CELL MOTION, CONTRACTILE NETWORKS, AND THE PHYSICS OF INTERPENETRATING REACTIVE FLOW

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ABSTRACT In this paper we propose a physical model of contractile biological polymer networks based on the notion of reactive interpenetrating flow. We show how our model leads to a mathematical formulation of the dynamical laws governing the behavior of contractile networks. We also develop estimates of the various parameters that appear in our equations, and we discuss some elementary predictions of the model concerning the general scaling principles that pertain to the motions of contractile networks.

INTRODUCTION

Interpenetrating flow is the motion of a dispersed mixture of two or more materials in which the components of the mixture move with significantly different velocity fields. Common phenomena where interpenetrating flow is important include the boiling of water, the "fizzing" of an agitated soft drink, the falling of rain, and the flow of blood.

It is widely appreciated among cytologists that the cortical cytoplasm of motile cells contains a complex filament network mixed with a solution of aqueous phase (Porter, 1984). Cytoplasmic motions are thought to come about because the interactions of actin and myosin produce tension within the filaments of the network. Such tensions cause the network material to contract and increase in density with a consequent displacement of aqueous material.

From these elementary considerations, it is evident that the motion of cytoplasm should be viewed as an interpenetrating flow of at least two distinct materials: the network and the solution. In this paper, we intend to give substance to the basic theoretical ideas implicit in such a view of cytoplasmic dynamics. Rather than pursue great generality, we will present a formulation that captures the essential complexity of interpenetrating motion with a minimum of extraneous detail. Because there is significant chemical interchange between matter in the network and solution, we call our formulation the "reactive flow" model.

In the reactive flow model, we will regard the material enclosing the cytoplasm (in most cases this will be the cell membrane) as comprising the stationary walls of a rigid reaction vessel. We will view the cytoplasm itself as a finely divided mixture of two distinct material phases: a contractile network of randomly oriented protein filaments and an aqueous solution. Finally, the model assumes that in a macrorheological sense, both phases of the cytoplasm behave as homogeneous Newtonian fluids. The operational meaning of these assumptions is discussed in the first appendix of this paper.

Average Fields and Excluded Volumes

To produce a tractable mathematical formulation of the reactive flow model, it is necessary to ignore the microscopic details of the motion and configuration of the network and solution phases and to focus instead on a representation in terms of average fields. To formulate such a field theory in the case of cytoplasm, one must somehow quantify the relative concentrations of network phase and solution phase near a particular location. In the reactive flow model we will do this by means of the fractional volumes of the networks and solution, $\theta_n(\mathbf{r}, t)$ and $\theta_s(\mathbf{r}, t)$.

Physically, the fractional volume of a particular phase represents the proportion of space near position $\mathbf{r}$ that is filled by that phase at time t. If network and solution are the only two phases present in the cytoplasm, then evidently θ_n and θ_s must satisfy the excluded volume relation

$$\theta_n(\mathbf{r}, t) + \theta_s(\mathbf{r}, t) = 1. \tag{1}$$

Incompressibility and Mass Conservation

Both the network and solution phase in cytoplasm are composed of highly incompressible materials (i.e., at normal pressures they each maintain constant density). The network phase is composed largely of protein (density $\approx$1.35 gm/ml), whereas the solution is largely aqueous but with important concentrations of dissolved salts, carbohydrates, and proteins (density $\approx$1.1 gm/ml). Since the density of the two phases is quite similar, the equations for conservation of mass of network and solution can be expressed in terms of volume fractions, i.e.,

$$\partial_t \theta_n = -\nabla \cdot (\theta_n \Omega_n) + J(\theta_n, \theta_s, \cdot) \tag{2a}$$

and

$$\partial_t \theta_s = -\nabla \cdot (\theta_s \Omega_s) - J(\theta_n, \theta_s, \cdot). \tag{2b}$$

In Eqs. 2a and 2b, Ω_n and Ω_s are the macroscopic velocity fields of the network and solution phases, and $J(\theta_n, \theta_s, \cdot)$ is the rate at which volume is transferred from the solution to the network due to chemical reactions of all types. If we add Eq. 2a and Eq. 2b and use Eq. 1 to cancel time derivatives, we obtain an alternative set of mass conservation equations:

$$\partial_t \theta_n = -\nabla \cdot [\Omega_n \theta_n] + J(\theta_n, \theta_s, \cdot) \tag{3a}$$

and

$$0 = \nabla \cdot [\theta_n \Omega_n + \theta_s \Omega_s]. \tag{3b}$$

Eq. 3a is simply a repetition of Eq. 2a, whereas Eq. 3b expresses the fact that because of overall incompressibility, the volume fluxes of network and solution in and out of a small region must cancel. Eq. 3b is sometimes called the law of volume conservation.

In order to actually carry out calculations with the mass conservation equations in whichever form, it is necessary to specify a formula for computing the unknown function, $J(\theta_n, \theta_s, \cdot)$. To do this, we first note that, because of the excluded volume relation, a dependence of $J(\theta_n, \theta_s, \cdot)$ on θ_s can be combined into the dependence on θ_n: $J_n(\theta_n, \theta_s, \cdot) = J(\theta_n, 1 - \theta_n, \cdot) = J(\theta_n, \cdot)$.

We must next notice that any chemically realistic process of network formation and breakdown will possess a stable equilibrium point at which the net rate of reaction vanishes. If $\tilde{\theta}_n(\cdot)$ is the value of θ_n corresponding to such an equilibrium point, then using Taylor's theorem we can express the reaction rate in the form $J(\theta_n, \cdot) = \tilde{J}'(\theta_n - \tilde{\theta}_n) + \frac{1}{2}\tilde{J}''(\theta_n - \tilde{\theta}_n)^2 + \cdots$. Neglecting terms that contain quadratic and higher powers of $(\theta_n - \tilde{\theta}_n)$, this expression can be put in the equivalent form

$$J(\theta_n, \cdot) \approx (\tilde{\theta}_n - \theta_n) / T_{eq}, \tag{4}$$

where $T_{eq} = -(\tilde{J}')^{-1}$.

T_{eq} corresponds to a characteristic relaxation time of the chemical reaction of network assembly and disassembly. If we start with an initial volume fraction that is not too far from equilibrium, then T_{eq} gives a measure of the time required for the difference $|\tilde{\theta}_n - \theta|$ to decrease by fixed percentage.

Momentum Conservation

In extremely small, highly damped mechanical systems, both convective momentum transport and inertia can be neglected. In this so-called "creeping flow" limit, the law of momentum conservation for both the network and solution phases reduces to an expression for force balance.

Let $\mathbf{F}_{pp}$ be the force exerted on the material of phase p in a given control volume by neighboring material of the same phase, and let $\mathbf{F}_{pq}$ be the force exerted by surrounding material of the other phase (i.e., phase q). Neglecting body forces, we conclude that if the forces on phase p are in balance, then

$$0 = \mathbf{F}_{pq} + \mathbf{F}_{pp}. \tag{5a}$$

If x_j is an arbitrary coordinate system, then the associated components of $\mathbf{F}_{pp}$ can be expressed in terms of the average stress tensor in phase p material, weighted by the volume fraction of this phase

$$f_i = \partial_{x_j} \theta_p \sigma_{ij}^{(p)}. \tag{6a}$$

(See Drew, 1971, and Drew and Segal, 1971, for a derivation.)

In order to use Eq. 6a, we must propose an appropriate stress-strain constitutive law for phase p material. In the current model we assume that both phases obey the simplest possible constitutive law, i.e., that for a Newtonian fluid

$$\sigma_{ij}^{(p)} = -P_p \delta_{ij} + \tfrac{1}{2}\Lambda_p \delta_{ij} e_{kk}^{(p)} + M_p e_{ij}^{(p)}. \tag{6b}$$

In Eq. 6b, δ_{ij} is the unit tensor, $e_{ij}^{(p)}$ is the macroscopic rate of strain tensor of phase p, P_p is the macroscopic intra-p-phase pressure, Λ_p is a coefficient characterizing the dilation viscosity of phase p and M_p is a coefficient characterizing the shear viscosity of phase p.

An equation for $\mathbf{F}_{pp}$ is obtained by inserting Eq. 6b into 6a. In terms of vector operator notation the result is

$$\mathbf{F}_{pp} = (\nabla \cdot 2M_p \theta_p \nabla)\Omega_p + \nabla \times (M_p \theta_p \nabla \times \Omega_p) + \nabla(\Lambda_p \theta_p \nabla \cdot \Omega_p) - \nabla \theta_p P_p. \tag{6c}$$

The various terms on the right of Eq. 6c have simple physical interpretations. The first term gives the force on phase-p material due to relative translational motion of neighboring masses of primary-p material; the second term gives the force acting due to relative rotational motion of neighboring material; the third term gives the force acting due to dilations or contractions of neighboring material; and the final term gives the force due to static pressure or tension exerted by neighboring phase-p material.

The interphase force acting on phase p, F_{pq}, can be expressed as the sum of two components

$$\mathbf{F}_{pq} = P\nabla\theta_p + \Phi\theta_p\theta_q(\Omega_q - \Omega_p). \tag{7}$$

The first component, $P\nabla\theta_p$, represents the net static pressure exerted on material of phase p by material of phase q (for a derivation, see Appendix B of Drew and Segal, 1971). In this term, P (with no subscript) is the socalled interphase pressure. The second component in Eq. 7 represents the frictional drag due to relative motion of phases p and q (Φ is called the drag coefficient).

Combining Eqs. 6c and 7 and setting the subscript $p = s$, we obtain the law of momentum conservation for the

solution phase

$$0 = \nabla(\Lambda_s\theta_s\nabla \cdot \Omega_s) + 2(\nabla \cdot M_s\theta_s\nabla)\Omega_s + \nabla \times (M_s\theta_s\nabla \times \Omega_s) + \Phi\theta_s\theta_n(\Omega_n - \Omega_s) - \theta_s\nabla P - \nabla\Upsilon\theta_s, \quad (8a)$$

where $\Upsilon = (P_s - P)$.

The corresponding expression for the network phase is similar except that we must replace the subscript s by the subscript n,

$$0 = \nabla(\Lambda_n\theta_n\nabla \cdot \Omega_n) + 2(\nabla \cdot M_n\theta_n\nabla)\Omega_n + \nabla \times (M_n\theta_n\nabla \times \Omega_n) + \Phi\theta_n\theta_s(\Omega_s - \Omega_n) - \theta_n\nabla P - \nabla(\Upsilon - \Psi)\theta_n, \quad (8b)$$

where $\Psi = P_s - P_n$.

Υ (the solvation stress) is a measure of the difference between the static stress within the solution phase and the static stress acting across the network-solution interface. Υ is large and positive if the solution phase is a good solvent for the network and negative if it is a poor solvent.

Ψ (the contractile stress) is a measure of the static or isometric tension acting along the axial coordinate of network filaments (i.e., those stresses that act only within the network phase and not in the solution phase). Ψ is positive if network filaments are on average stretched, and negative if they are compressed.

Since solvation stresses are of a nonspecific chemical nature, we generally assume in the reactive flow model that Υ is a constant independent of θ_n. However, since axial stresses have at least some component that arises from specific ATP-dependent interactions, the contractile stress will, in general, depend on the density of network filaments.

The functional dependence of Ψ on θ_n could be empirically described by means of a simple Maclaurin series in θ_n. However, for reasons which will become apparent, a more natural description is obtained if one expands Ψ as Maclaurin series in the quantity $-2(1 + \theta_n^{-1} \ln \theta_s)$. (Note that $-2(1 + \theta_n^{-1} \ln \theta_s) \sim \theta_n$ unless θ_n is quite close to one.) This approach yields the constitutive law

$$\Psi(\theta_n) = \Psi(0) - 2\Psi'(0)(1 + \theta_n^{-1} \ln \theta_s), \quad (8c)$$

where $\Psi'(0)$ is the derivative of Ψ with respect to θ_n evaluated at $\theta_n = 0$.

The three coefficients Υ, $\Psi(0)$, and $\Psi'(0)$ are the basic physical quantities needed to quantify contractile and solvation stresses in the reactive flow model. However, because pressure in the reactive flow model is implicitly determined by the incompressibility constant and because of the form chosen for Eq. 8c, it turns out that for most purposes, it is sufficient to specify only two lumped coefficients.

To bring about this reduction in complexity, we must introduce a socalled effective pressure,

$$P_F \equiv P + \Upsilon + \Upsilon \ln \theta_s = P_s + \Upsilon \ln \theta_s. \quad (9a)$$

Physically, P_F is the total mechano-chemical potential energy per unit volume of solution phase material.

Changing variables and using the assumption that Υ is constant, we express the last two terms of Eq. 8a in the form

$$-\theta_s\nabla P - \nabla(\Upsilon\theta_s) = -\theta_s\nabla(P + \Upsilon + \Upsilon \ln\theta_s) = -\theta_s\nabla P_F. \quad (9b)$$

In similar fashion the final two terms of 8b take the form

$$-\theta_n\nabla P - \nabla[(\Upsilon - \Psi)\theta_n] = -\theta_n\nabla P_F + \nabla\{[\Psi(0) - \Upsilon]\theta_n\} + \nabla\{[\Upsilon - 2\Psi'(0)](\theta_n + \ln\theta_s)\} = -\theta_n\nabla P_F + \nabla\{\Psi_F[\theta_n + \sigma(\theta_n + \ln\theta_s)]\}, \quad (9c)$$

where $\Psi_F \equiv \Psi(0) - \Upsilon$ is called the effective contractile stress, and $\sigma \equiv [\Upsilon - 2\Psi'(0)]/[\Psi(0) - \Upsilon]$ is a nondimensional ratio called the swelling number.

If we substitute Eq. 9b and 9c into 8a and 8b, we obtain a reduced form of the momentum equations wherein the lumped coefficients, Ψ_F and σ, are the only quantities required to describe the dynamical effects of solvation and contractile stresses. The price of this simplification is that, in its reduced form, the reactive flow model does not directly yield the true solution phase pressure. Rather, the dependent variable predicted by the model is the effective pressure P_F. Since direct pressure measurements on cytoplasm have never been attempted, this is not currently a significant problem. If it is necessary to compute the solution pressure, the reduced model must be supplemented by Eq. 9a. This latter computation is the only step that requires all three coefficients, Υ, $\Psi(0)$, and $\Psi'(0)$.

Boundary Conditions

As in the case of the Navier-Stokes equations, the tangential components of the solution velocity field can be subject to either free-slip boundary conditions, or to no-slip boundary conditions. If $\mathbf{t}$ and $\mathbf{n}$ are, respectively, unit tangent and normal vectors to the boundary, then, in terms of the standard vector notation, the solution velocity near a free slip-type boundary must satisfy the constraint $(\mathbf{n} \cdot \nabla)(\mathbf{t} \cdot \Omega_s) = 0$; whereas near a no-slip boundary, the solution velocity field must obey the constraint $(\mathbf{t} \cdot \Omega_s) = 0$.

To formulate these two boundary conditions in a combined expression, it is convenient to introduce an index function for whether free-slip or no-slip conditions hold along various surfaces. Thus if $\mathbf{R}_B$ is an arbitrary point on the boundary of the reaction vessel, we let $\mathrm{SLP}_s(\mathbf{R}_B) \equiv -1$ if no-slip conditions apply for the solution phase at $\mathbf{R}_B$, and $\mathrm{SLP}_s(\mathbf{R}_B) = +1$ if free-slip conditions apply. Use of this index yields a single tangent boundary condition for the solution phase

$$(1 + \mathrm{SLP}_s)(\mathbf{t} \cdot \Omega_s) + (1 - \mathrm{SLP}_s)(\mathbf{n} \cdot \nabla)(\mathbf{t} \cdot \Omega_s) = 0. \quad (10a)$$

The behavior of the normal component of the solution velocity field at an arbitrary boundary point will be

determined by the local pressure field across the reaction vessel wall. Thus if $H_c(\mathbf{R}_B)$ is the hydraulic conductivity of the boundary, and if $P_{ex}(\mathbf{R}_B)$ is the mechano-chemical potential per unit volume of the solution on the external surface of the boundary, then the normal boundary condition on the solution is

$$\Omega_s \cdot \mathbf{n} = H_c(P_s + \Upsilon \ln \theta_s - P_{ex}) = H_c(P_F - P_{ex}). \quad (10b)$$

Notice that if thermal equilibrium exists, then the solution pressure in the reaction vessel must balance both the solvation force and the external pressure. The excess pressure required to balance the solvation force is commonly called the osmotic pressure.

As with the solution phase, it is possible for the tangential component of the contractile network velocity to obey either free-slip or no-slip conditions at a boundary. If $SLP_n(\mathbf{R}_B)$ is an index function of whether the network phase obeys free-slip or no-slip conditions at various boundary points, then the tangent boundary condition on the network can be stated in the form

$$(1 + SLP_n)(\mathbf{t} \cdot \Omega_n) + (1 - SLP_n)(\mathbf{n} \cdot \nabla)(t \cdot \Omega_n) = 0. \quad (11)$$

The normal component of the network velocity can be influenced by the boundary in at least two qualitatively different ways. One possibility is to have a boundary at which the normal component of the velocity must vanish

$$\Omega_n \cdot \mathbf{n} = 0. \quad (12a)$$

We shall refer to Eq. 12a as the "stick" condition since physically it corresponds to a situation in which a thin layer of network adjacent to the walls of the reaction vessel is prevented from moving either outward or inward.

The other possibility, the opposite of a stick boundary condition, is a no-stick boundary condition. Physically, such a boundary condition corresponds to an inert wall that blocks outward motions of the network but offers no resistance if network is moving in an inward direction.

To formulate a precise mathematical statement of what happens to the network velocity field at a no-stick boundary, we first recognize that the normal component of network velocity at this kind of boundary can be zero or less than zero, but cannot be positive

$$\mathbf{n} \cdot \Omega_n \leq 0. \quad (12b)$$

Suppose that at some boundary point $\mathbf{R}_b$, $\mathbf{n} \cdot \Omega_n$ is strictly less than zero. Since no network can enter from the exterior of the reaction vessel, we conclude that at the point $\mathbf{R}_b$, $\theta_n = \mathbf{t} \cdot \nabla\theta_n = 0$ and that $\mathbf{n} \cdot \nabla\theta_n \neq 0$.

Consider now the projection of the balance of force equation for the network phase (Eq. 8b) in a direction normal to a boundary surface at $\mathbf{R}_b$. If we take the limit of the resulting expression as θ_n and $\mathbf{t} \cdot \nabla\theta_n$ approach zero, then we obtain

$$0 = \Lambda_n \nabla \cdot \Omega_n + 2M_n(\mathbf{n} \cdot \nabla)(\Omega_n \cdot \mathbf{n}) + \Psi_F. \quad (12c)$$

Eq. 12c provides a normal boundary condition of Ω_n but only on the assumption that $\mathbf{n} \cdot \Omega_n$ was strictly less than zero. Thus in using 12c it must be implicitly understood that the result is superseded by the constraint (Eq. 12b). In other words, if use of Eq. 12c leads to a positive value of $\Omega_n \cdot \mathbf{n}$ at one or more points, then we ignore this result and simply set $\mathbf{n} \cdot \Omega_n = 0$ at such points.

In cases where certain portions of the reaction vessel wall contain adhesive sites for network, whereas other portions do not, it is necessary to combine boundary conditions 12a and 12c. We do this by the standard device of introducing an index function STL $(\mathbf{R}_b)$ such that $STK(\mathbf{R}_b) = +1$ at points where stick boundary conditions apply, and $STK(\mathbf{R}_b) = -1$ at points where no-stick conditions apply. In terms of such a stick/no-stick index function, the boundary condition on the normal component of the network velocity on any portion of the reaction vessel wall is

$$0 = (1 + STK)(\mathbf{n} \cdot \Omega_n) + (1 - STK)[\Lambda_n \nabla \cdot \Omega_n + 2M_n(\mathbf{n} \cdot \nabla)(\mathbf{n} \cdot \Omega_n) + \Psi_F]. \quad (12d)$$

If the normal component of Ω_n vanishes at the boundary, then the boundary condition on θ_n is irrelevant. On the other hand, if $\mathbf{n} \cdot \nabla\Omega_n < 0$, then from our previous discussion, $\theta_n = 0$ at the boundary. In either event, a uniformly valid boundary condition on the network density is

$$(1 + STK)(\mathbf{n} \cdot \nabla\theta_n) + (1 - STK)\theta_n = 0. \quad (13)$$

An independent boundary condition on the solution density is redundant since θ_s at the boundary follows directly from the excluded volume-relation and the value of θ_n.

For a closed system, the pressure field is only meaningful up to an arbitrary additive constant (i.e., only gradients of pressure matter). Thus a boundary condition on the pressure is unnecessary unless the boundary is permeable to the solution phase. In the latter case the function P_{ex} must be specified along all permeable portions of the boundary (see Eq. 10).

In summary, in order to uniquely determine the motion of the network and solution phases in a fixed domain, we must specify boundary conditions for the network density and for the normal and tangent components of both the solution and the network velocity fields. For the tangent components of the solution and network velocities, we must decide on either slip or no-slip conditions. For the normal component of the solvent field we have only one possible type of behavior, but we must specify the external pressure and the hydraulic conductivity of the boundary. Finally, for the normal component of the network velocity field we must decide on either stick or no-stick conditions.

The various boundary conditions we have discussed, together with the freedom to choose size and shape, provides for an enormous number of different configurations of the reaction vessel. However, even this variety does

not cover all situations of interest. Of particular importance is the case of periodic boundaries and the case of an open boundary, (i.e., a reaction vessel with an opening into a large well-mixed reservoir). The case of deformable boundaries will be given some further examination in Appendix A.

Estimation of Parameters

For easy reference, the various equations that constitute the reaction flow model are summarized in the second appendix of this paper (Eqs. B1–B10). These 10 equations constitute a proposed device for taking various isolated bits of knowledge about individual factors of importance in cell motion and integrating this information in such a way as to make definite predictions about complex multifactor systems.

From the point of view of experimental biology, the qualitative form of input information required by the reactive flow model is in itself instructive. Table I gives a list of the nine coefficients associated with the differential equations describing mass and momentum transport. In order to have a well-defined mathematical problem, we must specify these coefficients. We must also specify the size and shape of the reaction vessel, and the five functions SLP_s, SLP_n, STK, H_c, and P_{ex} that describe the properties of the reaction vessel. Finally, we must specify the initial distribution of network at all points in the reaction vessel.

If the reactive flow model is to be taken seriously, then it must be possible (at least in principle) to independently measure the various pieces of input information required by the model. Thus it is essential to systematically survey the currently available information and to suggest practical methods for obtaining any missing pieces.

TABLE I
PARAMETERS OF THE REACTIVE FLOW MODEL OF CONTRACTILE NETWORKS*

Parameter	Symbol	Range§	CGS Units‡
Fractional volume of network at chemical equilibrium	$\tilde{\theta}_n$	10^{-4}–10^{-2}	—
Relaxation time near chemical equilibrium	T_{eq}	10–10^3	s
Shear viscosity of solution	M_s	0.01–0.05	Poise
Dilatation viscosity of solution	Λ_s	(0.01–10) × M_s	Poise
Shear viscosity of network	M_n	10^2–10^6	Poise
Dilatation viscosity of network	Λ_n	(0.1–10) × M_n	Poise
Network-solution drag coefficient	Φ	10^7–10^{11}	Poise/cm^2
Effective contractile stress	Ψ_F	0–10^6	dyn/cm^2
Swelling number	σ	(10–10^3)	—

*Parameters associated with the boundary conditions or initial conditions are not included.
‡Poise ≡ dyns/cm^2.
§Range is meant to indicate the reasonable limits of biological variation. Range does not connote the experimental uncertainty of any particular measurement.

Network Density at Chemical Equilibrium, $\tilde{\theta}_n$. The notion of chemical equilibrium in a closed reaction vessel has both a global and a local aspect. Globally, chemical equilibrium implies that the total mass of network in the reaction vessel is constant in time. Even if this condition is satisfied, in most amoeboid cells the network is concentrated in regions such as the cell cortex, and the bulk of the cell has a very low network density. Such an arrangement cannot be in chemical equilibrium because entropy is maximized only when the density distribution is uniform over the available volume. Thus, $\tilde{\theta}_n$ is the network volume fraction that would occur if the steady-state mass of network in a cell were uniformly distributed.

In various motile cells where measurements have been attempted, the reported actin content of the cytoplasm is in the range of 2–20 mg/ml (see Bray and Thomas [1975], Bray and Thomas [1976a,b], and Hinssen [1979]). If we make the assumption that network components other than actin are of negligible mass and that virtually all cellular actin is incorporated into network at equilibrium, then we obtain an upper limit on $\tilde{\theta}_n$ of $\sim 2 \times 10^{-2}$. This figure is probably high, however, because under physiological conditions, between 90 and 50% of the actin of motile cells seems to be in unpolymerized form (Bray and Thomas, 1976; Wang et al., 1982). After adjusting for such unpolymerized actin, we conclude that $\tilde{\theta}_n$ is $<10^{-2}$ and could reasonably be as small as 10^{-4}.

Relaxation Time, T_{eq}. A number of complementary approaches can be used to estimate the chemical relaxation time of the network assembly and disassembly reaction. The simplest method is to introduce a rapid perturbation to the reaction and to observe how long it takes for the system to re-equilibriate. An elegant study of this kind has used the microinjection of a saturating dose of actin-capping protein into tissue culture cells (Fuchtbauer et al., 1983). As assayed by the percent of cells still containing microfilament bundles at various time after injection, this technique yields a half-maximal response time of ~5 minutes. Another study that can be interpreted according to a similar principle, concerns the behavior of isolated droplets of physarum endoplasm (Isenberg and Wohlfarth-Bottermann, 1976). In this study, a drop of endoplasm was protruded by puncturing a vein of physarum. The subsequent reaction, which ultimately results in resorption of the drop, can be followed by sectioning techniques as well as by other means. Immediately after protrusion, the endoplasm contains no detectable F-actin. Within a period of 5 min, F-actin filaments are quite prominent, and by 10 min the actin filaments have become

aggregated into a network which undergoes periodic contractions. As with the results of Fuchtbauer et al., these data strongly indicate that the value of T_{eq} falls in the general range of 1–10 min.

A third methodology for estimating the chemical relaxation time of contractile network under physiological conditions is based on the microinjection of fluorescently labeled actin monomer into living cells. In due course the labeled actin is incorporated into the contractile network, and the chemical relaxation time of the network is estimated from the time required for fluorescence recovery after small patches of the labeled network are photobleached (Kreis et al., 1982). In agreement with the two previous techniques, the results obtained with fluorescence photobleaching recovery indicate a relaxation time in the range of 5–10 min.

Another approach to estimating the chemical relaxation time is based on systems that display cycles of network formation and contraction. It is easy to see that in such phenomena the period of a cycle gives an upper bound on the chemical relaxation time (i.e., we neglect the portion of the cycle devoted to contraction or to some other processes). An elegant system that has been recently reported involves the contraction of actomyosin gels from amphibian eggs (Ezzell, et al., 1983). The interval between contractions in this study again indicates a relaxation time on the order of 5 min. Another periodic phenomenon where network assembly and disassembly are definite parts of the cycle is the shuttle streaming of the accellular slime mold, *Physarum polycephalum* (Gotz von Olenhusen and Wohlfarth-Bottermann, 1979). In the case of shuttle streaming the periodicity indicates a relaxation time of only 1 min.

A final approach to estimating the relaxation time of network formation is based on direct in vitro studies of the kinetics of actin polymerization. In principle, this approach yields a lower bound on T_{eq} since actin polymerization is only one component of network formation; on the other hand, it is difficult to be sure that in vitro studies have physiological relevance. At any rate, many studies (see the review by Korn, 1982; also see Pollard, 1984) have demonstrated a time course that is consistent with a value of T_{eq} between 0.2 and 10 min. These studies also indicate that the polymerization of actin is an autocatalytic reaction.

Autocatalysis is thought to come about because the initial nucleation of actin filaments is slow compared with the elongation of filaments. Thus, as the mass of polymerized actin increases, fragmentation events create more and more nucleation sites, and the whole process accelerates. To describe such an autocatalytic reaction, it is necessary to regard T_{eq} as an increasing function of θ_n.

The Solution Shear Viscosity, M_s. The shear viscosity of the solution phase in cytoplasm will be greater than the viscosity of pure water because of the presence of dissolved proteins and ions. On the other hand, the diffusion of small molecules injected into the cytoplasm indicates that the decrease in mobility over that observed in pure aqueous solution is no more than a factor of three (Wang et al., 1982). We thus conclude that the lower limit of M_s is 0.01 poise and the upper limit is 0.05 poise.

The Drag Coefficient, Φ. The most direct way to measure Φ is by studies of the water permeability of immobilized or gelled contractile networks (cf., discussion of hydraulic conductivity). Unfortunately, we are not aware of any direct experiments of this kind. Nevertheless it is possible to arrive at a rough estimate of Φ on the basis of hydrodynamical arguments.

Since the network density is low (see estimate of $\tilde{\theta}_n$), it is reasonable to estimate the Stokes drag between the network and solution by regarding the network as being composed of a sparse distribution of hydrodynamically independent unit elements, each of fixed shape. By definition, Φ is the frictional coefficient per unit volume of network. Thus, the assumption of independent unit elements leads to the expression

$$\Phi \approx f/v, \tag{14a}$$

where f is the frictional coefficient of an individual unit element, and v is the volume of a unit element.

As a simple geometric model for the shape of a unit element of a contractile network, we consider a long cylinder of radius a and length b. Elementary texts on hydrodynamics derive an asymptotic expression for the frictional coefficient of such an object

$$f \approx \tfrac{1}{2}\pi M_s b/\ln(b/a). \tag{14b}$$

Since the volume of a cylinder is $v = \pi a^2 b$, we can combine Eqs. 14a and 14b to obtain

$$\Phi \approx (\tfrac{1}{2}) M_s/[a^2 \ln(b/a)]. \tag{15}$$

To compute an upper bound on Φ we assume that the unit element of the network has the radius of only a single actin filament; i.e., $a \approx 4 \times 10^{-7}$ cm. We also assume that b is only 10 times larger than a and that M_s is 0.05 poise. From these estimates we conclude that $\Phi \lesssim 10^{11}$ poise/cm^2.

To compute a lower bound on Φ we assume that the unit element is a bundle of actin filaments with cross-sectional area of $\sim 10^3$ single filaments; i.e., $a = 10^{-5}$ cm. We also assume that $b/a = 10^4$ and that M_s is only 0.01 poise. From these estimates we conclude that $\Phi > 10^7$ poise/cm^2.

The Network Shear Viscosity, M_n. In order to determine the shear viscosity of the network it is instructive to consider the limiting case of the reactive flow model wherein the contractile force is turned off (i.e., $\Psi_F = 0$). In this case, the very large value of the drag coefficient (see estimate of Φ) will ensure that the velocities of the solution

and network phases are approximately equal ($\Omega_s \sim \Omega_n$). Furthermore, the chemical reaction terms will ensure that $\theta = \tilde{\theta}_n$ is constant throughout the reaction vessel. Taken together, these constraints imply that the mixture of network and solution will behave like an ordinary one-phase incompressible fluid with macrorheological shear viscosity

$$M_{eff} = M_s\theta_s + M_n\tilde{\theta}_n. \tag{16}$$

Using this expression, M_n is readily obtained by measuring the elevation of effective viscosity caused by the presence of network.

In vivo studies of effective cytoplasmic shear viscosity have frequently been based on the motion of small particles. Such studies have yielded values of M_{eff} in the range 10–10^5 poise (see Table I of Valberg and Albertini, 1985, for an excellent summary of the data). Although these results are very important, it should be understood that the analysis of probe particle motion in a multiphase system is subject to severe theoretical difficulties. First, it is impossible to know whether the network phase obeys slip or no-slip boundary conditions at the particle surface. Second, since the network is actively contractile, the presence of a sticky (or slippery) particle can lead to clumping (or retraction) of network in the neighborhood of the particle.

An independent technique for in vivo measurement of cytoplastic viscosity is based on micropipette aspiration. Studies of this kind have been carried out by Sung et al. (1982) and, more recently, by E. Evans (private communication). Measurements based on aspiration of human leukocytes indicate values of M_{eff} for the cortical network on the order of 10^2–10^5 poise.

In vitro rheological studies of solutions containing various densities of actin filaments under conditions of incompressible flow have been quite extensive (see the recent review by Stossel, 1984). As predicted by Eq. 16, it has been found that M_{eff} is a linear function of actin density. It is also found that M_{eff} depends linearly on the average length of actin filament. At low shear rates M_{eff} is inversely related to the 0.8 power of the velocity gradient, but at high shear rates, M_{eff} approaches a constant (see discussion of non-Newtonian rheology in Appendix A).

In his review article, Stossel has estimated M_{eff} for a 10 mg/ml actin filament solution with average filament length of 10^{-5} cm under shear conditions typical of cell motions (shear rate $\approx 0.3\ s^{-1}$). For these conditions M_{eff} is ~3 poise, corresponding to M_n of 300 poise. This value of M_n should be viewed as a lower limit since in an actual cytoplasm M_n would be increased due to the presence of actin binding proteins. In large quantities, the presence of such proteins has been shown to increase the effective shear viscosity by up to a factor of 10^3 (McLean et al., 1980; Griffith and Pollard, 1982; Rockwell et al., 1984). Studies have not been done to determine the effect of actin binding proteins on the shear rate dependence of M_{eff}.

The Dilation Viscosities, Λ_n, Λ_s. Λ_n is the coefficient that relates frictional resistance to the speed of network contraction or expansion. Λ_s is a similar parameter associated with expansions or contractions of the solution phase. Because of volume conservation, expansion or contraction of network and/or solution are necessarily of opposite sign. In other words, dilatation motions are motions that change the proportions of the network-solution mixture. Despite the importance of this class of motions, almost all experimental work on the rheology of cytoplasm has been concerned with simple shearing motions that have no dilatational component whatsoever. An important exception to this statement is the socalled meniscus depletion assay (Rockwell et al., 1984) that involves separation of the network and solution phases by centrifugation. Unfortunately, even the meniscus depletion assay does not yield direct information on Λ_n and Λ_s because a number of other parameters can also have an important effect on the outcome.

In the absence of reliable experimental studies, the most we can do is to tentatively estimate that values of Λ_n will be of the same general order of magnitude as values of M_n (and likewise with Λ_s and M_s). Of course, this procedure is based on the dangerous supposition that strains caused by changes in composition are similar in type and magnitude to the strains caused by shear at constant composition.

The Solvation Coefficient, Υ. Strictly speaking, the solvation coefficient is not required input information for the reactive flow model (see section on momentum conservation). Nevertheless, it is desirable to discuss what is known of this parameter because it makes a contribution to the lumped coefficients Ψ_F and σ and because information on Υ is necessary in order to convert between the effective pressure and the solution pressure (see Eqs. 9a–9c).

The most direct way to estimate Υ is by means of osmotic pressure measurements. For such studies one requires a semipermeable membrane that will permit passage of all components of the solution phase (including G actin and other dissolved proteins) but which will not allow passage of the network phase. In the absence of contractile activity, the pressure jump required to prevent volume flux across such a membrane is $\Delta P_{os} = -\Upsilon \ln \theta_s$. For low network densities we can also use the approximate expression $\Delta P_{os} \approx \Upsilon\theta_n$.

Unfortunately, we know of no published osmotic pressure studies of contractile networks. Thus we are forced to consider indirect measurements of Υ. First let us consider the possibility that $\Upsilon < 0$. Negative values of Υ correspond to the existence of unfavorable solution-network interactions that tend to cause spontaneous phase separation. Thus, since spontaneous shrinkage of contractile network in the absence of contractile activity has never been reported, we conclude that Υ must be positive.

Positive values of Υ have the indirect effect of causing

spontaneous expansion or swelling of network material. Obviously, if such a tendency were large, it would have to be overcome, at the cost of much ATP, in order for network tension to produce contraction. Thus from a shamelessly teleological point of view, it can be argued that Υ "should" be as close to zero as possible. Serious evidence that this argument has some validity comes from the fact that, as with spontaneous shrinkage, spontaneous expansion of contracted network clumps has never been reported even though the size of such clumps has been followed for several days.

The Effective Contractile Stress, Ψ_F. The preceding discussion indicates that, at least in many cases, solvation stresses are negligible. If this is assumed, then $\Psi_F \sim \Psi(0)$ can be estimated from measurements of the force required to prevent a contractile network from moving.

If the volume fraction of the network is θ_n, if the cross-sectional area perpendicular to the axis of contraction is A, and if the force required to prevent contraction is F, then the contractile stress is obtained from the relation

$$\Psi_F \approx \frac{F}{A\theta_n}. \tag{17}$$

In the case of the contractile ring of dividing sea urchin eggs, all the information necessary to estimate Ψ_F is available. The force required to prevent contraction of the circumference is $\sim 3 \times 10^{-3}$ dyn; the cross section of the ring is $\sim 1.5 \times 10^{-8}$ cm^2; and the packing of filaments corresponds to $\theta_n \approx 0.1$ (see Hiramoto, 1978; Schroeder, 1975). Substituting these numbers into Eq. 17, we conclude that Ψ_F for the contractile ring is $\sim 10^6$ dyn/cm^2. A similar value of Ψ_F can be deduced from tension measurements on synthetic actomyosin threads (Sugino and Matsumura, 1983) and from the tension required to deform resting granulocytes (Evans and Kukam, 1984).

The filaments of the contractile ring probably represent an extreme level of contractile activity. Thus 10^6 can be taken as a practical upper limit on the value of Ψ_F, at least for nonmuscle cells. In less active contractile networks, Ψ_F could be lowered due to decreases in the activity of myosin caused by changes in calcium or other control factors. Thus zero is the only practical lower limit to the value of Ψ_F.

The Swelling Number, σ. As a network shrinks under the influence of contractile tensions, there can be changes in the net driving force for contraction due to solvation stresses and due to changes in the orientation and packing of network filaments. The strength of these countervailing factors, relative to the effective contractile stress, is quantified by the swelling number. For any positive value of the swelling number, there will be a unique network density at which the mechanical stresses tending to cause expansion and contraction of the network exactly balance. The network density of this point of mechanical equilibrium is given by the nonzero root of the transcendental equation

$$\hat{\theta}_n + \sigma[\hat{\theta}_n + \ln(1 - \hat{\theta}_n)] = 0. \tag{18a}$$

For a given value of the swelling number, mechanical stresses will favor expansion or constriction of a network depending on whether or not its density is greater or less than $\hat{\theta}_n$. Thus, $\hat{\theta}_n$ represents the practical upper limit of network density that can occur in the cytoplasm of an amoeboid cell. Typical values of $\hat{\theta}_n$ can be estimated from published electron macrographs of the hylan ectoplasm or cortex of various cells. Such estimates indicate that $\hat{\theta}_n$ is in the range of 0.1–10% by volume.

To convert estimates of $\hat{\theta}_n$ into estimates of σ, it is necessary to solve Eq. 18a. This could be done numerically, but fortunately a highly accurate algebraic approximation to the nonzero root of Eq. 18a can be obtained by the method of Pade' approximation. The analytic result is

$$\hat{\theta}_n \sim (1 + 2\sigma)/(1 + \sigma)^2. \tag{18b}$$

Comparison with numerical solutions demonstrates that Eq. 18b is accurate to within 7% for all positive values of σ. Furthermore, the percent error approaches zero as $\sigma \to 0$ and as $\sigma \to \infty$. Using Eq. 18b, it is easy to see that if $\hat{\theta}_n$ is in the range 10^{-3}–10^{-1}, then σ is in the range 10–1,000.

Hydraulic Conductivity, H_c. The various boundaries commonly encountered in biological systems can be divided into four groups with respect to hydraulic conductivity. The first group is comprised of surfaces that are totally impermeable to water ($H_c = 0$). Examples of such surfaces are planes of mirror symmetry or interfaces between cytoplasm and solid substances such as glass.

The second group of surfaces consists of lipid bilayers of various compositions. Water is able to pass through these barriers only if it diffuses through the lipid phase; consequently, the hydraulic conductivity of such membranes is very low, but not actually zero. The review by Anderson (1978) lists many reported measurements of conductivity for artificial membranes of different lipid composition; the values fall in the range 0.5×10^{-12} to 5.0×10^{-12} cm^{3}dyn^{-1}s^{-1}.

The third important group of boundary surfaces consists of the natural members of plants and animal cells. Such membranes typically contain small protein channels that allow water molecules to pass without entering the lipid. The hydraulic conduction of the membrane of the Alga, *Chara corallina*, is $1.2 \pm 0.2 \times 10^{-11}$ cm^{3}dyn^{-1}s^{-1} (Steudle and Tyerman, 1983). Remarkably, the conductivity of the human red cell is almost identical; $1.8 \pm 0.1 \times 10^{-11}$ cm^{3}dyn^{-1}s^{-1} (Terwillinger and Solomon, 1981). In kidney cortical collecting tubules, the hydraulic conductivity is controlled by antidiuretic hormone (ADH) (Frindt et al., 1982). In the presence of ADH the conductivity is 1–

5×10^{-11} $cm^3dyn^{-1}s^{-1}$ (i.e., a value similar to that of the red cell). In the absence of ADH the conductivity falls to a value of $\sim 0.8 \times 10^{-12}$ $cm^3dyn^{-1}s^{-1}$ (i.e., a value characteristic of a lipid bilayer with no channels).

A final important class of boundary surfaces is comprised of gel-sol (or endoplasmic-ectoplasmic) interfaces. The flow of water between a sol phase and a gel phase can be approximated if we replace the gel by an equivalent semipermeable membrane. The effective hydraulic conductivity of such an equivalent membrane can be estimated by considering the special case of the reactive flow model wherein the network is held stationary (i.e., $\Omega_n = 0$). When this is done, it is easy to show that the hydraulic conductivity of a uniform gel slab of thickness δl is $H_{cgel} = [\Phi\theta_n\theta_s\delta l]^{-1}$, where Φ is the drag coefficient and θ_n and θ_s are the volume fractions of network and solution in the gel. To obtain a reasonable estimate of H_{cgel}, we must let δl be $\approx \frac{1}{2}$ the characteristic path length that solution follows in percolating through the gel; we must let Φ be within the limits previously discussed, and we must take $\theta_n \approx \tilde{\theta}_n$. As an illustration, we take $\Phi \approx 10^9$ poise/cm^2, $\theta_n \approx 3 \times 10^{-3}$, and $\delta l \approx 3 \times 10^{-2}$ cm. From these numbers we calculate $H_{cgel} \sim 10^{-5}$ $cm^3dyn^{-1}s$. This result demonstrates the relative ease with which the solution phase can filtrate through gelled material, even over large distances.

The Effective Pressure of the External Solution, P_{ex}. This parameter is self explanatory. It should be realized, however, that as far as the observable behavior of the reactive flow model is concerned, only gradients of external pressure matter. Thus, in the most usual case where the external pressure is constant, the actual value of the pressure is completely irrelevant.

The Solution Slip/No-Slip Index, SLP_i. Given our long experience with flow of aqueous materials, the value of SLP_s is usually fairly obvious from the physical nature of the material comprising the reaction vessel wall. If a position of the boundary is formed by a rigid or highly viscous material, then the tangential boundary conditions on the solution should be of the no-slip type ($SLP_s = -1$). On the other hand, an interface with a fluid material (e.g., solution-air) or a place of mirror symmetry will be of the free-slip type ($SLP_s = +1$).

The Network Slip/No-Slip Index, SLP_n. In some circumstances it is easy to decide whether $SLP_n = 1$ or -1 at a particular boundary point. For example, in the case of a boundary formed by a plane or mirror symmetry, one must use free-slip conditions. On the other hand, it is very difficult to decide with confidence whether the inside of a cell membrane is slippery with regard to tangential sliding of contractile network or whether the network immediately adjacent to the inner surface of the membrane is held stationary. The answer depends on the degree of fluidity of the membrane and on the density, lateral mobility, and binding affinity of network anchoring sites on the inner surface of the membrane.

Network Stick/No-Stick Index, STK_n. Stick-type boundary conditions (i.e., $STK_n = +1$) imply that adhesive sites are present on the inner surface of the boundary and that these sites anchor a layer of network to the inner surface and prevent this boundary layer from moving in a perpendicular direction. It is important to remember that stick boundary conditions are not the same as no-slip conditions. It is perfectly possible for network to stick in the sense of motions perpendicular to the boundary and yet to be capable of slipping freely in directions tangential to the boundary. One can understand this distinction by thinking of adhesive sites that are anchored to the plane of a fluid lipid membrane and yet are capable of translation diffusion within this plane. Once again, we know of no way of telling in advance whether or not a particular boundary is sticky or not sticky towards the network phase.

Scaling Laws

In order to examine the scaling principles implicit in the reactive flow model, we must introduce nondimensional variables. Thus, if L_v is a characteristic linear dimension of the reaction vessel, then it is natural to express all distances in units of L_v. Similarly, we choose to introduce $(M_n + \Lambda_n)/\Psi_F$ as the natural unit of time, $(M_n + \Lambda_n)$ as the natural unit of viscosity, and $\Psi_F\tilde{\theta}_n$ as the natural unit of pressure. After making the appropriate substiutions and rearrangements, it is apparent that the scale-invarient formulation of Eqs. B1–B5 contains only seven nondimensional parameters (see Table II). Provided that the initial conditions and boundary conditions are not changed, any choice of the dimensional parameters in Table I that yield the same values of the nondimensional parameters in Table II will produce the same qualitative motion, although on a different distance and time scale.

Examination of Table II reveals that the size of the reaction vessel enters into only one of the nondimensional parameters, $[L_v^2\Phi/(M_n + \Lambda_n)]$. This is also the only nondimensional parameter in which the drag coefficient

TABLE II
NON-DIMENSIONAL PARAMETERS OF THE REACTIVE FLOW MODEL

1) Equilibrium network volume fraction	$\tilde{\theta}_n$
2) Contraction-reaction (C-R) number	$T_{eq}\Psi_F/(M_n + \Lambda_n)$
3) Relative solution shear viscosity	$M_s/(M_n + \Lambda_n)$
4) Relative solution dilatation viscosity	$\Lambda_s/(M_n + \Lambda_n)$
5) Relative network dilatation viscosity*	$\Lambda_n/(M_n + \Lambda_n)$
6) Rending number‡	$L_v^2\Phi/(M_n + \Lambda_n)$
7) Swelling number	σ

*Note that the relative network shear viscosity is not an independent parameter.

‡L_v is a characteristic linear dimension of the reaction vessel.

appears. We thus conclude that, according to our model, magnifying the size of the reaction vessel by a factor of 10 will produce the same nondimensional behavior as increasing the network-solution drag coefficient by a factor of 100. Superficially, this seems like a curious, but rather untestable prediction. Nevertheless, if we look at this scaling law from another point of view, we conclude that if the reaction vessel is very small, then the predicted behavior of the contractile network contained in the vessel will approach the behavior of a limiting special case of the reactive flow model, namely, the case $\Phi = 0$. We also observe that in this limiting case the nondimensional behavior of our model becomes independent of reaction vessel size.

To see how these considerations lead to an experimentally testable prediction, suppose that we cut a reaction vessel (e.g., a small cell) into a number of pieces such that each piece receives a proportional share of the original contents of the vessel. Our scaling arguments predict that if the original reaction vessel is sufficiently small and if the initial conditions and boundary conditions remain unchanged, then the behavior of the contractile network in the various parts will be precisely scaled miniature replicas of the behavior of the original system. This is quite remarkable, but we would also like to know exactly how small the reaction vessel has to get before this kind of invarience will emerge. Unfortunately, the answer to this question is not immediately apparent from scaling arguments alone.

An additional aspect of Table II that deserves some comment involves the scaling behavior of our model with respect to time. To see this behavior we need only note that increasing T_{eq} by a fixed percentage will produce exactly the same values of the nondimensional parameters as decreasing Ψ_F by the same percentage. Physically, this means that slowing the chemical reaction of network synthesis and breakdown is equivalent to increasing the strength of contractile activity. This is an interesting result, particularly when one considers the possible implications for controlling the behavior of contractile networks by changes in the rate of the chemical reaction.

Scaling laws such as the ones we have discussed are quite general and can be deduced with virtually no effort. Nevertheless, they obviously provide only a limited amount of insight into the predictions of a complex model. Another methodology for studying the reactive flow model is to consider approximate analytical treatments of various simplified special cases. Simplified cases that retain a maximum degree of realism can be derived by considering the general order of magnitude of the various nondimensional numbers in Table II. For example, if the viscosity estimates in Table I are somewhat reliable, then the nondimensional solution viscosities in Table II are both on the order of 10^{-5}. This suggests that we might try to simplify the reactive flow model by neglecting the viscosity of the solution phase (i.e., setting $M_s = \Lambda_s = 0$). This procedure yields a great reduction in complexity since it involves a decrease in the degree of a partial differential equation. An additional reduction in complexity can be achieved by assuming that flow occurs only along one spatial dimension, assuming that the network is highly dilute ($\tilde{\theta}_n \to 0$), and/or by neglecting swelling stress ($\sigma \to 0$). Yet another important special case arises if the characteristic time for chemical reaction (T_{eq}) is slow compared with the characteristic time for network motion $(M_n + \Lambda_n)/\Psi_F$. In this case the contribution of the chemical formation and breakdown of the network can be neglected (i.e., the contraction-reaction number $\to 0$). We, as well as some others, have managed to obtain a few analytical results relevant to some of these special cases (see, for example, Dembo et al., 1984; Alt, 1985). These results are interesting, but they are very limited, and it is difficult on the basis of such results to have a firm idea of how the reactive flow model really behaves under realistic circumstances.

CONCLUSION

The reactive flow model is an attempt to apply the concepts of multifield continuum mechanics to biological contractile networks. Throughout our discussion we have focused on physical issues of a fundamental sort. It seems likely that approaches of this kind are necessary if one is to understand the dynamical basis of cell motion. Nevertheless, the important details of how to resolve various technical issues are subject to considerable uncertainty (see Appendix A). Thus, while the reactive flow model seems inherently plausible, the model must still be rigorously tested and refined.

In order to test the reactive flow model, the first necessity is to devise a method of solving the rather formidable-looking system of equations summarized in Appendix B. Although analytic solutions and results are attainable in certain special cases, for the most part we must rely on numerical solutions. A method that we have devised for obtaining numerical solutions is described in the second paper of this sequence. In the third paper, we will present evidence that the reactive flow model is applicable to understanding the behavior of a simple biological system, i.e., isolated cytoplasm from amoeba and slime molds. In subsequent papers we expect to discuss the analysis of more complex systems.

APPENDIX A: CRITIQUE OF THE MODEL

The model of contractile network dynamics discussed in the body of this paper makes a number of simplifying assumptions. Some of the assumptions are of relatively little significance. Examples of such trivial assumptions include the use of a Taylor's series expansion to express the chemical reaction rate of network formation and breakdown, the use of the creeping flow limit of the momentum transport equations, and the assumption that both phases are of equal density. In addition to such relatively uncontroversial approximations, the treatment employs a number of important assumptions that deal with fundamental issues of physics and biology. Some important examples of this latter category of assumptions are (*a*)

the boundaries of the reaction vessel are stationary, (*b*) the cytoplasm contains only two phases, (*c*) the solution and network phases are "homogeneous," (*d*) the network and solution phases are "Newtonian fluids."

It is advisable to critically examine assumptions *a–d* with a view towards indicating the conditions where they should be expected to break down and what must be done to correct matters if such conditions are encountered.

(*a*) Stationary Boundaries

With few exceptions the motile activity of cells is accompanied by some degree of displacement of the cell membrane. Despite this, the assumption of fixed boundaries is warranted provided the boundary displacements are not a significant perturbation to the underlying cytoplasmic dynamics. For example, in the crawling locomotion of cells over flat surfaces, the cell membrane undergoes many rapid and seemingly random fluctuations in detailed configuration. Nevertheless, the motion of the cytoplasm inside a crawling cell and the resulting forces that drive locomotion can be studied by neglecting the wall fluctuations and assuming that the shape of the cell is constant, at least in so far as average behavior over large distances is concerned.

The assumption of fixed boundaries is inadequate if one is interested in fine details of cell membrane motion or if one is interested in very large motions that occur rapidly. To have confidence in a model of such motion, it would be necessary to understand in detail the rheology of the cell membrane. In addition, it would be necessary to describe the transmission of forces to the membrane from the cytoplasm and from external objects. Such forces are quite complicated because, at the minimum, a membrane that binds to the contractile network on its inner surface will experience inward contractile forces from the network, tangential viscous shear forces from the network and solution, and inward or outward forces produced by pressure gradients across the membrane.

(*b*) Two Phases

In addition to the solution and network phases, the cytoplasm frequently contains a third phase of matter that consists of microtubules, intermediate filaments, and other structural materials. The assumption of the present modeling approach is to view these structural materials as comprising a porous architectural scaffolding that has the rheology of an elastic or viscoelastic solid and that can be described by a continuous density distribution.

If θ_m is the fractional volume of the structural matrix phase, then, in the presence of structural matrix, the law of excluded volume takes the form

$$\theta_m + \theta_n + \theta_s = 1. \tag{A1}$$

In view of the structural role of the matrix, it is plausible to assume as a starting approximation that the flow velocity of the matrix material is negligible and that there is negligible assembly and disassembly of matrix material. If the matrix is also isotropic, then aside from Eq. A1, the only consequence of the presence of the structural matrix is to produce a frictional drag that slows the flow of the other two phases of the cytoplasm. Such a frictional drag will give rise to additional interphase forces of the form $-\Phi_{ms}\theta_m\theta_s\Omega_s$ and $-\Phi_{mn}\theta_m\theta_n\Omega_n$ in the momentum equations for the solution and network, respectively.

Homogeneous Composition

To understand the meaning of the assumption of homogeneity and the implications of inhomogeneous composition, let us start by studying the equation governing transport of the major raw material of the contractile network, i.e., G-actin. If we consider that G-actin is free to diffuse in the solution phase, then

$$\partial_t G = D_g\nabla^2(G/\theta_s) - \nabla\cdot G\Omega_s - \rho J(\theta_n, G, \cdot), \tag{A2}$$

where G is the macroscopic mass density of G-actin and ρ is the mass density of the cytoplasm. The various terms on the right side of Eq. A2 represent the difussion of G-actin in the solution phase, the convection of G-actin due to flow of the solution phase, and the consumption (or production) of G-actin due to formation (or breakdown) of contractile network. In the latter term we have explicitly indicated that the network formation rate will depend on G as well as on θ_n and on other variables.

If we want to solve for the motion of the cytoplasm, including the distribution of G-actin, then we must supplement Eqs. 17a–17c by adding Eq. A2 and solving the resulting system of simultaneous equations for the four scaler fields, θ_s, θ_n, G, and P; and for the two vector fields, Ω_s and Ω_n. Fortunately this is not usually necessary since under many conditions the solution phase of the cytoplasm will be well-mixed (i.e., homogeneous) so that significant changes in the distribution of G-actin do not occur. Thus, homogeneity really means that the solutions to Eq. A2 are of the simple form

$$G/\theta_s \approx \text{constant}. \tag{A3}$$

To see when Eq. A3 will be valid, we combine Eq. A2 with Eq. 6b to obtain

$$\partial_t g = \frac{1}{\theta_s}\partial_t G - \frac{G}{\theta_n^2}\partial_t\theta_s$$

$$= \frac{D_g}{\theta_s}\nabla^2 g - \Omega_s\cdot\nabla g - \frac{1}{\theta_s}J[\rho - g], \tag{A4}$$

where $g \equiv G/\theta_s$.

If L_V is a characteristic linear dimension of the reaction vessel, and if $\|\Omega\|$ is a characteristic flow speed of the solution phase, then using Eq. A4 it can be shown that a sufficient condition for homogeneity of G actin density is $D_g/L_V^2 + \|\Omega_s\|/L_V \gg \text{MAX}[J(1 - g/\rho)]$. Note that this inequality will be satisfied if J is not too different from 0 (i.e., if the assembly-disassembly reaction is never too far from equilibrium) or if $g \approx \rho$ (i.e., if the solution consists of almost pure G-actin). Homogeneity will also occur if the flow of solvent is very fast (i.e., convective mixing) or if the size of the reaction vessel is small (diffusive mixing).

Let us next consider the equations governing transport of a substance, X, that unlike G-actin is not a major structural component of the contractile network. To be specific, X could be a substance like calcium ion that causes phosphorylation of myosin and thus influences the strength of contractile activity. Alternatively, X could be a molecule-like filamin that causes crossbridging of actin filaments and thus influences the network viscosity. In general, any or all of the nine coefficients in Table I could be functions of X.

As a general rule, X will not be restricted to one phase of the cytoplasm but will be able to reversibly partition between the network and solution. Taking this into account, it is easy to show that if X_n and X_s are the macroscopic mass densities of X contained in the network and solution phases respectively, then

$$\partial_t X_n = D_n\nabla^2(X_n/\theta_n) - \nabla\cdot\Omega_n X_n + k_B\theta_n X_s - k_r\theta_s X_n \tag{A5a}$$

and

$$\partial_t X_s = D_s\nabla^2(X_s/\theta_s) - \nabla\cdot\Omega_s X_s - k_B\theta_n X_s - k_r\theta_s X_n, \tag{A5b}$$

where k_B and k_r are the rate constants for binding and release of X from the network, and D_n and D_s are the diffusion constants of X in the network and solution phases.

To be rigorous, one should solve Eqs. A5a and A5b simultaneously with the five fundamental equations of the reactive flow model (i.e., Eqs. B1–B10). However, if the assumption of homogeneity holds, then the solutions of Eqs. A5a and A5b will obey the relations

$$X_n/\theta_n \approx K_p X_s/\theta_s \approx \text{constant}, \tag{A6}$$

where $K_p \equiv k_b/k_r$ is the partition coefficient of X. Equation A6 implies that X_n and X_s are simply proportional to θ_n and θ_s, respectively. Thus if A6 holds, X_n and X_s need not be regarded as independent dynamical variable.

To see when the solutions of Eqs. A5a and A5b satisfy Eq. A6, we combine A5a and A5b with Eqs. B2 and B3 to obtain

$$\partial_t x_n = \theta_n^{-1} D_n \nabla^2 x_n - \Omega_n \cdot \nabla x_n + [k_B x_s - k_r x_n - J x_n/\theta_n\theta_s]\theta_s \quad \text{(A7a)}$$

$$\partial_t x_s = \theta_s^{-1} D_x \nabla^2 x_s - \Omega_s \cdot \nabla x_s - [k_B x_s - k_r x_n - J x_s/\theta_n\theta_s]\theta_n \quad \text{(A7b)}$$

where $x_s = X_s/\theta_s$, and $x_n = X_n/\theta_n$.

These equations show that a sufficient condition for homogeneity with respect to X_n and X_s is that partitioning of X between the phases be fast compared to the assembly and dissembly reactions: $\mathrm{MIN}(k_B, k_n) \gg \mathrm{MAX}(J/\theta_n\theta_s)$.

If this condition is violated, then homogeneity might still hold separately in one or the other of the phases if diffusion plus convection in this phase were sufficiently fast. In such a case the condition for homogeneity is entirely analagous to the previously derived condition for homogeneity of G-actin in the solution phase.

In conclusion, homogeneity is a reasonable assumption under a variety of conditions, but not under all conditions. If inhomogeneities occur, then one must supplement the basic equations of excluded volume, mass conservation, and momentum conservation with equations describing the transport of the inhomogeneous material.

Non-Newtonian Rheology

For our purposes a Newtonian fluid can be defined as an isotropic material in which the stress is a linear function of the rate of strain. In the case of the solution phase, the model of a Newtonian fluid is almost certainly adequate. On the other hand, various experiments on whole cytoplasm have detected evidence of non-Newtonian rheology in the network phase (Taylor and Condeelis, 1979). Non-Newtonian behavior of the network could arise because of a dependence of the stress on the past history of deformation (as in the Maxwell fluid); nonlinear dependence of stress on strain (as in a pseudoplastic fluid) or because of nonrandom orientation of network filaments (anisotropic fluid or liquid crystal). A detailed discussion of the constitutive laws of the Maxwell fluid, the pseudoplastic fluid, and other non-Newtonian fluids is beyond the scope of this appendix but can be found elsewhere (e.g., Schowalter, 1978).

For the present, we do not feel that there is much basis for the claim that non-Newtonian effects are important in understanding the physiological function of contractile networks. Furthermore, non-Newtonian models are difficult to analyze, and it is even more difficult to obtain realistic estimates of the rheological coefficients in the models. Thus, we defend the simplicity of our assumption about network rheology, but we recognize that a more complex approach may eventually be justified.

APPENDIX B: SUMMARY OF EQUATIONS

In this appendix we summarize the various dynamical equations that constitute the reactive flow model of contractile networks. The equations are:

the excluded volume relation

$$\theta_n + \theta_s = 1; \quad \text{(B1)}$$

the mass conservation equations

$$0 = \nabla \cdot [\theta_s \Omega_s] + \nabla \cdot [\theta_n \Omega_n] \quad \text{(B2)}$$

and

$$\partial_t \theta_n = -\nabla \cdot [\theta_n \Omega_n] + [\tilde{\theta}_n - \theta_n]/T_{eq}; \quad \text{(B3)}$$

the momentum conservation equations

$$0 = \nabla[\Lambda_s \theta_s \nabla \cdot \Omega_s] + 2[\nabla \cdot M_s \theta_s \nabla]\Omega_s + \nabla \times [M_s \theta_s \nabla \times \Omega_s] + \Phi\theta_s\theta_n(\Omega_n - \Omega_s) - \theta_s \nabla \mathrm{P}_F \quad \text{(B4)}$$

and

$$0 = \nabla[\Lambda_n \theta_n \nabla \cdot \Omega_n] + 2[\nabla \cdot M_n \theta_n \nabla]\Omega_n + \nabla \times [M_n \theta_n \nabla \times \Omega_n] + \Phi\theta_s\theta_n(\Omega_s - \Omega_n) - \theta_n \nabla \mathrm{P}_F + \nabla\{\Psi_F[\theta_n + \sigma(\theta_n + \ln \theta_s)]\}; \quad \text{(B5)}$$

the tangent boundary conditions on solution and network

$$(1 + \mathrm{SLP}_s)(\mathbf{n} \cdot \nabla)(\Omega_s \cdot \mathbf{t}) + (1 - \mathrm{SLP}_s)(\Omega_s \cdot \mathbf{t}) = 0 \quad \text{(B6)}$$

and

$$(1 + \mathrm{SLP}_n)(\mathbf{n} \cdot \nabla)(\Omega_n \cdot \mathbf{t}) + (1 - \mathrm{SLP}_n)(\Omega_n \cdot \mathbf{t}) = 0; \quad \text{(B7)}$$

the normal boundary conditions on the solution and network

$$\mathbf{n} \cdot \Omega_s = H_c(\mathrm{P}_F - \mathrm{P}_{ex}) \quad \text{(B8)}$$

and

$$0 = (1 + \mathrm{STK})(\mathbf{n} \cdot \Omega_n) + (1 - \mathrm{STK}) \cdot (\Lambda_n \nabla \cdot \Omega_n + 2M_n(\mathbf{n} \cdot \nabla)(\mathbf{n} \cdot \Omega_n) + \Psi_F); \quad \text{(B9)}$$

and finally, the boundary condition on the network density

$$(1 + \mathrm{STK})(\mathbf{n} \cdot \nabla\theta_n) + (1 - \mathrm{STK})\theta_n = 0. \quad \text{(B10)}$$

The authors with to thank Wolfgang Alt of the University of Heidelberg and George I. Bell of Los Alamos National Laboratory for useful comments, criticisms, and discussions.

This work was supported by National Institutes of Health grant R01-AI21002-01 and by National Institutes of Health Research Career Development Award K04-AI00966-02 to Dr. Dembo. This work was also supported by the United States Department of Energy.

Received for publication 26 February 1985 and in final form 22 January 1986.

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