Smooth muscle's stress equals that of skeletal muscle with less myosin. Thus, under isometric conditions, smooth muscle myosin may spend a greater fraction of its cycle time attached to actin in a high force state (i.e., higher duty cycle). If so, then smooth muscle myosin may also have a higher duty cycle under unloaded conditions. To test this, we used an in vitro motility assay in which fluorescently labeled actin filaments move freely over a sparsely coated (5–100 μg/ml) myosin surface. Actin filament velocity (V) was a function of the number of cross-bridges capable of interacting with an actin filament (N) and the duty cycle (f), \( V = (a \times V_{max}) \times (1 - (1 - f)^N) \) (Uyeda et al., 1990; Harada et al., 1990). N was estimated from the myosin density on the motility surface and the actin filament length. Data for V versus N were fit to the above equation to predict f. The duty cycle of smooth muscle myosin (4.0 ± 0.7%) was not significantly different from that of skeletal muscle myosin (5.8 ± 0.5%) in agreement with values estimated by Uyeda et al. (1990) for skeletal muscle myosin under unloaded conditions. The duty cycles of smooth and skeletal muscle myosin may still differ under isometric conditions.

Eukaryotes contain an array of motor proteins which vary in function from muscular contraction and cytokinesis (i.e., myosin I and II) (Harrington and Rodgers, 1984; Warrick and Spudich, 1987; Kiehart, 1990) to intracellular transport of macromolecules and vesicles (i.e., dynein and kinesin) (Vale, 1987; Schroer and Sheetz, 1991). Myosin II and kinesin are distinct, unrelated proteins that share striking structural and functional similarities. Both have globular amino-terminal head domains that contain the enzymatic site for ATP hydrolysis and a binding site for a cytoskeletal polymer (actin in the case of myosin and microtubules for kinesin), which upon binding increases the ATP hydrolysis rate (Warrick and Spudich, 1987; Vale and Goldstein, 1990). In addition, the ATP hydrolysis by these two molecular motors occurs through a series of biochemical intermediates that are qualitatively similar and may relate to similar mechanical events.

Myosin and kinesin are believed to generate force and/or motion by mechanical cycles during which the motors repetitively attach to the appropriate cytoskeletal polymer, undergo a conformational change (i.e., power stroke) and then detach (Warrick and Spudich, 1987; Block et al., 1990). A fundamental aspect of this cycle is the fraction of the cycle time during which the motor is attached and generating force or motion (i.e., the duty cycle). Differences in duty cycle, for instance, have been proposed to explain smooth muscle's ability to generate as much isometric force per cross-sectional area as skeletal muscle with only 20% as much myosin (Murphy et al., 1974). A higher smooth muscle cross-bridge duty cycle, under isometric conditions, relative to skeletal muscle myosin would yield a greater average smooth muscle cross-bridge force. However, the duty cycle may be force dependent and thus different under loaded and unloaded conditions (Huxley, 1957).

In vitro motility assays, in which cytoskeletal polymers are observed moving over surfaces coated with molecular motors (Kron and Spudich, 1986; Harada et al., 1987; Howard et al., 1989) or in which beads coated with molecular motors are observed moving over tracks of cytoskeletal polymers (Sheetz and Spudich, 1983; Block et al., 1990), provide an unique opportunity to determine the duty cycles of various molecular motors at zero load (Howard et al., 1989; Block et al., 1990; Harada et al., 1990; Uyeda et al., 1990). In this study, we have observed fluorescently labeled actin filaments moving over smooth and skeletal muscle myosin-coated surfaces to directly compare the duty cycles of these two molecular motors under unloaded conditions.

**MATERIALS AND METHODS**

**Contractile Protein Isolation and Preparation**—Smooth (turkey gizzard) and skeletal (chicken pectoralis) muscle myosin were prepared as previously described (Warshaw et al., 1990). Smooth muscle myosin was 100% thiophosphorylated by incubation with myosin light chain kinase, calmodulin, 1.5 mM CaCl\(_2\), and ATP\(_S\). Both myosins were stored in monomeric form at ~20 °C in 50% glycerol solution. Myosin concentrations were determined both by extinction coefficient and protein assay (Bio-Rad).

Actin was isolated from chicken pectoralis acetone powder as previously described (Pardue and Spudich, 1982), and stored in filamentous form at 4 °C. Actin was fluorescently labeled by incubation with tetramethylrhodamine isothiocyanate-labeled phalloidin (Sigma) overnight prior to each experiment (Warshaw et al., 1990). Where short actin filaments were required, the actin-containing solution was sonicated for 10–30 s immediately prior to use.

**In Vitro Motility Assay**—The in vitro motility assay was carried out essentially as previously described (Warshaw et al., 1990). Flow-through 30-μl chambers were constructed from a nitrocellulose-coated coverslip and glass microscope slide. First, 30 μl of myosin (5–100 μg/ml) in Myosin Buffer (300 mM KCl, 25 mM imidazole, 1 mM EGTA, 4 mM MgCl\(_2\), 1 mM DTT; pH 7.4) was introduced and equilibrated in the chamber for 60 s. Then 60 μl of bovine serum albumin (BSA) (0.5 mg/ml) in Myosin Buffer was perfused through to remove unbound myosin and to block any exposed nitrocellulose. Next, 60 μl of fluorescently labeled actin (0.5–5 μg/ml) in Assay Buffer (25 mM KCl, 25 mM imidazole, 1 mM EGTA, 4 mM MgCl\(_2\), 10 mM DTT; pH 7.4), containing an oxygen scavenger system (0.1 mg/ml glucose oxidase, 0.018 mg/ml catalase, 2.3 mg/ml glucose) to retard photo-

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*This work was supported in part by National Institutes of Health Grants AR34872 and HL45161 (to D. M. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: ATP\(_S\), adenosine 5'-O-(thiotriphosphate); DTT, dithiothreitol; BSA, bovine serum albumin.
bleaching of the actin fluorescence, was introduced for 60 s, allowing actin to bind to the myosin. Then, 50 µl of the Assay Buffer, containing 1% methyl cellulose (Uyeda et al., 1990) and 1 mM ATP, was introduced to initiate actin filament movement on the myosin-coated coverslip. An objective heater (Vermont Technologies, Burlington, VT) controlled temperature at 30 °C.

The addition of 1% methyl cellulose to the final assay buffer was required to prevent filament from diffusing away from the coverslip coated with low myosin densities. Methyl cellulose up to 1% has previously been shown to increase the solution viscosity and retard the filament's lateral Brownian motion without affecting sliding velocity over skeletal muscle myosin (Uyeda et al., 1990; Kron et al., 1990). For this analysis, we considered filament velocity at 0.5 and 1% methyl cellulose over both smooth and skeletal muscle myosin (100 µg/ml) and found no difference (data not shown).

Recording and Analysis of Actin Filament Motion—Actin filaments were viewed through an inverted fluorescence microscope (Zeiss IM) fitted with a 100-watt HBO mercury discharge lamp, a Hamamatsu image filter set, and a high numerical aperture objective (63× Zeiss planachromat, NA = 1.4). An intensified video camera system (Hamamatsu C2400-97), dedicated image processor (Hamamatsu, Argus 10), and videorecorder (Panasonic AG7900) allowed recording of the images, following background subtraction, on sVHS video tape for later analysis.

Filaments were digitized with a video grabber card (Oculus 200, Corceco Inc., Ville Saint-Laurent, Quebec, Canada). The interval between snapshots was varied from 0.2 to 3 s so that actin filaments moved approximately half their length between consecutive images, and 10–15 images were grabbed for each filament. Actin filament velocity and length were measured by computer (Work and Warshaw, 1992). Only those filaments that were at least 0.5 µm in length, moved a distance > 2 µm, and displayed a consistent velocity (i.e. having a standard deviation of velocity 0.33 × mean velocity) were included in the data sets. The 0.5-µm actin filament length cutoff was chosen to minimize the error in actin filament length measurements based on our pixel resolution of 0.165 µm.

Estimating Cross-bridge Head Density on the Coverslip—The amount of myosin bound to the coverslip was estimated by comparing NH₄⁺-EDTA ATPase rates (Chalovich and Eisenberg, 1982) of known amounts of myosin in solution with similar ATPase rates obtained on the coverslip. This analysis assumes that the NH₄⁺-EDTA ATPase rate of myosin bound to nitrocellulose is the same as that of myosin free in solution.

In solution, varying amounts of smooth and skeletal muscle myosin (0–0.375 µg) were assayed for NH₄⁺-EDTA myosin ATPase activity in a buffer containing 2 mM EDTA, 0.4 mM NH₄Cl, 0.2 mM succrose, 25 mM Tris, 1 mM DTT, 2.5 mM ATP, 0.2% BSA, pH 8.0, at 37 °C. The reaction was stopped at various times by adding SDS to a final concentration of 3.3%. ATPase activity was assessed by measuring inorganic phosphate release colorimetrically (Tauskay and Shorr, 1955).

To determine ATPase activity on the coverslip (Fig. 1), varying concentrations of both smooth and skeletal muscle myosin (5–100 µg/ml) were introduced into flow-through chambers constructed from two nitrocellulose-coated coverslips. The myosin was allowed to bind to the nitrocellulose under standard assay conditions except that MgCl₂ was eliminated from the Myosin Buffer. After the 60-s equilibration and BSA rinse, the NH₄⁺-EDTA ATPase assay buffer (described above) was introduced into the chamber. After various periods of time, the chamber was perfused with stop solution (3.3% SDS). The effluent was collected and assayed for inorganic phosphate, as described above.

The amount of myosin bound to the coverslip was estimated by dividing the phosphate release rate for myosin bound in the chamber (slopes in Fig. 1) by the phosphate release rate per microgram of myosin in solution. Then, dividing the amount of myosin bound by the total area of the two nitrocellulose surfaces (i.e. 540 mm²), and multiplying by 2 for the number of heads/myosin, we obtained the density of myosin heads on the coverslip.

To determine the number of cross-bridges available to interact with a unit length of actin, we assumed, as suggested previously (Uyeda et al., 1990), that a crossbridge-bound to the motility surface up to 10 nm away from the actin filament could attach to that filament. Since actin filaments are 6 nm in diameter (Wakabayashi et al., 1975), all myosin cross-bridge heads within a 26-nm-wide band could interact with an actin filament as it passes (Uyeda et al., 1990). Given this assumption, we calculated the number of cross-bridges that were capable of interacting with a 1-µm length of actin filament for each concentration of both smooth and skeletal muscle myosin introduced into the flow chamber (Table 1).

Duty Cycle Estimation—The duty cycles for both smooth and skeletal muscle myosin were estimated by adopting the approach pioneered by Spudich and co-workers (Uyeda et al., 1990). Briefly, if cross-bridges interact in a stochastic manner with actin, then an actin filament will move at maximum velocity (V<sub>max</sub>) in vitro, when at least one cross-bridge is bound and going through its power stroke at all times. The probability of this occurring is related to both the cross-bridge duty cycle (f) and the total number of cross-bridges (N) capable of interacting with the actin filament: (1 - (1 - f)^N) (Uyeda et al., 1990; Harada et al., 1990). Therefore, over sparsely coated myosin surfaces, where this probability is low, actin filament velocity (V) will no longer be maximum but rather decrease as a function of f and N, as shown by the following equation.

\[ V = (\alpha \times V_{max}) \times \left[1 - (1 - f)^N\right] \]  

(1)

The term α × V<sub>max</sub> relates to the efficiency of transmitting along an actin filament at low myosin densities (Uyeda et al., 1990) (see "Results").

To estimate the cross-bridge duty cycle, an actin filament's velocity (V) and length were measured for varying myosin concentrations (5-100 µg/ml) introduced to the chamber (Fig. 2). Based on cross-bridge density estimates, we plotted actin filament velocity versus number of cross-bridge heads available to interact with that actin filament at each myosin concentration. These data were then fitted to Equation 1. The best fit regression analysis (Sigmastat, Jandel Scientific, Corte Madera, CA), with the product (α × V<sub>max</sub>) and the duty cycle (f) determined as parameters of the fit.

Using this approach, all biochemically active cross-bridges on the motility surface are assumed to be capable of interacting optimally with the actin filament regardless of cross-bridge orientation (Uyeda et al., 1990). This assumption is supported by the finding that oriented rows of skeletal muscle heavy meromyosin propel actin filaments in both directions at the same velocity (Toyoshima et al., 1989). Head orientation does have an affect on actin filament velocity when measured over native clam myosin filaments (Sellers and Kachar, 1990). However, this effect may result from the loss of cross-bridge rotational freedom when myosin monomers polymerize into filaments.

RESULTS

Myosin Density on the Coverslip—NH₄⁺-EDTA ATPase rates in solution for both smooth and skeletal muscle myosin were directly dependent on the amount of myosin present with rates of 6.66 and 11.57 nmol of phosphate/min/µg of myosin for smooth and skeletal muscle myosin, respectively. NH₄⁺-EDTA ATPase measurements conducted on myosin bound to the nitrocellulose surface showed linear relationships between amount of phosphate released and incubation time for both smooth and skeletal muscle myosin (Fig. 1). ATPase rates, determined by the slopes of these relationships, were a function of myosin concentration (5–100 µg/ml) introduced into the chamber (Table 1). Using these solution and motility surface ATPase rates (see above), we estimated the maximum number of heads available to interact per micrometer length of actin for the various myosin concentrations introduced into the experimental chamber (Table 1). These estimates were then used to convert actin filament lengths into number of interacting heads as seen in Fig. 2.

Actin Filament Motion at Low Myosin Densities—At high myosin densities, actin filaments move in a continuous and directed manner. However, at low myosin densities, even in the presence of methyl cellulose, actin filaments exhibit random motion parallel to the filament's long axis (i.e. reptation). These random movements are even more pronounced in the absence of myosin on the coverslip (Fig. 3). When few cross-bridges interact with the actin filament, periods of time must exist where cross-bridges are not attached and the actin filament is free to reptate. To estimate the effect that this random motion might have on measured actin filament velocity, we analyzed the random motion of filaments not undergoing directed motion from myosin (Fig. 3). We added this motion to the predicted motion of an actin filament being
moved at constant velocity by 25 cross-bridges of either smooth (0.4 μm/s) or skeletal (4.0 μm/s) muscle myosin (computed from the equations of the fits to the data in Fig. 2d) to create mock motility data sets. Then we sampled these mock movement data sets at appropriate time intervals to determine the effect of the random motion on measured actin filament velocity.

A mock actin filament moving at 4.0 μm/s was estimated to move at 4.0 ± 0.5 μm/s with the random motion added and sampled at 0.2 s, whereas a slower filament moving at 0.40 μm/s was estimated at 0.40 ± 0.17 μm/s when sampled at 2-s intervals. Thus, reptation in the presence of methyl cellulose could introduce a source of variability that is larger for slower moving filaments than for faster moving ones. This may explain the greater variability in actin filament velocities moving over smooth compared to skeletal muscle myosin (Fig. 2). To minimize the effect that such variability might have on duty cycle estimates, at least 200 filaments were analyzed at each myosin concentration at which motility data were fit to Equation 1.

Smooth and Skeletal Muscle Myosin Duty Cycles—Actin filament velocity was plotted as a function of the number of cross-bridges available to interact with that filament for both smooth and skeletal muscle myosin (Fig. 2). For myosin concentrations between 5 and 20 μg/ml infused into the chamber (Fig. 2, b-e), shorter actin filaments, which interacted with fewer cross-bridges, moved more slowly than longer filaments, with velocity saturating for actin filaments interacting with greater than 75 heads. This is in marked contrast to results at higher myosin density (Fig. 2a), where all actin filaments moved at the same average velocity. The lack of any dependence of velocity on filament length at high myosin densities might be expected given our criterion that actin filaments be greater than 0.5 μm in length. Even these short filaments would still interact with large numbers of cross-bridges.

The actin filament velocity versus number of heads data for [myosin] infused into the flow chamber less than 100 μg/ml were well fitted by Equation 1 (solid lines in Fig. 2, b-e) with the smooth and skeletal muscle myosin duty cycles (f) a parameter of the fit (Table I). Data when [myosin] infused into the flow chamber was 100 μg/ml (Fig. 2a) could not be fitted because even very short actin filaments interacted with enough myosin heads to move at maximum velocity. The quality of fit can be appreciated in Fig. 2b, when duty cycle estimates half and twice the predicted value are substituted. As predicted by the model, halving the duty cycle results in estimates half and twice those of skeletal muscle myosin (2.9-4.3%). These duty cycle estimates were not a function of myosin concentration infused into the chamber, suggesting that varying the myosin concentration only altered the cross-bridge density on the coverslip and not the duty cycle. By pooling the duty cycle estimates for each myosin, the average smooth muscle myosin duty cycle (4.0 ± 0.7%) was not statistically different from that of skeletal muscle myosin (3.8 ± 0.5%).

Efficiency of Motion Transmission—For any density of myosin concentrations between 5 and 20 μg/ml (Fig. 2), actin filament velocities were best fit value ± 95% confidence limits for data in Fig. 2 fitted to Equation 1. Velocity data at 100 μg/ml myosin were not fitted to Equation 1 because even very short actin filaments moved at maximum velocity (see text). Effective intermonomer distance is the inverse of the number of available monomers/1 μm of actin filament length.

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**TABLE I**

**Myosin binding and function in flow-through chambers**

| [Myosin] | ATPase rate | Myosin density | Available heads | Duty cycle | a × V_max |
|----------|-------------|----------------|-----------------|------------|-----------|
| μg/ml    | Smooth      | Skeletal       | Smooth          | Skeletal   | Smooth    | Skeletal |
| 5        | 0.35        | 0.72           | 270             | 276        | 7.0       | 7.2      | 285      | 279      | 4.5 ± 0.5 | 4.1 ± 0.3 | 0.32 ± 0.02 | 3.9 ± 0.1 |
| 10       | 0.67        | 0.94           | 514             | 361        | 13        | 9.4      | 150      | 212      | 3.6 ± 0.3 | 4.2 ± 0.3 | 0.45 ± 0.01 | 5.0 ± 0.2 |
| 15       | 0.95        | 1.52           | 737             | 585        | 19        | 15       | 104      | 131      | 4.6 ± 0.6 | 3.1 ± 0.1 | 0.47 ± 0.02 | 6.6 ± 0.1 |
| 20       | 1.30        | 1.81           | 1003            | 696        | 26        | 18       | 77       | 110      | 3.2 ± 0.2 | 3.7 ± 0.2 | 0.58 ± 0.01 | 6.6 ± 0.1 |
| 100      | 2.97        | 5.45           | 2328            | 2092       | 61        | 54       | 33       | 37       | —         | —         | —         | —        |

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**FIG. 1.** Inorganic phosphate release (P_I) as a function of time for smooth (o) and skeletal muscle myosin in flow-through chambers (b). Data for [myosin] = 5 ( ), 10 ( ), 15 ( ), 20 (Δ), and 100 (V) μg/ml are shown. Linear regressions through the data at each myosin concentration and used to determine ATPase rates shown in Table I.
Smooth and Skeletal Myosin Duty Cycle in Vitro

**FIG. 2.** Actin filament velocity as a function of the number of smooth and skeletal muscle myosin cross-bridge heads available to interact with the actin filament. Data for [myosin] = 100 (a), 20 (b), 15 (c), 10 (d), and 5 (e) μg/ml infused into the flow chamber are shown. Solid lines in b-e are the fits to Equation 1. Parameters of the fit are given in Table I. Calculation of "N" is discussed under "Materials and Methods." Dashed lines in b illustrate goodness of the fit by showing the effect of doubling (long dash) and halving (short dash) the duty cycle "7.

**FIG. 3.** Displacement from initial position of an actin filament undergoing motion parallel to its long axis (i.e. reptation) in the absence of myosin. Sampling rate = 15/s.

Myosin on the motility surface, actin filament velocity was a saturable function of the number of cross-bridge heads that can interact with the filament (Fig. 2). Furthermore, in agreement with previous determinations (Uyeda et al., 1990), the velocity at which saturation occurred (a × Vmax) falls as the myosin density is reduced (Table I). Actin filaments are highly compliant structures, which bend easily during Brownian motion (Yanagida et al., 1984). Therefore, at low myosin densities, where the spacing between cross-bridges may be large, motion imparted to the actin filament may not be fully translated into the filament’s forward motion. If so, then the efficiency of motion transmission from the myosin head to the actin filament would fall as the distance between myosin heads increases.

It is possible to estimate the efficiency of motion transmission by comparing the parameter of the fit (a × Vmax) to the average actin filament velocity when 100 μg/ml myosin is infused into the flow chamber (Vw = 0.62 ± 0.09 μm/s, n = 115 for smooth and 6.11 ± 0.77 μm/s, n = 60 for skeletal muscle myosin). Based on the estimated myosin densities, the distance between myosin monomers can also be calculated (Table I). The efficiency of motion transmission from myosin to actin appears to fall dramatically when the distance between monomers is greater than approximately 80 nm for smooth muscle myosin and approximately 130 nm for skeletal muscle myosin. It is of interest that cross-bridges are helically arranged on myosin filaments in vivo, with cross-bridges positioned every 14.3 nm along the helix which repeats every 43.2 nm (Huxley, 1969). Our data suggest that in vivo, cross-bridges are spaced appropriately to give maximum efficiency of motion transmission and that it may not be surprising that the efficiency falls in vitro at intermonomer distances greater than 80 nm.

**DISCUSSION**

What effect might alterations in the cross-bridge duty cycle have on mechanical indices of the actomyosin interaction? Under unloaded conditions, as in the motility assay, an in-
crease in cross-bridge duty cycle results in fewer cross-bridges being required to move an actin filament at maximum velocity (see Fig. 2d). However, under isometric conditions, duty cycle differences between molecular motors could result in differences in the average force per motor, given that this average force should be a product of the motor's unitary force and duty cycle. Therefore, the apparently enhanced average force per cross-bridge in smooth muscle, as suggested by tissue data (Murphy et al., 1974), may be due to differences in its duty cycle relative to skeletal muscle myosin.

**Duty Cycle Estimates at Zero Load**—Solution biochemical evidence indicates that smooth muscle S-1 may have a higher fraction bound to actin than does skeletal S-1 (Rosenfeld and Taylor, 1984). However, in the motility assay individual cross-bridges bear positive or negative strain and only the sum of all cross-bridge strains is zero (Warshaw et al., 1990), whereas in solution cross-bridges may not be strained. Since the rate constant for cross-bridge detachment is thought to be strain dependent (Huxley, 1957), the duty cycle for myosin in the motility assay and in muscle fibers shortening at zero load may be different from that seen in solution. To address this concern, investigators have attempted to estimate the skeletal muscle cross-bridge duty cycle under unloaded conditions by using the in vitro motility assay to limit the number of cross-bridges (i.e., <20) that can interact with an actin filament and observing the effect on actin filament velocity (see Equation 1). Yanagida and co-workers (Harada et al., 1990) computed a duty cycle of 80% by observing the motion of very short (40 nm) actin filaments over high myosin densities. However, these filaments could be visualized only during brief pauses in motion (Harada et al., 1990). Thus, they could have undergone undetected tumbling, producing inaccurately high velocities and duty cycle measurements. By contrast, Spudich and co-workers (Uyeda et al., 1993) estimated a 5% duty cycle using low myosin densities. We estimated that the skeletal muscle myosin duty cycle was 4%, similar to that of Uyeda et al. (1990), and that the smooth muscle myosin duty cycle was similarly low. This low myosin duty cycle is not an artifact of measurements conducted over low motor densities. The motility of microtubules over sparsely coated kinesin surfaces (Howard et al., 1989) and that of silica beads coated with one or two kinesin molecules (Block et al., 1990) suggest that kinesin remains attached to the microtubules for almost its entire mechanical cycle (i.e., duty cycle >90%). This high duty cycle is functionally reasonable since, in vivo, kinesin must move cytoplasmic organelles too small to bind more than a few motor proteins (Miller and Lasek, 1985) over long distances on microtubular tracts without the organelle escaping from the tract (Spudich, 1990).

**Smooth and Skeletal Myosin Duty Cycles under Load**—Does the similarity in smooth and skeletal muscle myosins' duty cycle, under unloaded conditions, preclude a difference under maximally loaded conditions, where data suggest that the average smooth muscle cross-bridge force is higher than in skeletal muscle (Murphy et al., 1974)? Based on energetic and mechanical data, the kinetics for several cross-bridge transitions appear to be dependent on cross-bridge strain with this feature being incorporated into models of muscle contraction (Huxley, 1957; Eisenberg et al., 1980). The effect of this strain dependence translates into model predictions for the cross-bridge duty cycle that will be as low as 25% at zero load (i.e., at maximum shortening velocity) and increase to 80% under maximally loaded conditions (i.e., isometric contraction). This proposition is supported by stiffness data from skeletal muscle fibers (Goldman and Simmons, 1977; Higuchi and Goldman, 1991) and single smooth muscle cells (Harris and Warshaw, 1990).

If the myosin duty cycle is load-dependent, then the value estimated in this study at zero load may be quite different under isometric conditions. In addition, if the load dependence is different for smooth and skeletal muscle myosin, then an increased duty cycle in smooth muscle could still be one explanation for an apparently high average smooth muscle cross-bridge force. At a minimum, in vitro force measurements, using a compliant glass microneedle (Kishino and Yanagida, 1988; VanBuren et al., 1993), will be required to determine if differences do exist in the average cross-bridge force between smooth and skeletal muscle myosin. However, direct measurements of the cross-bridge duty cycle will not be available until an in vitro system with sufficient force and time resolution is developed. Preliminary data using the laser optical trap do offer promise that these measurements will be attainable in the near future (Finer et al., 1992).

**Acknowledgments**—We thank Dr. Kathleen Trybus of Brandeis University for teaching us the NH4EDTA ATPase assay and for comments on this manuscript. We also thank Steven Work for the computer programs used to track actin filament motion.

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