Mechanical Properties of Brain Tubulin and Microtubules

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Abstract. We measured the elasticity and viscosity of brain tubulin solutions under various conditions with a cone and plate rheometer using both oscillatory and steady shearing modes. Microtubules composed of purified tubulin, purified tubulin with taxol and 3× cycled microtubule protein from pig, cow, and chicken behaved as mechanically indistinguishable viscoelastic materials. Microtubules composed of pure tubulin and heat stable microtubule-associated proteins were also similar but did not recover their mechanical properties after shearing like other samples, even after 60 min. All of the other microtubule samples were more rigid after flow orientation, suggesting that the mechanical properties of anisotropic arrays of microtubules may be substantially greater than those of randomly arranged microtubules. These experiments confirm that MAPs do not crosslink microtubules. Surprisingly, under conditions where microtubule assembly is strongly inhibited (either 5G or at 37°C with colchicine or Ca++) tubulin was mechanically indistinguishable from microtubules at 10–20 μM concentration. By electron microscopy and ultracentrifugation these samples were devoid of microtubules or other obvious structures. However, these mechanical data are strong evidence that tubulin will spontaneously assemble into alternate structures (aggregates) in nonpolymerizing conditions. Because unpolymerized tubulin is found in significant quantities in the cytoplasm, it may contribute significantly to the viscoelastic properties of cytoplasm, especially at low deformation rates.

Three major protein polymers, actin filaments, intermediate filaments and microtubules, are thought to be responsible for the complex, viscoelastic mechanical properties of cytoplasm. One way to establish this relationship between the cytoskeleton and the physical properties of cells is to characterize the mechanical properties of each of the elements of the cytoskeleton. Detailed studies of purified actin have established that both actin filaments and nonfilamentous actin form weak viscoelastic materials (Jen et al., 1982; Zaner and Stossel, 1983; Sato et al., 1985, 1986; Oppermann and Jaberg, 1985) and that actin filament cross-linking proteins increase both the elasticity and viscosity (Zaner, 1986; Sato et al., 1987) at least at high rates of deformation. In this study we used quantitative rheological methods (Ferry, 1970) to obtain comparable mechanical information of unpolymerized tubulin and microtubules composed of either pure tubulin or tubulin plus microtubule-associated proteins (MAPs). The idea was to learn whether microtubules diffuse freely in solution and rebound elastically when they collide or whether they bind to each other strongly enough to form some sort of a network. Most of our measurements were made by low amplitude oscillatory shear methods over a range of deformation frequencies, shear strains and shear rates including those expected in the cytoplasm of living cells. By using minute strains, the measurements were nondestructive and had no detectable effect on the mechanical properties of the samples. On the other hand, the cytoplasm of living cells is known to be shear sensitive (Sato et al., 1983, 1984; Valberg and Albertini, 1985), so that we also examined microtubules under various conditions where they were sheared continuously at homogeneous shear rates at every point in the sample.

We found that microtubules with or without MAPs are remarkably similar, shear-sensitive viscoelastic materials, showing that MAPs do not crosslink microtubules. On the other hand, the rheological properties of microtubules with or without MAPs are more complex than expected for noninteracting rigid rods, behavior that may be explained by weak interactions between microtubules. Surprisingly, solutions of unpolymerized tubulin have mechanical properties similar to microtubules, indicating a tendency of the molecules to aggregate in a way that might possibly contribute to the structure of cytoplasm. Our measurements were made at microtubule concentrations where the material is expected to be isotropic. Buxbaum et al. (1987) recently reported somewhat

1. Abbreviation used in this paper: MAPs, microtubule-associated proteins.
different mechanical properties of microtubules; the differences are most likely attributable to their use of high protein concentrations where liquid crystal formation is possible.

**Materials and Methods**

**Rheological Principles**

In oscillatory experiments using minute deformations, a Newtonian liquid, such as water, exhibits the maximum stress at the greatest shear rate. Therefore, the stress of a Newtonian liquid oscillates ~90° out of phase with an input sinusoidal deformation (strain) (Ferry, 1970). Conversely, the stress of a Hookean solid, such as steel, oscillates in phase with the input strain (Ferry, 1970). The stress of a viscoelastic material, like tubulin, oscillates between 0° and ~90° depending on how solid- or liquid-like it is. The dynamic elasticity (G') is the in-phase component of the sinusoidal material response and the dynamic viscosity (η') is the ~90° out of phase component of the material response. By definition, a Newtonian liquid has a G' of zero and a frequency-independent viscosity, η. Hookean solids have a frequency-independent elastic modulus, called the equilibrium elastic modulus, G₀, and an infinite viscosity, η (Ferry, 1970). Shear viscosity (η), the frictional resistance of a sample to steady flow (shear rate) (Walters, 1975), is defined as the ratio of the shear stress to the shear rate: \( \eta = T / \dot{\gamma} \), where T, the shear stress (resistance), is expressed in dynes per square centimeter; \( \dot{\gamma} \), the shear rate, is expressed in inverse seconds; and η, the shear viscosity, is in units of Poise (dyne·s·cm⁻²). The shear viscosity of a Newtonian liquid is independent of both the shear rate and the extent of displacement (strain) (Ferry, 1970). On the other hand, for biological materials, the shear viscosity is a complex function of shear rate (which is the resultant velocity gradient determined by the rheometer geometry) and changes with the extent of the strain. This and the following techniques disrupt the sample and should not be confused with the nondisruptive conditions used in oscillatory analysis. Yield stress and recovery time are empirical parameters used to confirm the existence of structure in a complex material (Walters, 1975). A yield stress can be defined as the maximum stress a solid (or viscoelastic material) can sustain without disruption (flow) when deformed continuously at a constant shear rate. Recovery time is a measure of the time required for self-healing of the structure to 90% of the pre-sheared dynamic elasticity (Sato et al., 1985). Both parameters are dependent on the rate and extent of deformation (strain). Newtonian liquids have neither a yield stress nor recovery time.

**Rheometry**

An R8 Weissenberg rheogoniometer (Sangamo Controls, Ltd., Bognor Regis, Sussex, England) was used in both small-amplitude forced oscillation and continuous shear modes. The cone and plate geometry and the equations used to derive G', η', yield stress, strain and shear rate are detailed in Sato et al. (1985). Tubulin proteins polymerizing between the cone and plate generally took 90 minutes to equilibrate to 37 ± 0.01°C.

**Protein Purification and Assays**

Microtubule protein, consisting of tubulin and microtubule-associated proteins, was prepared by cycles of polymerization and depolymerization from pig cerebral cortex (Murphy, 1982; Selden and Pollard, 1983) or cow cerebral cortex (Murphy and Hiebsch, 1979). Chicken brain tubulin and phos- phoceullulose purified chicken tubulin were the generous gift of Drs. S. Roth- well and D. Murphy, Johns Hopkins University School of Medicine (Baltimore, Maryland). Pellets from the second cycle of polymerization were frozen drop-wise in liquid nitrogen and stored at -70°C. Before use, tubulin pellets were thawed in the appropriate buffer, sonicated briefly on ice, and held on ice for a total of 15 min. These solutions were clarified in an Eppendorf Microfuge at 5°C for 20 min. Supernatans were assayed for protein content with the Bradford (1976) assay with ovalbumin as standard. Some pig tubulin was further purified by DEAE-Sephadex A-50 as detailed by Murphy et al. (1977). Heat stable MAPs consisting of about 61% MAP-2 were obtained from pig brain by the methods of Nishida et al. (1981) and Selden and Pollard (1983). In some experiments we used MAP-2 isolated to greater than 95% purity from heat stable pig brain MAPs (Selden and Pollard, 1983). The concentration of MAPs was determined with the Hartree (1972) method with bovine serum albumin as the standard. The com-
Figure 1. Dynamic elasticity of tubulin as a function of time. Dynamic elasticity (G', □) measured at 0.6 Hz was followed from the time tubulin stored at 0°C was placed on the rheometer plates (time = 0). Samples in their respective tubulin buffers took ~90 min to equilibrate to 37°C. (A) Three times cycled microtubule protein (2 mg/ml, ~16 μM tubulin) from cow brains at 37°C. The plot is typical for microtubules from pig, cow, and chicken. Polymerization was complete within 30 min judging from Ostwald capillary viscometry of parallel samples. The presence or absence of 1.07 mg/ml (3.7 μM) MAP-2, 2 mg/ml unfractionated heat stable MAPs or 20 μM taxol with purified 20 μM tubulin did not significantly affect this time course. (B) DEAE tubulin (20 μM) from pig brains with 20 μM colchicine. This plot is also typical for microtubule protein from brains of pig, cow and chicken in various nonpolymerizing conditions including 20 μM colchicine, 1.4 mM CaCl2 and 5°C.

log dynamic elasticity was directly proportional to log frequency over the whole range tested and did not become constant at the lowest measurable frequencies as expected for a viscoelastic solid.

It is remarkable that the absolute values of these physical properties were nearly the same for both polymerized and unpolymerized pure tubulin at 10–20 μM concentrations (Fig. 2). Neither the animal source of the microtubule protein, the presence or absence of taxol or MAPs nor the method used to prevent polymerization had a substantial influence on the dynamic elasticity or viscosity at any frequency (data not shown). At 30 μM concentration, the dynamic elasticity and viscosity of pure tubulin microtubules were three times the corresponding values for nonfilamentous tubulins (see legend to Fig. 2 A).

Shear Viscosity

When microtubule samples at 20 μM concentration were sheared (rather than oscillated) by impulsively rotating the bottom plate at 0.09 RPM for 1 min, the cone and plate geometry imposed a constant shear strain rate of 0.546 s⁻¹. The microtubules responded with a quasi-linear increase in stress with strain until structure was disrupted at the yield stress of 14 ± 1 dyne/cm² (mean ± SD) (Fig. 3 A). For comparison, Fig. 3 B illustrates the behavior of a Newtonian fluid where the stress responds nearly as a step function to the initiation of a constant rate of strain.

The shear viscosity (η), a measure of the frictional resistance to flow, depended greatly on the shear rate (κ') for both tubulin and microtubules (Fig. 4). Microtubules at 20 μM concentration composed of microtubule protein, pure tubulin, or pure tubulin plus MAPs had similar properties. At shear rates between 0.06 and 5 s⁻¹, log η was linear with log κ' with a slope of −0.99 ± 0.03. Below a shear rate of 0.06 s⁻¹, the slope was notably greater. This change in slope at κ' = 0.06 ± 0.02 s⁻¹ was reproducible for all microtubule samples in the absence of taxol. In the presence of taxol there was no change in the slope which was −1 even at the lowest shear rates. At shear rates >200 s⁻¹, the shear viscosity tended toward an upper limiting value of 0.08 P independent of the shear rate.

The shear viscosities of all of the 20 μM tubulin samples that were prevented from polymerizing (5°C or 37°C with 1.4 mM CaCl2 or colchicine) were similar to each other and
Comparison of Rheological Data on Microtubules

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Figure 3. Shear stress as a function of strain. Samples were incubated for 200-300 min at 37°C on the plattens and then strained (deformed) from time = 0 at a constant shear rate of 0.546 s⁻¹ for 1 min by rotating the bottom rheometer plate at 0.09 rpm. The response of the tubulin shear stress was monitored by the freely suspended top cone. (A) Three times cycled microtubule protein from chickens (2.2 mg/ml, ~16 μM tubulin) at 37°C. Microtubules in various conditions had a yield stress of 14 ± dyne/cm² (mean ± SD). Nonfilamentous tubulin (20 μM) at 5°C or with 20 μM colchicine responded similarly to strain with a yield stress of 8 ± 3.6 dyne/cm². The quasi-linear stress response before the yield stress is typical of viscoelastic materials and is evidence for structure formation in both kinds of samples. No differences were observed among microtubule protein from pig, cow, and chicken. (B) Viscosity standard oil (Cannon Instruments, State College, PA). The stress of this and other Newtonian liquids respond as a step function of strain.

Figure 4. Shear viscosities of microtubules and tubulin as a function of shear rate. Isotropic samples were incubated for at least 200 min at 37°C and then sheared sequentially at shear rates of 10⁻² to 10³ s⁻¹. The dramatic inverse dependence of shear viscosity on shear rate for both samples is typical of viscoelastic materials and is evidence for shear sensitive structures in both types of samples. (A) Microtubules from 20 μM pig DEAE tubulin (○). This plot is representative of microtubules formed from purified tubulin, purified tubulin with heat stable MAPs and 3× cycled microtubule protein. (B) 20 μM pig DEAE tubulin with 20 μM colchicine (○). This plot is representative of tubulin in various nonpolymerizing conditions.

Recovery of Structure After Shearing

After shearing at 0.546 s⁻¹ for 1 min, the mechanical properties of microtubules recovered over 60 min (Fig. 5 A). The new dynamic elasticity was 50% larger than that of equilibrated samples prior to shearing (Fig. 5 A). During the recovery period, short fragments of microtubules were replaced by longer intact microtubules judging from electron microscopy of samples taken at 0, 5, 15, and 30 min after shearing. The larger dynamic elasticity of these recovered samples suggests that microtubules oriented by shearing form a structure more rigid than the random networks formed during spontaneous polymerization. Ordered arrays were not obvious in the negatively stained samples of sheared microtubules, probably because any order was lost during staining.

In contrast, when microtubules reconstituted from pure tubulin and heat stable MAPs or purified MAP-2 were sheared at 0.546 s⁻¹, η' did not recover over more than 60 min. By electron microscopy these samples consisted of heterogeneous mixtures of fragmented and fractured microtubules even after 60 min. When these reconstituted microtubules were sheared at a higher rate (56 s⁻¹), η' recovered by 10-20%. Short fragments of microtubules persisted for at least 30 min judging from electron microscopy. These observations show that heat stable MAPs inhibit the rate of recovery of fragmented microtubules.

Nonfilamentous tubulin sheared at 0.546 s⁻¹ for 1 min recovered only partially (Fig. 5 C). By electron microscopy, these samples contained no microtubules or other obvious structures before or after shearing.

Discussion

Comparison of Rheological Data on Microtubules

Our shear viscosity data for microtubules is slightly different from that reported recently by Buxbaum et al. (1987). Both groups used the same type of rheometer. Buxbaum et al. tested prepolymerized samples of unfractionated microtubule protein at concentrations of 6-12 mg/ml. We polymerized the microtubules in the rheometer to avoid shearing of the material and used protein concentrations three to sixfold greater.
To our knowledge, this paper reports the only data available on other aspects of microtubule and tubulin rheology. The new information includes data on the changes in elasticity and viscosity with time, on nonperturbing oscillation measurements and on the time course of recovery from shearing.

The oscillation data suggest that microtubules under non-disruptive conditions behave as a weak viscoelastic material. The dependence of the dynamic elasticity and dynamic viscosity on frequency shows that the mechanical properties of this material depend greatly on the rate of deformation. The yield stress measurements (Fig. 3) and the power law relation between the shear viscosity and the shear rate (Fig. 4) show that microtubules are weak, shear sensitive structures. At high shear rates the material disrupts into small fragments, so that shear viscosity eventually becomes independent of the shear rate like a Newtonian liquid (Fig. 4).

**Physical Models for Solutions of Microtubules**

Suspensions of microtubules might conceivably assume a variety of different physical states, including liquid crystalline at one extreme (Buxbaum et al., 1987) and randomly oriented rods with more or less flexibility and affinity for each other at the other extreme. Alignment of the microtubules within domains can account for the shear viscosity data of Buxbaum et al. (1987) and accordingly they observed such ordered rods with more or less flexibility and affinity for each other in the shear rate like a Newtonian liquid (Fig. 4).

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![Figure 5](https://example.com/figure5.png)

**Figure 5.** Recovery of the dynamic elasticity after disruption by shearing. Equilibrated samples were sheared at 0.546 s⁻¹ for 1 min and then ζ' was monitored at 0.6 Hz beginning at time = 0. The extreme left value corresponds to the pre-sheared dynamic elasticity. (A) Three times cycled bovine microtubules (2.2 mg/ml, ~16 μM tubulin) at 37°C. This shear recovery was typical for microtubules from the 3 sources. (B) DEAE tubulin (20 μM) from pig with heat-stable MAP-2 (3.7 μM) at 37°C. This absence of shear recovery over 60 min was reproduced for two separate batches of pig microtubules and also with heat-stable, unfractionated MAPs at 2 mg/ml. (C) DEAE tubulin (20 μM) from pig with 20 μM colchicine at 37°C. This shear recovery was typical of pure tubulin under the various nonpolymerizing conditions.

Table I. Theoretical Calculation of Critical Volume Fractions for Phase Transitions of Rod-like Polymers

<table>
<thead>
<tr>
<th>Polymer length (μm)</th>
<th>Axial ratio</th>
<th>Theoretical Critical Volume Fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Φ*</td>
</tr>
<tr>
<td>8</td>
<td>266</td>
<td>0.0298</td>
</tr>
<tr>
<td>12</td>
<td>400</td>
<td>0.0199</td>
</tr>
<tr>
<td>20</td>
<td>667</td>
<td>0.0120</td>
</tr>
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</table>

This table lists theoretical values for the concentrations of rod-like polymers required for phase transitions. These concentrations are expressed as critical volume fractions (Φ) and depend on the axial ratio of the polymers. Φ* is the minimum for the appearance of liquid crystalline domains in an isotropic fluid. Above Φ* all of the polymers are in ordered domains. Below Φ* all of the polymers are isotropic. Between Φ* and Φ+ the 2 phases coexist. The Flory (1956) approximate equations are:

Φ* = \( \frac{8}{\pi} \) (1 - 2/α)  \( \Phi^+ = 12.5/\alpha \)

The Doi and Edwards (1986) approximate equations are:

Φ* = 3.34/α  \( \Phi^+ = 4.49/\alpha \)

The axial ratio α is the length divided by the diameter (30 nm). Under our conditions the mean length of the microtubules is about 12 nm (Selden and Pollard, 1986), so Φ is less than 0.006, a value well below Φ* for either model. The volume fractions used by Buxbaum et al. (1987) are in the range of 0.016 and 0.031 where liquid crystals are expected.
between random rods and a liquid crystal should occur at microtubule concentrations between those used by ourselves and Buxbaum et al.

Several different considerations argue that solutions of microtubules are not simple, noninteracting rigid rods. First, the theoretical calculations summarized in Table II reveal that rigid, noninteracting rods of uniform length, would have different rheological behavior than we observed for microtubules. Second, the complex changes in the rheological parameters with time (Fig. 1) cannot be explained by noninteracting rigid rods of constant length. Third, noninteracting rigid rods would not become stiffer after recovery from shearing (Fig. 5). Length heterogeneity, length variation with time and/or flexibility of microtubules may explain, in part, the complex rheological properties. However, it seems more likely to us that most of complex properties will eventually be explained by low affinity interactions between microtubules, such that collisions between microtubules lead to transient binding rather than to elastic recoil. Such weak interactions may also explain the excess stiffness of flow oriented samples, the fact that cytoplasm containing parallel arrays of microtubules, such as axoplasm, resists deformation more along the long axis than transversely (Sato et al., 1984), and the tendency of microtubules to form liquid crystals (Buxbaum et al., 1987). Clearly, much additional work will be required to formulate and test a quantitative, predictive model that can account for the complex physical properties of microtubules.

The MAPs that project from the surface of microtubules are the logical candidates for promoting interactions between microtubules, but we found that they have little or no effect on the rheological properties. This is consistent with pelleting experiments (Brown and Berlin, 1985) showing that MAPs repel microtubules from each other, and biochemical evidence that there is no tubulin-binding site on the projective model that can account for the complex physical properties of globular proteins. For example, cytochrome C (20 \mu M) behaves as a Newtonian liquid indistinguishable from water with a viscosity that is independent of both frequency ($10^{-4}$ to 2 Hz) and shear rate ($10^{-2}$ to $10^{3}$ s$^{-1}$). However, since nonfilamentous actin, profilin and nonfilamentous tubulin are prominent constituents of cytoplasm, they and other globular proteins may contribute substantially to the rigidity of cytoplasm, especially at low rates of deformation (Sato et al., 1986).

The similarity in the shear viscosity of tubulin and microtubules differs from previous measurements by Ostwald capillary viscometry (Olmsted and Borisy, 1973) and

### Table II. Comparison of Observed Rheological Values with Theoretical Values for Rigid, Noninteracting Rods of Uniform Length

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Theoretical</th>
<th>Observed</th>
</tr>
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<tbody>
<tr>
<td>$G'$ (Dynes-cm$^{-2}$)</td>
<td>0.019 ($\omega \rightarrow \infty$)</td>
<td>30 ($f = 1$ Hz)</td>
</tr>
<tr>
<td>$G''$ (Dynes-cm$^{-2}$)</td>
<td>0.015 ($f = 6 \times 10^{-4}$Hz)</td>
<td>15 ($f = 6 \times 10^{-4}$Hz)</td>
</tr>
<tr>
<td>$\eta'$ (Dynes-cm$^{-2}$)</td>
<td>0.15 ($\omega \rightarrow \infty$)</td>
<td>4 ($f = 1$ Hz)</td>
</tr>
<tr>
<td>$\eta''$ (Dynes-cm$^{-2}$)</td>
<td>24.6 ($f = 6 \times 10^{-4}$Hz)</td>
<td>1,000 ($f = 6 \times 10^{-4}$Hz)</td>
</tr>
<tr>
<td>$\eta$ (Poise)</td>
<td>41.4 ($\kappa \rightarrow 0$)</td>
<td>400 ($\kappa' = 0.03$ s$^{-1}$)</td>
</tr>
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</table>

These theoretical calculations were made using the corrected equations of Jain and Cohen (1981) and Doi and Edwards (1986) which predict 2 asymptotic relationships. First, as $\omega / 6D$, tends toward zero (where $\omega$ is the angular frequency and $D$, is the rotational diffusion coefficient)

$$G(\omega) \sim \frac{3}{5} \frac{cKbT}{\alpha} \frac{\kappa}{\sigma}$$

In these equations $c$ is the number density of microtubules/cc, $\sigma_b$ is $1.38 \times 10^{6}$ erg/K (Boltzmann’s constant), $T$ is the absolute temperature, $\eta$, is the solvent viscosity estimated at 0.02 $P$ L is the length of the rods and $b$ is a constant empirically estimated to be in the range of $10^{-10}$ by Doi and Edwards. We use a value of $10^{1}$. As $\omega / 6D$, tends toward infinity:

$$G(\infty) \sim \frac{3}{5} \frac{cKbT}{\alpha}$$

The theoretical polymers in these calculations were uniform rigid rods 12-$\mu$m long and 30-nm wide. $G'$ is the dynamic elastic modulus; $\eta'$ is the dynamic viscous modulus; $\eta$ is the shear viscosity, $f$ is the oscillation frequency and $k'$ is the shear rate.
requires some comment. First, both the recent history and sample conditions during measurement greatly differ between the rheometer and the capillary viscometer. In the rheometer, samples were either undisturbed or previously sheared at lower rates before measurements, while in a capillary viscometer the samples are usually sheared at higher rates just before measurement (especially when repeatedly sucked through the capillary during a time course experiment). Second, the shear rate is homogeneous throughout the sample in the rheometer as defined by the cone and plate geometry but not in the capillary. Third, the asymptotic stress of the material was used to calculate shear viscosity while in the capillary experiments a complex average of the total stress response (including the transient yield stress) is used. Finally, the mechanical properties of microtubules are larger after flow orientation, while the opposite is true for unpolymerized tubulin. Because these complex, time dependent processes occur simultaneously during capillary viscometry, it is not surprising that the measurements by the two methods differ and that capillary measurements give higher viscosities for microtubules than unpolymerized tubulin or microtubule protein.

We do not yet have a physical model that accounts for the viscoelastic properties of unpolymerized tubulin, actin and profilin. We think that they may have a common basis because the mechanical properties are similar in both oscillation and continuous shear experiments. For all three proteins the relationship of log shear viscosity and log shear rate has a slope of -1 that may reflect similar weak interactions between the molecules. Despite obvious differences in the chemical bonds, we suggest that colloidal aggregation (Tadros, 1984; Nelson and Glatz, 1985) may provide a useful model for the interaction between these globular protein molecules.

We gratefully thank S. Rothwell and D. Murphy for the chicken brain tubulin and for helpful discussions. We also thank George Leimbach, Washington University School of Medicine (St. Louis, MO), and David Chan, Harvard University (Boston, MA) for assistance in collecting some of the data. This work was supported by National Institutes of Health Research grants, GM-26338 and GM-2632 (T. D. Pollard) and a Postdoctoral Fellowship from the Muscular Dystrophy Association (MS).

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