PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Cytoplasmic Structure and Contractility: The Solation-Contraction Coupling Hypothesis [and Discussion]

D. L. Taylor, M. Fechheimer and D. M. Shotton

Phil. Trans. R. Soc. Lond. B 1982 **299**, 185-197
doi: 10.1098/rstb.1982.0125

Phil. Trans. R. Soc. Lond. B **299**, 185–197 (1982)
Printed in Great Britain

Cytoplasmic structure and contractility: the solation–contraction coupling hypothesis

By D. L. Taylor† and M. Fechheimer
Department of Cellular and Developmental Biology, Harvard University,
16 *Divinity Avenue, Cambridge, Massachusetts* 02138, *U.S.A.*

We have briefly described our studies of cytoskeletal and contractile elements in intact cells, in cell extracts, and in mixtures of purified proteins. Changes in the concentration of calcium and of protons have been found to modulate both gelation and contraction in all of these preparations. The distribution of calcium, protons and actin has been studied in intact amoeboid cells. Using these results, we have refined our working model of the relation of cytoskeletal and contractile proteins: the solation–contraction coupling hypothesis. The model is also supported by quantitative analysis of the rates of contraction in a soluble extract of *Dictyostelium discoideum* amoebae allowed to gel in a capillary and stimulated by the addition of calcium ions at one end. A plausible interpretation of the most prominent cytological features of amoeboid locomotion is obtained by application of the principles of our model. In addition, we propose that the solation–contraction coupling hypothesis may be useful in further study of a variety of motile phenomena observed in many types of cells.

1. Introduction

Any concept proposed to explain the mechanism of amoeboid cell movement must be based on spatial and temporal information about the distribution of contractile proteins and potential secondary messengers in cells. We present a brief review of our efforts to understand the consistency and contractility of cytoplasm, to isolate and to characterize cellular constituents that contribute to cell shape and motility, and to understand how the function of these components is modulated spatially and temporally to produce movement. The solation–contraction coupling hypothesis has evolved as a working model describing our present view of the relation between cytoplasmic structure and contractility in amoeboid cells. The experiments and ideas reported in this paper have been produced during collaboration and interaction with R. D. Allen, J. S. Condeelis, P. Moore, S. Hammond, J. Rhodes, S. Hellewell, Y.-L. Wang, J. Heiple, M. Rockwell, J. Brier and P. L. McNeil.

Our laboratory has employed several fluorescence techniques to study the question of cytoplasmic structure and contractility at the levels of pure proteins, crude cell extracts and living cells. The first stage of this approach involved the preparation of a functional fluorescent derivative of actin (Wang & Taylor 1980). The molecular details of actin structure and assembly have been investigated initially by measurement of resonance energy transfer (Taylor *et al.* 1981; Wang & Taylor 1981). Resonance energy transfer and photobleaching techniques have also been used to define the effects of various actin-associated proteins on the structure and assembly of actin. Furthermore, the fluorescent analogues of actin have been employed in studies with cell extracts and with living cells (fluorescent analogue cytochemistry). The preparation of fluorescent derivatives of actin-binding proteins will facilitate the study both of the interaction of these proteins with actin *in vitro*, and of their distribution and function in cells.

† Present address: Department of Biological Sciences, Center for Fluorescence Research, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213, U.S.A.

2. Consistency and contractility of cytoplasm *in vitro*

Earlier studies of cytoplasmic structure employed intact cells. The existence of structural elements in cytoplasm was inferred from measurements of the Brownian motion of particles in cells, from the velocity of movement of particles during cytoplasmic streaming, from the movement of particles subjected to centrifugal or magnetic force, and by physical perturbation induced with microneedles. The presence of both anisotropic and isotropic arrays of fibrous elements in cells was also demonstrated direct by the use of light and electron microscopic techniques (see Allen (1961) and Taylor & Condeelis (1979) for reviews).

The preparation of single-cell models facilitated experimental analysis of the properties of cytoplasm. The observation of streaming of cytoplasm in *Chaos carolinensis* amoebae ruptured in capillaries showed that structural and contractile elements of cells could be investigated in isolated cytoplasm (Allen *et al.* 1960). It was subsequently shown that the behaviour of cytoplasm isolated from these cells is sensitive to the free [Ca^{2+}] of the buffer in which it is isolated (Taylor *et al.* 1973). Cytoplasm isolated in the presence of 10 nM free Ca^{2+} is non-motile, gelated, and optically isotropic. Cytoplasm subjected to stress formed anisotropic actin-containing fibrils, presumably through the alignment of randomly oriented cross-linked filaments present in the cytoplasm. The birefringence of the stressed preparations did not return completely to the original value after release of the fibrils, suggesting that gelated cytoplasm is viscoelastic (Taylor *et al.* 1973). Elevation of the free [Ca^{2+}] of the solution to *ca.* 1 μM caused rapid shortening of the anisotropic fibrils, streaming of cytoplasm, and extension of pseudopodium-like structures in the isotropic cytoplasmic droplets (Taylor *et al.* 1973).

The preparation of soluble extracts from large quantities of cells allowed more detailed analysis of the structural and contractile properties of cytoplasm (Thompson & Wolpert 1963; Kane 1975). Extracts from *Dictyostelium discoideum* formed a stable, non-motile and isotropic gel when warmed to room temperature if prepared in the presence of *ca.* 10 nM free Ca^{2+}, and a pH of 6.8–7.0. Elevation of the free [Ca^{2+}] or the pH of the extract induced contraction. However, if the free [Ca^{2+}] or the pH of the extract was increased before warming, the extent of gelation was decreased and the warmed extracts exhibited a superprecipitation without forming a contracted mass. Force-producing contraction apparently required the presence of at least a partly structured gel to transmit tension (Taylor *et al.* 1977; Condeelis & Taylor 1977). It was also shown that factors that form a gel when mixed with actin are present in significant quantity in the fraction that is soluble after sedimentation of the contracted mass (Condeelis & Taylor 1977). Removal of myosin from the extract of *D. discoideum* allowed the study of gelation in the absence of contraction (Condeelis & Taylor 1977; Hellewell & Taylor 1979). Solation was induced by elevation of the free [Ca^{2+}], or elevation of the pH in the myosin-free fractions. The same treatments induced contractions in preparations to which purified *D. discoideum* myosin had been added. Since conditions that induced solation in the absence of myosin induced contraction when myosin was present, it was postulated that the maximally gelated state might inhibit active contraction (Condeelis & Taylor 1977; Hellewell & Talyor 1979).

Lysed cell preparations and soluble extracts also formed an isotropic gel after warming in microscope observation chambers (Taylor *et al.* 1976, 1977) or in glass capillaries (Taylor 1977). Addition of a solution containing *ca.* 1 μM free Ca^{2+} to the preparation induced contraction, judged by formation of anisotropic fibrils at the interface of the extract with the solution. These fibrils shortened while forcing less structured cytoplasm away from the contracting

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

mass (Taylor *et al.* 1976, 1977). It has been postulated that the decrease in gel structure induced by calcium is related to the initiation of contraction of the extract (i.e. contraction is coupled to solation).

More recently, we have attempted to determine the rate and location of contracting regions in the *D. discoideum* extract. The extract was allowed to gel in a glass capillary, and then stimulated by addition of a solution containing *ca.* 1 μM Ca^{2+} to one end. To allow visualization

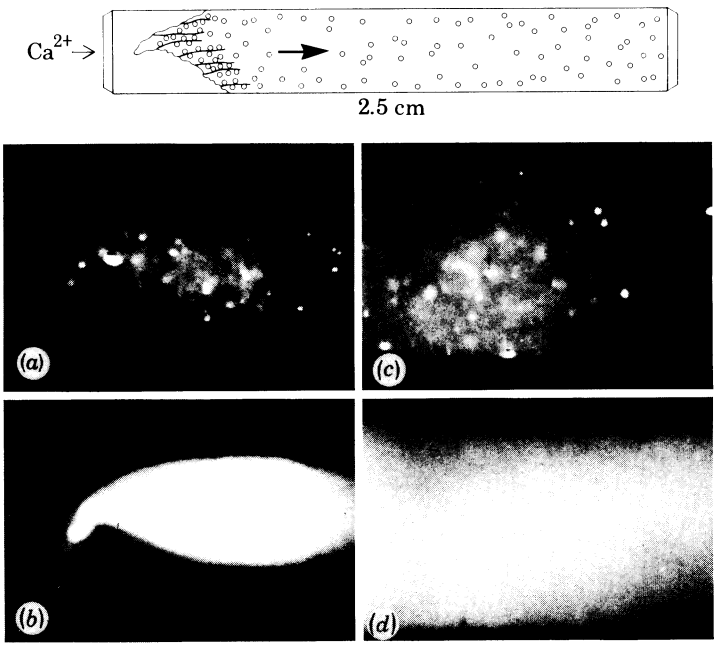


FIGURE 1. Observation of gelation and contraction of an extract of *D. discoideum* in glass capillaries. Glass capillaries 2.5 cm in length were filled with an extract of *D. discoideum* containing rhodamine-labelled beads and fluorescein-labelled actin at 4 °C. The extract was warmed to room temperature for 20 min to allow gelation. A solution containing 1 μm free Ca^{2+} was applied to one end of the capillary as shown. The extract contracted and moved away from the side of the capillary to which calcium had been added. Contraction, assessed by the rate of movement of single beads or clusters of the rhodamine-labelled beads (*a*), and the fluorescein-labelled actin (*b*), occurred at the end to which calcium had been added. In contrast, the rhodamine-labelled beads (*c*), and the fluorescein-labelled actin (*d*) at the end of the capillary opposite to that to which calcium was added offered no indication of contraction.

of actin in the extract, we added actin labelled with fluorescein to a final concentration of 0.1 mg ml^{-1} (AF-actin) (Wang & Taylor 1980) to the extract before allowing it to gel in the capillary. To allow measurement of the rate of shortening in various regions of the capillary, rhodamine-labelled beads (0.46 μm in diameter, fluoresbrite microspheres, Polysciences, Warrington, Pennsylvania) were also added to the extract before gelation. Before the addition of calcium, the AF-actin and the rhodamine beads were uniformly distributed in the gelated extract, and no shortening was detected. After the addition of calcium to one end of the capillary, the gelated extract contracted and moved away from the end to which calcium had been added. Formation of distinct actin fibrils (fluorescein fluorescence) and an increase in the density of the rhodamine-labelled beads were observed in the contracting region of the capillary (figure 1*a*, *b*). The rate of movement of the beads was measured by use of videotape recordings

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of the beads in various regions of the capillary (figure 2). Beads located at the end to which calcium was added moved away from that end, while no movement of beads in the central and distal regions of the capillary was detected (figure 1*c*, *d*; figure 2). In the first 9 min after addition of calcium, the interface of the gelated extract with the calcium solution moved 700 μm from its initial position at the end of the capillary. No shortening was detected in central and distal regions of the capillary during this experiment. This experiment is consistent with the interpretation that contraction, assessed by movement of non-specifically attached or trapped beads, occurred in the region of the gelated extract whose consistency had been decreased by the presence of elevated free [Ca^{2+}]. A statement and discussion of the solation–contraction coupling hypothesis is presented in § 5.

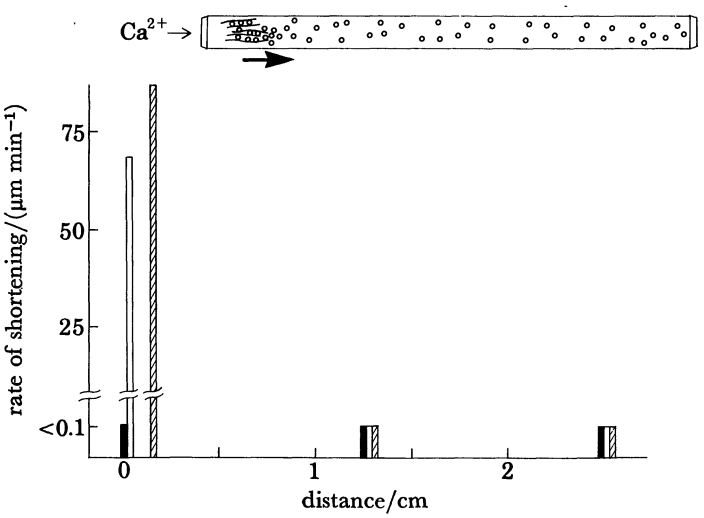


FIGURE 2. Measurement of the rate of contraction of an extract of *D. discoideum*. The rate of movement of the rhodamine-labelled beads was measured by the use of videotape recordings in various regions of the capillary. Before the addition of calcium, no movement of the beads was detected anywhere along the capillary (solid bars). Three minutes after the addition of the calcium, both the movement of the beads and the formation of actin fibrils was observed at the site of addition of calcium (open bars). However, no shortening was measured in the middle or at the opposite end of the capillary at this time (open bars). Nine minutes after the addition of the contraction solution, movement of the beads was measured at the interface of the gelated extract with the solution *ca.* 700 μm from the site of addition of calcium to the end of the capillary (hatched bars). However, movement was still undetected in the middle or the opposite end of the capillary.

3. POTENTIAL SECONDARY MESSENGERS AND ACTIN: SPATIAL AND TEMPORAL INFORMATION IN LIVING CELLS

Observations of the behaviour of cytoplasm in cell extracts have stimulated further analysis of cytoplasm in intact cells. Because changes in the concentration of either calcium or of protons in cell extracts appear to regulate the viscoelasticity and contractility of these preparations, the effect of micro-injection of solutions with a pH of 7.5 or containing *ca.* 1 μM Ca^{2+} into *C. carolinensis* amoebae was determined (Taylor 1977). Microinjection of small volumes of a solution containing *ca.* 1 μM free Ca^{2+} caused immediate strong contraction if delivered to the ectoplasm, moderate contraction and cytoplasmic streaming if placed in the anterior endoplasm, and relatively weak contraction in the uroid endoplasm. The injection of a relatively

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

large volume of 1 μM free Ca^{2+} caused the cytoplasm to contract into a central mass, leaving the plasma membrane intact at the cell periphery. Injection of a 'relaxation solution' (5 mM EGTA with no calcium at pH 7.0) caused cell rounding, cessation of movement, and loss of differentiation between endoplasm and ectoplasm. Injection of the same solution adjusted to pH 7.5 induced contractions similar to those observed after the injection of 1 μM free Ca^{2+} (Taylor 1977).

To investigate the potential physiological role of calcium and protons in the regulation of the consistency and contractility of cytoplasm during amoeboid movement, the concentrations of these ions have been measured in locomoting cells. Aequorin luminescence (Blinks 1978) has been used as an indicator of the free [Ca^{2+}] in single *C. carolinensis* amoebae (Taylor *et al.* 1980*a*). Luminescence measured from whole cells by using a photon-counting system attached to the microscope (Allen & Blinks 1979; Blinks *et al.* 1979) was characterized by a continuous luminescence that varied between 5 and 10 times the dark current of the photomultiplier, and by intermittent pulses of luminescence of variable amplitude. The two types of luminescence were localized by use of a four-stage image intensifier (Reynolds 1968). The continuous luminescence was localized in the tails of actively motile cells, and the intermittent pulses occurred primarily over the anterior regions of cells. The pulses of luminescence were sometimes correlated with extension of pseudopods, and both the pulses of luminescence and the extension of pseudopods ceased upon removal of extracellular calcium. By measurement of the luminescent signal as a function of the free [Ca^{2+}], it was shown that the free [Ca^{2+}] increased to not less than *ca.* 0.1 μM during amoeboid movement (Taylor *et al.* 1980*a*).

Cytoplasmic pH has been determined by measuring the fluorescence of fluorescein thiocarbamyl-ovalbumin injected into single specimens of *C. carolinensis*. Since the excitation spectrum of fluorescein is pH-sensitive, the cytoplasmic pH may be determined by measuring the ratio of fluorescence emission following excitation at two different wavelengths. The ratio of fluorescence intensity was related to the pH by use of a standard curve generated *in situ* (Heiple & Taylor 1980). The average cytoplasmic pH in *C. carolinensis* amoebae was 6.75 ± 0.3. The pH in different amoebae ranged from 6.3 to 7.4. Measurements of pH in pseudopod tips and in tails of locomoting amoebae are not significantly different at the level of spatial (aperture of 50 μm) and temporal (1.3 s) resolution available in this study.

These studies of intact cells are consistent with our studies of the effects of calcium and pH on cell extracts and purified proteins. The average cytoplasmic pH (6.75) is in the range of pH at which regulation of solation and contraction by calcium has been observed (see above). Thus, changes in the free [Ca^{2+}] to not less than *ca.* 0.1 μM could control the structure and contractility of cytoplasm. It is necessary to determine whether fluctuations in cytoplasmic pH occur during amoeboid movement, and whether the effects of calcium and protons are independently or coordinately controlled and expressed.

Observations on the distribution and function of cytoskeletal and contractile proteins in cell extracts and reconstituted systems must also be extended by the study of intact cells. Immunofluorescence microscopy has been used for this purpose, although results obtained with this approach may be affected by fixation, extraction, the availability of antigenic determinants, and variations in path length or accessible volume in different regions of cells. An alternative approach is to prepare a functionally competent fluorescent analogue of the protein under investigation, and to observe its distribution after microinjection into single cells (Taylor & Wang 1978, 1980; Wang *et al.* 1982*a*). The distribution of 5-iodoacetamidofluorescein-labelled

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

actin in living *C. carolinensis* amoebae has been determined in this way (Taylor *et al.* 1980*b*). The images of AF-actin were compared with the images of ovalbumin labelled with rhodamine, to evaluate the effects of path length and accessible volume on the observed distribution. Local increases in fluorescence intensity suggestive of the presence of bundles or fibrils of actin were observed in the ectoplasm in the tails of these cells, and in the plasmagel sheets in the pseudopods of locomoting amoebae. Yet the most fundamental result of this analysis is that actin is rather uniformly distributed in these actively motile cells. A uniform distribution of actin and myosin has also been observed by the use of immunofluorescence microscopy in motile HeLa cells (Herman *et al.* 1981).

The mobility of the fluorescent analogue of actin has been characterized in *C. carolinensis* by fluorescence photobleaching recovery experiments (Lanni & Ware 1982; Wang *et al.* 1982*b*). Some 90% of the actin has a mobility one half to one eighth of that of G-actin in aqueous solution (high-mobility fraction), and approximately 10% of the actin has a mobility comparable with that of F-actin in aqueous solution (low-mobility fraction). Therefore no more than 10% of the actin in the cytoplasm can exist as static filaments. The highest proportion of low-mobility actin was measured in the tails of motile cells. Actin associated with isolated plasma membranes (Taylor *et al.* 1976) had the lowest mobility. These results are consistent with the qualitative observation of distinct actin structures primarily in the tails of amoebae.

In addition, the continuous aequorin luminescence was identified in the tails where both distinct actin fibrils and a low-mobility fraction of actin were observed. The pulses of aequorin luminescence were also detected primarily at the tips of pseudopods where we identified transient actin fibrils in the plasmagel sheets. The correlation of the spatial and temporal distributions of a potential secondary messenger (calcium) and of actin supports the hypothesis that changes in functional activity and organization of contractile proteins in cells are induced by fluctuations in concentrations of secondary messengers.

4. Cytoskeletal and contractile proteins in *D. discoideum*

The above observations on the fundamental properties of cytoplasm naturally lead to an interest in the individual components of the cytoskeleton. In this section we review the growing list of proteins isolated from *D. discoideum* amoebae that may participate in changes in consistency and contractility of the cytoplasm of these cells. Actin isolated from *D. discoideum* amoebae is similar in physical and chemical properties to rabbit skeletal muscle actin (Uyemura *et al.* 1978). *Dictyostelium* myosin is composed of polypeptides with apparent molecular masses of 210, 18 and 16 kDa, forms bipolar thick filaments at low ionic strength, and exhibits activation of its ATPase activity in the presence of actin (Clarke & Spudich 1974). Both the 18 and 210 kDa polypeptides of *Dictyostelium* myosin are phosphorylated. Phosphorylation of the heavy chain of *Dictyostelium* myosin modulates both its ability to form thick filaments and its actin-activated ATPase activity (Kuczmarski & Spudich 1980). The contracted mass that forms upon the addition of calcium to a gelated extract contains 34% of the actin and 90% of the myosin present in the soluble extract of *D. discoideum* amoebae (Condeelis & Taylor 1977; Hellewell & Taylor 1979).

A number of actin-binding proteins have also been isolated from *Dictyostelium*. A 120 kDa protein has been shown to induce a large increase in viscosity when added to a solution of rabbit skeletal muscle actin (Condeelis 1981*a*, *b*). It has been suggested that this protein cross-links actin filaments in the cortical region of *Dictyostelium* amoebae (Condeelis *et al.* 1981). Two

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

fractions isolated from the contracted pellet fraction of *Dictyostelium* amoebae were shown to increase the viscosity of a solution of rabbit skeletal muscle actin in the presence of *ca.* 10 nM free Ca^{2+}, but not at elevated free [Ca^{2+}] (Hellewell & Taylor 1979). These properties are also demonstrated by purified 30 and 95 kDa proteins isolated from these cells (Taylor *et al.* 1982; Fechheimer *et al.* 1982). A decrease in the viscosity of mixtures of the 95 kDa protein with actin is induced by a free [Ca^{2+}] as low as 0.1 μM at pH 7.0. In addition, a significant increase in the apparent viscosity of a solution of actin induced by the purified 95 kDa protein is observed at pH 6.7, but not at pH 7.2. The 95 kDa protein bears a structural resemblance to α-actinin (Burridge & Feramisco 1981), since it is a dimeric, rod-shaped molecule with a length of 38 nm and a Stokes radius of 7.4 nm. Reversible interaction of this protein with actin to form a cross-linked network of actin filaments is explained by the fact that binding of the dimeric molecule to actin assessed by co-sedimentation with actin is observed in the presence of *ca.* 10^{-8} M, but not *ca.* 1 μM free Ca^{2+}. The 95 kDa protein does not restrict the length of actin filaments in the presence of *ca.* 1 μM free Ca^{2+} (Fechheimer *et al.* 1982).

A 40 kDa protein isolated from *D. discoideum* amoebae does restrict the length of actin filaments in the presence of calcium (Brown *et al.* 1982). Other proteins with this activity, such as gelsolin and villin, have been shown to decrease the viscosity of mixtures of actin with filamin from macrophages or chicken gizzard (Yin *et al.* 1980; Nunnally *et al.* 1981).

A partly purified preparation from *Dictysotelium* that contains a prominent polypeptide with a molecular mass of 47 kDa has dramatic effects on the viscosity, polymerization, depolymerization and reannealing of actin filaments (Taylor *et al.* 1982). This molecule may be functionally similar to the actin filament capping protein isolated from *Acanthamoeba castellanii* (Isenberg *et al.* 1980).

Calmodulin has also been isolated from *D. discoideum* (Bazari & Clarke 1981). This calmodulin is structurally, functionally and antigenically similar to mammalian calmodulin, although its precise function in *Dictyostelium* has not been defined.

The problem of defining the complex interactions of these and other as yet unidentified components of the contractlie–cytoskeletal apparatus of these cells will be quite challenging. Quantitative fluorescence studies should help to define the molecular interactions in reconstituted contractile systems. Description of the interaction of these molecules *in vitro* provides a foundation for speculation and study of their interactions in cells.

5. The solation–contraction coupling hypothesis

The basic ideas leading to the solation–contraction coupling hypothesis were presented by Taylor *et al.* (1977) and Condeelis & Taylor (1977). The detailed hypothesis has been developed in several succeeding papers (Hellewell & Taylor 1979; Taylor *et al.* 1979; Condeelis 1981*a*, *b*; Taylor & Fechheimer 1982). The postulates of the hypothesis are presented below, and discussed in relation to experimental results described in the preceeding sections.

1. The gel is a significant structural component of cytoplasm that consists of actin filaments cross-linked by one or more types of actin-binding proteins.

2. The gel, in its most rigid form, is an antagonist to contraction.

3. The gel may be weakened by dissociation of one type of cross-linking protein from actin, or by the action of proteins that restrict the length of actin filaments in the gel, or both.

4. A weakened (partly solated) gel cannot inhibit contraction, and aids in transmitting the tension during contraction.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

5. Contractions are initiated selectively in regions of decreasing gel structure.

6. Solation–contraction is a 'self-destructing process'. Extensive contraction releases a fraction of the cytoskeletal-contractile proteins into the soluble pool.

A schematic drawing illustrating some of the concepts of the solation–contraction coupling hypothesis is shown in figure 3. The gel is represented by a random array of actin filaments cross-linked by both extended, flexible, calcium-insensitive (filamin type) and rod-shaped calcium-sensitive (α-actinin type) actin-binding proteins. This most rigid form of the gel is

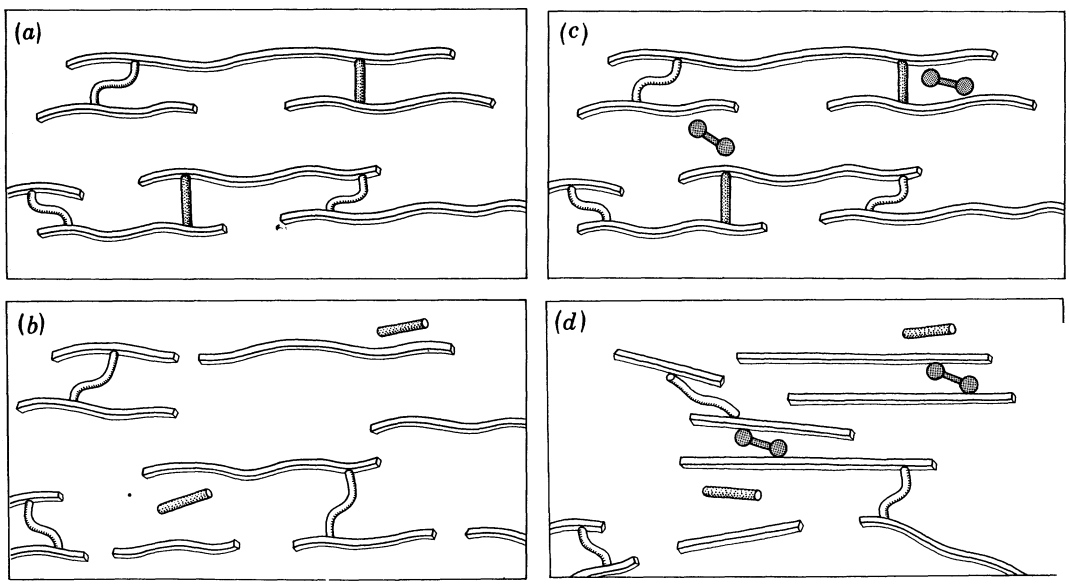


FIGURE 3. Schematic drawings illustrating some of the concepts of the solation–contraction coupling hypothesis. Actin filaments, represented by the long bars, are cross-linked to form a gel by both flexible, calcium-insensitive (filamin type), and rod-shaped, calcium sensitive (α-actinin type) actin-binding proteins (*a*). Solation of the gel, in the absence of myosin, occurs by two possible mechanisms. In the presence of elevated free [Ca^{2+}], the calcium-sensitive actin-binding proteins are dissociated from actin, and the lengths of the actin filaments are shortened by the effect of factors such as villin and gelsolin (*b*). The gel with myosin present (dumb-bell shaped structures) is non-motile (*c*). Solation of the gel in the presence of myosin reduces the antagonistic action of the gel on contraction. The resistance to shortening in the solating gel decreases, and contraction occurs (*d*).

not motile in either the presence of absence of myosin. Myosin is represented by dumb-bell shaped structures, although its state of assembly and activation (i.e. stoichiometry of phosphorylation of both heavy and light chains) is not presumed. Study of myosin-depleted fractions has shown that elevation of the free [Ca^{2+}] induces a local and partial decrease in gel structure. Two mechanisms of solation may be involved. Activation by calcium of factors such as gelsolin and villin, which decrease the length of actin filaments, solates the gel by causing a change in the ratio of cross-links to filaments (Yin *et al.* 1980; Nunally *et al* 1981). In addition, dissociation of calcium-sensitive actin-binding proteins on addition of calcium decreases the number of cross-links (figure 3). The relative contribution of these two potential mechanisms of solation has not been determined.

During local and partial solation induced by addition of calcium to the myosin-containing gel, contraction is initiated. Contraction, measured by the rate of movement of rhodamine-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

labelled beads, and by the formation of actin-containing fibrils, occurs at the site of the solation, and not in highly gelated domains distant from the site of application of calcium (figure 2). We speculate that cross-links present in the partly solated gel, which serve to transmit tension during contraction, are predominantly the calcium-insensitive (filamin) type, since these molecules remain bound to actin in the presence of calcium.

The progressive action of proteins that 'cut' actin filaments leads to release of a portion of the actin and actin-binding proteins from the contracting mass. This aspect of the proposed solation–contraction process could help to explain why large transcellular redistributions of actin are not characteristic of cell movements such as cytokinesis or amoeboid locomotion (Wang & Taylor 1979; Taylor *et al.* 1980*b*).

The solation–contraction coupling hypothesis differs from the 'tug-of-war', or gelation–contraction hypothesis (Stendahl & Stossel 1980; Hartwig *et al.* 1980; Stossel *et al.* 1979). This latter hypothesis is based primarily on studies of reconstituted mixtures of actin, myosin, filamin and gelsolin placed in capillaries. Contraction of these mixtures was not inhibited by formation of a gel. Rather, the addition of increasing quantities of actin-binding protein under the experimental conditions employed increased the rate of contraction. Moreover, the addition of calcium to one end of a capillary containing the reconstituted protein mixture caused contraction to proceed in an asymmetric fashion, with a net displacement of the contracting mass away from the end to which calcium was added. It was proposed that movement away from the end stimulated with calcium was due to an increased rate of contraction in the more highly gelated region of the capillary in the end opposite to that to which calcium was added. That is, contraction of the highly gelated actomyosin was postulated to pull the more solated material away from the end stimulated by addition of calcium (Stendahl & Stossel 1980).

Our results with whole cell extracts and partly reconstituted models support the solation–contraction coupling hypothesis and are inconsistent with the tug-of-war hypothesis. Both sets of experiments describe the movement of mass from the site of calcium addition towards the opposite end. The difference is simply the mechanism by which contraction is related to the state of gelation. The results presented in figures 1 and 2 demonstrate that contraction, measured by shortening, is initiated at the site of calcium addition.

Our hypothesis does agree with one postulate suggested by the work of Stossel and colleagues. The presence of some actin-binding proteins can amplify the contraction by transmitting the forces. This concept is supported by our observation that superprecipitation rather than contraction is observed if calcium is added to an extract of *D. discoideum* before gel formation (Taylor *et al.* 1977; Condeelis & Taylor 1977), and by the results of Stendahl & Stossel (1980). However, the behaviour of the whole extract of *D. discoideum* is complex, and cannot be explained simply by the fact that an actin cross-linking protein enhances the rate of contraction in a reconstituted mixture. First, the gelled extract is stable, and does not contract unless the gel structure is partly disrupted by physical or chemical perturbation (Condeelis & Taylor 1977). Second, the rate of contraction in the gelated extract stimulated by the local addition of calcium is highest at the point of application of calcium, whereas the most highly gelated regions display no detectable contraction. Thus the solation–contraction coupling hypothesis is an acceptable working hypothesis of the experimental results now available.

The behaviour of cytoplasm postulated by the solation–contraction coupling hypothesis depends critically on the mechanism by which the gelled extract acts as an antagonist to contraction. It is possible that myosin is sterically inhibited from interaction with actin in the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

gel. Alternatively, a highly cross-linked network of actin filaments may provide resistance to shortening without inhibiting interaction of actin with myosin. In addition, it is possible that myosin is maximally active at sites of local solation induced by elevation of the free [Ca^{2+}], since phosphorylation of myosin by calcium-dependent myosin light chain kinase has been shown to enhance the actin-activated ATPase activity of myosin isolated from a number of cell types (Adelstein & Eisenberg 1980). The experimental analysis of these possible mechanisms may require the isolation of other components of the extract that contribute to the behaviour observed in the intact system.

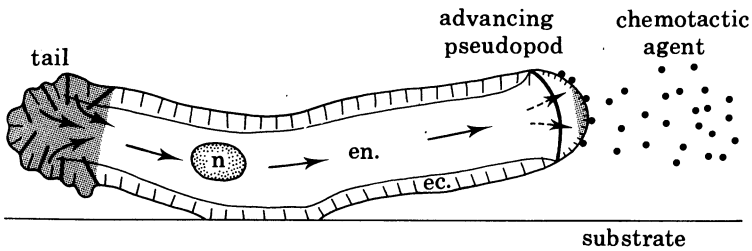


FIGURE 4. Schematic diagram of an amoeboid cell moving toward a source of a chemotactic agent. The distribution of distinct actin fibrils, detected by microinjection of fluorescein-labelled actin, is indicated by the heavy lines in the tail ectoplasm and the heavy line at the site of the plasmagel sheet at the tip of the advancing pseudopod. The presence of elevated (*ca.* 0.1 μM) free Ca^{2+} is indicated by the stippled regions in the tail and at the tip of the advancing pseudopod. The nucleus (*n*), endoplasm (en., clear area), and ectoplasm (ec. hatched area) are indicated. Arrows indicate the direction of cytoplasmic streaming.

The solation–contraction coupling hypothesis can be used to interpret and integrate a number of observations concerning the movements of the giant free-living amoebae (figure 4). The continuous elevated free [Ca^{2+}] in the tail may cause solation–contraction of the tail cortex. Forces produced by the contractions may form actively shortening fibrils in the tail. The solation of the cortex may not only weaken the gelated cortex to permit contraction, but may produce streaming endoplasm by a 'self-destructing' solation–contraction process in the tail. This maximally solated endoplasm may be pushed forward by the force of the contractions (Mast 1926; Taylor 1977). The increasing gradient of viscoelasticity from uroid endoplasm to anterior endoplasm to ectoplasm (Taylor 1977) may be due to a decreasing gradient of the free [Ca^{2+}] in the same regions.

Pseudopod extension may occur when a transient and localized change in the secondary messengers (i.e. free [Ca^{2+}] or pH) causes a solation of the cortex. The plasmagel sheet is probably formed during this event. Destabilizing the cortex may make this site weak relative to the remaining ectoplasm, permitting cytoplasmic streaming in this direction.

This working hypothesis can be applied to other cell movements such as cytokinesis. It has been shown that dividing cells contain a contractile ring containing numerous actin filaments (Schroeder 1973), and that myosin is required for cleavage to occur (Mabuchi & Okuno 1977). Partial solation of the cortex along a localized ring around the cell might permit contraction, and solation may continue throughout cleavage. The cortex may be progressively solated and dispersed by the solation–contraction in the contractile ring as the membranes are pulled together for fusion. This suggestion is consistent with the observation that the rigidity of the gelated cortex of sea-urchin eggs decreases during the latter stages of cleavage, as measured

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

by suction applied to the surface with a cell elastimeter (Mitchison & Swann 1955; Wolpert 1966), and as measured by a compression technique (Hiramoto 1963). In addition, fluorescent actin injected into sea-urchin eggs is not extensively concentrated in the cleavage furrow during cytokinesis (Wang & Taylor 1979). Thus we suggest that a 'self-destructing' solation–contraction in the contractile ring offers an explanation of the mechanism by which the rigidity of the entire cortex is decreased during cleavage without the progressive formation of a dense contracted mass as contraction proceeds.

The solation–contraction coupling hypothesis may also help to explain other motile events including the contraction of stress fibres in cultured mammalian cells and even the contraction of smooth muscle cells. In general, cells that contain a cross-linked network of actin filaments must be able to balance the requirements for transmitting tension and for decreasing the resistance to shortening.

References

Adelstein, R. S. & Eisenberg, E. 1980 Regulation and kinetics of the actin–myosin–ATP interaction. *A. Rev. Biochem.* **49**, 921–956.

Allen, D. G. & Blinks, J. R. 1979 The interpretation of light signals from aequorin injected skeletal and cardiac muscle cells – a new method of calibration. In *Detection and measurement of free calcium in cells* (ed. C. C. Ashley and A. K. Campbell), pp. 155–174. Amsterdam: Elsevier/North-Holland Biomedical Press.

Allen, R. D. 1961 Amoeboid movement. In *The cell*, vol. 2 (ed. J. Brachet & A. E. Mirsky), pp. 135–216. New York: Academic Press.

Allen, R. D., Cooledge, J. W. & Hall, P. J. 1960 Streaming in cytoplasm dissociated from the giant amoeba, *Chaos chaos*. *Nature, Lond.* **187**, 896–899.

Bazari, W. L. & Clarke, M. 1981 Characterization of a novel calmodulin from *Dictyostelium discoideum*. *J. biol. Chem.* **256**, 3598–3603.

Blinks, J. R. 1978 Applications of calcium-sensitive photoproteins in experimental biology. *Photochem. Photobiol.* **27**, 423–432.

Blinks, J. R., Mattingly, P. H., Jewell, B. R., Van Leeuwen, M., Harrer, G. C. & Allen, D. G. 1979 Practical aspects of the use of aequorin as a calcium indicator: assay, preparation, microinjection, and interpretation of signals. *Methods Enzymol.* **57**, 292–328.

Brown, S. S., Yamamoto, K. & Spudich, J. A. 1982 A 40,000 dalton protein from *Dictyostelium discoideoum* affects assembly properties of actin in a Ca^{2+}-dependent manner. *J. Cell Biol.* **93**, 205–210.

Burridge, K. & Feramisco, J. R. 1981 Non muscle α-actinins are calcium-sensitive actin-binding proteins. *Nature, Lond.* **294**, 565–567.

Clarke, M. & Spudich, J. A. 1974 Biochemical and structural studies of actomyosin-like proteins from non-muscle cells. Isolation and characterization of myosin from amoebae of *Dictyostelium discoideum*. *J. molec. Biol.* **85**, 209–222.

Condeelis, J. S. 1981*a* Reciprocal interactions between the actin lattice and cell membrane. *Neurosci. Res. Prog. Bull.* **19**, 83–99.

Condeelis, J. S. 1981*b* Microfilament–membrane interactions in cell shape and motility. In *International cell Biology, 1980–1981* (ed. H. G. Schweiger), pp. 306–320. Berlin: Springer-Verlag.

Condeelis, J. S., Salisbury, J. & Fujiwara, K. 1981 A new protein that gels F-actin in the cell cortex of *Dictyostelium discoideum*. *Nature, Lond.* **292**, 161–163.

Condeelis, J. S. & Taylor, D. L. 1977 The contractile basis of amoeboid movement. V. The control of gelation, solation, and contraction in extracts from *Dictyostelium discoideum*. *J. Cell Biol.* **74**, 901–927.

Fechheimer, M., Brier, J., Rockwell, M., Luna, E. J. & Taylor, D. L. 1982 A calcium and pH regulated actin binding protein from *D. discoideum*. *Cell Motility* **2**, 287–308.

Hartwig, J., Yin, H. & Stossel, T. 1980 Contractile proteins and the mechanism of phagocytosis in macrophages. In *Mononuclear phagocytes. Functional aspects*, pt 2 (ed. C. Van Furth), pp. 971–996.

Heiple, J. M. & Taylor, D. L. 1980 Intracellular pH in single motile cells. *J. Cell Biol.* **86**, 885–890.

Hellewell, S. B. & Taylor, D. L. 1979 The contractile basis of ameboid movement. VI. The solation–contraction coupling hypothesis. *J. Cell Biol.* **83**, 633–648.

Herman, I. M., Crisona, N. J. & Pollard, T. D. 1981 Relation between cell activity and the distribution of cytoplasmic actin and myosin. *J. Cell Biol.* **90**, 84–91.

Hiramoto, Y. 1963 Mechanical properties of sea urchin eggs. II. Changes in mechanical properties from fertilization to cleavage. *Expl Cell Res.* **32**, 76–88.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Isenberg, G., Aebi, U. & Pollard, T. D. 1980 An actin-binding protein from *Acanthamoeba* regulates actin filament polymerization and interactions. *Nature, Lond.* **288**, 455–459.

Kane, R. E. 1975 Preparation and purification of polymerized actin from sea urchin egg extracts. *J. Cell Biol.* **66**, 305–315.

Kuczmarski, E. R. & Spudich, J. A. 1980 Regulation of myosin self-assembly: phosphorylation of *Dictyostelium* heavy chain inhibits formation of thick filaments. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7292–7296.

Lanni, F. & Ware, B. 1982 Modulation detection of fluorescence photobleaching recovery. *Rev. scient. Instrum.* (In the press.)

Mabuchi, I. & Okuno, M. 1977 The effect of myosin antibody on the division of starfish blastomeres. *J. Cell Biol.* **74**, 251–263.

Mast, O. 1926 Structure, movement, locomotion, and stimulation in *Amoeba*. *J. Morph. Physiol.* **41**, 347–425.

Mitchison, J. M. & Swann, M. M. 1955 The mechanical properties of the cell surface. III. The sea urchin egg from fertilization to cleavage. *J. exp. Biol.* **32**, 734–750.

Nunnally, M. H., Powell, L. D. & Craig, S. W. 1981 Reconstitution and regulation of actin gel-sol transformation with purified filamin and villin. *J. biol. Chem.* **256**, 2083–2086.

Reynolds, G. T. 1968 Image intensification applied to microscope systems. *Adv. Opt. Electron Microsc.* **2**, 1–40.

Schroeder, T. E. 1973 Actin in dividing cells: contractile ring filaments bind heavy meromyosin. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1688–1692.

Stendahl, O. I. & Stossel, T. P. 1980 Actin-binding protein amplifies actomyosin contraction, and gelsolin confers calcium control on the direction of contraction. *Biochem. biophys. Res. Commun.* **92**, 675–681.

Stossel, T., Hartwig, J., Yin, H. & Davies, W. 1979 Actin binding protein. In *Cell motility: molecules and organization* (ed. S. Hatano, H. Ishikawa & H. Sato), pp. 189–209. University of Tokyo Press.

Taylor, D. L. 1977 The contractile basis of amoeboid movement. IV. The viscoelasticity and contractility of *Amoeba* cytoplasm *in vivo*. *Expl Cell Res.* **105**, 413–426.

Taylor, D. L., Blinks, J. R. & Reynolds, G. 1980*a* Contractile basis of ameboid movement. VIII. Aequorin luminescence during ameboid movement, endocytosis, and capping. *J. Cell Biol.* **86**, 599–607.

Taylor, D. L. & Condeelis, J. S. 1979 Cytoplasmic structure and contractility in amoeboid cells. *Int. Rev. Cytol.* **56**, 57–144.

Taylor, D. L., Condeelis, J. S., Moore, P. L. & Allen, R. D. 1973 The contractile basis of amoeboid movement. I. The chemical control of motility in isolated cytoplasm. *J. Cell Biol.* **59**, 378–394.

Taylor, D. L., Condeelis, J. S. & Rhodes, J. A. 1977 The contractile basis of amoeboid movement. III. Structure and dynamics of motile extracts and membrane fragments from *Dictyostelium discoideum* and *Amoeba proteus*. In *Proceedings on the Conference on Cell Shape and Surface Architecture*, pp. 581–603. New York: Alan Liss.

Taylor, D. L. & Fechheimer, M. 1982 Calcium regulation in amoeboid movement. In *Recent advances in* Ca^{++} *and cell function: calmodulin and Intracellular calcium receptors* (ed. S. Kakiuchi & H. Hadaka). New York: Plenum. (In the press.)

Taylor, D. L., Heiple, J., Wang, Y.-L., Luna, E. J., Tanasugarn, L., Brier, J., Swanson, J., Fechheimer, M. Amato, P., Rockwell, M. & Daley, G. 1982 Cellular and molecular aspects of amoeboid movement. In *Cold Spring Harb. Symp. quant. Biol.* **46**, 101–111.

Taylor, D. L., Hellewell, S. B., Virgin, H. W. & Heiple, J. 1979 The solation–contraction coupling hypothesis of cell movements. In *Cell motility: molecules and organization* (ed. S. Hatano, H. Ishikawa & H. Sato), pp.363–377. University of Tokyo Press.

Taylor, D. L., Reidler, J., Spudich, J. A. & Stryer, L. 1981 Detection of actin assembly by fluorescence energy transfer. *J. Cell Biol.* **89**, 362–367.

Taylor, D. L., Rhodes, J. A. & Hammond, S. A. 1976 The contractile basis of amoeboid movement. II. Structure and contractility of motile extracts and plasmalemma-ectoplasm ghosts. *J. Cell Biol.* **70**, 123–143.

Taylor, D. L. & Wang, Y.-L. 1978 Molecular cytochemistry: incorporation of fluorescently labeled actin into living cells. *Proc. natn. Acad. Sci. U.S.A.* **75**, 857–861.

Taylor, D. L. & Wang, Y.-L. 1980 Fluorescently labelled molecules as probes of the structure and function of living cells. *Nature, Lond.* **284**, 405–410.

Taylor, D. L., Wang, Y.-L. & Heiple, J. M. 1980*b* Contractile basis of amoeboid movement. VII. The distribution of fluorescently labelled actin in living amoebas. *J. cell Biol.* **86**, 590–598.

Thompson, C. M. & Wolpert, L. 1963 The isolation of motile cytoplasm from *Amoeba proteus*. *Expl Cell Res.* **32**, 156–160.

Uyemura, D. G., Brown, S. S. & Spudich, J. A. 1978 Biochemical and structural characterization of actin from *Dictyostelium discoideum*. *J. biol. Chem.* **253**, 9088–9096.

Wang, Y.-L., Heiple, J. M. & Taylor, D. L. 1982*a* Fluorescent analog cytochemistry of contractile proteins. In *Methods in cell biology*, vol. 24, pt. B (ed. L. Wilson). New York: Academic Press. (In the press.)

Wang, Y.-L., Lanni, F., NcNeil, P., Ware, B. & Taylor, D. L. 1982*b* Mobility of cytoplasmic and membrane associated actin in living cells. *Proc. natn. Acad. Sci. U.S.A.* (In the press.)

Wang, Y.-L. & Taylor, D. L. 1979 Distribution of fluorescently labelled actin in living sea urchin eggs during early development. *J. Cell Biol.* **82**, 672–679.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Wang, Y.-L. & Taylor, D. L. 1980 Preparation and characterization of a new molecular cytochemical probe: 5-iodoacetamidofluorescein-labeled actin. *J. Histochem. Cytochem.* **28**, 1198–1206.

Wang, Y.-L. & Taylor, D. L. 1981 Probing the dynamic equilibrium of actin polymerization by fluorescence energy transfer. *Cell* **27**, 429–436.

Wolpert, L. 1966 The mechanical properties of the membrane of the sea urchin egg during cleavage. *Expl Cell Res.* **41**, 385–396.

Yin, H. L., Zaner, K. S. & Stossel, T. P. 1980 Ca^{2+} control of actin gelation. Interaction of gelsolin with actin filaments and regulation of actin gelation. *J. biol. Chem.* **255**, 9494–9500.

Discussion

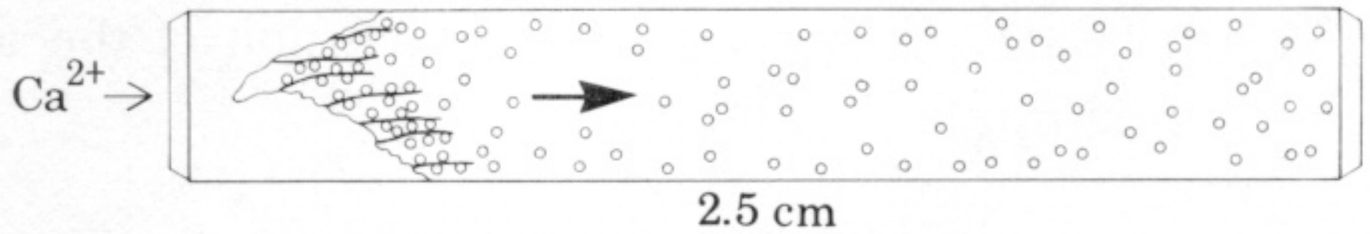
D. M. Shotton (*Department of Zoology, University of Oxford, U.K.*). There is a report in the literature (Cobbold, P. H. 1980, *Nature, Lond.* **285**, 441–446) which claims that differences in Ca^{2+} concentration cannot be detected in amoebae by using aequorin. Would Dr Taylor comment on this, please?

D. L. Taylor. First of all, Dr Shotton's statement is incorrect. Cobbold stated that the free [Ca^{2+}] varied between *ca.* 50 and 200 nM. However, he indicated that the rounded, non-motile cells exhibited the higher free [Ca^{2+}]. His major interpretation was that it was unlikely that the free [Ca^{2+}] reached 1 μM. Therefore a regulatory mechanism for 1 μM free Ca was not supported. We published a paper at the same time (Taylor *et al.* 1980*a*) stating that the free [Ca^{2+}] was less than 1 μM. We did see a correlation between motility and fluctuations in the free [Ca^{2+}]. Our recent studies with a calcium-sensitive actin-binding protein (Fechheimer *et al.* 1982) demonstrated that fluctuations of the free [Ca^{2+}] at less than 1 μM could regulate gelation *in vitro*.

D. M. Shotton. Has Dr Taylor now abandoned the frontal contraction theory of amoeboid movement, which he formerly espoused?

D. L Taylor. We published one paper (Taylor *et al.* 1973) supporting the frontal contraction model. Since that time our laboratory has published numerous papers describing the evidence for tail contractions. At present we are confident that contractions do occur in the tail. We have no evidence refuting the presence of contractions at the tips of pseudopods. However, the simplest interpretation of our results is presented in the foregoing paper.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



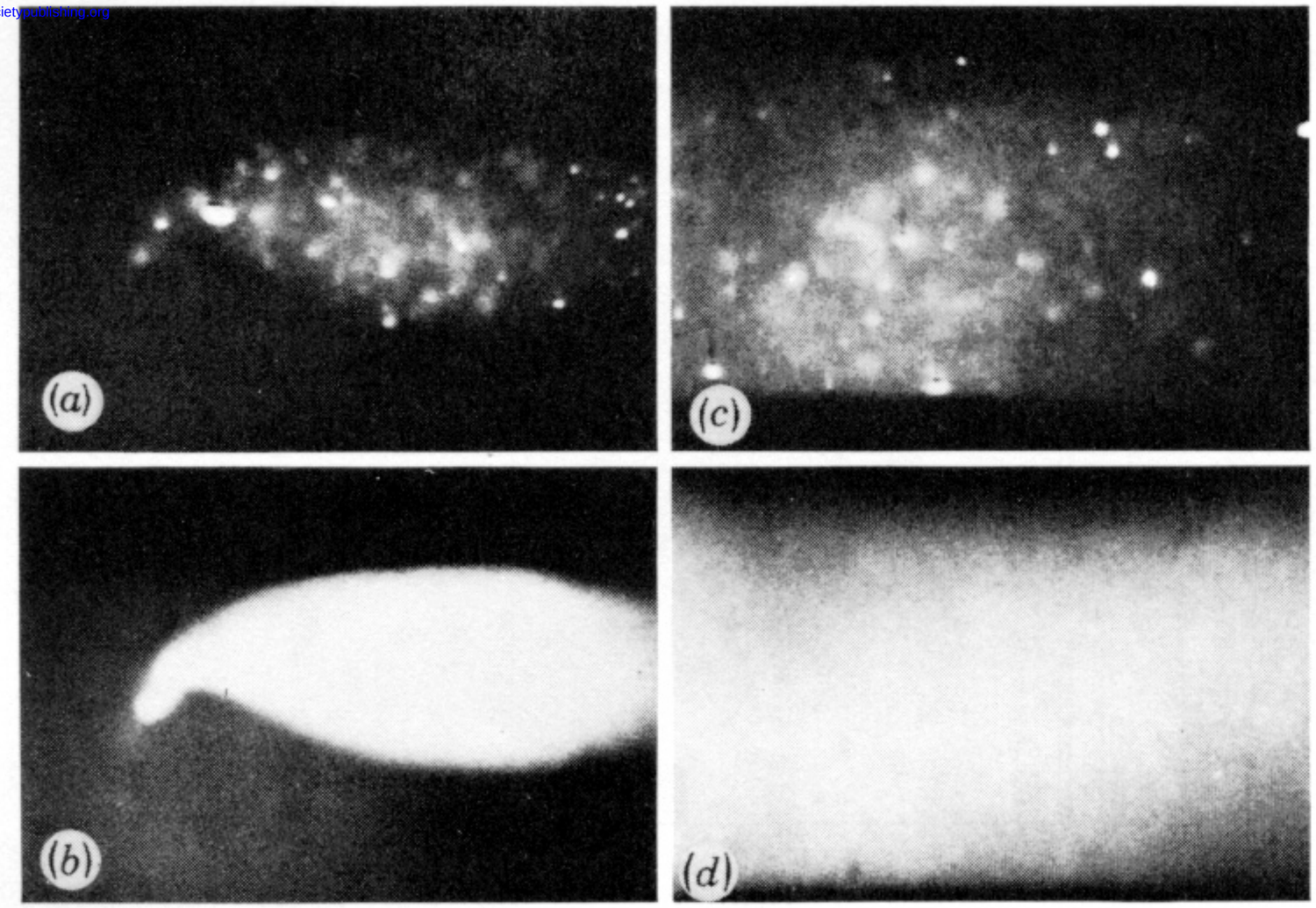


FIGURE 1. Observation of gelation and contraction of an extract of *D. discoideum* in glass capillaries. Glass capillaries 2.5 cm in length were filled with an extract of *D. discoideum* containing rhodamine-labelled beads and fluorescein-labelled actin at 4 °C. The extract was warmed to room temperature for 20 min to allow gelation. A solution containing 1 μm free Ca^{2+} was applied to one end of the capillary as shown. The extract contracted and moved away from the side of the capillary to which calcium had been added. Contraction, assessed by the rate of movement of single beads or clusters of the rhodamine-labelled beads (*a*), and the fluorescein-labelled actin (*b*), occurred at the end to which calcium had been added. In contrast, the rhodamine-labelled beads (*c*), and the fluorescein-labelled actin (*d*) at the end of the capillary opposite to that to which calcium was added offered no indication of contraction.