PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Structure of Cortical Cytoplasm

T. P. Stossel

Phil. Trans. R. Soc. Lond. B 1982 **299**, 275-289
doi: 10.1098/rstb.1982.0132

Phil. Trans. R. Soc. Lond. B **299**, 275–289 (1982)
Printed in Great Britain

The structure of cortical cytoplasm

By T. P. Stossel
Hematology–Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114, *U.S.A.*

[Plates 1–3]

Actin-rich cortical cytoplasm of phagocytic leucocytes forms pseudopodia and controls cell shape and movement by generating directional propulsive and contractile forces. Proteins purified from leucocytes form and deform an actin matrix. Actin-binding protein (ABP) cross-links actin filaments into a three-dimensional lattice with perpendicular branches. This structure, which can be visualized in the electron microscope, is consistent with physical properties of actin–ABP matrices. Gelsolin binds one end of actin filaments with high affinity in the presence of calcium; acumentin, another protein, constitutively binds the other end with low affinity. Together these proteins can control actin filament length and thereby regulate expansion (propulsion) or collapse of the actin network. The assembly state of the network also controls myosin-based contractile forces. A tug-of-war decides the direction of lattice movement, regions of lesser structure tending to move toward regions of greater structure.

Introduction

A region of cytoplasm beneath the plasma membrane controls the structure, shape and movement of eukaryotic cells (Just 1924). The ability of isolated granulocyte cortices totally separated from the body of the cell to migrate actively is a particularly compelling demonstration of the motor function of this distinct subcellular organelle (Keller & Bessis 1975).

This discussion reviews the evidence that the assembly, dissolution and deformation of an actin filament lattice determine the structure and function of the cortical cytoplasm and that gradients of free calcium within the cortex coordinate these events. Results from studies of proteins isolated from phagocytic leucocytes, specifically rabbit pulmonary macrophages and human peripheral blood granulocytes, constitute the basis of this evidence. Proteins very similar in structure and function to the cortical components of phagocytic leucocytes exist in other eukaryotic cells. Interactions of these proteins might therefore be of general importance for cell structure, shape and movement.

Evidence for an actin network in cortical cytoplasm

The notion that some kind of dynamic colloidal gel occupies the volume of cortical cytoplasm and changes its shape is old (see, for example, Kopac 1951). Since the discovery that actin fibres are a component of cortical cytoplasm, such ideas began to have meaning in molecular terms.

The initial documentation of actin in phagocytic leucocytes is ascribable to Senda *et al.* (1969) and to Allison *et al.* (1971). However, the biochemical and morphological description of actin in other non-muscle cells (reviewed by Pollard & Weihing 1974) predated these discoveries.

The labelling of cytoplasmic proteins by means of immunofluorescence has established that actin and its associated proteins are localized or at least concentrated in cortical cytoplasm. A biochemical analysis of cortical fragments of phagocytic leucocytes confirmed that actin is a major component of this organelle. It comprises up to 20 % of the total protein in the fragments by mass (Davies & Stossel 1977; Hartwig *et al.* 1977). The components of the other major cytoplasmic fibre systems, the microtubules and intermediate filaments, are much less prominent in cortical cytoplasm of phagocytic leucocytes, as determined by immunofluorescence microscopy (Oliver *et al.* 1978; Stendahl *et al.* 1980; Valerius *et al.* 1981; Painter *et al.* 1981; J. H. Hartwig & K. Weber, unpublished). These findings are consistent with previous observations of others investigating the localization of proteins related to cell motility in other motile non-muscle cells (reviewed by Groeschel-Stewart 1980).

The cortical cytoplasm of phagocytic leucocytes viewed in electron micrographs of thin sections appears to contain a large mass of overlapping actin filaments originally described as a 'microfilament network' (Keyserlingk 1968; Reaven & Axline 1973). Recently, cell remnants demembranated by means of shear stress or detergents have yielded improved ultrastructural images of these filaments (Boyles & Bainton 1979, 1981; Trotter 1981). Figure 1, plate 1, shows an electron micrograph of a region of cortical cytoplasm in a human polymorphonuclear leucocyte that had spread out on a surface. The specimen was prepared for electron microscopy by extraction with a detergent, fixation, critical-point drying and rotary shadowing with platinum. The picture reveals marked branching of fibres that have the diameter of actin filaments. A striking tendency of the filaments to branch at right angles is best appreciated when such images are viewed stereoscopically. The average distance between points where the filaments cross over is about 0.16 μm. In general, the appearance of these filaments also resembles peripheral cytoplasmic architecture visualized by means of similar techniques in fibroblasts and cultured epithelial cells (see, for example, Heuser & Kirschner 1980).

If the junctions between actin filaments seen in these electron micrographs are sufficiently strong to inhibit diffusion and deformation of the fibres by applied stresses, then the perpendicular branching appearance of actin in cortical cytoplasm is the colloidal 'gel' that gives form to the cortex. Variations in this structure could account for cortical shape changes and movements. The evidence supporting the validity of this conclusion is presented below.

Mechanical properties of actin

The large number of actin filaments in cortical cytoplasm raises the questions as to whether actin fibres alone could account for the structure of this region, whether other factors come into play, and, if so, how. The evidence reviewed in this section suggests that actin by itself is not responsible for the architecture of cortical cytoplasm.

Almost all information about the network properties of actin is based on studies with skeletal muscle actin. Actin in phagocytic leucocytes has a more basic isoelectric point than skeletal muscle actin (Anderson 1979; F. S. Southwick, unpublished). This 'beta' mobility of the leucocyte actin is characteristic for mammalian cytoplasmic actins (Vanderkerckhove & Weber 1978). However, at the time of writing, no important differences are evident between muscle and leucocyte actins with respect to the mechanical properties to be summarized below.

Purified actin forms double-helical filaments in neutral salt solutions by means of an initiated assembly process (Oosawa & Asakura 1975). The filaments formed at the end of the reaction

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

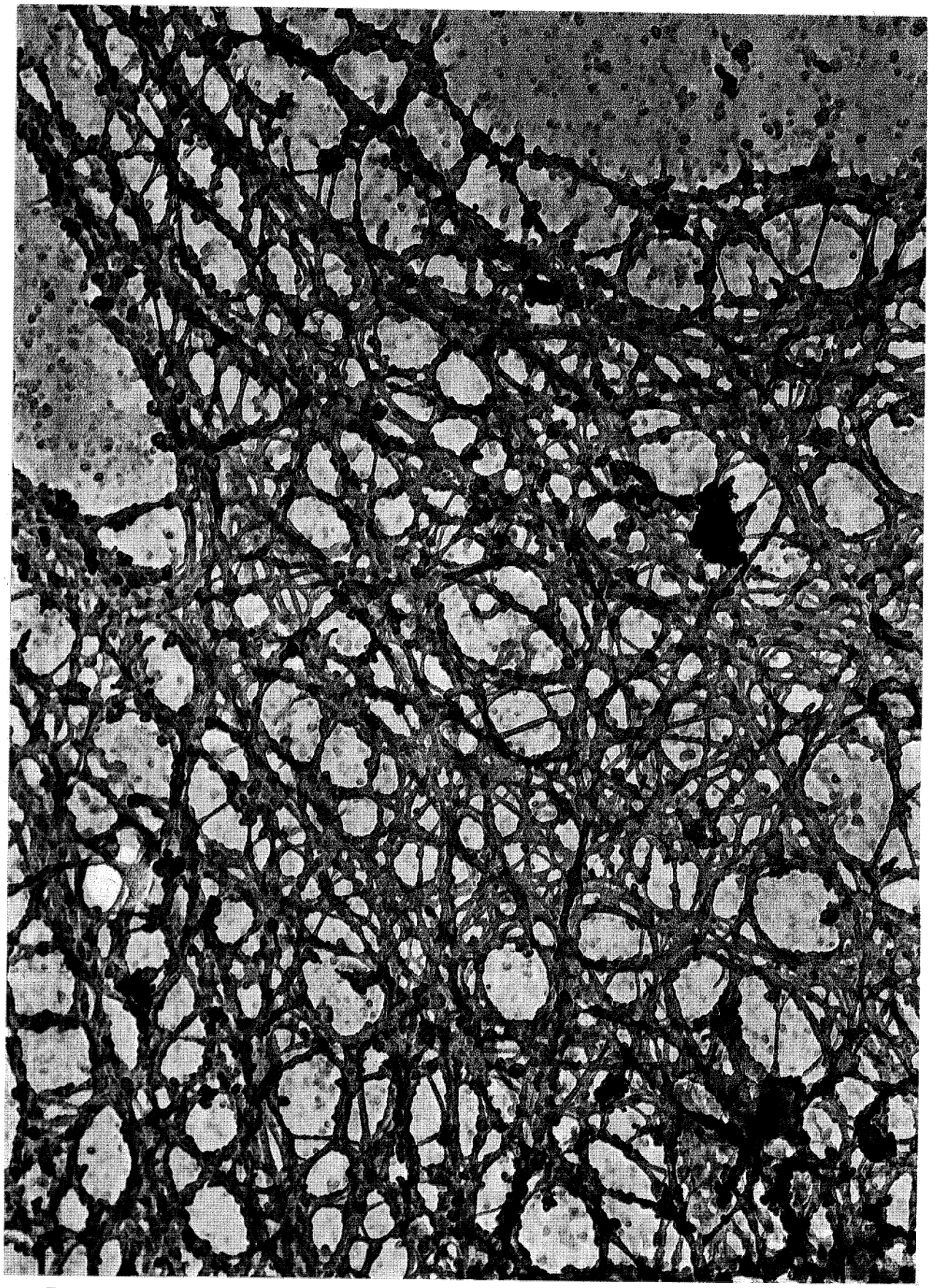

FIGURE 1. Residue of a human polymorphonuclear leucocyte adherent to a glass surface. The cell was extracted with Triton X-100, fixed with glutaraldehyde, critical-point dried and rotary-shadowed with platinum. The photomicrograph is of a region of the cortical cytoplasm. (Magn. ×80000.)

(*Facing p.* 276)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

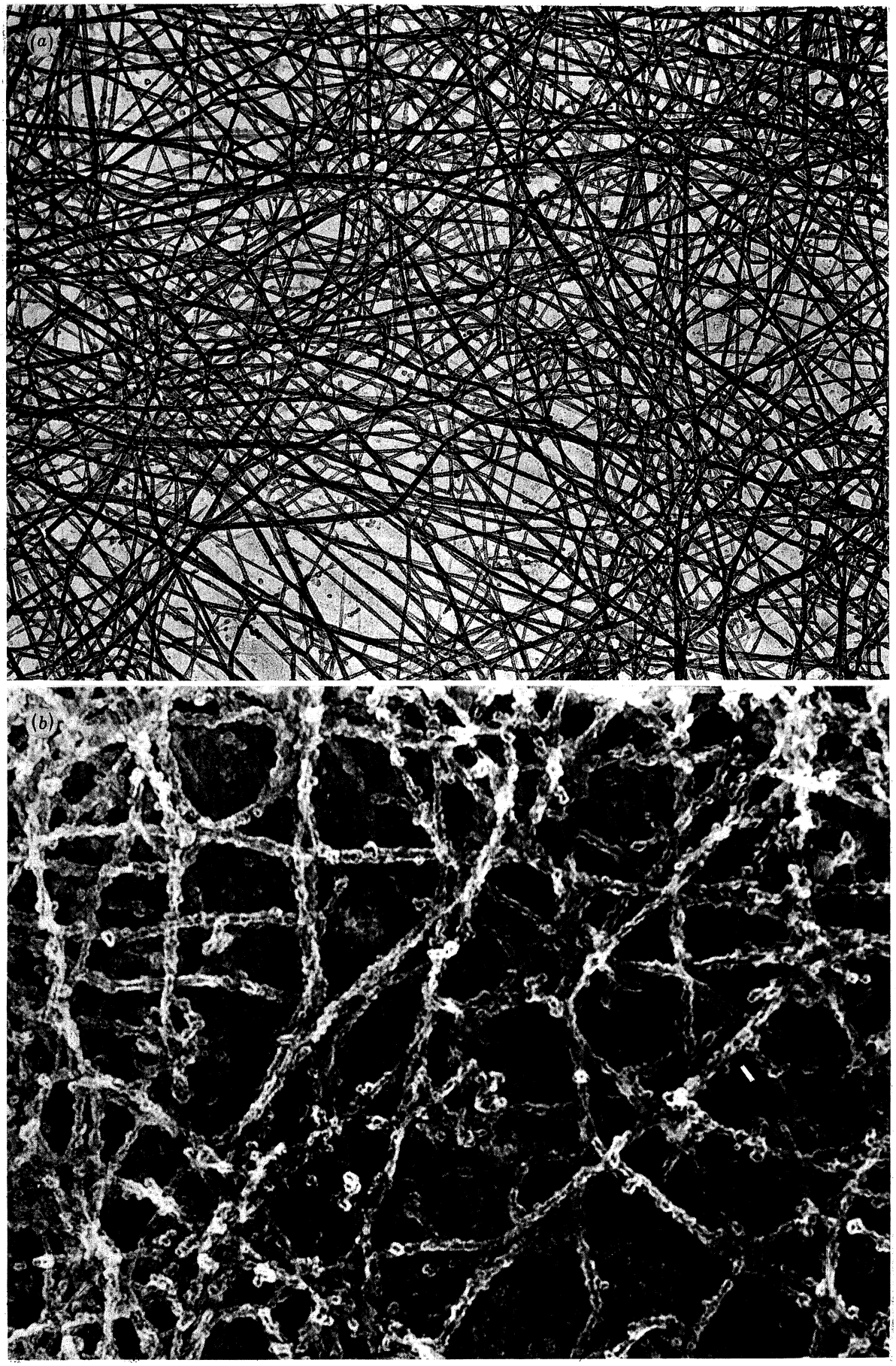

FIGURE 3 (*a*, *b*). For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

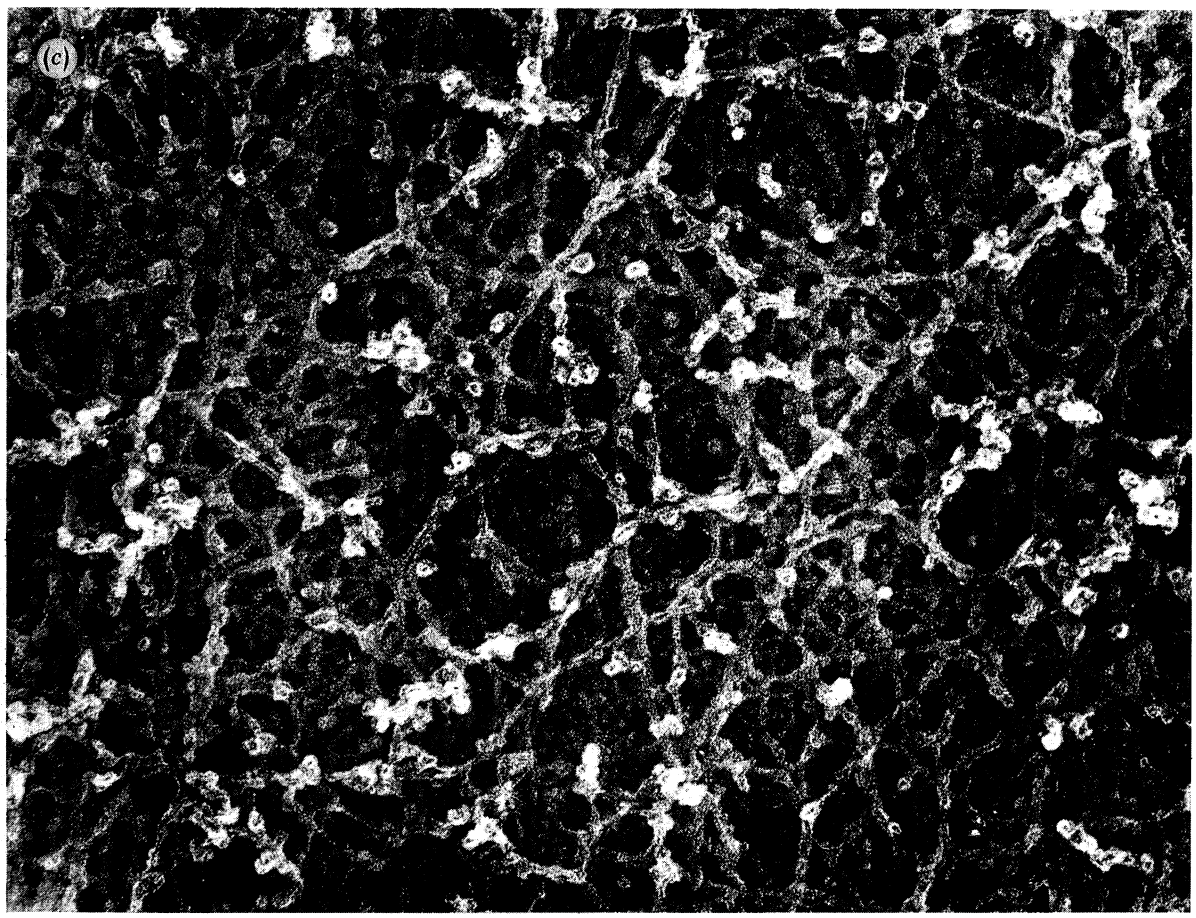

FIGURE 3. Morphology of actin filaments alone (*a*) and when assembled in the presence of macrophage actin-binding protein (*b*, *c*). The actin concentration was 0.05 mg ml^{-1}, the actin-binding protein: actin molar ratios were 1:50 (*b*) and 1:25 (*c*). The proteins were placed on copper grids, fixed, critical-point dried and rotary-shadowed as described in figure 1. (Magns: (*a*), ×53000; (*b*, *c*), ×120000.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

are quite long. The average filament length is 2–4 μm and some filaments are as large as 10 μm in length (Kawamura & Maruyama 1970). The actin filaments are also relatively stiff (Nagashima & Asakura 1980). The length and stiffness of pure actin filaments render them likely to collide with one another, even at very low concentrations. They are therefore in a favourable situation to link up to form some kind of network.

It has been known for some time that the viscosity of actin filament (F-actin) solutions can be very high, and that this viscosity diminishes as the stress increases (Kasai *et al.* 1960; Maruyama *et al.* 1974). This phenomenon has led previous investigators to conclude that F-actin is a 'gel', on the assumption that its viscosity would be infinite at zero stress. They also classified F-actin as 'thixotropic', meaning a substance capable of undergoing reversible isothermal sol–gel transformations (Kasai *et al.* 1960; Maruyama *et al.* 1974).

To evaluate the possible importance of F-actin in cytoplasmic structure, it is necessary to know the nature of the junctions between actin fibres that account for its 'gel'-like properties and to know what happens to these junctions under stress. If F-actin forms a gel because of interpolymer junctions, it is necessary to specify the strength of these bonds and how they might be regulated in the cell. It is also a requirement to determine why F-actin solutions flow in response to stress.

If F-actin is a true gel, then stress could simply break some type of bonds connecting the polymers to each other. Alternatively, an applied force could fragment the polymers between these junctions. Actin filaments fragment easily in the presence of mechanical stresses, and they anneal spontaneously when the stress ceases (Asakura *et al.* 1963). The 'thixotropic' behaviour of F-actin is consistent with reversible destruction of junctions between filaments by stress. On the other hand, the mechanical behaviour of F-actin could just as likely result from the effects of entanglements between the long stiff actin chains. Entanglements can produce topological constraints to diffusion to polymers such that they exhibit elastic properties for short times after the application of a stress. At longer times, the chains can disentangle, orient and flow. Theories concerning the rheological behaviour of entangled polymers are well developed, and there are many experimental examples of synthetic polymers exhibiting behaviour conforming with the theories (see, for example, Graessley 1974). The experimental data concern measurements of the strain of polymers at various times in response to varied stresses.

The extreme length of pure actin filaments has made it difficult to study their rheology. The relaxation times of such filaments are so slow that very low stresses are required to obtain meaningful strain values. Recent investigations employing a viscoelastometer capable of exerting low stresses (less than 0.1 μN cm^{-2}) and measuring strain (Zaner *et al.* 1981; Zaner & Stossel 1982; Stossel *et al.* 1982) have begun to provide this information. The data demonstrate that entanglements and not 'gelation' are responsible for the network properties of F-actin in physiological solvents. Three separate experimental manipulations lead to this conclusion.

First, the data confirm the previously noted decrease in the viscosity of F-actin as the stress increases. Furthermore, this dependence follows quantitatively an exponential relation universally observed for entangled synthetic polymers and defined as 'power-law' behaviour (Graessley 1974). For polymers displaying power law behaviour, the shear dependence of viscosity is assumed to result from an orientation of entangled chains in response to stress. Power-law behaviour is restricted to a finite range of stresses, and the relation between shear and viscosity deviates at stresses above this range (Graessley 1974).

With F-actin, high stresses fragment the polymers, causing filament populations of different

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

lengths to appear more similar. For example, the Ostwald-type viscometer exerts a considerable stress and therefore fragments actin filaments (Zaner & Stossel 1982).

Second, the compliance, defined as the strain measured at fixed stresses, of an F-actin solution follows a time course expected for entangled polymers (figure 2). The curve exhibits an initial lag phase, a rapid increase, a plateau and finally a rising slope. The lag phase at the early interval after the application of stress is interpreted to reflect the initial resistance of interpolymer bonds to deformation. The rapid transient increase in compliance observed next is due to short-range deformations of the polymers when the continuous application of stress overcomes

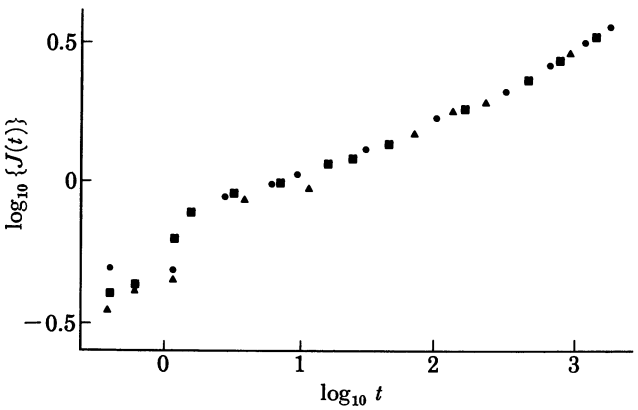

FIGURE 2. Compliance, $J(t)$, of a solution of F-actin (2 mg ml^{-1}) in 0.1 M KCl, 0.02 M imidazole–HCl solution, pH 7.0, as a function of time, t. The compliance was measured in a viscoelastometer (Zaner *et al.* 1981) at the following stresses: ●, 0.39 μN cm^{-2}; ▲, 0.78 μN cm^{-2}; ■, 1.56 μN cm^{-2}.

the initial resistance of the chains to bending. These deformations then become hindered by entanglements, as expressed by the flattening of the curve. However, the absence of a stable plateau at this point and the upward deviation of the curve indicate the presence of polymer movements that would not be expected in a true gel. Furthermore, the shape of the compliance curve does not vary over an eightfold range of stresses. This invariance indicates that the pattern is not the result of destruction of a gel by the applied stress (Ferry 1980). If the flow of the actin filaments resulted from the destruction of a true gel by stress, the upward inflexion of the curve would take place at earlier times as the stress increased. To argue that the destruction of a gel occurred maximally at the lowest stress applied in these experiments would require that the junctions linking polymers together in the 'gel' had an essentially negligible energy of the order of 40 kJ mol^{-1}.

A third piece of evidence that the mechanical properties of pure F-actin arise from entanglements is obtained from analysing the strain in an actin sample subjected to an oscillating stress. The ratio of the amplitudes of stress/strain, the strain in phase with the stress, defines the dynamic modulus of rigidity (Osaki 1973). The dynamic modulus of rigidity of F-actin decreases as the oscillation frequency diminishes (Maruyama *et al.* 1974; K. S. Zaner & T. P. Stossel, unpublished). The findings are explained by the greater time that chains have to disentangle at low frequencies of oscillation than at high frequencies. The disentanglement at low frequencies leads to a lower measured dynamic rigidity.

In summary, pure F-actin in solvents of physiological ionic composition is not a true gel. Its elastic properties result from entanglements between chains. The 'thixotropy' and flow properties of F-actin are explained by chain disentanglement and orientation and, at high

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

stresses, by chain fragmentation. In theory, a highly concentrated pure F-actin solution composed of very long filaments could be so viscous as to behave phenomenologically like a 'gel' with sufficient rigidity to give form to cortical cytoplasm. However, the average length of actin filaments of cortical cytoplasm is much shorter than that of pure actin filaments. As amplified below, the viscosity of actin filaments subjected to stress in the power-law range is directly proportional to the filament length. If, as estimated from electron micrographs of cortical cytoplasm of phagocytic leucocytes, the average actin filament length is 0.2 μm, the viscosity of F-actin at a concentration of 18 mg ml^{-1} would be about 17 P (1.7 Pa s).

Deviations of the solvent ionic conditions from physiological values can promote the formation of ionic interpolymer interactions, such that the chains may form some kind of polyelectrolyte gel (see, for example, Yamamoto *et al.* 1975). However, such ionic gelation would be rather non-specific and of little obvious biological value.

In conclusion, the cortical cytoplasm of phagocytic leucocytes appears to contain a structure composed of a network of short and highly branched actin filaments very different from the long entangled chains of pure F-actin.

Actin-binding protein and the branching actin network of the cortical cytoplasm

In phagocytic leucocytes the primary molecule responsible for the cross-linking of actin into a true gel is a protein of high molecular mass called actin-binding protein (Hartwig & Stossel 1975; Stossel & Hartwig 1975, 1976; Boxer & Stossel 1976). A related protein has been isolated from platelets (Rosenberg *et al.* 1981), cultured epithelial cells (Schloss & Goldman 1979), smooth muscle (where it has been called 'filamin') (Wang *et al.* 1975) and toad eggs (H. L. Corwin & J. H. Hartwig, unpublished).

The conclusion that actin-binding protein is responsible for the gelation of actin in the cortex of phagocytic leucocytes is based on the following information. First, 75% of the F-actin cross-linking activity in homogenates of phagocytic leucocytes co-purifies with actin-binding protein when assayed by its capacity to cause pure F-actin to sediment rapidly at low *g* values (Brotschi *et al.* 1978). Second, actin-binding protein purified from phagocytic leucocytes is a very efficient actin cross-linking protein. This efficiency is gauged by the critical cross-linker concentration, v_c, at which an abrupt increase occurs in the apparent viscosity or rigidity of an F-actin solution (Brotschi *et al.* 1978; Hartwig & Stossel 1979, 1981). The phase transition between sol and gel states at the critical cross-linker concentration is characteristically abrupt and represents the formation of a giant cross-linked structure. The critical cross-link concentration at incipient gelation is inversely related to the mass-average molecular mass of the polymer chains, $\bar{M}_w$. Specifically, the theoretical critical cross-linker concentration, v_c, is equal to $c/\bar{M}_w$, where c is the polymer concentration in grams per litre (Flory 1953). The measured v_c of actin-binding protein is close to the theoretical value (Hartwig & Stossel 1979; Yin *et al.* 1980). This agreement between experiment and theory implies that actin-binding protein can cross-link F-actin with high efficiency.

Third, actin-binding molecules can be visualized cross-linking actin filaments in the cortex of phagocytic leucocytes in electron micrographs. The structure of the cortical cytoplasm closely resembles the morphological appearance of actin polymerized in the presence of actin-binding protein. The following paragraphs elaborate on this evidence.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The efficiency of actin-binding protein in cross-linking actin filaments stipulates that each added cross-linker molecule join two new filaments. For this purpose the cross-linker must have a high binding affinity for F-actin. The association constant of actin-binding protein for F-actin is 2×10^6 mol^{-1} (Hartwig & Stossel 1981). In addition, redundant cross-linking, the insertion of cross-links between previously cross-linked chains must be avoided. Such incestuous cross-linking can be minimized if the chains can be kept from aligning in parallel. In many synthetic polymers that orient as random coils and whose gelation has been studied, the flexibility of the chains prevents such parallel orientation. However, actin filaments are stiff in comparison with the synthetic polymers. The cross-linker molecule must therefore be large and flexible enough to bind specific domains in the actin filaments whenever they collide. The actin-binding protein molecule is a dimer with large and flexible subunits. The subunits each have a contour length of 80 nm, and they are linked together at one end defined as the 'head'. The opposite ends, the 'tails', are the domains of the protein that bind to F-actin, possibly in the filament helix grooves (Hartwig & Stossel 1981).

The most remarkable feature of actin cross-linking by actin-binding protein is that the interaction can lead to *perpendicular* branching of actin filaments. This perpendicularity is most easily appreciated when actin is polymerized in the presence of actin-binding protein, and the complexes are viewed in the electron microscope early in the polymerization process (Hartwig *et al.* 1980). Actin-binding protein nucleates the assembly of actin, as evidenced by the fact that actin-binding protein shortens the lag time usually observed when actin assembles in the presence of salts (Hartwig *et al.* 1980). The nucleation of the branching assembly allows the initial growth of filaments to occur at perpendicular branch points, thereby preventing the formation of parallel bundles, even in the presence of high concentrations of actin-binding protein. The mechanism by which actin-binding protein nucleates actin assembly is unclear. Possibly the 'tails' of actin-binding protein dimers bind to several monomers in the helix groove, thereby conferring stability on spontaneously forming nuclei.

Recently, the three-dimensional branching network of actin cross-linked by actin-binding protein has been visualized in stereo pairs of electron micrographs of specimens prepared by critical-point drying and rotary shadowing with platinum (figure 3, plates 2 and 3). The morphology of this structure is strikingly different from that of purified F-actin alone shown for comparison and much more similar to the appearance of cortical cytoplasm. As the ratio of actin-binding protein to actin increases, the distances between filament branch points decreases. In contrast, the pure actin filaments are very long and straight and tend to line up in parallel.

Similarly, the mechanical properties of actin cross-linked by actin-binding protein differ from those of F-actin alone. As shown in figure 4, the compliance curve as a function of time reaches a plateau characteristic of a true gel. After being deformed by the applied stress to their equilibrium lengths, the cross-linked chains cannot disentangle, and the strain remains constant (Ferry 1980).

Estimates of the total cell content of actin and actin-binding protein in phagocytic leucocytes indicate that they compose 10% and 1% respectively of the total cell protein by mass (Hartwig & Stossel 1975). On the assumptions that the major fraction of these proteins resides in the cortical cytoplasm and that the bulk of the actin is polymerized, there would be one actin-binding protein molecule for 120 actin monomers, equivalent to one cross-link for every 0.32 μm of filament length. This estimate of the ratio of actin-binding protein to actin is probably a lower limit, because some of the actin in the cell is likely to be unpolymerized, as indicated by

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the results of extraction experiments mentioned above. In any case, the filament segment lengths predicted from this ratio are of a magnitude observed in the electron micrographs of cortical cytoplasm.

In summary, actin-binding protein transforms F-actin into a true gel. This gel differs from many polymer gels studied in the past, which are composed of chains oriented as random

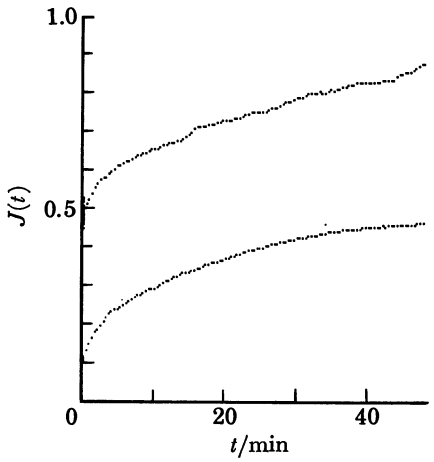

FIGURE 4. Compliance, $J(t)$, of a solution of F-actin (2 mg ml^{-1}) (upper curve) and F-actin assembled in the presence of actin-binding protein (100 μg ml^{-1}) (lower curve). The measurement and solvent conditions were as in figure 2.

coils and cross-linked at intervals. The actin network cross-linked by actin-binding protein is a perpendicularly branching structure. The actin chains are relatively stiff, and the cross-linking actin-binding protein molecules are relatively flexible. Such a network might act like a leaf spring in series with a coil spring.

This type of network may have several advantages over the 'classical' polymer gels. First, it can achieve maximal extension with a minimum of mass. Second, it very rigorously minimizes rendundant cross-linking. Third, it may provide for precise control of gel pore size.

A three-dimensional perpendicularly branching network composed of F-actin cross-linked by actin-binding protein is visible in electron micrographs of the cortical cytoplasm of phagocytic leucocytes. The appearance of this network closely resembles the morphology of actin assembled in the presence of actin-binding protein. The physical properties of this network are those of a gel.

REGULATION OF CORTICAL NETWORK STRUCTURE BY CONTROL OF ACTIN FILAMENT LENGTH

Length is a powerful determinant of the viscosity of linear polymers (Graessley 1974). Figure 5 shows that the viscosity of an F-actin solution subjected to stresses in the power-law range is directly proportional to the mass-average filament length to the 0.6 power. As described above, there is an inverse relation between the length of polymers and the concentration of a cross-linker required for incipient gelation (Flory 1953). This relation has been verified experimentally for actin filaments undergoing cross-linking by actin-binding protein and related proteins (Hartwig & Stossel 1979; Yin *et al.* 1980; Nunnally *et al.* 1981). The control of actin filament length can therefore regulate F-actin's viscosity and its sol–gel transformations.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The mechanisms for changing filament lengths are reversible polymerization and reversible fragmentation. In the former the length varies without changing the number of filaments; in the latter the number changes.

Reversible fragmentation presents three advantages over reversible polymerization as a control mechanism. First, for a given degree of filament shortening, fragmentation can occur much faster than depolymerization. It is therefore more efficient. Second, fragmentation can decrease filament length without increasing the concentration of free monomers

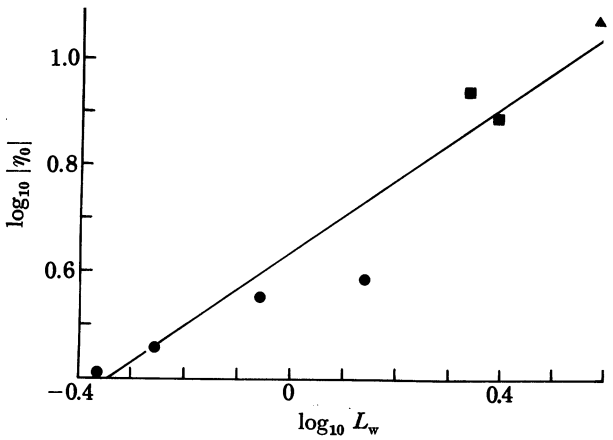

FIGURE 5. Dynamic viscosity (η_0) of F-actin as a function of actin filament length. The dynamic viscosity was measured in a viscoelastometer (Zaner *et al.* 1981). The mass-average actin filament lengths were determined from gel point determinations and the concentration of macrophage actin-binding protein required for incipient gelation. The symbols indicate different methods employed to alter actin filament lengths. These procedures included the addition of agents that fragment F-actin, i.e. gelsolin–calcium (●) (Yin & Stossel 1979) and cytochalasin B (■) (Hartwig & Stossel 1979) and the removal of minor contaminants from purified F-actin by means of an additional purification step (▲). The highly purified actin filaments are about 40% longer than actin filaments not subjected to this additional purification (Zaner & Stossel 1982).

significantly, thereby preventing the possibility of uncontrolled assembly of new filaments from a pool of monomers. Third, in considering the sol–gel transformation, fragmentation but not depolymerization changes the critical cross-link concentration. The term $c/\bar{M}_w$ in the equation $v_c = c/\bar{M}_w$ increases during fragmentation but does not change during depolymerization.

Phagocytic leucocytes contain at least two proteins that affect the length of actin filaments by a mechanism related to fragmentation. The proteins, named gelsolin and acumentin, have several common features. Both proteins bind to actin during or after assembly and shorten the filament length distribution without significantly altering the total polymer mass. The mechanism of shortening is in part kinetic. Both proteins promote the nucleation step of actin assembly, thereby leading to a population of short filaments relative to actin assembled in the absence of these proteins. Short actin filaments at equilibrium can be maintained by proteins that 'cap' the ends of the filaments, because the 'capped' filaments cannot anneal. Gelsolin and acumentin 'cap' actin filaments. When added to F-actin, the proteins also shorten the actin filament length distribution without changing the total polymer mass. This process is equivalent to fragmentation of the filaments (Yin *et al.* 1980; Southwick & Hartwig 1982).

The mechanisms of nucleation and fragmentation of F-actin are not defined. The proteins may complex with actin monomers to nucleate their assembly. They may also stabilize spontaneously forming actin nuclei. Therefore, these proteins may both actively cut actin fibres and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

also 'cap' spontaneously fragmenting filaments. Because most experiments involving these proteins require mechanical mixing, the second mechanism is difficult to exclude. Acumentin and gelsolin differ from one another with respect to the potency with which they shorten actin filaments, their binding sites on actin, and the regulation of the binding.

Acumentin is a 65 kDa globular protein first purified from human granulocytes and subsequently from rabbit-lung macrophages (Southwick & Stossel 1981; Southwick & Hartwig 1982). It derives its name from the Latin word acumen, meaning 'point', because it binds to the end of actin filaments reacted with heavy meromyosin to which the heavy meromyosin 'arrowheads' (Huxley 1963) point. In solvents of physiological ionic strength, acumentin binds constitutively to actin filaments. The affinity of acumentin for actin is lower than that of gelsolin, because higher concentrations are required to produce comparable degrees of actin filament shortening (figure 6). However, acumentin is present in high concentrations in extracts of phagocytic leucocytes in which one molecule of acumentin is present for every five actin monomers.

Gelsolin is a 90 kDa globular calcium-binding protein originally purified from rabbit-lung macrophages (Yin & Stossel 1979, 1980) and subsequently identified in a variety of mammalian cells and tissues, including blood plasma (Yin *et al.* 1981*a*). Gelsolin differs from acumentin in several important features. First, it binds to the 'barbed' ends of actin filaments decorated with heavy meromyosin (Yin *et al.* 1981*b*). Second, the action of gelsolin is regulated positively by the free calcium concentration, whereas the action of acumentin is equivalent at high and low free calcium concentrations. In the presence of free calcium concentrations above 2×10^{-7} M. gelsolin fragments F-actin. The effect is maximal at a free calcium concentration of about 10^{-5} M, Third, as mentioned above, the gelsolin–calcium complex is more potent on a molar basis than acumentin (figure 6). Gelsolin is present in lower concentrations than acumentin in extracts of phagocytic leucocytes: 1 molecule of gelsolin for every 50 actin monomers, in contrast to 1 molecule of acumentin for every 5 actin monomers (Southwick & Stossel 1981). This protein derives its name from the opinion that together with calcium it regulates the actin sol–gel transformation of cortical cytoplasm.

The differences between acumentin and gelsolin permit the two proteins to act cooperatively and to confer a high degree of control over actin filament length. This control is shown schematically in figure 7. Regardless of the starting point, either monomeric or polymeric actin, the gelsolin–calcium complex shortens the filament length and is bound to the 'barbed' end of the shortened filaments. Acumentin binds to the 'pointed' ends of actin filaments. The number of pointed ends obligatorily increases after the action of gelsolin–calcium. The covering of both filament ends prevents the dissociation of monomers from the ends of filaments. It also prevents the annealing of filaments. After a fall in the free calcium concentration, gelsolin dissociates from the 'barbed' ends of the actin filaments. The free 'barbed' ends have a higher affinity than acumentin for 'pointed' filament ends. Therefore filaments anneal, and a longer filament length distribution emerges. The acumentin concentration is expected to increase somewhat when filaments anneal. This rise in acumentin concentration would in effect buffer the extent of the filament length increase. Therefore, the filaments do not become as long as expected for pure F-actin. In the total control of actin filament length, gelsolin can be viewed as a regulator and acumentin as a buffer.

It remains to be determined whether phagocytic leucocytes also contain molecules that sequester actin monomers, thereby adding an additional dimension of control over the length

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

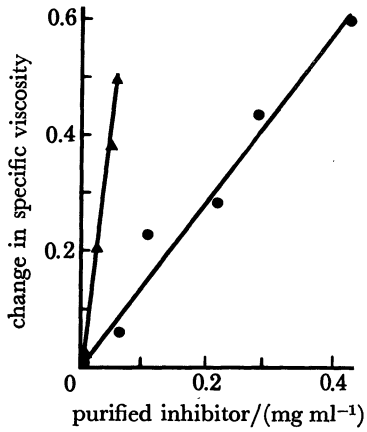

FIGURE 6. Effect of concentration of purified acumentin and of gelsolin–calcium on the final viscosity of F-actin. Actin at a concentration of 1 mg ml^{-1} was assembled by addition of KCl in the presence of the indicated concentrations of acumentin (●) and gelsolin (▲). The calcium concentration was 1 mM. Viscosity, measured with an Ostwald-type viscometer, was determined after 1 h and compared with that of actin assembled in the absence of the regulatory proteins. The vertical axis indicates the diminution in viscosity of the actin sample in the presence compared with the absence of these proteins.

and number of actin filaments in the cortical cytoplasm. The reported existence of such molecules in other cells (Lindberg *et al.* 1979) and the small fraction of non-sedimentable actin in detergent extracts of macrophages suggest that this may be the case.

THE ACTIN NETWORK IN THE STRUCTURE AND MOVEMENT OF CORTICAL CYTOPLASM

Cell movements have directionality. How can a three-dimensional perpendicularly branching actin network be involved in directional movements, especially when many movements depend on oriented structures like the sarcomere of striated muscle? The information summarized in the preceding sections of this discussion can be integrated to create a working hypothesis as to how the 'directionless' actin network moves the cortical cytoplasm of phagocytic leucocytes. The basic premise of the hypothesis is that the free calcium concentration regulates the state of assembly of the cortical cytoplasmic actin network and secondarily its structure and response to stress.

The structural basis of the hypothesis is the reversible growth of the actin network under the influence of actin-binding protein, acumentin and gelsolin. Under physiological conditions, actin-binding protein, acumentin and actin always interact. Free calcium concentrations therefore determine the state of activity of gelsolin. This activity in turn regulates the actin filament length, as outlined in figure 7.

The assembly of an actin network on a fulcrum of pre-existing actin network (or some other cell structure) could generate propulsive force. Propulsive movements produced by network assembly in the cortex require no special filament–membrane connections, because the growing filament network could simply push on the lipid membrane bilayer or its subjacent lamina. The predictions follow that protrusions of cytoplasm will occur in regions where the free calcium concentration is relatively low. Furthermore, the calcium concentration should be lowest at the advancing tip of the protrusion. A requirement for such propulsion is that actin monomers or

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

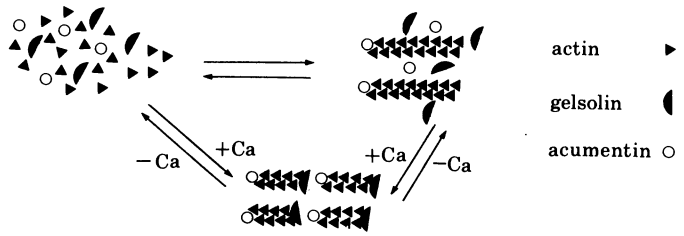

FIGURE 7. Control of actin filament length in phagocytic leucocytes. During or after assembly of actin at low calcium concentrations, acumentin binds to the 'pointed' ends of growing actin filaments. At calcium concentrations in the submicromolar range, gelsolin fragments and binds to the 'barbed' ends of actin filaments.

fragments, or both, are available for assembly onto the scaffolding. These building blocks could diffuse or be actively propelled from regions with higher free calcium concentrations.

The existence of myosin molecules in the cortical cytoplasm of phagocytic leucocytes suggests an additional 'contractile' or retractive element in the movements of cortical cytoplasm (Davies & Stossel 1977; Hartwig *et al.* 1977; Stendahl *et al.* 1980; Painter *et al.* 1981; Valerius *et al.* 1981). The myosin molecules of these cells are hexameric proteins each containing two large and four small subunits. They are similar in structure to vertebrate muscle and non-muscle cell myosins and are capable of generating contractile forces energized by ATP hydrolysis (Hartwig & Stossel 1975; Boxer & Stossel 1976; Stossel & Hartwig 1976; Trotter & Adelstein 1979). By inference, the force-generating mechanism involves cyclic cross-bridge formation between globular actin-binding domains in the two large myosin subunits and actin monomers in filaments. Based on evidence predominantly obtained from studies with platelet and smooth muscle myosins, reversible phosphorylation of two of the small myosin subunits by a kinase enzyme may regulate the rate of cross-bridge formation. The activity of the kinase may in turn be regulated in a positive fashion by the free calcium concentration and by calmodulin (Adelstein & Eisenberg 1980).

There is little information at the present about the quaternary structure of myosin in phagocytic leucocytes. Like most muscle and non-muscle cell myosins, myosins of phagocytic leucocytes form bipolar filaments, a structure in which helical 'tail' domains of the large myosin subunits aggregate, leaving the globular actin-binding 'heads' protruding from the ends of the aggregates. If information recently reported about myosin of lymphoid cells is applicable to phagocytic leucocytes, it is possible that the formation of myosin filaments also requires the phosphorylation of the small myosin subunits (Scholey *et al.* 1980).

Putting together the available facts and some speculation, one might envisage myosin filaments as inactive in cross-bridge cycle formation and disaggregated in areas of low free calcium concentration.

This state would permit myosin molecules to diffuse into other regions where the free calcium concentration is higher. Approaching this region, the myosin molecules might assemble and begin to generate movements of actin filaments in that part of the actin lattice. The predicted net direction of actin filament movement would be from regions of high to regions of low free calcium concentration. This prediction has been verified experimentally in a model system in which actin filaments were assembled in horizontal capillaries in the presence of myosin, gelsolin and actin-binding protein. The ends of the capillaries were inserted into wells containing buffer solutions with either low or high calcium concentrations. The protein mixtures

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

contracted. When a calcium gradient was in existence, the direction of contraction was invariably from high to low calcium concentrations (Stendahl & Stossel 1980).

The effect of cross-linking of the actin filaments by actin-binding protein on the contraction of actin by myosin was also shown in this system. Cross-linking of F-actin by actin-binding protein decreased the quantity of myosin required to produce a gross contraction. The results indicate that the amount of stress generated by the interaction of actin and myosin filaments under these conditions is sufficient to disentangle the actin filaments but is insufficient to disrupt the gel state imposed upon the actin by actin-binding protein. Cross-linking is therefore required for the contraction to propagate throughout a given volume. Myosin filaments contribute to this cross-linking, but they are less potent than actin-binding protein molecules in this activity (Brotschi *et al.* 1978).

In the intact cell, the cortical actin network can be pictured as engaged in a 'tug of war' around the cell periphery. The cortical actin network builds in areas of relatively low free calcium concentrations at the expense of actin network in regions of higher calcium concentration. This formulation can account for the 'conservation of mass' of the intact cortex during cell movements, which has been documented frequently (see, for example, Trinkaus 1980; Chen 1981). This phenomenon in which the appearance of new protrusions of cortex is precisely coordinated with the retraction of pre-existent pseudopodia has previously been likened to a tug of war (Weiss & Garber 1952). The dissolution of the cortical actin network in regions where the calcium concentration rises can lead to the collapse of overlying plasma membrane which is no longer supported by the actin network. In this case, the function of myosin is simply to transport the cortical cytosol. Because the membrane is a continuous structure, there is no need to pull it actively. It can move passively from places where the actin network is collapsing, into enlarging pseudopodia where the network is building. On the other hand, segments of plasma membrane may also be pulled actively from local attachments of actin filaments that are being retracted by myosin toward regions where the actin network is growing.

Selective exclusion is a property of gels, and variable organelle exclusion from the cell periphery is one of the first phenomena invoked as evidence for gel–sol transformations in cortical cytoplasm. Changes in the stability of the cortical actin network occurring in response to fluctuations in the free calcium concentration could regulate the size of the gel pores. Together with corresponding effects of these network variations on myosin-based contractions, the cell could generate movements of organelles. This mechanism of organelle movement has the simplicity of requiring no specific attachments between the organelle membranes and actin filaments. In phagocytic leucocytes there are sometimes quite violent oscillating and saltatory movements of cortical granules in regions of the cortex where secretion occurs (Hirsch 1962) and behind the leading lamella of granulocytes in locomotion (Fukushima *et al.* 1954). These motions may be the consequence of a loosening of the cortical actin network in these regions. In these domains (presumed to have relatively high free calcium concentrations), the disrupted network would permit greater freedom of movement of the granules. At the same time, the filaments in this region would be undergoing motions generated by actin–myosin interactions, which would be creating a tug of war between regions of tighter network structure on either side of the disrupted zone.

In summary, calcium gradients may guide the direction of growth and the regional stability of a perpendicular branching actin network in the cortex of phagocytic leucocytes. Retractive forces exerted on the actin filaments by myosin draw actin filaments from regions of lower

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

network stability to areas with greater strength. In this way cortical actin flows to domains where it is needed to generate new pseudopodia. Plasma membranes can be pushed by propulsive forces generated by the growth of the lattice and collapse upon dissolution of the network. In some cases, actin filaments may be attached to membrane-binding sites and actively pull the membrane. Changes in the pore size and flow of the cortical actin network may move the internal organelles.

The work reviewed in this paper was done in collaboration with Drs J. H. Hartwig, Dr Helen L. Yin, Dr F. S. Southwick and Dr K. S. Zaner. It was supported by grants from the United States Public Health Service (nos HL 19429, Ca 06032), from the Council for Tobacco Research, U.S.A., The American Heart Association, the Muscular Dystrophy Association, and the Edwin S. Webster Foundation.

References

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

Anderson, N. L. 1979 The β and γ cytoplasmic actins are differentially thermostabilized by MgADP; γ actin binds MgADP more strongly. *Biochem. biophys. Res. Commun.* **89**, 486–490.

Allison, A. C., Davies, P. & DePetris, S. 1971 Role of contractile microfilaments in macrophage movement and endocytosis. *Nature, new Biol.* **232**, 153–155.

Asakura, S., Taniguchi, M. & Oosawa, F. 1963 Mechanico-chemical behaviour of F-actin. *J. molec. Biol.* **7**, 55–69.

Boxer, L. A. & Stossel, T. P. 1976 Interactions of actin, myosin, and an actin-binding protein of chronic myelogenous leukemia leukocytes. *J. clin. Invest.* **57**, 964–976.

Boyles, J. & Bainton, D. F. 1979 The changing patterns of plasma-membrane-associated filaments during the initial phases of polymorphonuclear leukocyte adherence. *J. Cell Biol.* **82**, 347–368.

Boyles, J. & Bainton, D. F. 1981 Changes in plasma-membrane-associated filaments during endocytosis and exocytosis in polymorphonuclear leukocytes. *Cell* **24**, 905–914.

Brotschi, E. A., Hartwig, J. H. & Stossel, T. P. 1978 The gelation of actin by actin-binding protein. *J. biol. Chem.* **253**, 8988–8993.

Chen, W.-T. 1981 Mechanism of retraction of the trailing edge during fibroblast movement. *J. Cell Biol.* **90**, 187–200.

Davies, W. A. & Stossel, T. P. 1977 Peripheral hyaline blebs (podosomes) of macrophages. *J. Cell Biol.* **75**, 941–955.

Ferry, J. D. 1980 *Viscoelastic properties of polymers*. New York: Wiley.

Flory, P. J. 1953 *Principles of polymer chemistry*. Ithaca, N.Y.: Cornell University Press.

Fukushima, K., Senda, N., Ishigami, S., Murakami, Y. & Nishian, K. 1954 Dynamic pattern in the movement of leukocyte. II. The behaviour of neutrophil immediately after commencement and removal of stimulation. *Med. J. Osaka Univ.* **5**, 47–56.

Graessley, W. W. 1974 The entanglement concept in polymer rheology. *Adv. Polym. Sci.* **9**, 3–178.

Groeschel-Stewart, U. 1980 Immunochemistry of cytoplasmic contractile proteins. *Int. Rev. Cytol.* **65**, 193–254.

Hartwig, J. H., Davies, W. A. & Stossel, T. P. 1977 Evidence for contractile protein translocation in macrophage spreading, phagocytosis and phagolysosome formation. *J. Cell Biol.* **75**, 956–967.

Hartwig, J. H. & Stossel, T. P. 1975 Isolation and properties of actin, myosin and a new actin-binding protein in rabbit alveolar macrophages. *J. biol. Chem.* **250**, 5699–5707.

Hartwig, J. H. & Stossel, T. P. 1979 Cytochalasin B and the structure of actin gels. *J. molec. Biol.* **134**, 539–554.

Hartwig, J. H. & Stossel, T. P. 1981 Structure of macrophage actin-binding protein molecules in solution and interacting with actin filaments. *J. molec. Biol.* **145**, 563–581.

Hartwig, J. H., Tyler, J. & Stossel, T. P. 1980 Macrophage actin-binding protein promotes the bipolar and branching polymerization of actin. *J. Cell Biol.* **87**, 841–848.

Heuser, J. E. & Kirschner, M. W. 1980 Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. *J. Cell Biol.* **86**, 212–234.

Hirsch, J. G. 1962 Cinemicrophotographic observations on granule lysis in polymorphonuclear leucocytes during phagocytosis. *J. exp. Med.* **116**, 827–833.

Huxley, H. E. 1963 Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. molec. Biol.* **3**, 281–308.

Just, E. E. 1924 *The biology of the cell surface*. Philadelphia: Blakiston.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Keyserlingk, D. G. 1968 Elektronenmikroskopische Untersuchungen über die Differenzierungsvorgänge im Cytoplasma von segmentierten Neutrophilen leukocyten während der Zellbewegung. *Expl Cell Res.* **51**, 79–91.
Kopac, M. J. 1951 Probable ultrastructures involved in cell division. *Ann. N.Y. Acad. Sci.* **51**, 1541–1546.
Kasai, M., Kawashima, H. & Oosawa, F. 1960 Structure of F-actin solutions. *J. Polym. Sci.* **44**, 51–69.
Kawamura, M. & Maruyama, K. 1970 Electron microscopic particle length of F-actin polymerized *in vitro*. *J. Biochem. Tokyo* **67**, 437–457.
Keller, H. U. & Bessis, M. 1975 Migration and chemotaxis of anucleate cytoplasmic, leukocyte fragments. *Nature, Lond.* **258**, 723–724.
Lindberg, U., Carlsson, L., Markey, F. & Nystroem, L. E. 1979 The unpolymerized form of actin in nonmuscle cells. *Meth. Achiev. exp. Path.* **8**, 143–170.
Maruyama, K., Kaibaıa, M. & Fukada, E. 1974 Rheology of F-actin. 1. Network of F-actin in solution. *Biochim. biophys. Acta* **371**, 20–29.
Nagashima, H. & Asakura, S. 1980 Dark-field light microscopic study of the flexibility of F-actin complexes. *J. molec. Biol.* **136**, 169–182.
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.
Oliver, J. M., Kraweic, J. A. & Becker, E. L. 1978 The distribution of actin during chemotaxis in rabbit neutrophils. *J. Reticuloendoth. Soc.* **24**, 697–704.
Oosawa, F. & Asakura, S. 1975 *Thermodynamics of the polymerization of protein.* London, etc.: Academic Press.
Osaki, K. 1973 Viscoelastic properties of dilute polymer solutions. *Adv. Polym. Sci.* **12**, 1–63.
Painter, R. G., Whisenand, J. & McIntosh, A. T. 1981 Effects of cytochalsin B on actin and myosin associated with particle binding sites in mouse macrophages: implications with regard to the mechanism of action of the cytochalasins. *J. Cell Biol.* **91**, 373–384.
Pollard, T. D. & Weihing, R. R. 1974 Actin and myosin and cell movement. *CRC crit. Rev. Biochem.* **2**, 1–65.
Reaven, E. P. & Axline, S. G. 1973 Subplasmalemmal microfilaments and microtubules in resting and phagocytizing cultivated macrophages. *J. Cell Biol.* **59**, 12–27.
Rosenberg, S., Stracher, A. & Lucas, R. C. 1981 Isolation and characterization of actin and actin-binding protein from human platelets. *J. Cell Biol.* **91**, 201–211.
Schloss, J. A. & Goldman, R. D. 1979 Isolation of a high molecular weight actin-binding protein from baby hamster kidney (BHK-21) cells. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4484–4488.
Scholey, J. M., Taylor, R. A. & Kendrick-Jones, J. 1980 Regulation of nonmuscle myosin assembly by calmodulin-dependent light chain kinase. *Nature, Lond.* **287**, 233–234.
Senda, N., Shibata, N., Tatsumi, N., Kondo, K. & Hamada, K. 1969 A contractile protin from leucocytes. Its extraction and some of its properties. *Biochim. biophys. Acta* **181**, 191–200.
Southwick, F. S. & Hartwig, J. H. 1982 Acumentin, a protein in macrophages which caps the 'pointed' end of actin filaments. *Nature, Lond.* **297**, 303–307.
Southwick, F. S. & Stossel, T. P. 1981 Isolation of an inhibitor of actin polymerization from human polymorphonuclear leukocytes. *J. biol. Chem.* **256**, 3030–3036.
Stendahl, O. I., Hartwig, J. H., Brotschi, E. A. & Stossel, T. P. 1980 Distribution of actin-binding protein and myosin in macrophages during spreading and phagocytosis. *J. Cell Biol.* **84**, 215–224.
Stendahl, O. I. & Stossel, T. P. 1980 Actin-binding protein amplifies actomyosin contraction, and gensolin confers calcium control on the direction of contraction. *Biochem. biophys. Res. Commun.* **92**, 675–681.
Stossel, T. P. & Hartwig, J. H. 1975 Interactions of actin, myosin and an actin-binding protein of rabbit alveolar macrophages. Macrophage myosin Mg-adenosine triphosphatase requires a cofactor for activation by actin. *J. biol. Chem.* **250**, 5706–5712.
Stossel, T. P. & Hartwig, J. H. 1976 Interactions of actin, myosin and a new actin-binding protein of rabbit pulmonary macrophages. II. Role in cytoplasmic movement and phagocytosis. *J. Cell Biol.* **68**, 602–619.
Stossel, T. P., Hartwig, J. H., Yin, H. L., Zaner, K. S. & Stendahl, O. I. 1982 Actin gelation and the structure of cortical cytoplasm. *Cold Spring Harb. Symp. quant. Biol.* **46**, 569–578.
Trinkaus, J. P. 1980 Formation of protrusions of the cell surface during tissue cell movement. In *Tumor cell surfaces and malignancy*, pp. 887–906. New York: Alan R. Liss.
Trotter, J. A. 1981 The organization of actin in spreading macrophages. The actin-cytoskeleton of peritoneal macrophages is linked to the substratum via transmembrane connections. *Expl Cell Res.* **132**, 235–248.
Trotter, J. A. & Adelstein, R. S. 1979 Macrophage myosin. Regulation of actin-activated ATPase activity by phosphorylation of the 20,000-dalton light chain. *J. biol. Chem.* **254**, 8781–8785.
Valerius, N. H., Stendahl, O., Hartwig, J. H. & Stossel, T. P. 1981 Distribution of actin-binding protein and myosin in polymorphonuclear leukocytes during locomotion and phagocytosis. *Cell* **24**, 195–202.
Vanderkerckhove, J. & Weber, K. 1978 At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. *J. molec. Biol.* **126**, 783–802.
Wang, K., Ash, J. F. & Singer, S. J. 1975 Filamin, a new high-molecular-weight protein found in smooth muscle and non-muscle cells. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4483–4486.
Weiss, P. & Garber, B. 1952 Shape and movement of mesenchyme cells as functions of the physical structure of the medium. Contributions to a quantitative morphology. *Proc. natn. Acad. Sci. U.S.A.* **38**, 264–280.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Yamamoto, K., Yanagida, M., Kawamura, M., Maruyama, K. & Noda, H. 1975 A study on the structure of paracrystals of F-actin. *J. molec. Biol.* **91**, 463–469.

Yin, H. L., Albrecht, J. H. & Fattoum, A. 1981*a* Identification of gelsolin, a Ca^{2+}-dependent regulatory protein of actin gel–sol transformation and its intracellular distribution in a variety of cells and tissues. *J. Cell Biol.* **91**, 901–906.

Yin, H. L., Hartwig, J. H., Maruyama, K. & Stossel, T. P. 1981*b* Ca^{2+} control of actin filament length. Effects of macrophage gelsolin on actin polymerization. *J. biol. Chem.* **256**, 9693–9697.

Yin, H. L. & Stossel, T. P. 1979 Control of cytoplasmic actin gel–sol transformation by gelsolin, a calcium-dependent regulatory protein. *Nature, Lond.* **281**, 583–586.

Yin, H. L. & Stossel, T. P. 1980 Purification and structural properties of gelsolin, a Ca^{2+}-activated regulatory protein of macrophages. *J. biol. Chem.* **255**, 9490–9493.

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

Zaner, K. S., Fotland, R. & Stossel, T. P. 1981 A low-shear, small volume viscoelastometer. *Rev. scient. Instrum.* **52**, 85–87.

Zaner, K. S. & Stossel, T. P. 1982 Some perspectives on the viscosity of actin filaments. *J. Cell Biol.* **93**, 987–991.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

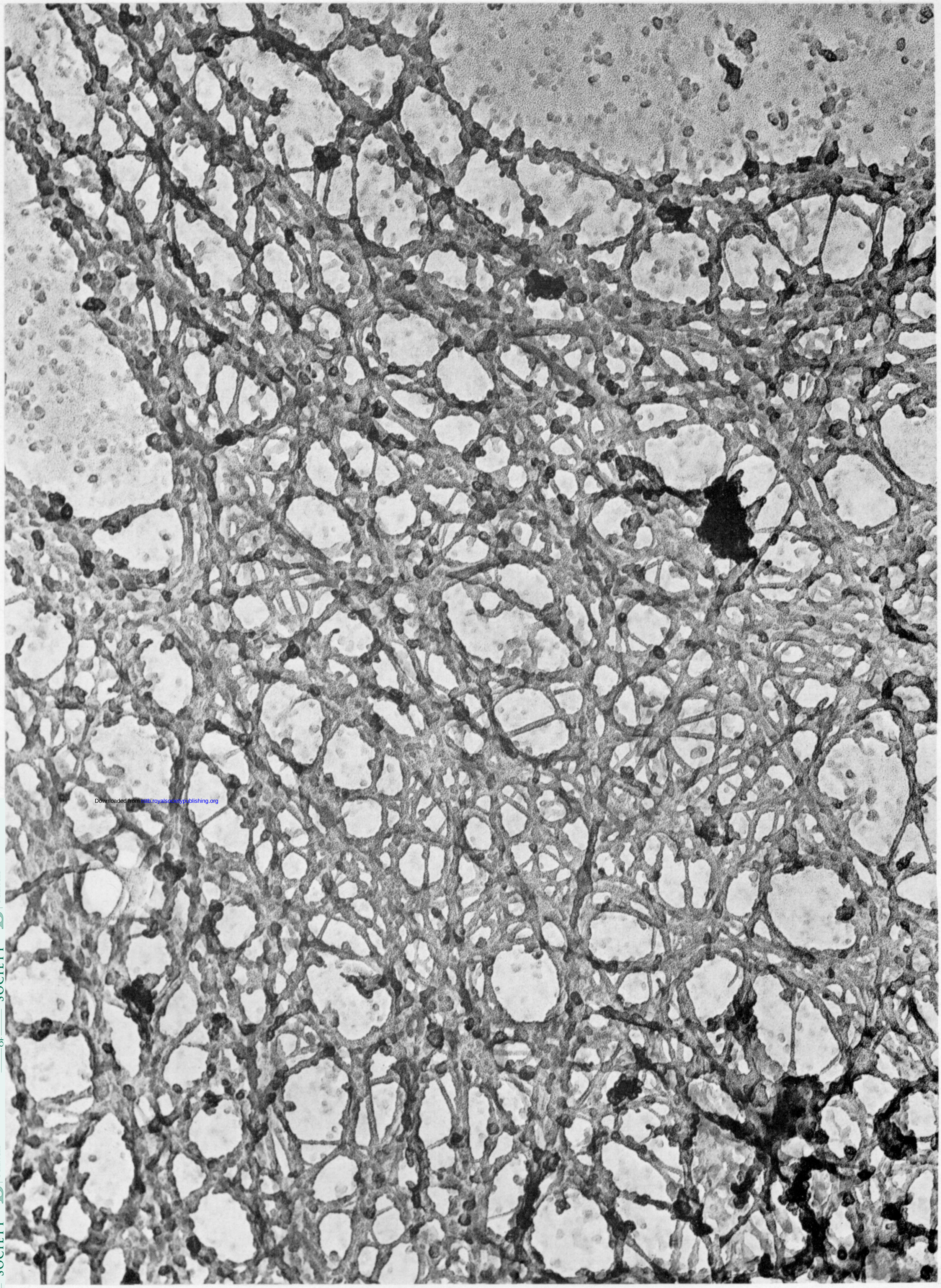

FIGURE 1. Residue of a human polymorphonuclear leucocyte adherent to a glass surface. The cell was extracted with Triton X-100, fixed with glutaraldehyde, critical-point dried and rotary-shadowed with platinum. The photomicrograph is of a region of the cortical cytoplasm. (Magn. ×80000.)

FIGURE 3 (*a*, *b*). For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 3. Morphology of actin filaments alone (*a*) and when assembled in the presence of macrophage actin-binding protein (*b*, *c*). The actin concentration was 0.05 mg ml^{-1}, the actin-binding protein: actin molar ratios were 1:50 (*b*) and 1:25 (*c*). The proteins were placed on copper grids, fixed, critical-point dried and rotary-shadowed as described in figure 1. (Magns: (*a*), ×53000; (*b*, *c*), ×120000.)