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F-Actin and Microtubule Suspensions as Indeterminate Fluids

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The viscosity of F-actin and microtubule suspensions has been measured as a function of shear rate with a Weissenberg rheogoniometer. At shear rates of less than 1.0 per second the viscosity of suspensions of these two structural proteins is inversely proportional to shear rate. These results are consistent with previous in vivo measurements of the viscosity of cytoplasm. This power law implies that shear stress is independent of shear rate; that is, shear stress is a constant at all shear rates less than 1.0 per second. Thus the flow profile of these fluids is indeterminate, or nearly so. This flow property may explain several aspects of intracellular motility in living cells. Possible explanations for this flow property are based on a recent model for semidilute suspensions of rigid rods or a classical friction model for liquid crystals.

ACTIN AND MICROTUBULES ARE among the most widely conserved structural proteins within eukaryotic cells. It has long been suspected that the viscoelastic properties of these polymers (particularly F-actin) play an important role in the maintenance and development of cell shape and in cellular motility (*1*). Maruyama *et al.* (*2*) found that the measured viscosity of F-actin was precisely inversely proportional to the shear rate. These workers reported on the uniqueness of this inverse proportionality and speculated that it may be the result of the formation of an actin network. Subsequent rheological studies confirmed that F-actin suspensions are shear thinning but did not find the same shear-viscosity dependence or did not discuss this relationship (*3, 4*). We confirm the observation of Maruyama *et al.* for actin and observe a similar inverse proportionality for suspensions of microtubules. Also, we show that as a result of this dependence, the fluid flow profiles for such suspensions are indeterminate. That is, a given force does not fix the velocity of movement in these fluids.

Figure 1 is a logarithmic plot of viscosity versus shear rate for two runs of actin at different concentrations, two runs of microtubules with and without taxol [a stimulator of microtubule assembly (*5*)], and one run of the viscosity standard. The values reported in Fig. 1 and throughout this report are the stable values obtained after shearing the fluids for several seconds. As found previously for actin (*2*) in both actin and microtubule suspensions, the torque rises sharply as shear begins but then declines to a stable value over a time period that depends on the shear rate. In addition to the shear-thinning behavior characteristic of all polymer suspensions, these plots show that the power law dependency for actin and microtubules is nearly -1 for all concentrations. That is, actin and microtubule suspensions behave as power law fluids with

$$\eta = A\dot{\gamma}^n \qquad (1)$$

where η is viscosity, A is a constant, $\dot{\gamma}$ is shear rate, and n is the power law exponent. In 12 runs of actin at concentrations from 2 to 6 mg/ml the exponent varied from -0.85 ± 0.02 ($\pm$ standard error of regression coefficient) to -1.15 ± 0.01 with a mean of -1.00. Both extreme values occurred for actin at 2 mg/ml with the rheogoniometer at its lower limit of resolution. In seven microtubule runs the exponent varied from -0.90 ± 0.01 to -1.03 ± 0.03 with a mean of -0.94. The -1 slope (on log-log plots) was found to be nearly independent of the shearing history of the sample, as shown in Fig. 2. The slope of the line for increasing shear (-1.00 ± 0.02) is nearly identical to that for decreasing shear (-1.01 ± 0.02). In a run of chromatographed actin (3 mg/ml) in which shear was varied at random the power law dependence was -0.99 ± 0.09. The power law dependence of the microtubule suspension viscosity was similarly insensitive to shear history. Our measurements varied only slightly. The small standard errors of regression coefficients indicate little variation of points around the calculated line. The slope from run to run also varied little at nominally the same conditions. In five runs of actin at 6 mg/ml from three different actin preparations the mean slope was 1.01 ± 0.04 (SEM). The possibility that our results are due to slipping at rheometer surfaces is eliminated by the finding that actin and microtubule suspensions pour easily and are well mixed after the shearing process in the rheometer. The observed viscosities for suspensions of these two filamentous constituents of the cytoplasm are consistent with previous in vivo measurements of cytoplasmic viscosity. Figure 3 shows a comparison of data obtained from various cytoplasmic studies with that of F-actin suspension at 3 mg/ml. This similarity of the viscosity function in vitro and in vivo is particularly interesting in view of the lack of true cross-links within purified F-actin (*6*) and the highly cross-linked structure of cytoplasm (*7*).

Power law observations in this range are significant because they contradict Graessley's classic random-coil-entanglement explanation that suggests the minimum n value is $-9/11$ (*8*). Typically, data show n between -0.4 and -0.85 for polymer solutions (*9*). Significantly, since shear stress is viscosity multiplied by shear rate

$$\tau = \eta\dot{\gamma} = (A\dot{\gamma}^n)\dot{\gamma} = A\dot{\gamma}^{(n+1)} \qquad (2)$$

where τ is the shear stress (force per unit area). Our observation that $n = -1$ means that the shear stress is independent of shear rate. In this case, fluid motion is completely indeterminate. In Fig. 4 we plot shear stress data for actin, microtubules, and the Newtonian fluid standard. One's intuition, for example, on the basis of flooded rivers, is that increased shear increases shear stress. As shown in Fig. 4, the Newtonian standard

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follows the intuitive expectation. However, for both actin and microtubules the shear stress remains constant at all shear rates tested. This constant is the value A in Eqs. 1 and 2.

In these experiments we controlled the shear rate and measured the force. In most biological (and other) situations, however, the interaction is reversed: a pump, a gravitational potential, or a cell supplies a particular shear stress, and the flow pattern accommodates it. However, as shown in Fig. 4, a force alone cannot fix the flow patterns for actin and microtubule (that is, indeterminate) suspensions. For any geometry, only one force pattern gives reasonable flow rates; push with less force and there is no motion, push with more force and the fluid velocity is limited by inertia alone.

Actin and microtubules are widely regarded as important in determining the physical properties of cytoplasm (*1*). Energy-dependent movement of particles through the cytoplasm occurs in all eukaryotic cells, and cytoplasm itself is capable of rapid, energy-dependent movement in some cells (*10, 11*). Our data suggest that the velocity of movement is not limited by force, and the low Reynolds number characteristic of living systems (*12*) suggests that inertia is not responsible. By deduction then, the observed velocities are fixed by the chemical kinetics of the biological motors. For example, structures within cells move suddenly over moderate (~30 μm) distances at constant velocity, and they halt equally suddenly (*10*). One explanation is that no motion occurs until a threshold force is reached, then a constant velocity is maintained by the stable kinetics of the mechanochemical motor.

The unusual fluid dynamics of nearly indeterminate fluids may also explain the flow properties that characterize cytoplasmic streaming in various cells (*11*). In these cells shearing flows are contained by cell membrane-cortex structures or, in the case of algae, by cytoplasmic walls. The flowing cytoplasm and the containing cytoplasm are fundamentally similar in their content of water and proteins. Flow indeterminacy may explain how these two regions of similar chemistry have such different velocities while bearing exactly the same stresses. Possibly, the cell pushes the cytoplasm at constant force in some part of the cell and drags on the cytoplasm in the rest of the cell (necessarily with the same force per unit area). If the area pushing is smaller than the area dragging, there will be no cytoplasmic flow. If it is larger, flow is rapid. Thus, for finite flows and equal shear on the cytoplasm and membrane, small differences in

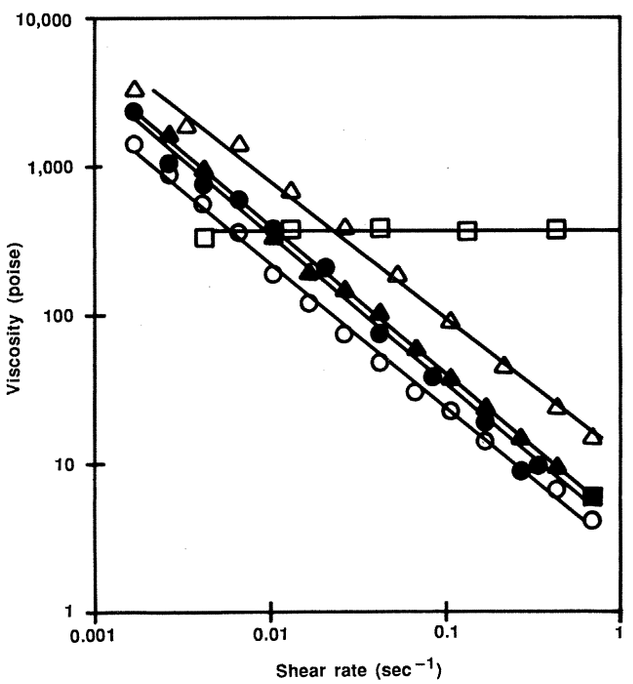

Fig. 1. Viscosity as a function of shear rate at 23°C on a log-log scale for suspensions of cytoskeletal proteins and a viscosity standard. Actin was purified from rabbit skeletal muscle by the method of Spudich and Watt, or by their method and further purification by chromatography on Sephacryl S-200 (Pharmacia, Piscataway, NJ) (*19*). Purified actin was maintained in dialysis at 4°C against buffer A [2.0 m*M* tris-HCl, *p*H 8.0, 0.2 m*M* ATP (adenosine triphosphate), 0.5 m*M* mercaptoethanol, and 0.2 m*M* $CaCl_2$] for no more than 6 days. Microtubule protein was purified from beef brain in the presence of glycerol according to the method of Shelanski *et al.* (*20*) with the modification that 0.1*M* Pipes (piperazine ethanesulfonic acid), *p*H 6.6, was used in all reassembly buffers. A model R.18 modified (*21*) Weissenberg rheogoniometer was used for all rheometry experiments. Actin was polymerized by adding KCl to a final concentration of 50 m*M* and $MgSO_4$ to a final concentration of 2 m*M* to G actin at various concentrations in buffer A; the mixture was applied quickly to the bottom cone (2°, 5 cm in diameter) of the rheogoniometer, and the actin was polymerized for 1 hour at 23°C before measurements were made. Microtubule protein suspended in 0.1*M* Pipes, *p*H 6.9, 1 m*M* $MgCl_2$, 1 m*M* EGTA, 0.1 m*M* EDTA, and 0.5 m*M* GTP (guanosine triphosphate) was polymerized at 37°C for 20 minutes and applied to the cone of the rheogoniometer. A humidified atmosphere was maintained around the cone and plate assembly by adding a small pool of water to the floor of the instrument's temperature control chamber. For each sample of protein loaded into the rheometer, 10 to 14 shear rates (drive settings) were chosen to obtain torque readings. After each torque reading the drive was turned off and the torque allowed to return to baseline before we reset the drive for the next reading. Measurements on a single sample of protein took approximately 2 hours. The rheogoniometer was calibrated with a Newtonian viscosity standard (R12000, Cannon Instrument Company). Measurements of viscosity and shear were calculated from rheogoniometer drive settings and torque readouts by standard formulas. (●) F-actin, 6 mg/ml; slope, −1.00 ± 0.02; (○) chromatographed F-actin, 2 mg/ml; slope, −0.98 ± 0.02; (▲) polymerized microtubule protein, 12 mg/ml; slope, −1.00 ± 0.01; (△) microtubule protein, 12 mg/ml, polymerized in the presence of 5 μ*M* taxol; slope, −0.95 ± 0.03; (□) viscosity standard, slope, 0.00 ± 0.01.

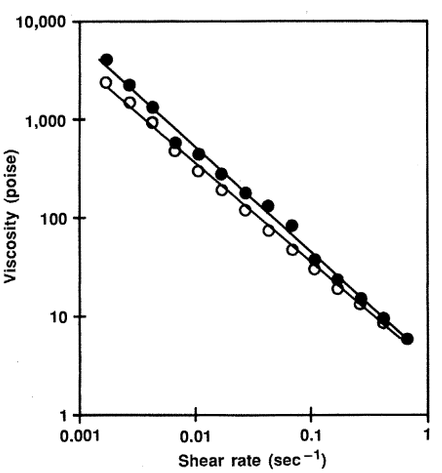

Fig. 2. Two shear protocols for chromatographed F-actin, 3 mg/ml, from low to high rates of shear (●), slope, −1.00 ± 0.02, and from high to low shear (○), slope, −1.01 ± 0.02.

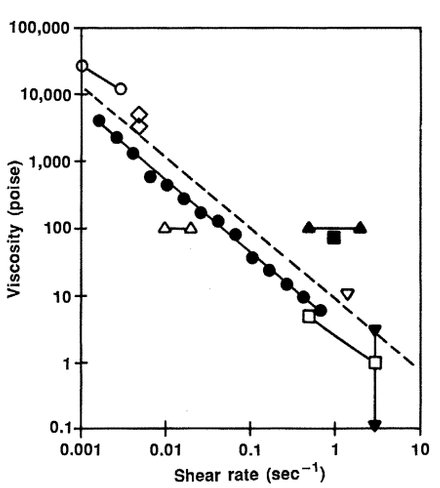

Fig. 3. Viscosity of chromatographed F-actin, 3 mg/ml (●) compared with viscosity measurements of cytoplasm by various in vivo methods: (○) Valberg and Albertini (*22*), (◇) Nemoto (*23*), (△) Sung *et al.* (*24*), (▲) Hiramato (*25*), (■) King and Macklem (*26*), (□) Sato *et al.* (*27*), (▽) Crick and Hughes (*28*), and (▼) Yagi (*29*). The linear regression for the in vivo data (----) gives a slope of −1.02 ± 0.18.

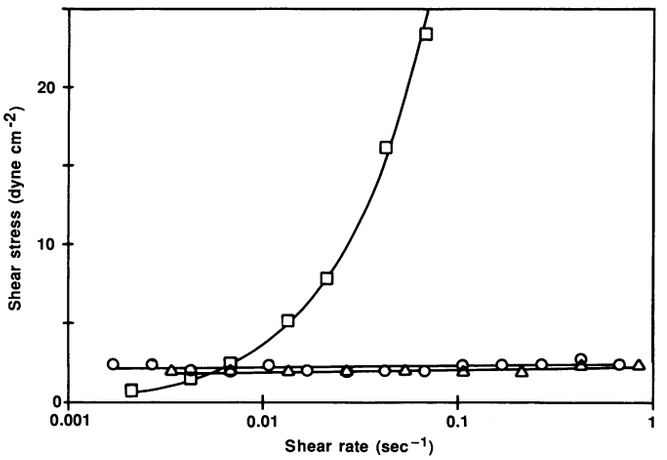

Fig. 4. Shear stress as a function of shear rate for chromatographed F-actin, 3 mg/ml (○); polymerized tubulin, 6 mg/ml (△); and the R12000 viscosity standard (□).

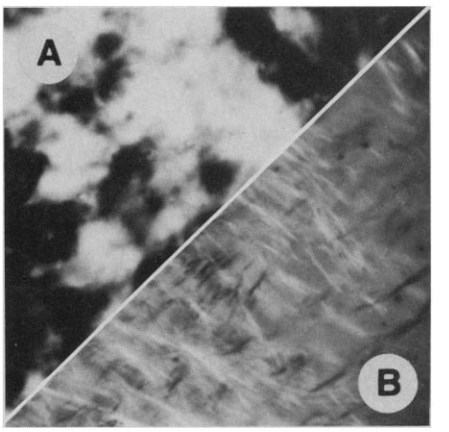

Fig. 5. Polarization micrographs of suspensions of actin and microtubules (Leitz Ortholux I Pol microscope, ×1200). (**A**) F-actin at 25 mg/ml. (Similar domains were observed at concentrations of 8 mg/ml, but the birefringence was too dim to photograph successfully.) (**B**) Microtubules at a concentration of 6 mg/ml.

chemistry can produce enormous differences in flow.

This viscosity relation may also play a role in slow axonal transport, which involves a flow of cytoskeletal elements down the neural axon at a variety of different rates characteristic of the different cytoskeletal elements (*13*). If the fluid flow is nearly indeterminate and the axonal pressure drop is constant, slight changes in the pushing pressure or entrance region pressure drop will greatly affect the transport rate down the axon.

Another possibility from shear-thinning behavior that does not require flow indeterminacy concerns organisms that locomote upon a slime trail. In most instances this slime is a polymer solution that would be shear thinning; most crawling results from sustained oscillations of contraction and extension. Two models are possible: (i) an asymmetric oscillation in space so that the moving area is always smaller than the stationary area or (ii) an asymmetric oscillation in time both with the condition that the drag on the front of the organism over the substrate is determined by Newtonian viscosity (in water, for example) and the drag on the rear of the creature is determined by shear-thinning slime. Then, as in Fig. 1, if the cell extends faster than it contracts, a net force will move the organism forward. Snail slime possesses "solid-like" properties at low shear rates (*14*) in concert with a shear-thinning fluid model.

The rheology of actin suspensions has generally been thought to be the result of network destruction by shearing (*2, 6, 15*). Although microtubule suspensions do not form gels or the looping entanglements required by network theories (*4, 8*), they have the rheological properties of actin suspensions. Further, the limiting power law exponent for entangled polymers is calculated to be −9/11 (*8*). Our data (Fig. 2) also indicate that shearing history has a relatively small effect on the viscosity of actin and microtubule suspensions. These observations suggest that network destruction is not primarily responsible for the rheology of these filamentous proteins. We have formulated two alternative models for these viscosity observations.

One model follows a suggestion of Zaner and Stossel (*3*) for actin suspensions and assumes that semidilute suspensions of rigid rods are at concentrations below the liquid crystal formation (that is, an isotropic distribution of the rods in space before shearing). In this model, as treated by Doi and Edwards (*16*) and by Jain and Cohen (*17*), a large obstructional effect is caused by the constraint that rods cannot pass through each other. Assuming that the frequency of rod-rod obstructions is due to shear-independent Brownian motion, Doi and Edwards (*16*) found regions where shear stress is independent of shear rate. To the extent that this model is appropriate, the phenomenon that is typically called "gelation" (true solidification) in the biological literature is seen as the "glass" (liquid with solid properties) transition induced by this obstructional constraint. This glass transition explains why the viscosity of cytoplasm and actin suspensions is so similar, that is, why cross-linkers in cytoplasm have so little effect on viscosity, as shown in Fig. 3. It also explains why actin "gels" behave as liquids. This "log jam model" can predict the observed −1 power law dependence of the viscosity and also that the power can be smaller than −1 over small ranges of shear.

We propose a second model to explain the observations with relatively flexible actin filaments and to extend the applicable concentrations above those for liquid crystal formation. This model differs from the network model and rigid rod model in that the fluid is locally anisotropic. Fluid motion is not continuous but occurs among discrete domains in a classical analogy to solid surface friction or superfluidity. Anderson (*18*) has proposed this model to describe large-scale density waves in smectic B liquid crystals. If one domain slides over another, two roughnesses encountering one another must either be completely overwhelmed by the forces driving them or must stop the motion entirely. A roughness cannot provide a continuous source of linear dissipation because any roughness that causes a purely elastic deformation does not dissipate energy. Instead, it returns as much mechanical energy to the lattice as was present in the original deformation. Viscous stress is thus viewed as the (shear rate independent) force necessary to substantially kink or break those protein strands that extend from one solid-like domain to another. The effect that biologists call gelation is, presumably, liquid crystal domain formation. This solid friction analogy is supported by the finding of ourselves and others (*2*) that the initial shear stress (analogous to static friction of solids) is always greater than the steady-state stress (analogous to sliding friction of solids). Additional evidence for this model is shown in Fig. 5 in which actin and microtubule protein suspensions reveal distinct domains under polarized light in contradiction to the assumptions of the rigid rod model described above.

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Two Mammalian Genes Transcribed from Opposite Strands of the Same DNA Locus

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This report describes the characterization of a genomic locus in the rat that encodes overlapping genes occupying both strands of the same piece of DNA. One gene (strand) encodes gonadotropin-releasing hormone (GnRH). A second gene, SH, is transcribed from the other DNA strand to produce RNA of undefined function. The RNAs transcribed from each DNA strand are spliced and polyadenylated, and share significant exon domains. GnRH is expressed in the central nervous system while SH transcripts are present in the heart. Thus, the genome of a mammalian organism encodes two distinct genes by using both strands of the same DNA.

INHERENT TO THE CONCEPT OF A EUkaryotic gene is that the function of the DNA strand opposite the transcribed strand is to serve as a necessary conduit for double-stranded, semiconservative DNA replication. Likewise, although the discovery of introns brought about modifications in the concept of genes as linear discrete units along the chromosome, introns have been regarded as raw genetic material susceptible to high levels of evolutionary drift with regard to nucleotide sequence and even to size fluctuation. Exons have been regarded as the biologically relevant portions of a gene, existing under varying degrees of selective pressure, reflective of the relative advantage they confer upon the organism possessing the particular allele. However, a number of recent reports have indicated a need to reexamine these concepts. The *Gart* locus of *Drosophila melanogaster*, known to encode three purine pathway enzymatic activities, has been shown to contain an entire gene encoding a cuticle protein, "nested," within the first *Gart* intron and transcribed from the opposite DNA strand (*1*). The cuticle protein gene itself is divided by an intron. Another Drosophila locus, encoding dopa decarboxylase, has been shown to share an 88-bp region at its 3′ end with the 3′ end of a transcript arising from an unknown gene on the opposite strand (*2*). A comparable situation has been described for a region of mouse DNA from tissue culture cells, BALB/cTS-A-3T3, which encodes two 3′ overlapping transcripts of undetermined functions (*3*). The data presented here extend these observations to describe the virtual cohabitation of a genomic locus within mammalian DNA by two distinct genes. Both DNA strands serve as templates for transcription, and the resulting polyadenylated [poly(A)$^+$] RNAs contain a significant amount of shared exonic sequences.

The gonadotropin-releasing hormone (GnRH) gene is a single-copy gene exhibiting a similar arrangement of four exons in both rats and humans (*4*). In the maturation of human placental preproGnRH messenger RNA (mRNA), the first intron is not removed and thus the human placental GnRH mRNA possesses an exceptionally long 5′ untranslated region (*5*). During experiments to characterize the hypothalamic form of GnRH mRNA, human placental GnRH complementary DNA (cDNA) was nick-translated and used as a hybridization probe to screen rat hypothalamic cDNA clones. DNA sequence analysis demonstrated that ten of the clones showing hybridization to this probe represented the hypothalamic form of preproGnRH mRNA. Surprisingly,

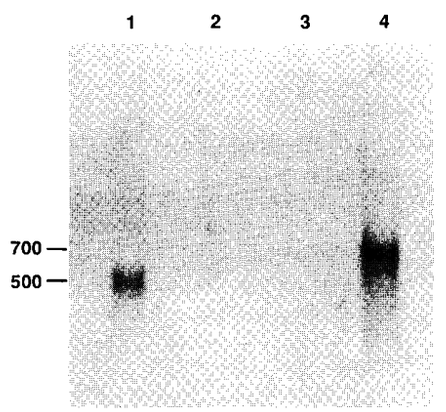

Fig. 1. Northern analysis of rat hypothalamic and heart poly(A)$^+$ RNA. Duplicate samples of heart (lanes 2 and 4) and hypothalamic (lanes 1 and 3) poly(A)$^+$ RNAs were prepared as a Northern blot and probed with an SP6-generated GnRH (lanes 1 and 2) or SH (lanes 3 and 4) strand-specific probe. In control hybridizations to M13 template DNAs of defined sequence the SP6 probes were shown to be completely strand-specific. Size markers are indicated to the left. Total cellular RNA was prepared from the indicated rat tissues by the guanidinium/CsCl method (*27*) and poly(A)$^+$ RNAs selected on oligo(dT)-cellulose. Glyoxal-denatured poly(A)$^+$ RNA (2.5 μg per lane) was fractionated on a 1.4% agarose gel, run in 10 m*M* $NaPO_4$ (*p*H 6.7) (*28*), and subsequently transferred to Nytran. SP6 strand-specific RNA probes were generated as described (*7*). Probes were labeled to a specific activity of 5×10^8 counts per minute per microgram of RNA. Blots were probed at 60°C in standard hybridization solution in the presence of 50% formamide. Filters were washed in .07× standard saline citrate/5 m*M* EDTA/0.5% SDS at 70°C and exposed to x-ray film for 10 hours.

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*This work is dedicated to the memory of Edward Herbert.