$E_T = T \sum_{\mathbf{x}} \left( \frac{1}{2} \sigma_{\mathbf{x}}^2 + \frac{1}{12} \sigma_{\mathbf{x}}^4 + \dots \right),$$

to within an additive constant, where  $\sigma_{\mathbf{x}} \equiv 2\rho_{\mathbf{x}} - 1$ .

We conclude that, in terms of the field  $\sigma_{\mathbf{x}}$  with limiting values

$$\sigma_{\mathbf{x}} = \begin{cases} -1 & \text{no cell at } \mathbf{x}, \\ 1 & \text{cell at } \mathbf{x}, \end{cases}$$

but intermediate values as well, the system energy can be written approximately as

$$E = \sum_{\mathbf{x}} \left( \frac{1}{12} \sigma_{\mathbf{x}}^4 + \frac{1}{2} \sigma_{\mathbf{x}}^2 + \left( \frac{\lambda}{2} - \frac{Jc}{4} \right) \sigma_{\mathbf{x}} \right) - \frac{J}{8} \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} \sigma_{\mathbf{x}} \sigma_{\mathbf{y}} - \frac{\gamma}{2} \sum_{\text{bd}} \sigma_{\mathbf{x}},$$

to within an additive constant. Here  $T$  has been chosen as the unit of energy, and  $c$  is the potential number of neighboring cells of a given cell, so that  $\sum_{\langle \mathbf{x}, \mathbf{y} \rangle} \sigma_{\mathbf{x}} = c \sum_{\mathbf{x}} \sigma_{\mathbf{x}}$ , etc. Because of the fluctuation energy approximation,  $\sigma_{\mathbf{x}}$  is no longer automatically bounded by  $-1$  and  $1$ , so that we must interpret an increase from  $0$  simply as the increasing likelihood of a cell, and a decrease from  $0$  as the increasing likelihood of no cell.

Suppose now that the energy was able to adjust itself locally. Then in any region—away from the boundary—of uniform cell density corresponding to “spin”  $\sigma$  (a term deriving from the use of  $\sigma$  in assemblies of magnetic dipoles), we would have an energy per grid point or *energy density*

$$E(\sigma) = \frac{1}{12} \sigma^4 + \left( \frac{1}{2} - \frac{Jc}{8} \right) \sigma^2 + \left( \frac{\lambda}{2} - \frac{Jc}{4} \right) \sigma$$

to within an additive constant. What does this look like? If  $Jc/4 < 1$ , then

$$\frac{\partial^2 E}{\partial \sigma^2} = \sigma^2 + \left( 1 - \frac{Jc}{4} \right) > 0,$$

so that there is just one minimum; see Figure 1.4(a). If  $\sigma$  is constrained to a high value  $\approx 1$  at the egg boundary, it must fall rapidly to the homogeneous medium minimum, creating a thin shell. The sign of the minimum  $\sigma$  is precisely the reverse of that of the constant in  $E(\sigma)$ , i.e.,  $\frac{Jc}{4} - \frac{\lambda}{2}$

If  $\frac{Jc}{4} > 1$ , it depends. Let us write

$$E = \frac{1}{12} \sigma^4 + \frac{1}{2} u \sigma^2 + \frac{1}{3} v \sigma;$$

the uniform cell density  $\sigma$  is now controlled by the two parameters  $u$  and  $v$ : the stationary points are given by  $E' = \frac{1}{3} \sigma^3 + u \sigma + \frac{1}{3} v = 0$ . If  $u \ll 0$  or  $\frac{Jc}{4} \gg 1$ , there are three real roots, corresponding to two minima of  $E(\sigma)$ ; see Figure 1.4(b). If the

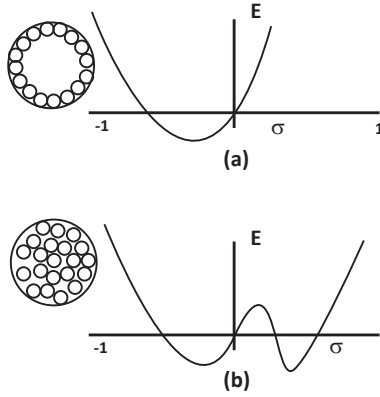


FIGURE 1.4. Stationary points of the energy function.

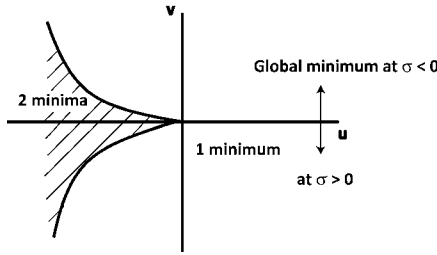


FIGURE 1.5. Regimes of blastulation.

highest one is selected by the boundary condition, we can readily have a morula structure, but the lowest root produces a blastocoel again. Once more, the lowest minimum, due to skewing by the linear term, is at  $\pm$  values of  $\sigma$  according to the sign of  $\frac{Jc}{4} - \frac{\lambda}{2}$ .

The dividing line between the above regimes occurs when a maximum and minimum coincide, producing an inflection point:  $E'' = \sigma^2 + u = 0$ . Eliminating  $\sigma$  in  $E' = \frac{1}{3}\sigma^3 + u\sigma + \frac{1}{3}v = 0$ , the stationary character of  $E$  with respect to  $\sigma$  undergoes a sudden (*catastrophique* in French) change when the *control variables*  $u$  and  $v$  satisfy

$$v^2 + 4u^3 = 0.$$

We can express this diagrammatically as shown in Figure 1.5.

If it turns out that  $\lambda$  is increasing in time, a typical sample of developmental paths in parameter space from morula to blastula is indicated. It is clear, however, that there can be a catastrophic change in the initial state as well, as a function of evolution.

**1.3.2. Global Behavior.** In the presence of nonuniformity, and we have already distinguished between boundary and inside, the local analysis is insufficient. We must solve for the full three-dimensional density  $\sigma_x$ , again with two control

parameters. Returning to the full energy of the system,

$$E = \sum_{\mathbf{x}} \left( \frac{1}{12} \sigma_{\mathbf{x}}^4 + \frac{1}{2} \sigma_{\mathbf{x}}^2 + \left( \frac{\lambda}{2} - \frac{Jc}{4} \right) \sigma_{\mathbf{x}} \right) - \frac{J}{8} \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} \sigma_{\mathbf{x}} \sigma_{\mathbf{y}} - \frac{\gamma}{2} \sum_{\text{bd}} \sigma_{\mathbf{x}},$$

let us first suppose that  $\gamma$  is sufficiently high that it simply enforces the condition  $\sigma_{\mathbf{x}} = 1$  at the boundary; the boundary cells are guaranteed to be stuck to the hyaline layer. Further, we observe that

$$\begin{aligned} - \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} \sigma_{\mathbf{x}} \sigma_{\mathbf{y}} &= \frac{1}{2} \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} (\sigma_{\mathbf{x}} - \sigma_{\mathbf{y}})^2 - \frac{1}{2} \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} (\sigma_{\mathbf{x}}^2 + \sigma_{\mathbf{y}}^2) \\ &= \frac{1}{2} \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} (\sigma_{\mathbf{x}} - \sigma_{\mathbf{y}})^2 - c \sum_{\mathbf{x}} \sigma_{\mathbf{x}}^2. \end{aligned}$$

Hence we have

$$E = \sum_{\mathbf{x}} \left( \frac{1}{12} \sigma_{\mathbf{x}}^4 + \left( \frac{1}{2} - \frac{Jc}{8} \right) \sigma_{\mathbf{x}}^2 + \left( \frac{\lambda}{2} - \frac{Jc}{4} \right) \sigma_{\mathbf{x}} \right) + \frac{J}{16} \sum_{\langle \mathbf{x}, \mathbf{y} \rangle} (\sigma_{\mathbf{x}} - \sigma_{\mathbf{y}})^2,$$

where  $\sigma_{\mathbf{x}} = 1$  at the boundary.

The inhomogeneity correction is a positive energy that must be supplied any time that  $\sigma_{\mathbf{x}}$  changes in space; i.e., a surface tension results from breaking cell-cell adhesion contacts (see Chapter 4) and must be justified globally. Now, taking  $\partial/\partial\sigma_{\mathbf{x}}$ , we have at the minimum, in the previous  $uv$ -notation

$$\frac{1}{3} \sigma_{\mathbf{x}}^3 + u \sigma_{\mathbf{x}} + \frac{1}{3} v = \frac{J}{4} \sum_{\mathbf{y}}^{\langle \mathbf{x}, \mathbf{y} \rangle} (\sigma_{\mathbf{y}} - \sigma_{\mathbf{x}}).$$

The right-hand side here is essentially the finite difference analogue of the Laplace operator, i.e., the mean deviation of a function on a surface surrounding the point in question. To see this, we introduce the average  $Au$  over nearest neighbors and write, for a slowly varying field  $\sigma_{\mathbf{x}}$ ,

$$\begin{aligned} \frac{J}{4} \sum_{\mathbf{y}}^{\langle \mathbf{x}, \mathbf{y} \rangle} (\sigma_{\mathbf{y}} - \sigma_{\mathbf{x}}) &= c A v_{\mathbf{y}} (\sigma_{\mathbf{y}} - \sigma_{\mathbf{x}}) \\ &= c A v_{\mathbf{y}} \left( \mathbf{y} - \mathbf{x} \cdot \nabla \sigma_{\mathbf{x}} + \frac{1}{2} (\mathbf{y} - \mathbf{x})(\mathbf{y} - \mathbf{x}) : \nabla \nabla \sigma_{\mathbf{x}} + \dots \right). \end{aligned}$$

But if  $|\mathbf{y} - \mathbf{x}| = a$ , then for an arbitrary vector  $w$ ,  $A v (\mathbf{y} - \mathbf{x}) \cdot w = 0$  and  $A v ((\mathbf{y} - \mathbf{x}) \cdot w)^2 = \frac{1}{3} a w^2$ , and so we may write

$$\frac{1}{3} \sigma_{\mathbf{x}}^3 + u \sigma_{\mathbf{x}} + \frac{1}{3} v = \frac{J}{12} a^2 c \nabla^2 \sigma_{\mathbf{x}},$$

or simply

$$E'(\sigma_{\mathbf{x}}) = \frac{J}{12} a^2 c \nabla^2 \sigma_{\mathbf{x}},$$

where  $\nabla^2$  is the continuous limit of the lattice Laplacian,  $\nabla \cdot \nabla = \partial^2/\partial x^2 + \partial^2/\partial y^2 + \partial^2/\partial z^2$ , and  $E$  denotes the non-interacting energy density.



Suppose now that  $\sigma$  varies only in one direction, which we can imagine as radial position  $r$  in a spherical egg; we then replace  $\nabla^2$  by  $\partial^2/\partial r^2$ , which causes obvious difficulties for small  $r$ . Since our nonlinear partial differential equation is thereby replaced by the ordinary differential equation

$$E'(\sigma) = \frac{J}{12} a^2 c \frac{d^2 \sigma}{dr^2},$$

its solution is readily obtained. We simply multiply by  $\frac{d\sigma}{dr}$  and integrate, obtaining

$$\frac{1}{24} J a^2 c \left( \frac{d\sigma}{dr} \right)^2 - E(\sigma) = K,$$

where  $K$  is a suitable constant, necessarily greater than  $-E(\sigma)$  at each  $\sigma$  attained. This relation can be shown to be equivalent to the minimization of

$$E_1 = \int \left( E(\sigma) + \frac{1}{24} J a^2 c \left( \frac{d\sigma}{dr} \right)^2 \right) dr,$$

the cell continuum one-dimensional version of our original expression.

The value of the constant  $K$  is, of course, determined by boundary conditions. At the surface of the egg,  $r = R$ , we require  $\sigma = 1$ . Inside, we want the “motion” to stop,  $\frac{d\sigma}{dr} = 0$ , at  $r = 0$ . Since

$$dr = \frac{d\sigma}{d\sigma/dr} = \text{const} \frac{d\sigma}{\sqrt{K + E(\sigma)}},$$

$K$  is thereby determined. It will be convenient for us to use a numerically indistinguishable but somewhat different criterion for purposes of illustration. The one-dimensional version of a three-dimensional egg makes more sense if we never have to go to  $r = 0$  because the cell density has stabilized by then—has become asymptotic. Hence we shall use the condition that  $\frac{d\sigma}{dr} = 0$  is reached at  $r = -\infty$ , but only look at what happens until  $r = 0$ . This requires that  $K + E(\sigma)$  not only vanish at the small  $\sigma$  endpoint but in fact become stationary (for then  $r = \int dr$  will diverge). We can now go through the two major sequences as  $J$  decreases,  $\lambda$  increases, or both. In each case, we draw the curve  $-E(\sigma)$  (dropping the Taylor expansion approximation in  $\sigma$  steepens the sides) and the level line  $K$ . As  $\lambda$  increases to bias a portion of the egg towards  $\sigma = -1$ ,  $J$  may start out low enough that only the single-minimum regime holds throughout, and a steady cavity formation results; see Figure 1.6(a).

On the other hand, if we start in the two-minimum region, a jump in  $\sigma_{\min}$  can occur as soon as the left peak of  $-E$  dominates the right one. There are then two regions of relatively uniform  $\sigma$ , but the outer one takes on a shell appearance as  $-E$  degenerates to a single peak. The lesson we learn at this stage then is that the control parameters  $J$  and  $\lambda$  determine the global  $\{\sigma_x\}$  behavior in just the fashion that they determine the local  $\sigma$ -behavior—what matters is position relative to the singularities in the  $J\lambda$ -plane; see Figure 1.6(b).

There remains the problem of finding  $J$  and  $\lambda$  as functions of time. The former is in principle known (but perhaps not in units of “temperature”  $T$ —is the pulsatile sea urchin activity equivalent to high  $T$  and/or low  $J$ ?), but the latter depends upon

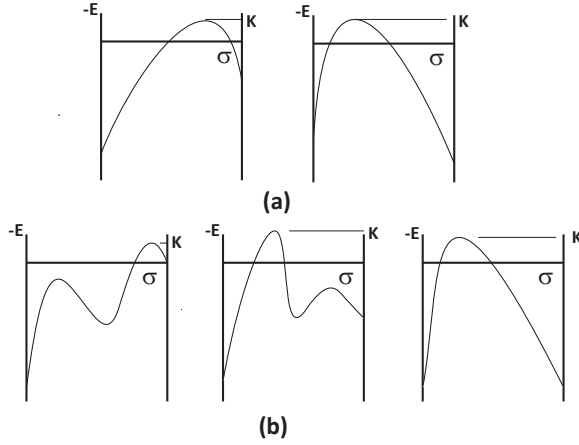


FIGURE 1.6. Cavity formation via the energy function.

the radius  $R$  of the egg, itself the result presumably of an energy minimization, contingent upon knowledge of the surface adhesivity  $\gamma$ .

Let us write  $E(\lambda, R)$  to emphasize the unknown parameters. First fix  $R$ . Then we can write

$$E(\lambda) = \min(E_0 + \lambda g),$$

where  $g = 0$  expresses the condition that the total number of cells is given. For degrees of freedom  $q_\alpha$ , the minimizing values  $q_\alpha(\lambda)$  thus satisfy  $\partial(E_0 + \lambda g)/\partial q_\alpha = 0$ . The value of  $\lambda$  should be chosen such that  $g = 0$  at the minimum. One way of accomplishing this is to set

$$\frac{dE(\lambda)}{d\lambda} = 0,$$

for then

$$\frac{dE(\lambda)}{d\lambda} = \left( \frac{\partial}{\partial \lambda} + \sum \frac{dq_\alpha}{d\lambda} \frac{\partial}{\partial q_\alpha} \right) E = g + \sum \frac{dq_\alpha}{d\lambda} \frac{\partial E}{\partial q_\alpha} = 0$$

implies  $g = 0$ . Furthermore,

$$\begin{aligned} \frac{d^2E}{d\lambda^2} &= \frac{dg}{d\lambda} + \sum \frac{d^2q_\alpha}{d\lambda^2} \frac{\partial E}{\partial q_\alpha} + \sum \frac{dq_\alpha}{d\lambda} \frac{\partial g}{\partial q_\alpha} + \sum \left( \frac{\partial^2 E}{\partial q_\alpha \partial q_\beta} \right) \frac{dq_\alpha}{d\lambda} \frac{dq_\beta}{d\lambda} \\ &= \sum \frac{\partial^2 E}{\partial q_\alpha \partial q_\beta} dq'_\alpha dq'_\beta, \end{aligned}$$

so that  $E$  is a minimum with respect to  $\lambda$ , too.

Now  $\lambda$  is a function of  $R$ , and we want  $\frac{\partial}{\partial R} E(\lambda(R), R) = 0$  or  $\frac{\partial \lambda}{\partial R} \frac{\partial E}{\partial \lambda} + \frac{\partial E}{\partial R} = 0$ . We conclude that

$$\frac{\partial E(\lambda, R)}{\partial R} = \frac{\partial E(\lambda, R)}{\partial \lambda} = 0$$

and  $E$  is a minimum.

Thus the control variables  $J$ ,  $N$ , and  $\gamma$  determine the parameters  $\lambda$  and  $R$ , which then serve as control variables for the cell distribution. Let us consider this piece of the problem briefly, oversimplifying greatly to clarify the point to be made. We choose to build the system energy out of surface adhesion and internal surface tension alone, regarding the volume energy at fixed total cell volume  $2\pi V$  as fixed. If the spherical cavity goes from  $R - d$  to  $R$ , we take  $\frac{1}{d}$  as a measure of the cell density gradient, so that the surface tension energy is of the form  $VJ/d^2$ . For a thin shell, we have  $2\pi V = \frac{1}{2}4\pi R^2 d$ . Hence parametrizing the surface adhesion in the obvious way,

$$\Delta E = -\gamma R^2 + \frac{VJ}{d^2} \quad \text{where } R^2 d = V.$$

Eliminating  $d$  rather than using a Lagrange parameter,

$$\Delta E = \frac{J}{V} \left( R^4 - \frac{\gamma V}{J} R^2 \right).$$

This is a degenerate case ( $v = 0$ ) of the quartic expression we previously had to minimize, and always has precisely two minima at

$$|R| = \left( \frac{\gamma V}{2J} \right)^{1/2},$$

with a maximum at 0—a simple intuitively obvious  $\frac{\gamma}{J}$  dependence, valid only for small  $d$ . The degeneracy is a result of radial symmetry, and if the more appropriate variable  $R^2$ , or  $\frac{1}{d}$ , is used, the basic quadratic minimization is recovered. The structure of the minimum never changes, and in terms of the variable  $R^2 - \frac{\gamma V}{2J}$ , there are no control variables at all.