Mechanics of Cytogels I: Oscillations in Physarum

G.F. Oster and G.M. Odell

Department of Biophysics and Entomology, University of California, Berkeley (G.F.O.); Department of Mathematics, Rensselaer Polytechnic Institute, Troy (G.M.O.)

The contractility of actomyosin gels is the basis for a variety of cellular motility phenomena. We present here a mechanical analysis of contractile gels. By making certain hypotheses on the chemical regulation of cytogel contraction we formulate a model for the rhythmic contractions of plasmodia in the slime mold Physarum polycephalum which is in accord with a number of experimental observations.

Key words: cytogel, actomyosin, Physarum, oscillations, mechanics

1. INTRODUCTION

The relationship between the molecular structure of cytoplasm and its mechanical properties has been the subject of intensive study, and recently a coherent picture is beginning to emerge. While the molecular constitution of cytoplasm is complex, it is clear that its contractile activity derives from the interaction between actin and myosin in a manner analogous to that in muscle fibers.

A critical difference between the contractile ability of cytoplasm and that of striated muscle is that muscle cells have their actomyosin fibrils oriented in parallel arrays, while the contractile machinery in cortical cytoplasm is more randomly oriented. This raises the question of how directed mechanical action can arise from a more or less isotropic medium. One suggestion for resolving this puzzle depends on coordinating a gel-sol transformation of the cytoplasm with the activation of myosin ATPase activity [see Taylor et al., 1979; Condeelis and Taylor, 1977].

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Address reprint requests to G.F. Oster, Department of Biophysics and Entomology, University of California, Berkeley, CA 94720.

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If, however, we are interested in the behavior of cytogels at the cellular level, then we do not need a detailed molecular description. Instead, we can take a macroscopic view and attempt to characterize cytogel behavior in terms of its viscous and elastic properties. This is the approach we shall take in the following sections; a detailed mathematical treatment of the theory is quite complex and will be presented elsewhere.

The paper is organized as follows. In section 2 we first state our rationale for approaching cytogel behavior from the viewpoint of continuum mechanics, and then we present a qualitative description of certain aspects of the physical chemistry of gels that are relevant to contractile actomyosin gels.

In section 3 we construct a mechanical model for contractile cytogel. This model is necessarily mathematical in form, and so on first reading the nonmathematical reader may skip directly to section 6 without loss of continuity. However, in order to make the discussion as accessible as possible, we shall present the description of the model in heuristic terms, relegating the quantitative discussion to the Appendices.

In section 4 we introduce a simplified model for the chemical regulation of cytogel contraction by free calcium. Here we specialize our discussion to the well-studied case of Physarum where there is a more general consensus about the nature of contraction regulation than in other systems.

Section 5 summarizes our discussion of the mechanochemical properties of cytogel by presenting a qualitative “response surface,” which shows how the stresses developed by a piece of cytogel are related to the strains it experiences and to the local free calcium concentrations.

Finally, in section 6, we apply our mechanochemical model to the puzzle of plasmodial oscillations in Physarum and show that many of the observed behaviors of that system can be understood on the basis of the physical chemistry of contractile gels.

2. THE MECHANOCHEMICAL PROPERTIES OF CYTOGELS

How Should the Mechanochemical Behavior of Cytogel be Described?

What constitutes an adequate description of cytogel depends on the questions one asks. Our purpose here is to characterize the “macroscopic” mechanical aspects of cytogel contractility. That is, we shall regard cytogel as a material whose properties are to be described by macroscopic quantities such as viscous and elastic parameters. Such a description need not deal explicitly with the molecular details of contraction, regulation, metabolism, and so forth.

The purpose of characterizing cytogel—or of any material—in terms of mechanical properties is that from such a description one may deduce its dynamic properties by applying the methods of continuum mechanics: writing a force balance equation and mass conservation equations allows one to compute all possible dynamical behaviors of a material.

What we require is a description of how the stresses developed by a piece of cytogel depend on its state of mechanical strain and its local “chemical state.” The stress-strain behavior can be measured, in principle, by conventional mechanical means. For example, one could hang a series of weights on a strip of cytogel and measure its elongations, and so obtain a stress-strain curve for the material. However,
such an experiment would have to be performed while holding the “chemical state” of the cytogel constant.

Unfortunately, the “chemical state” of a piece of cytogel is much more difficult to characterize than the mechanical state, since it comprises the concentrations of calcium, ATP, cAMP, light chain kinase, phosphatase, and a host of other substances. Imagine for a moment, however, that one could indeed control all of the concentrations somehow. Then one could perform a series of stress-strain experiments as above, while incrementally varying each of the chemicals. The resulting family of stress-strain curves would then constitute a complete mechanochemical description of the elastic behavior of the cytogel strip. In the situation where only one chemical was important, then the results of these experiments could be presented as a “surface,” giving the graph of stress as a function of strain and the concentration of the single chemical substance. With such a surface in hand, it is then possible to compute all of the possible dynamic behaviors the cytogel could perform. In this regard the situation is somewhat analogous to computing action potential conduction from the Hodgekin-Huxley equations using only measurements such as voltage-clamp data.

Possibly, this kind of experiment could be performed holding only one or two concentrations constant; for example, using EGTA to buffer calcium levels. However, it is doubtful that a complete set of buffers could be devised and implemented that could “clamp” all of the relevant chemical substances. Therefore, an alternative strategy is called for.

In the absence of an empirical “constitutive surface,” one can try to deduce what such a surface might look like using molecular models of the cytogel. It is not likely that one can obtain anything more than a qualitative picture using microscopic models, for experience with even simple gels has demonstrated the complexity of such calculations [cf Flory, 1953]. However, perhaps no more than a qualitative description is necessary for understanding many cytogel phenomena. Therefore, we shall proceed as follows.

First, we shall try and give a qualitative picture of the various forces which act within a piece of cytogel. The object of this will be to deduce some general features of the empirical—but as yet unmeasured—stress-strain relation. Next, we shall attempt to model certain aspects of the “chemical state” of cytogel, the object being to see how the stress-strain behavior varies with chemical composition.

It would be hopelessly complicated to try and model the kinetics of all of the substances which affect the behavior of cytogel, and the value of such a model would be dubious. If we are interested primarily in the mechanical dynamics of cytogel, the most sensible approach is to treat the chemistry phenomenologically, in a manner analogous to the way we treat the mechanics, i.e., just as the elastic and viscous parameters summarize all of the relevant molecular details, similarly, a phenomenological treatment of the chemistry of contraction must either focus on a single rate-controlling step, or endeavor to characterize the entire chemical chain by a small number of surrogate chemicals.

In many systems, the central chemical substance controlling mechanical activity appears to be calcium ions. Therefore, in our discussion, we will be aiming at deducing the shape of the stress-strain-calcium surface shown in Figure 6. This surface shows the way we believe total force generated by a piece of cytogel depends on strain and Ca\(^{++}\) concentration.
Of course, we must bear in mind that the symbol “c” we use for Ca\(^{++}\) in Figure 6 stands for the aggregate chemical state of the cytogel; Ca\(^{++}\) is but the surrogate, or rate-controlling substance.

The constitutive behavior of cytogel implicit in Figure 6—or in any qualitatively similar surface—endows cytogel with a rich dynamical behavior, capable of supporting a diverse spectrum of temporal and spatial patterns. Therefore, it is crucial to an understanding of cytogel mechanics that the response function be experimentally determined somehow. One of our principle objectives in this paper is to urge experimental cell biologists to attempt the required measurements, for once the correct version of Figure 6 is known, all of the dynamic behavior of cytogel can be calculated by standard techniques.

The Physical Chemistry of Cytogels

Before presenting the model for cytoplasmic viscoelasticity, it is helpful to have an intuitive picture of the possible underlying molecular events. We emphasize that a macroscopic theory such as we shall present is not tied to a particular molecular picture—that is, quite different microscopic phenomena can generate identical macroscopic models. However, our model must at least be consistent with the known physical chemistry of actin gels. The object of this discussion will be to provide a rationale for the constitutive surface shown in Figure 6.

A Gel is a Fibrous Network

Consider a small volume element of cytoplasm that is in mechanical equilibrium with its surroundings, as shown in Figure 1. This volume element can sustain a steady

![Fig. 1. A schematic microscopic view of a block of cytogel: actomyosin fibers are cross-linked by gelation factors, and severed (and capped) by solation factors. The activity of both actin binding proteins can be affected by Ca\(^{++}\), which also activates the actomyosin contractile machinery. The volume element is always in mechanical equilibrium with the surrounding cytogel; ie, viscous forces exactly balance elastic forces, since inertia is negligible.](image-url)
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The total osmotic pressure within a gel is the sum of these forces:

\[ P_{\text{OSM}} = P_{\text{MIX}} + P_{\text{ION}} + P_{\text{ATTR}} \]

Note that \( P_{\text{ATTR}} \) is a negative quantity: forces that tend to compress the gel are counted as negative, while expansive forces are positive.

If the polymers of the gel were not interconnected into a network structure, then the osmotic forces would disperse the fibers outward indefinitely. However, because of the cross-links between fibers, this inflationary pressure is limited by the elasticity of the network.

The elasticity of a gel arises from the thermal motions of the fibers: as the polymer strands writhe about they assume configurations that tend to bring the cross-links closer together. This results in an elastic stress, sometimes called an “elastic pressure,” \( P_{\text{ELAS}} \), which opposes the inflationary pressure of the osmotic forces (and so is a negative quantity). Thus the total force tending to swell a volume of gel is the difference between the osmotic and elastic pressures:

\[ P_{\text{SWELLING}} = P_{\text{OSM}} + P_{\text{ELAS}} \]

The gel will be in mechanical equilibrium when the swelling pressure is zero; that is, when the osmotic pressure is just equal to the elastic pressure:

\[ P_{\text{OSM}} + P_{\text{ELAS}} = 0 \text{ or } P_{\text{MIX}} + P_{\text{ION}} + P_{\text{ATTR}} + P_{\text{ELAS}} = 0 \]

In an actomyosin gel there is another source of compressive stress: the active contraction force generated by the myosin cross-bridges. We shall discuss this below. For now, note that if the actomyosin fibers in a volume element of cytogel commence to contract, the stresses they generate are transmitted to the neighboring volume elements. These, in turn, are forced to expand until the system reaches a new mechanical equilibrium. In this new strained state the actin network in the surrounding elements is stretched and polarized toward the contraction site. Mechanical equilibrium is reached when the active contraction of the original volume element is balanced by the osmotic and elastic forces of its strained neighbors and by the osmotic pressure within the volume element itself. This mechanical balance between active and passive elastic forces is a crucial feature of cytogels.

It is also worth pointing out that, since inertial effects are negligible, contractile forces equilibrate instantly over large distances. This means that a volume element far away from the contraction site senses the deformation “instantly” on the time scale of the gel contraction. Thus mechanical forces are global influences on the cellular scale, a fact that plays an important role in coordinating large-scale deformations of cytogel [cf Odell et al., 1981].

The molecular theory of gel osmotic forces is quite complex [cf Flory, 1953; Tager, 1978], and has only been worked out for relatively simple gel systems. A heuristic description of how the above forces conspire to create gel collapse and expansion can be found in Tanaka [1981]; we shall discuss the role of gel osmotic pressure more completely in a separate publication (see also Oster [1984] for a discussion of osmotic forces in cell motility).

**Cytogel Gets Stronger as it Contracts**

When a fibrous system is subjected to stresses, the fibers tend to align along the stress directions. When the fibers are more aligned, their elastic forces are more colinear, and so the effective modulus of elasticity in that direction increases. For example, if a drop of water is injected into the middle of an initially isotropic ball of wool, the wool will contract toward the droplet as the wool absorbs the water and shrinks. As the wool shrinks toward the point of injection, the fibers will align, and
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the strength of the fibers opposing the contraction center is reinforced. When the new elastic equilibrium is reached, the fibers will be radially oriented toward the focus of contraction.

The above effect will operate in any fibrous system; however, a second effect is unique to cytogels that contract by a sliding filament mechanism. As the fibers slide past one another more myosin cross-bridges come into play and the strength of contraction initially increases. In this regard, the cytogel fiber acts like muscle fiber. Of course, this reinforcing effect cannot continue indefinitely, and the force saturates at some level.

Thus a cytogel volume element that commences to contract can gain an initial mechanical advantage over its neighbors. Eventually, of course, a mechanical equilibrium is reached between the active and the passive fibers.

Cytogel is a Dynamic Structure

In contrast to muscle, contraction of cytogel is usually accompanied by dissociation of the fiber network [Condeelis, 1983]. Indeed, during the plasmodial oscillations in Physarum, nearly 30% of the cortical cytogel solates and regels in each cycle. Thus the assembly and disassembly of the actomyosin network is an important feature of cytogel mechanochemistry [cf. Oster, 1984]. Although the model equations given in Appendix C include the effects of gel-sol transitions, in our discussion here and in our Physarum simulation, we shall neglect this aspect of cytogel dynamics since it turns out that a number of interesting phenomena do not depend on it. We shall address the consequences of network lability in a separate publication.

The Mechanical State of Cytogel Is Regulated by Calcium

The above properties of cytogel are simple consequences of the physics of fibrous materials. The picture of how cytogel contraction is chemically controlled is much more unsettled. In most systems studied so far calcium ion concentrations play a central role in regulating both contractility and the sol-gel state of the actomyosin network. Ca\(^{2+}\), usually in concert with calmodulin, is instrumental in phosphorylating the myosin light chain kinase that commences the contraction cycle. Moreover, Ca\(^{2+}\) levels influence—and perhaps regulate—the activity of several actin binding proteins; some cross-link the actin fibers, causing the system to form a gel, while other enzymes sever the fibers and cap the polymer ends, thus solating the network (see, for example, Condeelis [1983] and Stossell [1983] for reviews and detailed references). The specifics of this regulatory machinery have not been completely worked out yet; consequently, we shall attempt to separate those aspects of our analysis that depend only on the mechanics, and those that depend on regulation of contractility. This separation is artificial in some respects: we shall discuss several mechanisms that contribute to the stress generated by an element of cytogel, and we shall see that the contribution of each mechanism changes as the cytogel element deforms and the internal chemical state varies. However, for clarity of exposition, we shall adopt the viewpoint that the chemical and mechanical events can be conceptually separated.

3. A QUANTITATIVE MODEL FOR CYTOGEL MECHANICS

For the benefit of those readers who digest mathematics with difficulty, we shall present the physics as “word equations.” In Appendix A we parallel our discussion here with the mathematical formulation; to facilitate comparison we will include some
equations along with our heuristic discussion—but these can be safely ignored by nonmathematical readers.

**The Stress-Strain Behavior of a Cytogel Cylinder**

Consider a one-dimensional cylinder of cytogel, which for intuitive purposes we can imagine as consisting of a collection of discrete viscous and elastic elements (“springs and dashpots”) as shown in Figure 2. The elastic behavior of the cytogel is represented by the “spring” and the viscous properties by the “dashpot.” We shall discuss each of these components separately.

**Cytoplasmic viscosity.** If the cylindrical volume element is originally \( L_0 \) cm long, and it is stretched to a length \( L \), then the strain is defined as the fractional elongation: \( \varepsilon = (L - L_0)/L_0 \). As the length of the cylinder shrinks to infinitesimal length, \( \varepsilon \to \partial u/\partial x \), where \( u = (L - L_0) \), the displacement from equilibrium.

For small strain rates the viscous stresses in the cytoplasm are proportional to the local velocity gradients. For the viscous element shown in Figure 2 the force resisting motion is given by

\[
\sigma_v = \mu \frac{\partial \varepsilon}{\partial t}
\]

![Figure 2](image_url)

Fig. 2. A strip of cytogel under tension \( F \): its viscoelastic properties can be represented schematically as spring and dashpot elements in parallel with an active contraction element. The strain is the fractional elongation, \( \varepsilon = (L - L_0)/L_0 \) of the strip, and the stress is the force per unit area transmitted through the strip, \( \sigma = F/\text{area} \). The strip resists the elongating stresses via elastic stresses, \( \sigma_E \), and viscous stresses, \( \sigma_v \). The viscous stress generated by the strip is proportional to the rate of strain, \( \partial \varepsilon / \partial t \), the proportionality constant being the viscosity, \( \mu \). The active stress, \( \tau \), is controlled by the local calcium concentration. At mechanical equilibrium, the internal forces generated by the cytogel equals the forces imposed by neighboring volume elements.
where the stress, $\sigma$ (dyne/cm$^2$), is the force per unit area applied to the cylinder and $\partial \epsilon/\partial t$ is the rate at which the element is being deformed.

Since cytogel is a polymer network, it cannot be modeled accurately by a simple viscoelastic substance as Equation 1 implies; that is, the viscosity, $\mu$, is not a constant, but itself depends on the strain rate [Stossell, 1983]. That aspect of cytogel mechanics need not concern us here; as we shall see later, however, the viscosity will depend on local chemical conditions.

**Passive cytoplasmic elasticity.** The elastic (“spring”) component of the cytoplasm element in Figure 2 derives from the entropic contraction of the gel, analogous to rubber elasticity. That is, the thermal motions of the gel fibers generate an “elastic pressure” that resists deformations.

An expression for the entropic contribution to the passive gel elasticity can be derived from the statistical mechanics of polymer chains [Flory, 1953; Acklonis and MacKnight, 1983]. It is a nonlinear (increasing) function of the strain; the formula, which is given in Appendix B, shows that the entropic elasticity is proportional to the number of fibers in the polymer network and their mean length. Moreover, the entropic term ensures that it is increasingly difficult to compress the cytogel toward zero volume. However, for our purposes, we commence with the simplest expression for the contribution of the passive elastic component of the fiber network given by Hooke’s law:

$$\sigma_p = E_p \epsilon$$  \hspace{1cm} (2)

The above expression only applies to one-dimensional stress situations; the complete expression for the elastic stress tensor in two and three dimensions is more complicated, requiring additional viscoelastic parameters, such as the Poisson ratio (cf. Appendix B).

Strain alignment of the gel fibers requires a minor modification of Equation 2. Because stretching the network aligns the fibers, the elastic modulus of the fiber network, $E_p$, is not a constant, but increases as the strain increases (“strain hardening”): $\sigma_p = E(\epsilon)\epsilon$ initially increases with strain. Technically, Equation 2 applies only to isotropic materials; however, for the situation we are discussing this need not trouble us.

Beyond a certain point the fibers eventually commence to yield, so that the elastic modulus first rises, then falls as the fiber network tears. This is typical behavior for fibrous materials; however, in our applications we rarely need consider behavior beyond the yield point.

Thus the passive stress-strain curve has the qualitative shape shown in Figure 3a. It is important to note that the precise form of this curve is not crucial to our conclusions—although that should be easily determined by conventional mechanical measurements.

**Active cytoplasmic elasticity.** In order to model accurately the active contribution to the cytogel elasticity, we would require a model for the sliding filament action of the actomyosin cross-bridges. Several models have been proposed for striated muscle systems where the contractile machinery is arrayed in parallel [cf. McMahon, 1983]; however, the calculation has not been carried out for a randomly oriented network of actin and myosin fibrils. For now we can model the contractile machinery phenomenologically as follows.
Fig. 3. a. The passive elastic stress, $\sigma_p$, as a function of strain, $\varepsilon$, for a fibrous material. In region 1 the material is in compression ($\varepsilon < 0$); the osmotic contribution ensures that the gel cannot be compressed to zero volume. As the material is stretched (region 2) it stiffens due to the alignment of the fibers. Eventually, the fibers commence to yield, and the curve peaks and then falls off (region 3). b. The active stress increases as an actomyosin fiber bundle shortens because the fibers align and additional myosin crossbridges come into play. The total elastic stress-strain curve for the cytogel strip is the sum of these two curves (see the constant c cross sections of the response surface in Fig. 6). This curve will not pass through the origin because of the active stress contribution; that is, at zero strain, actively contracting fibers can still generate a contractile force. c. The total elastic stress as a function of strain, holding all chemical concentrations constant. This curve is the sum of the elastic, osmotic, and active stress curves.
The essential features of the contractile force are the following: (i) the force increases with contraction owing to the sliding filament mechanism, as discussed above. (ii) The force increases with fiber density; that is, the more active fibers present the greater will be the contractile force. We can represent these two statements by requiring that the elastic stress tensor depend on fiber density. In symbols, we write

\[
\text{Active elastic stress } \equiv \sigma_a = \tau(\epsilon, n)
\]  

where \( n \) is the density of fibers (number of contractile fibers/unit volume), and \( \tau \) is the traction force generated per fiber. \( \tau \) should decrease as strain increases to account for the cross-bridge overlap effect, as shown in Figure 3b, and increase with \( n \). Since at least two actin fibers are required to form a “contractile unit,” \( \tau \) should increase nonlinearly with \( n \) (eg, as \( n^2 \)). We shall discuss later how \( \tau \) depends on the chemical state of the cytogel. The fiber density, \( n \), depends on the state of gelation of the actin network; however, in the present treatment we shall ignore sol-gel transitions.

**Osmotic pressure.** The final contribution to the stress developed in a volume element of cytogel is the osmotic pressure, \( P_{\text{osm}} \), which tends to inflate the gel. As discussed in the previous section, this has contributions from solvent mixing, polymer interactions, and ion pressures. The formula, given in Appendix B, shows that \( P_{\text{osm}} \) depends on the strain, the density of the gel, and the ionic constitution of the polymer and the solvent. We feel that osmotic effects are an important property of cytogels, and we will discuss their contribution more fully in a subsequent paper [cf Oster, 1984]. However, for our purposes here we shall neglect most of these complications and simply include osmotic pressure as an inflationary term of the form \( P_{\text{osm}} = \Pi(\epsilon, n) \) where \( \Pi(\epsilon, n) \) is a decreasing function of strain, and an increasing function of fiber density, \( n \).

**The Total Stress Developed by the Cytogel Fibers is the Sum of the Active and Passive Contributions**

The mechanical properties of contractile cytogel can now be summarized by adding together the active and passive contributions to the stress

\[
\sigma = E(\epsilon) \epsilon + \tau(\epsilon)n + \pi(\epsilon, n) + \mu \delta \epsilon / \delta t
\]

This, finally, is an algebraic description of how cytogel behaves mechanically. Figure 3c shows the total elastic stress, \( \sigma_p + \sigma_a \), as a function of strain, holding the chemical state of the gel constant.

**The Equation of Mechanical Equilibrium For a Volume of Cytogel**

Equation 4 contains all of the relevant properties of cytogel that are required to compute its dynamic behavior, providing that the various constitutive functions are known. The final step in constructing a model for the mechanical properties of cytogel

\[
\text{Total stress } = \text{passive stress} + \text{active stress} + \text{osmotic pressure} + \text{viscous stress}
\]
is to apply the equations of continuum mechanics to the model cytogel. This simply expresses the fact that each volume element of cytogel is always in mechanical equilibrium with its neighbors. That is, the force generated by our volume element is exactly balanced by the viscous and elastic forces exerted by the surrounding cytogel. We can write this as the equation:

\[ \text{div} \, (\sigma) = 0 \]  

If the volume element of cytogel is anchored to an external structure—say, a membrane or a microtubular structure—then we must add to the equation of mechanical equilibrium (5) the forces this structure exerts on the volume element.

Note that, since the fiber density appears in the expression (Eq. 5), we must include a separate equation to account for variations in \( n \). This equation, given in Appendix B, includes the processes of polymerization and depolymerization that accompany cytogel aggregation and disaggregation. However, in this study we shall ignore gel-sol transitions and consider only the simplest case wherein contractile fibers can only change their local density by moving along with the contracting gel.

The Qualitative Behavior of Cytogel Should Not be Sensitive to the Exact Values of the Parameters

All of the mechanical parameters introduced so far are, in principle, independently measurable by ordinary mechanical measurements, although designing the actual techniques may not be so straightforward. Most importantly, for many purposes we need not know the precise nonlinear dependence of the moduli on the strain and chemistry. Interesting calculations can be performed and predictions made that require only qualitative information, such as “the elastic modulus increases as the fibers contract”; the exact functional form of this increase may not be crucial.

4. THE CHEMISTRY OF CYTOGEL CONTRACTIONS IN PHYSARUM

In this section we discuss a few aspects of the mechanochemistry of cytogsels. Since there is presently no general consensus with regard to contraction regulation, we shall focus our attention on one particular system for which some agreement exists: Physarum polycephalum. Recent and comprehensive reviews by Tyson [1982], Kessler [1982], and Sauer [1982] document the evidence in support of the assumptions we shall make concerning contraction regulation.

ATP Fuels Cytogel Contraction and Calcium Can Solute the Gel and Initiate Contraction

If the actin network is too tightly gelled, the volume element may not be able to contract; it could remain in isometric tension even if it is not prevented from contraction by neighboring elements. Moreover, in a completely gelled state, there may be too few free ends to form actomyosin contractile units. Therefore, in order to perform mechanical work, the gel must be partially, but not wholly, solated; that is, some cross-links must be severed. \( \text{Ca}^{++} \) is known to activate certain solation proteins (eg, fragmin in Physarum and gelsolin in amoebae [Kessler, 1982; Taylor et al, 1979; Weeds, 1982; Stossel et al, 1980; Stossel, 1983; Condeelis, 1983] that sever the actin
Mechanics of Cytogels component of the gel network at points between the ABP junctions and/or cap the growing ends, and thus shorten the average fiber length. Therefore, exposed to a certain Ca\(^{++}\), the volume element partially solates and can contract against the tractions exerted by the surrounding volume elements.

In addition to solating the gel, Ca\(^{++}\) simultaneously activates myosin ATPase and initiates active contraction. Thus the net effect of the Ca\(^{++}\) signal is to cause the volume element to contract against the elastic resistance of its neighbors. Of course, a sufficient local supply of ATP is also necessary for active contraction, as well as other regulatory factors, including calmodulin and cAMP. Figure A1 (see Appendix A) shows a typical kinetic scheme for solation and contraction kinetics.

In this regard we note in passing that solation factors such as fragmin and gelsolin initially increase the traction force of a contracting network by generating new fiber ends that, via myosin bundles, can interact with other fibers to increase the net force of contraction. Too much fragmentation of the network fibers will, of course, finally reduce the effective contraction force as the fiber fragments become too short to support much tension.

**Some Possibilities For Ca\(^{++}\) Control of Cytogel Contraction**

Our phenomenological approach here begs the question of what the final arbiters of actomyosin contraction are, as well as the details of calcium control. However, various authors have suggested some possibilities, and since it is always worthwhile to have a molecular picture in mind, we record those suggestions here.

**Calcium-stimulated calcium cascades.** In a number of cytogel systems calcium is autocatalytically released from membrane-bound sequestering sites. This is familiar in smooth muscle [Bittar, 1981; Jaffe, 1980] and is implicated in neural synaptic transmission [Kuba and Takeshita, 1981]. Jaffe [1980] has documented a number of other systems in which calcium cascades appear to play an important role. In such systems a small amount of Ca\(^{++}\) introduced exogenously, or released endogenously, triggers a much larger release Ca\(^{++}\) from the sequestering vesicles. It is not clear whether this release is associated with voltage or ligand gated channels.

**Calcium Oscillations.** Rapp and Berridge [1977] proposed a scheme whereby the control loop involving Ca\(^{++}\) and cAMP would exhibit self-excited oscillations. Kuba and Takeshita [1981] modeled Ca\(^{++}\) oscillations via a membrane depolarization loop. These mechanisms, while plausible and having some experimental support [see Kessler, 1982], are still speculative. What is certain is that, in Physarum, the rhythmic contractions that drive shuttle streaming are accompanied by Ca\(^{++}\) oscillations that are in a fixed phase with the mechanical oscillation [Kamiya et al., 1982].

**Stretch activation.** Smooth muscle and certain insect flight muscles respond to mechanical stretching by Ca\(^{++}\) release, which then initiates contraction. In Physarum stretch activation of contraction is well documented [Kamiya, 1979, 1981; Wohlfarth-Bottermann, 1977], and Kessler [1982] presents evidence that plasmodial oscillations are synchronized by local stretch-stimulated Ca\(^{++}\) release.

**Assumptions About Chemical Regulation of Contraction**

The evidence is strong that, at least in Physarum, Ca\(^{++}\) is stored in a membrane vesicular system within the cytogel itself and that it is released and resequestered in phase with the mechanical oscillations. Therefore, we feel justified in assuming the following minimal functions concerning the chemical regulation of cytogel contraction:
Ca++ is an essential link in the control loop that initiates contraction of actomyosin gels. The stimulation for Ca++ release is provided by a “trigger chemical,” which we denote by c. This trigger chemical can be Ca++ itself (calcium-stimulated calcium release), or another chemical in the control loop (eg, the Ca++/cAMP loop model of Rapp and Berridge [1977]).

ii) Whatever the details of the control of Ca++ release, the result is a cascade of Ca++ release when the cytogel is “stimulated.”

iii) Ca++ release can also be stimulated by mechanical deformations of the cytogel. This “stretch-activation” can result, for example, from membrane depolarization accompanying deformation, which allows enough Ca++ to leak from its vesicular reservoirs to initiate the autocatalytic cascade.

iv) Ca++ levels help regulate the sol-gel equilibrium of the cytoplasm.

In what follows we shall represent the complicated sequence of chemical steps that regulates contraction by the dynamics of a single “trigger chemical,” c. As we have repeatedly emphasized, although we are thinking of Ca++ itself as the trigger chemical, c should be regarded as a surrogate variable that represents the rate-limiting step in the entire chemical chain—for example, the one depicted in Figure A1—that regulates contraction. While more complicated models can easily be constructed [see Kuba and Takeshita, 1981; Rapp and Berridge 1977, for example] these require additional assumptions concerning the chemistry which are unnecessary for modeling the mechanical aspects of the plasmodial oscillations.

A Model For Chemical Control of Cytogel Contraction

Consider a control volume of cytogel; we can write a mass balance for the concentration of the trigger chemical, c, as follows:

\[
\frac{dc}{dt} = \frac{\text{release of } c}{\text{volume of gel}} - \frac{\text{loss of } c}{\text{volume elements}} - \frac{\text{resequestering}}{\text{to neighboring volume of gel}} - \frac{\text{efflux of } c}{V \cdot J}
\]

The balance equation must be obeyed within each volume element regardless of details of the chemical scheme. We incorporate the above assumptions into the model by selecting particular forms for the terms in Equation 6.

a) According to assumption ii above, the release of c from its sequestering sites has the sigmoid shape characteristic of autocatalysis, as shown in Figure 4a. The curve in Figure 4a summarizes a complicated and as yet poorly understood kinetic scheme [Kato, 1979]; the only aspect of this control loop that concerns us here is the rapid release of Ca++ from its vesicular stores under the appropriate stimulus. This stimulus for release can be Ca++ itself (calcium-stimulated calcium release), another trigger chemical, or mechanical stretch, as we discuss below.

One simple way of arriving at the release curve in Figure 4a is to assume that the efflux from the vesicle compartment takes place by simple diffusion down a concentration gradient: efflux rate of c from vesicle compartment = P(c) (C0 - c), where C0 is the concentration of c sequestered inside the vesicle compartment and P(c) is the membrane permeability. P(c) is an initially increasing function of c that must eventually saturate, ie, a sigmoidal release curve typical of a “gated” channel.


b) Resequestering of $c$ takes place by a first-order process:

$$\text{Loss of } c \text{ per unit volume} = -rc$$

In a number of contractile systems the activity of the calcium transport system is controlled by phosphorylation catalyzed by a kinase that requires cAMP [Dedman et al, 1979]. Therefore, the rate constant, $r$, may depend on the local concentration of cAMP, which regulates the $Ca^{++}$ pump [Rapp and Berridge, 1977]. We will discuss the possible role cAMP plays in plasmodial oscillations below. However, to keep things as simple as possible, we shall assume for now that $r$ is a constant.
c) Stretch activation of c-release can be modeled most simply by including a term proportional to the strain in the vesicle permeability: \( P = P(c) + \gamma \varepsilon \); i.e., the permeability increases with strain. Again, we need not specify the molecular mechanism underlying stretch stimulated release of c—although it probably involves local membrane depolarization brought about by mechanical deformations.

d) The flux of c to and from neighboring volume elements has at least two components: (a) c is carried along convectively with solated cytoplasm, (b) c can diffuse away from regions of high concentration. These terms are modeled in Equation 6 by the usual expressions (see Appendix B).

In Figure 4b we have plotted the total rate of change of c by summing the effects of production, resequestration, stretch activation, and flux (the mathematical expression is given in Appendix A). The composite curve governing the endogenous kinetics of c is S-shaped; it turns out that this type of curve is generic: any single chemical autocatalytic model with two stable states will look like Figure 4b.

The above assumptions, cast in mathematical form, constitute our model for the regulation of free calcium (or “trigger chemical”) in Physarum. The chemical kinetics of c controls the mechanical contraction of the cytogel fibers via the parameter \( \tau \) in Equation 3. That is, in addition to its dependence on strain and fiber density, the traction is an increasing function of the concentration of trigger chemical, c:

\[
\tau = \tau(c).
\]

The only important feature of this curve is that increasing c increases the traction force. The curve must saturate at some point, and probably is sigmoidal, but this is not important for our conclusions. As before, the function \( \tau(c) \) summarizes the relevant features of a complicated kinetic scheme whereby \( \text{Ca}^{++} / \text{calmodulin} \) interacts with myosin ATPase to regulate contraction.

Referring to Figure 4c, we can see how the \( \text{Ca}^{++} \) trigger for contraction operates. The system has two stable equilibria—concentrations where \( \frac{dc}{dt} = 0 \). As the cytogel is strained, or a local influx of c occurs, the curve is lifted as shown. Eventually a critical value of \( \varepsilon = \varepsilon_c \) is reached whereupon the lower equilibrium disappears and the chemical concentration moves to the upper equilibrium value. This causes the tension, \( \tau(c) \) to increase as shown on the lower curve. This is the simplest kind of chemical “switch” for turning on contraction of the cytogel; we will see later that this simple mechanism needs to be elaborated to account for certain phenomena.

**Calcium Regulates the Cytoplasmic Viscosity**

The same micromolar \( \text{Ca}^{++} \) levels that stimulate gel contraction also initiates network disassembly by activating solating factors that regulate the degree of polymerization and cross-linking of the cytogel—and thereby its viscosity. We can model the solation effect by making the viscosity parameter \( \mu \) a function of \( \text{Ca}^{++} \), as shown in Figure 5a [Stossel, 1983].

The solation of the actin fibers ultimately attenuates the ability of the network to transmit stress. Therefore, the active component of the stress varies with calcium as shown in Figure 5b: first rising as the contraction machinery is turned on, then falling as the network liquefies.

**The Parameters Involved in the Chemical Trigger**

We require several parameters in order to write the balance equation for c as a mathematical equation (cf Appendix B). They include the diffusion constant for c, the
rate $c$ is resequestered, and parameters controlling the release of $c$. In principle, each is independently measurable.

In addition to the above quantities, we also must specify the curve $\tau(c)$, which models the activation of the myosin ATPase and the convective velocity, $\nu$, of the cytosol. Strictly, $\nu$ is not the same as the velocity of contraction of the gel, which we have denoted by $\partial \epsilon / \partial t$; $\nu$ must be calculated from a more complete model for cytogel, which accounts for the phenomenon of "syneresis," in which the contracting solid phase of the cytoplasm drives the flow of the liquid phase—much like squeezing water from a sponge. We address this issue in Oster and Odell [1983b].

$\tau(c)$ can be modeled using a two-parameter curve, as discussed in appendix B. As we stated above, we only require that $\tau(c)$ be increasing. In smooth muscle systems, cAMP appears to decrease the activity of the kinase that catalyzes the phosphorylation of the myosin light chains [Adelstein, 1980; Adelstein et al, 1978]. In order to account for this the contraction parameter, $\tau$ should be a decreasing function of cAMP; we shall ignore this effect in our simplified model.

5. SUMMARY: THE MECHANOCHEMICAL RESPONSE SURFACE OF CONTRACTILE CYTOGEL

We have endeavored to dissect the various mechanical and chemical contributions to the stress tensor of cytogel, and so the foregoing discussion may seem fragmented; however, we are now in a position to summarize the essential mechanochemical behavior of cytogel in an easily visualized way.

Our model for contractile cytogel consists of two parts. The purely mechanical aspects discussed in section 3 depended on the physics of fibrous gels, and the chemical regulation of contraction discussed in section 5 depended on some general assumptions about $Ca^{++}$ regulation of contraction.

The net outcome of the various contributions to the force cytogel can generate is contained in the dependence of the stress tensor, $\sigma$, on the strain, $\epsilon$, and the concentration of trigger chemical, $c$. If we consider a one-dimensional strip of cytogel, this is simply the force/unit area, and we can plot this force as a function of $c$ and the elongation of the strip as shown in Figure 6.
The essential feature of the $\sigma(\varepsilon,c)$ surface is its "hills," which means that there are chemical and mechanical "windows" for optimum force generation. The mechanical optimum—in a slice through the surface holding $c$ constant—comes about because the strip gets stronger as it contracts, up to the point where the cytogel is put into compression. Similarly, there is a chemical "window" for optimum force generation: at low $c$ the gel is rigid and the active contractile machinery is not excited; at high $c$ the gel becomes so solated that no effective tension can be produced.

Thus there is an "operating region" that functions, as we shall see, to regulate the contractile activity of the cytoplasm much as the optimum temperature-pH operating region does in enzymatic control systems. Due to mechanochemical coupling between volume elements, each region can push or pull its neighbors into (or out of) this window; this is the key to understanding how cytogel contractions can be coordinated. We note that Ueda and Kobatake [1977] have found an ATP optimum
Figure 6 suggests the kind of experiments that must be done to quantify the phenomenological constitution of cytogel. For example, a series of isometric experiments on a plasmodal strand of Physarum could be run at various calcium concentrations. Chelating agents (EGTA) could “clamp” the local calcium concentrations and isometric force measurements taken for various imposed extensions. Alternatively, series of isotonic or isometric experiments with calcium clamps could be used to map out the force surface. Such experiments would, in principle, replace the sorts of dynamic experiments performed heretofore, wherein tension, length and/or calcium concentrations are measured as a function of time. Given the static response surface $\sigma(\epsilon,c)$, these dynamic responses can be predicted from the mechanochemical equations of motion.

One important caveat concerning Figure 6 should be emphasized at this point: The graph of $\sigma(\epsilon,c)$ shown in the figure must be interpreted as a static relationship. That is, the surface is determined by a “calcium clamp” experiment as described above, so that the stress is measured after all transients have died out. In fact, as we shall discuss below, there may be a considerable delay between the rise in free calcium and the onset of active contraction. In this study we shall ignore the delay between the chemical trigger and the mechanical response of cytogel; however, we consider it an important feature of cytogel dynamics, and we shall study its effects in a subsequent paper.

In order to investigate the properties of the model we must resort to mathematical analysis and computer simulation. We relegate the description of these investigations to the appendices; in the next section we shall show how the cytogel model captures certain aspects of the phenomenon of plasmodal oscillations in Physarum.

**6. PLASMODIAL OSCILLATIONS IN PHYSARUM**

One of the most thoroughly studied of cell motility phenomena are the periodic plasmodial contractions that drive shuttle streaming in Physarum. Reviews by Kessler [1982], Tyson [1982], Kamiya [1981], and Sauer [1982] document the many experimental investigations on this system. Here we shall demonstrate that the simple cytogel model we have constructed can provide an explanation for certain aspects of this phenomenon, and correlate a number of puzzling observations.

**Simplified Model for a Plasmodial Strand**

We have constructed a model of a plasmodal strand using the model cytogel developed above. A complete numerical study of its dynamical behavior will be reported elsewhere. Here we shall present simplified version of that model that will serve to illustrate how one needs no more than the assumptions we have made concerning cytogel mechanochemistry to capture most of the qualitative features of plasmodal dynamics.

We model a section of the plasmodal strand as two hollow shells of cytogel connected by a flow resistance, as shown in Figure 7a. Between the volume elements flows solated cytoplasm, whose chemical properties are identical with the cytogel ectoplasm, save that its viscosity is much lower. In this simplified model we shall make two assumptions: (i) the plasmodium is composed of but two elements, rather
Fig. 7. a. A simplified model for a plasmodial strand of Physarum. Two cytogel shells are mechanically joined, and cytosol is allowed to flow between them. b. With stretch activation and cytosol the model exhibits self-sustained mechanical oscillations that pump cytosol back and forth (shuttle-streaming). The period of the mechanical and chemical oscillations are equal, but they will generally not be in phase. Note that the maximum stress occurs at the minimum radius, which is out of phase with the calcium oscillations.

than a continuously distributed cylinder of cytoplasm; (ii) we ignore the sol-gel transformation, which interconverts ectoplasm and endoplasm during the contraction cycle. These approximations affect the periods and phasing of the chemical and mechanical oscillations, but not their qualitative aspects. Mathematical analysis and computer simulation demonstrate the following properties of the model, which correlate with experimental observations. The mathematical equations used in the numerical simulations are derived in Appendix D, and a complete discussion of this simplified shuttle streaming model is given in Odell [1984].
**Stretch activation.** A strand of model plasmodium can be stimulated to contract rhythmically by mechanically stretching it [Kamiya, 1979, 1981; Yoshimoto and Kamiya, 1978]. Moreover, because $\tau$ is an increasing function of $c$, larger amplitude stretches will induce larger amplitude oscillations, in accordance with observations [Kamiya, 1981].

**Synchronization.** In order for the model plasmodium to sustain oscillations, endoplasm must be present; that is, the central core of the model must be filled with solated (low viscosity) cytoplasm. If the kinetics of the trigger chemical in the fluid core are inactivated the oscillations damp out: the calcium leaks from the ectoplasm and is diluted out in the endoplasm until there is not enough to trigger further contractions. This is in accord with the observations of Ueda et al [1978]; Ueda and Kobatake [1977], who employed calcium-EGTA buffers to control the level of free endoplasmic calcium in cycling plasmodia.

In addition to the required endoplasm, synchronization of plasmodial elements depends on stretch activation via mechanical coupling. By setting the stretch activation too low, the oscillations never synchronize and eventually damp out. The fact that that oscillations are not sustained without stretch activation is a result of our neglect of anything other than $\text{Ca}^{++}$ kinetics. If equations for cAMP and/or ATP are included as we discussed above, then it is easy to tune the parameters so as to achieve sustained chemical oscillations that will drive local mechanical contractions. Stretch activation, however, aids in synchronizing these local oscillations between neighboring sections of the plasmodium.

**Tension oscillations.** Real plasmodia have radial and longitudinally oriented actin fibers, which produce radial and longitudinal oscillations [Kamiya, 1979]. While our model has assumed an initially isotropic fiber orientation, when stretched, the fibers align. Thus both radial and longitudinal tension oscillations will occur, with concomitant fiber alignments in accord with those observed in vivo. Figure 7b shows a sample of the sorts of oscillations produced by the model. Because we have included but two volume elements, the waveform does not exactly match that observed in actual plasmodia; however, when more volume elements are simulated we hope that more realistic waveforms can be achieved.

**Calcium oscillations.** Numerous observations on plasmodia have shown that free cytoplasmic calcium levels oscillate with the same period as the tension oscillations. Given the model assumptions, it is no surprise that it too shows calcium oscillations whose period matches the contractions. What is more surprising, however, is the observation that the calcium cycles are almost 180° out of phase with the mechanical cycles [Yoshimoto et al, 1981; Yoshimoto and Kamiya 1982; Kamiya et al, 1982; Kamiya, 1981]. This is unexpected in light of the consensus that calcium stimulates contraction, and the absence of calcium quenches the rhythms. Examination of the model dynamics shows that it can reproduce this odd phase relationship between tension and calcium, as shown in Figure 7b. The reason is that the oscillations can take place on a "downhill" side of the force response surface in Fig. 6; so that the phase of maximum tension can be quite distinct from the chemical phase.

**Shuttle streaming.** As the ectoplasmic tension oscillates, it generates pressure rhythms that pump the fluid back and forth between the cytogel cylinders, simulating the passively driven shuttle streaming observed in real plasmodia. In the model we have assumed that the endoplasm has the same composition as the ectoplasm, except that its viscosity is much lower, ie, it is more fluid than solid (recall we have neglected sol-gel transitions in the simplified model). Moreover, we have assumed that the
cytosol flows as a Newtonian fluid—which cannot be strictly true given its polymer composition [cf Bird et al., 1977]. Notwithstanding these simplifications, the model can reproduce the phase relationships observed between tension oscillations and shuttle streaming.

**Limitations of the Model**

There are, of course, many aspects of plasmodial oscillations not addressed by the above model. However, it is gratifying that a few simple assumptions about the physicochemical constitution of cytogel can synthesize such a variety of observations. In a subsequent paper we shall present a far more realistic simulation; there we shall investigate the role of a number of phenomena we have omitted here, including syneresis, osmotic forces, actin depolymerization, and the role of cAMP and ATP kinetics. Here we briefly discuss the qualitative effect of some of our approximations.

In the kinetic scheme for calcium-stimulated contraction, we assumed that contractile forces were generated immediately by calcium release. However, it is clear from a number of systems that while solation (and osmotic swelling) occurs within seconds of free calcium increase, the contractile forces take much longer to develop [cf Oster, 1984]. This delay between calcium release and the onset of contraction has an important effect on the oscillation dynamics. Most of the observed phase difference between calcium and tension maxima may be attributable to this lag, although in the present simulations the phase difference was simply as a consequence of the nonlinear dynamics. A more important effect of the contraction delay has to do with the autonomy of the calcium oscillations.

Under certain conditions, calcium oscillations can be observed in ectoplasm homogenates, indicating that the chemistry may not be as tightly coupled to the mechanics as we have assumed [Yashimoto and Kamiya, 1982]. Indeed, when the contraction delay is incorporated into the kinetic equations, it is possible to realize mechanochemical oscillations for certain parameter values that have a different character from those simulated here. In particular, isolated plasmodial strands will oscillate for quite a while under isometric and isotonic conditions, even in the absence of endoplasm. The simplified model described herein cannot duplicate these observations. Furthermore, the oscillatory dynamics of the two-cortex model depends on (i) the property of the model cytogel that the active traction of a strand increases as the strand shortens, and (ii) the geometrical fact that a cortex with a smaller curvature produces a larger contractile stress [cf. Odell, 1984, for a more complete discussion of these aspects of the model’s dynamics]. Inclusion of the contraction delay allows the model to produce mechanochemical oscillations in the absence of these two effects.

By neglecting the osmotic pressure within the gel, we have seriously affected the mechanical response of the model. This force is rendered even more important by the large fraction of the ectoplasm that is solated during each contraction cycle.

**Another Cellular Oscillator**

A. Harris [1971; p. 120, Figs. B, C] has reported the following phenomenon, which appears to be a system to which the above model applies. After treating 3T3 fibroblasts with $10^{-5}$ molar vinblastine, he observed that cells would round up into a dumbbell-shaped configuration. Thereupon the cell commenced to oscillate: the lobes alternated in contraction and expansion cycles with a period of about 16 sec. These
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oscillations persisted for many cycles. It seems to us that this phenomenon is captured very well by the simplified two-lobe model described above and suggests that the same mechanochemical processes are at work as in the plasmodial strand. That is, the capacity to form a mechanochemical oscillator is an inherent property of cytogel, and not a phenomenon peculiar to Physarum.

DISCUSSION

The cellular cytoplasm is an active force-generating substance that confers upon the cell the ability to perform mechanical work. If one could understand how cytoplasmic contractility works, this would shed much light on the morphogenesis of cells and tissues. Here we have taken a small step in that direction by constructing a model for the viscoelastic properties of actomyosin gels. The model is “macroscopic” (or phenomenological) in that it does not deal directly with the molecular details of the active contraction process; nevertheless, it captures the macroscopic, observable aspects of the mechanical behavior of cytogel. The key features of the model are the following: (i) Cytogel is a fibrous material that can actively contract when stimulated. (ii) While contracting, the gel exhibits nonlinear elastic behavior. (iii) The contraction is stimulated chemically via a control loop in which calcium plays an essential role by activating the myosin ATPase and partially solating the gel. (iv) Contraction is triggered by a chemical cascade involving calcium release from sequestering vesicles. Together, these properties confer on the model cytoplasm the capability of reproducing a number of interesting phenomena; here we have focussed particularly on the plasmodial oscillations in Physarum, and we find that the model can provide a partial explanation for a variety of experimental observations: mechanical contraction rhythms, shuttle streaming, and the phase relationships between contraction and calcium cycles. In a subsequent paper we shall present a more elaborate version of the Physarum model, which incorporates more realistic assumptions than we have included in the simplified analysis presented here.

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APPENDIX A: THE CHEMICAL REGULATION OF CYTOGEL CONTRACTION—THE ROLE OF OTHER SUBSTANCES

In the above discussion we have focused our attention exclusively on the dynamics of the free calcium concentrations, considering this to be the proximal event regulating contraction. As we have stressed repeatedly, however, although we are regarding calcium as the major control substance in a complex chemical network, other substances play an important role. For example, a number of authors [e.g., Rapp and Berridge, 1977; Kessler, 1982] have placed great weight on the feedback loop that links calcium with cAMP. Kessler's model depends on an endogenous Ca\(^{++}\)/cAMP oscillator to drive the plasmodial contractions, and Yoshimoto and Kamiya [1982] have recently detected periodic calcium fluctuations in homogenates of Physarum plasmodia suggesting that there is indeed an endogenous oscillator. In our model, however, it is not necessary to invoke such a chemical oscillator: the mechanochemical system will generate the oscillations without recourse to an independent chemical oscillator. Nevertheless, since there is some evidence for cAMP/Ca\(^{++}\) oscillations that proceed independently of the mechanical oscillations, we show here how the model can be enlarged to include more elaborate chemical dynamics. Simulations based on these more realistic kinetics will be presented elsewhere.

Figure A1 summarizes one current scheme of how cAMP enters into the calcium control loop [cf Kessler, 1982; Sauer, 1982; Tyson, 1982]. Via calmodulin, calcium regulates (i) the activity of the kinase, which phosphorylates a cAMP-dependent calcium transport system; (ii) the enzymes that catalyze the production and degradation of cAMP (adenylate cyclase and phosphodiesterase, respectively); (iii) the activation of the myosin light chain kinase.; Calcium also binds directly to and activates the solvation factors which sever the actin strands.

The simplest kinetic scheme for regulating the levels of free calcium that captures these interactions goes something like this. The equation governing the release of Ca\(^{++}\) from the membrane compartment is

\[
dc/dt = P(c,e) (C_0 - c) - rc
\]  

(A1)

If we now allow the resequestering rate to be dependent on the cAMP concentration—\(r = r(a)\)—we can couple this to an equation for cAMP

\[
da/dt = k_1c - k_2a (1 + c)
\]  

(A2)
Sequestered calcium (CS)—usually complexed with calsequestrin—is released autocatalytically from membranous sites. Free calcium (c) follows three pathways: (I) Resequestration. The free calcium is resequestered by membranous pumps, which require cAMP (a) and ATP. cAMP, in turn, is produced by adenylate cyclase and degraded by phosphodiesterase, each of which requires calmodulin. cAMP is utilized in other pathways as well. (II) Solation. Free calcium binds to solating factors (SF) rendering them active (SF*), whereupon they bind to actin filaments and sever them (SOLUTION). The solation proteins may remain capped to the “barbed” end until the calcium level falls again below micromolar concentration. (III) Contraction. Free calcium binds to calmodulin (CM) inducing a conformational change to its active form (CM*); calmodulin binds up to four calcium ions, each inducing a conformation change. The activated calmodulin binds to the myosin light chain kinase (MLCK) inducing it to its active form (MLCK*). Meanwhile, myosin subunits assemble to thick filament-like structures, (M) and are then phosphorylated by the kinase. Also, actin subunits (A) (fragments and monomers, perhaps complexed with profilin) assemble to filamentous actin strands (AF) and bind to the activated myosin to form “contractile units”: segments of counter-oriented actin fibers crosslinked by activated myosin chains. These contractile units shorten to produce the traction stresses in the gel (CONTRACTION).

where a denotes the concentration of cAMP. Here synthesis and degradation of cAMP proceed at a calcium-dependent rate, and we allow for additional degradation pathways as well. (Kato et al. [1984] have investigated a somewhat different scheme for the calcium regulation of actomyosin contraction).

One can show that these two kinetic equations can, indeed, with the appropriate choice of parameters, sustain periodic solutions and so drive the mechanical oscillations.

To further complicate matters, Kamiya et al. [1982] also report periodic variations in ATP concentration that match the mechanical oscillations. Since ATP participates in the calcium pump, and ATP pyrophosphohydrolase (APPH), which depletes ATP and is calcium activated, is also found in plasmodia, it is possible that ATP dynamics can affect the calcium control loop. If we add a third equation for ATP, it is even easier to tune parameters so as to obtain oscillatory kinetics.

Adding complications to the chemical control of contraction tends to reduce the usefulness of kinetic models, since the proliferation of parameters almost ensures that one can produce whatever behavior is desired—even in light of the experimental difficulty in determining precise values for the kinetic constants. However, we can
obtain mechanical oscillations even if we ignore the details of the mechanism for Ca\(^{++}\) release. Therefore, it is not strictly necessary to include more chemical equations, and so we have eschewed the unnecessary complication. This is not to say purely chemical oscillations do not occur; indeed, Kuba and Takeshita [1981] have shown that Ca\(^{++}\) oscillations can be produced by the interaction between transport and sequestration kinetics. In a subsequent paper we shall investigate more fully how including additional chemical steps in the mechanochemical chain of events can affect the quantitative fit of the model to the data.

**APPENDIX B: MATHEMATICAL FORMULATION OF THE MODEL**

We model a small linear element of cytogel as a Kelvin solid as shown in Figure 2. The total stress tensor can be written as the sum of viscous, elastic, osmotic, and active contributions:

\[
\sigma = \sigma_V + \sigma_E + \sigma_A + P_{OSM}
\]  

where \(\sigma\) has the units of force per unit area per unit mass of gel.

The viscous stress is given by

\[
\sigma_V = \mu \frac{\partial \epsilon}{\partial t}
\]

where the infinitesimal strain, \(\epsilon\), is the spatial derivative of the displacement: \(\epsilon = \frac{\partial u}{\partial t}\).

For an ideal polymer network, the gel elastic stress is given by the expression for rubber elasticity [Flory, 1953; Aklonis and MacKnight, 1983].

\[
\sigma_E = G(\lambda - 1/\lambda^2) = G[(\epsilon^2 + 3\epsilon + 3) / (\epsilon + 1)^2] \epsilon \equiv E_E(\epsilon)\epsilon
\]

where \(G\) is the shear modulus, which is proportional to the concentration; \(\epsilon\) is the concentration of network chains in the gel; and \(\lambda\) is the stretch ratio: \(\lambda = \frac{\text{stressed length}}{\text{unstressed length}} = \frac{L}{L_0} = \epsilon + 1\).

In two and three dimensions Hooke's law has the form [Lin and Segel, 1974]

\[
\sigma_p = \frac{E(\epsilon)}{2(1 + \nu)} [\epsilon + \frac{\nu}{(1 - 2\nu)} \theta I]
\]
where $\nu$ is Poisson's ratio, $\epsilon$ is the (small displacement) strain tensor, $\epsilon \equiv [\nabla u + \nabla u^T]$, and $u$ is the displacement vector of the material from its initial position. $\theta = \text{Tr}(\epsilon) = \nabla \cdot u$ is the dilatation, and $I$ is the identity tensor. For fibrous materials, Poisson's ratio, $\nu$, can be considerably greater than 1.

The property of fiber alignment causes the effective elastic modulus to increase with dilation, as discussed in the text. Strictly speaking, when stressed a fibrous material can no longer be modeled by the isotropic constitutive relation (Eq. B4); however, we can obtain an approximate accounting for this phenomenon by making the elastic modulus, $E$, a nonlinear function of strain, as shown in Figure 3b. In the higher dimensional case, however, we must model the continuum as an orthotropic material, with separate elastic moduli in the directions of principle strains.

Note that

$$\lim_{\epsilon \to -1} \sigma = -\infty$$

That is, it is impossible to squeeze the cylinder to zero length, since the gel contains vesicles and other virtually incompressible components. However, at high strains, the gel eventually yields, so that

$$\lim_{\epsilon \to \infty} \sigma = 0$$

The gel osmotic pressure has contributions from (i) the entropy of mixing of the polymer with the solvent, (ii) the ion pressure of the counterions if the polymer is charged, (iii) the short range interactions between the polymer strands. An approximate formula for the osmotic pressure is given by [Hill, 1960; Flory, 1953]

$$P_{\text{OSM}} = \ln (1 - \phi) + \phi \text{ entropy of mixing}$$

$$+ \frac{z^2 \phi^2}{2x^2} \text{ ion pressure}$$

$$+ \chi \phi^2 \text{ polymer interaction}$$

where $\phi = \text{polymer volume fraction} (= 1/\lambda)$; $z = \text{ionic charge of the xth subunit of the polymer}$; $x = \text{number of subunits}$; $\tilde{\psi} = \text{molar volume of the solvent}$; $\Sigma = \Sigma_k \rho_k z_k^2$ = ionic strength of the solvent ($\rho = \text{density of solute molecules}$).

While osmotic swelling is an important feature of actomyosin gels [cf Oster, 1984], we shall not include the osmotic contribution to the stress tensor in this paper. We incorporate the active (sliding filament) contribution to the elastic stress tensor by including a functional dependence on the fiber density, $n(x,t)$ (Eq. 3):

$$\text{Active stress } = \tau(\epsilon, c, n)$$

where $\tau(n)$ is a function of $n$ that increases faster than linear, since it requires at least two actin strands to create a contractile actomyosin unit. $\tau(c)$ will be a sigmoidally
increasing function of the local calcium concentration; for simulation purposes we have employed a simple rational function:

$$\tau(c) = \eta + \text{constant}/[1 + (c/\xi)^2]$$  \hspace{1cm} (B8)

where \(\eta\) is the least value \(\tau\) can attain as \(c\) becomes large, and the parameter \(\xi\) controls the “threshold” value of \(c\) (ie, \(\tau\) drops to about \(1/2\) when \(c = \xi\).

Using the above expressions, we can write the total stress, \(\sigma = \sigma(\epsilon)\) as

$$\sigma = E(\epsilon) \cdot \epsilon + \mu \partial \epsilon/\partial t + n \tau$$  \hspace{1cm} (B9)

The elastic modulus, \(E(\cdot)\), also depends on the calcium concentration, \(c\). As a function of \(c\), \(E(c)\) must first increase as contraction is stimulated, then decrease as the gel becomes too solated to sustain tension. In the finite element version we take \(E(L,c)\) to be the following rational function

$$E(L,c) = \frac{1}{(1/K_{\text{max}}) + a_2 L_0 (c)^2 + a_3 L^2 + a_4 c^2}$$

where \(K_{\text{max}}\) is the largest value the modulus can attain.

In higher dimensions, the viscous part of the stress tensor is given by a form analogous to the Navier Stokes stress:

$$\sigma_v = \mu \partial \epsilon/\partial t + \lambda \partial \Theta/\partial t \mathbf{I}$$  \hspace{1cm} (B10)

where \(\mu\) and \(\lambda\) are the shear and bulk viscosities, respectively, and \(\tau\) is the dilatation, \(\nabla \cdot \mathbf{u}\). The finite element version is a simple linear dashpot: \(\mu \partial \epsilon/\partial t\). The calcium stimulated solation is modeled as a sigmoidal phase change curve (Fig. 5); in the simulations we have employed the equation

$$\mu(c) = \frac{1}{1 + (c/c_{\text{v}})^2}$$  \hspace{1cm} (B11)

The higher-dimensional generalization of the stress tensor (Eq. B9) is not so straightforward. For example, as mentioned above, we require more elastic parameters to account for fiber alignment, and we must account for the constraints of material frame indifference. However, for small strains, we can proceed by analogy with ordinary linear elasticity [eg, Landau and Lifshitz, 1970] and write an approximate stress tensor as

$$\sigma = \left[ \frac{E(\tau)}{2(1 + \nu)} \left[ \epsilon + \frac{\nu}{1 - 2\nu} \Theta \mathbf{I} \right] + \eta \nu \right] + \left[ \frac{\partial \epsilon}{\partial t} + \frac{\partial \Theta}{\partial t} \mathbf{I} \right]$$  \hspace{1cm} (B12)
where $N = \text{Poisson's ratio}$; $\Theta = \nabla \cdot u$, the dilatation; $\mu_1 = \text{the shear viscosity}$; $\mu_2 = \text{the bulk viscosity}$; $I = \text{the identity tensor}$; and the strain, $\epsilon$, is given by $\epsilon = \nabla u + \nabla u^T$. Here we have tacitly assumed that the fibrous gel is primarily a tension structure; ie, shear forces are negligible, and so we can use the dilatation, $\partial$, (a scalar quantity) to capture the nonlinearities due to fiber alignment.

The mechanical equations of motion for the cytogel are, in the continuum case, simply

$$\nabla \cdot \sigma + \rho F = 0$$

(B13)

$$\frac{\partial n}{\partial t} = -\nabla \cdot (n\partial \epsilon/\partial t)$$

(B14)

where $F$ is the body force per unit mass, $\rho$. The finite element version of (Eq. B8) is simply a force balance at each node of the element network, as described in Odell et al [1981].

$$\sum \text{all nodes Forces} = 0$$

(B15)

The chemical equations of motion we have employed in the simulations are given in the text:

$$\frac{\partial c}{\partial t} = P(c,\epsilon) - rc + \nabla \cdot J$$

(B16)

where $J$, the flux of $c$, is given as simple diffusion: $J = -Dvc$, and where $P(c,\epsilon)$ is given by

$$P(c,\epsilon) = \alpha c^2/(1 + \beta c^2)$$

(B17)

### Solation-Gelation Dynamics

During one cycle, nearly 30% of the ectoplasmic cortex of a plasmodial strand solates and regels. This is clearly an important feature of cytogel dynamics that we have omitted from the present model. This aspect will be treated in detail in a subsequent study; however, for completeness we sketch out here the equations governing the sol-gel dynamics [cf Oster, 1984].

The phase-transition from sol to gel is a complicated molecular event and is only partially understood for simple experimental systems. For the purposes of studying the plasmodial oscillations, however, we can proceed phenomenologically as follows.

We describe the material composition of the cytogel by two field variables: (1) $n(x,t) = \text{the density of gel fibers at position } x \text{ at time } t$. (2) $m(x,t) = \text{the density of sol \textbf{“monomers” at position } x \text{ at time } t}$. By \textbf{“monomers,”} we mean all of the nonfibrous components of the gel (fiber fragments, actin binding proteins, etc).
The two components are introconvertible according to the reaction \( J_r: n \rightleftharpoons m \):

\[
\text{Rate of solation} = J_r - k_S(c)n + k_G(c)m \\
\text{Rate of gelation} = -J_r
\]

where \( k_S(c) \) and \( k_G(c) \) are the rates of solation and gelation, respectively, per unit volume. The phase change between sol and gel is captured by imposing a sigmoidal dependence of \( k_S \) and \( k_G \) on calcium.

The stress balance equation (B13) is given per unit mass of gel, and so we must multiply it by \( n(x,t) \). The mass balance for \( n \) is given by equation (B14), augmented by the gel-sol reaction (Oster \& Odell, 1983a,b):

\[
\frac{\partial n}{\partial t} = -v \cdot (n \frac{\partial e}{\partial t}) + J_r \\
\frac{\partial m}{\partial t} = D_m v^2 m - J_r
\]

where we have assumed that the sol “monomers” can diffuse at a rate \( D_m \text{cm}^2/\text{sec} \).

Thus the total system of equations governing the cytogel dynamics consists of the stress balance equation and the mass balances for sol, gel, and calcium.

**APPENDIX C: DYNAMIC PROPERTIES OF THE MODEL CYTOGEL**

In this appendix we list some of the mechanical properties of the model cytogel that suggest applications to settings beyond those investigated here. A more complete discussion will be given in a subsequent publication.

In our simplified characterization of cytogel mechanochemistry there are but two dynamically varying quantities: the strain, \( \epsilon \) (or the length, \( L \), of the one-dimensional cytoplasmic strip) and the concentration of trigger chemical, \( c \). Therefore, we can understand the behavior of a volume element of cytogel by examining the dynamics of these two quantities. The result of that analysis is to demonstrate that the model cytogel can exhibit a number of interesting and unexpected dynamic properties, which we list here.

**Excitability**

The notion of an “excitable medium” is familiar from neurobiology: a nerve membrane will not fire unless it is stimulated (depolarized) beyond a certain threshold (Fitzhugh, 1960). Above the firing threshold the membrane potential makes a large excursion before eventually returning to its rest state. While firing, the membrane is “refractory”: it cannot refire until the rest state is regained. A more prosaic example of an excitable medium is a field of grass. If the grass is brought above its ignition threshold it will “fire”; moreover, it remains refractory to further firing until the grass regrows. An important property of an excitable medium is its ability to propagate waves of activity. Nerves propagate electrical depolarization waves, and a field of grass will propagate a fire front.

The model cytogel is also an excitable medium: if a strip of cytogel is excited by a local release of calcium—which may be brought about by stretching—then a contraction wave will propagate along the strip. The way this works is easy to see: when one section of the strip fires and contracts it can trigger its neighboring sections
to fire by igniting their calcium trigger. This ignition propagates by either stretch-induced release of calcium and/or by diffusion of calcium from firing regions.

**Oscillations**

We have not been able to make a single volume element exhibit self-sustaining oscillations using only the single \( \text{Ca}^{2+} \) kinetic equation. If, however, we add additional chemical kinetics (eg, the cAMP and/or ATP kinetics described in Appendix A), then it is possible to find kinetic parameters that sustain oscillations, although it turns out that the conditions for this appear to be rather stringent. However, self-sustained oscillations can easily be produced if we couple together more than one volume element. That is, a cytogel system of finite extent can sustain mechanical oscillations quite easily, while a very small volume requires rather fine parameter tuning.

**Bistability**

To achieve excitability and oscillatory behavior we gradually increased the parameter controlling the sensitivity of the chemical cascade curve shown in Figure 4a. If we continue to increase this parameter, we enter a region wherein the cytogel, once it has contracted, tends to remain in this state. In order to return to the relaxed state, one must reduce the parameter quite beyond the point at which it initially triggered contraction. That is, the system exhibits hysteresis, or bistability, much like

![Diagram](image)

Fig. A2. As the sensitivity of the chemical trigger is increased—ie, the parameter, \( \alpha \), controlling the shape of sigmoidal the release curve in Figure 4a—the model progresses through a number of dynamical regimes. In the excitable regime cytogel can propagate contraction waves, analogous to depolarization waves in nerve membranes. In the oscillatory regime the cytogel will exhibit self-sustaining contraction rhythms. In the bistable two stable equilibria coexist in a hysteresis loop, so that the cytogel can act as a mechanical “switch” between contracted and relaxed configurations.
a light switch which can also exist in two stable states. Previously, we have employed a bistable cytogel model to investigate the morphogenesis of epithelial sheets [Odell et al, 1981]; all of the phenomena we documented in that investigation can be reproduced exactly using the present cytogel model.

Figure A2 summarizes the various dynamical regimes for the model cytogel as the calcium trigger sensitivity is increased.

**APPENDIX D: DERIVATION OF THE EQUATIONS FOR THE SIMPLIFIED MODEL**

In this section we derive the actual equations used for the numerical simulations which produced Figure 7. A more complete discussion is given in Odell [1984].

The model consists of two thick cytogel shells connected by a flow resistance. In order to adjust for the effect of curvature we have included a parameter, m, which allows the geometry of the shell to vary between spherical (m = 3) and cylindrical (m = 2).

The model equations are derived by computing the strain rate in the cytogel shell along with the concentrations of Ca$^{++}$ in the shells and in the endoplasm. Since the endoplasm is incompressible, we need only compute the radius of one shell; the other one is then obtained algebraically. In principle, one could simply insert the stress tensor into the standard thick shell equations and perform the appropriate averages over the shell thickness. However, since the equations become rather cumbersome owing to geometrical nonlinearities, we shall sketch the procedure explicitly for the case of spherical shells.

Table I summarizes the various parameters; from these we define the following dimensionless parameters (* denotes dimensionless quantities)

\[
Q_m^* = \frac{3}{8} \left[ \frac{m - 1}{m(m + 1)} \right] \left[ \frac{N_0 \alpha^4 \mu}{\omega R_0^3 \mu_0} \right]: \text{flow resistance between shell 1 and 2}
\]

\[
2 \leq m \leq 3
\]

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_1$ = outer radius of cortical shell $i$, $i = 1, 2$</td>
</tr>
<tr>
<td>$c_i$ = concentration of Ca$^{++}$ in cortex $i$, $i = 1, 2$</td>
</tr>
<tr>
<td>$e_i$ = concentration of Ca$^{++}$ in the endoplasm within cortex $i = 1, 2$</td>
</tr>
<tr>
<td>$R_0$ = original outer radius of the cortex</td>
</tr>
<tr>
<td>$d_0$ = original thickness of the cortex</td>
</tr>
<tr>
<td>$m$ = parameter controlling the cortex geometry: $m = 3$: sphere; $m = 2$: cylinder</td>
</tr>
<tr>
<td>$N_0$ = fibers/unit cross-sectional area of cortex</td>
</tr>
<tr>
<td>$\mu$ = viscosity parameter of the cortical cytogel</td>
</tr>
<tr>
<td>$\mu_0$ = shear viscosity of the endoplasm</td>
</tr>
<tr>
<td>$\nu$ = diffusivity of Ca$^{++}$ between cortical gel and endoplasmic sol</td>
</tr>
<tr>
<td>$r$ = resequestration rate of Ca$^{++}$</td>
</tr>
<tr>
<td>$\gamma$ = release rate of Ca$^{++}$ from the vesicles per unit strain</td>
</tr>
<tr>
<td>$\lambda$ = leakage rate of Ca$^{++}$ from the vesicles</td>
</tr>
<tr>
<td>$a, \beta$ = parameters controlling the release kinetics of Ca$^{++}$</td>
</tr>
<tr>
<td>$\omega, r$ = length and radius of the flow resistance tube connecting the shell</td>
</tr>
</tbody>
</table>
The empirical input to the model is the stress surface \( \sigma(\epsilon, c) \) shown in Figure 6. We shall denote the dimensionless elastic stress by \( \Sigma = N \sigma(\epsilon, c^*)/\beta^{1/2} \), and henceforth drop the asterisks from the dimensionless variables. Thus, at each time step in the computation, \( \Sigma \) can be computed from the current values of \( \epsilon \) and \( c \).

The equations of motion may now be computed from the following recipe.

1) First, define the functions \( \chi(u) \) and \( \tilde{\chi}(u) \) by \( \chi(u) \equiv u/\rho(u) \), where

\[
\begin{align*}
\rho(u) & \equiv [\alpha^{m} - \delta]^{1/m} = \text{inner radius of cortex whose (dimensionless) outer radius is } u. \\
\delta & \equiv [1 - (1 - d)^{m}] \\
\tilde{\chi}(u) & \equiv B(u) + \chi(u)(1 - \delta)
\end{align*}
\]

where

\[
\begin{align*}
B(u) & \equiv 1 - \ln[\chi(u)]/\ln(1 - 1/u) \\
b(u) & \equiv \chi(u)\ln[\chi(u)]/\ln(1 - 1/u) - 1
\end{align*}
\]

2) Let \( H(u) \equiv H(R_1, \ldots, R_n) \), and

\[
\begin{align*}
\rho(R_1) & = \rho_1 = \text{inner radius of cortex 1} \\
\rho(R_2) & = \rho_2 = \text{inner radius of cortex 2} \\
\sigma(R_1 - 1, c_1) & = F_{R1} = \text{stress on outer surface of cortex 1} \\
\sigma(R_2 - 1, c_2) & = F_{R2} = \text{stress on outer surface of cortex 2} \\
\sigma(\rho_1/(1 - d) - 1, c_1) & = F_{\rho_1} = \text{stress on inner surface of cortex 1} \\
\sigma(\rho_2/(1 - d) - 1, c_2) & = F_{\rho_2} = \text{stress on inner surface of cortex 2}
\end{align*}
\]

We compute \( dR/dt \) as follows:

\[
\frac{dR}{dt} = F_1 = \frac{[B(R_2)F_{R2} + b(R_2)\cdot F_{\rho_2} - B(R_1)\cdot F_{R1} - b(R_1)\cdot F_{\rho_1}]}{R_1^{m-1}/Q_1 + \tilde{\chi}(R_1) + (R_1/R_2)^{m-1}\cdot \tilde{\chi}(R_2)}
\]  
(C1)

3) Compute the chemical equations as follows:

\[
\begin{align*}
\frac{dc_1}{dt} & = F_2 = \theta(R_1 - 1, c_1) + [D_m/\delta]\rho_1^{m-1}(e_1 - c_1) \\
\frac{dc_2}{dt} & = F_3 = \theta(R_2 - 1, c_2) + [D_m/\delta]\rho_2^{m-1}(e_2 - c_2)
\end{align*}
\]  
(C2)

4) Since convective flow of cytosol carries the upstream concentration of \( Ca^{++} \), in order to define the \( Ca^{++} \) concentration in the endoplasm, \( (e_1, e_2) \), we must know which way the cytosol is flowing. We do this as follows:
If $\partial R_1/\partial t > 0$, then $E_{F1} = mR_1^{m-1}F_1(e_2 - e_1)/\rho_1$
and $E_{F2} = 0$
If $\partial R_1/\partial t < 0$, then $E_{F1} = 0$
and $E_{F2} = mR_1^{m-1}F_1(e_2 - e_1)/\rho_2$

The endoplasm equations are

\[
\frac{\partial \mathbf{e}_1}{\partial t} = F_4 = [D_{m_1}/\rho_1](c_1 - e_1) + E_{F1} + \theta(1, e_1) \quad \text{(C4)}
\]
\[
\frac{\partial \mathbf{e}_2}{\partial t} = F_5 = [D_{m_2}/\rho_2](c_2 - e_2) + E_{F2} + \theta(1, e_2) \quad \text{(C5)}
\]

Equations C1–5 define the vector field $F(x)$. 