The Dependence of Unloaded Shortening Velocity on Ca\(^{++}\), Calmodulin, and Duration of Contraction in “Chemically Skinned” Smooth Muscle

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SUMMARY. Unloaded shortening velocity, a mechanical parameter associated with the rate of cross-bridge cycling, was investigated in chemically skinned guinea pig taenia coli and hog carotid artery. Shortening velocity was measured by the technique described by Edman, whereby large length steps are rapidly imposed on the muscle and the time under unloaded conditions is determined from the isometric myograms. Shortening velocity determined in this manner was similar to \(V^\text{m}_\text{ax}\) from the Hill force-velocity relations reported for both living and skinned taenia coli, and, in the case of carotid artery, was at least as large as that reported for living muscle. The behavior of shortening velocity was qualitatively similar for both preparations. Shortening velocity was strongly temperature dependent, with a \(Q_{10}\) of approximately 3.6. Shortening velocity was found to be dependent on both the Ca\(^{++}\) and calmodulin concentration. In contrast to the dependence of isometric force on Ca\(^{++}\)-calmodulin, shortening velocity could be increased further by the addition of Ca\(^{++}\) and/or calmodulin under conditions when isometric force was maximized. Incubation with ATP-\(\gamma\)S, which presumably maximizes the phosphorylation of myosin, did not increase shortening velocity beyond the maximum value obtained in the presence of Ca\(^{++}\)-calmodulin alone. The development of shortening velocity after exposure to high Ca\(^{++}\) solution was found to precede that of isometric force. The steady state value tended to be slightly lower than the maximum shortening velocity, the largest difference observed being less than 1.5-fold. Thus, whereas both isometric force and shortening velocity are dependent on the Ca\(^{++}\)-calmodulin concentration in skinned smooth muscle, the dependencies are not identical, differing with respect to temporal development and concentration. These differences may underlie the decline in velocity with maintained isometric force observed in living smooth muscle. (Circ Res 53: 342-351, 1983)

IN recent studies (Sparrow et al., 1981; Rüegg and Paul, 1982), we reported that isometric force in “chemically skinned” smooth muscle was dependent on both Ca\(^{++}\) and calmodulin concentrations. These observations are consistent with the hypothesis that the level of isometric force is related to myosin phosphorylation (Adelstein and Eisenberg, 1980; Hartshorne and Siemankowski, 1981). However, studies on living smooth muscle (Dillon et al., 1981) have shown that—although able to maintain constant levels of force—both shortening velocity and myosin phosphorylation decrease with the contraction duration. They have suggested that shortening velocity is related to the phosphorylation state of the myosin light chain (cf. Murphy et al., 1983). We have utilized chemically skinned smooth muscle preparations in which the myosin light chain phosphorylation state can be altered by experimental conditions. For example, myosin light chain kinase (MLCK) may be activated by addition of Ca\(^{++}\) or calmodulin to the bathing solution or by the use of ATP-\(\gamma\)S to thiophosphorylate myosin (Hoar et al., 1979). If phosphorylation of myosin light chains regulates only the number of activated cross-bridges, no change in \(V^\text{m}_\text{ax}\) with the level of isometric force would be expected. We have tested under various conditions whether unloaded shortening velocity \(V^\text{u}_\text{ax}\) is independent of the level of isometric force.

In addition, whereas it has been suggested that the decrease in \(V^\text{m}_\text{ax}\) with time observed in living tissues reflects a decline in intracellular Ca\(^{++}\) (Murphy et al., 1983), we have measured the dependence of shortening velocity on the duration of contraction under conditions in which Ca\(^{++}\) and calmodulin are held constant. To provide a basis for comparison to the living smooth muscle preparations, the dependence of \(V^\text{m}_\text{ax}\) on temperature has also been measured. Both hog carotid artery, representative of tonic vascular smooth muscle, and guinea pig taenia coli, representative of phasic smooth muscle, were studied.

Methods

Skinned Smooth Muscle Preparation

Strips of hog carotid media were dissected as described by Glück and Paul (1977) and skinned by a modification (Rüegg and Paul, 1982) of the procedure of Gordon (1978). Taenia coli was prepared in a similar fashion, as previously described (Sparrow et al., 1981). Preparations were treated...
similarly except where noted. Briefly, they were incubated in 20 mM imidazole, 5 mM EGTA, 50 mM KCl, and 150 mM sucrose (pH 7.4) for 30 minutes at 0°C, then incubated for a further 30 minutes, in the case of carotid artery, or between 4 and 12 hours, in the case of taenia coli, in the same solution with the addition of 1% Triton X-100, 0.5 mM dithioerythritol (DTE). They were then incubated for 5 minutes at 0°C in a solution of 20 mM imidazole, 4 mM EGTA, 10 mM MgCl₂, 7.5 mM ATP, 1 mM NaN₃, 0.5 mM DTE at pH 6.7, mixed with 50% glycerol (cf. Peterson, 1981), and then stored at -25°C in this solution. Strips used after overnight storage and those stored for up to 4 weeks showed similar behavior.

Mechanical Measurements

Bundles of fibers about 5 mm long, whose width and thickness were between 100 and 200 μm, were teased out of the strips. Fiber bundles were attached horizontally with a cellulose-based glue between an AME 801 force transducer (Aksjeselskapet Micro-Electronics) and, on the other end, to a device for rapid imposition of length changes. For experiments in Heidelberg, a Ling Dynamics vibrator (101) was used which had a maximum step time of 5 msec. For experiments in Cincinnati, a Cambridge Technology device was used which had a step time of 1.5 msec for releases of up to 1.5 mm. Length and tension were monitored on a digital oscilloscope ( Nicolet) and chart recorders, the length change being calibrated with an optical micrometer. For small length changes, the time elapsed before redevelopment of force was measured from the oscilloscope traces. For larger steps, and elapsed times greater than 1 second, the chart recorder gave a better estimate of the redevelopment of force, which was extrapolated to the baseline to determine the time elapsed before force redevelopment following the step decrease in length. The reproducibility of the measurement of elapsed time before redevelopment of force (tₑ) was on the order of 10%.

Experimental Protocol

Fibers were bathed in a “relaxing solution” containing (mm): K⁺, 21; Na⁺, 36; Mg (total), 10; EGTA, 4; ATP, 7.5; imidazol, 20; Cl⁻, 35; azide, 1; and an ATP-regenerating system consisting of 10 mM phosphocreatine and 10 U/ml of creatine phosphokinase; pH of the final solution was 6.7. Calmodulin, when used, was prepared from bovine testis according to the method of Teo et al. (1973). Tissues were bathed in appropriate solutions in 0.4–0.8 ml Lucite chambers, the temperature of which was regulated by a circulating water bath. Fibers were slowly stretched, then allowed to equilibrate; this was repeated in steps over a period of a few minutes until a stable force of approximately 0.5 mN was maintained. The fiber then was shortened and allowed to equilibrate at a negligible load (<0.02 mN). The length under these conditions was measured with an optical micrometer and was used as a reference length (l₀) for the standardization of length changes. Active force at this length was approximately 80% of the maximum force at the optimal length (Pfizer et al., 1982). This length was chosen to avoid the complications in the velocity measurements associated with the appreciable passive tensions at the length for optimal active isometric force development. Contraction velocity is expressed in units of this muscle length per second (l/sec).

Contraction was induced by partly replacing EGTA with Ca-EGTA, thereby increasing the free Ca++. The concentration of free Ca++ was calculated from the apparent binding constant for EGTA of 1.2 × 10⁻⁵ M⁻¹ (Portzehl et al., 1964). ATP-γS refers to adenosine 5’-O-(3-thiotriphosphate), which was obtained from Boehringer-Mannheim.

The relation of the magnitude of active stress, i.e., force/cross-section area, of these skinned fibers to that of the living fibers was not directly addressed in this work. Peterson (1980, 1982) and Arner (1982) focused on this question in similar preparations and reported stresses on the order of 30% to 90% of that in the living preparation. Based on typical fiber dimensions and absolute forces, stresses in the range of 50–100 mN/mm² were observed, which are similar to that reported in the above-mentioned studies. Absolute force production by the fiber bundles was 0.5–4 mN. After imposition of the length step and the associated measurements, the fiber was placed in relaxing solution and restretched back to its original length only after force was negligible. Thus, shortening velocity measurements were made on separate contractions. An exception to this was experiments after exposure to ATP-γS, in which the fibers by necessity had to be restretched under activated conditions (see Results). Rapid restretches appeared to be less destructive than slow stretches, in terms of returning to the original levels of isometric force.

In general, when differences were to be measured, a protocol was utilized in which a test contraction was preceded and followed by control contractions. Thus, each fiber served as its own control which reduced variability due to differences between fibers. The results of this type of experimental design are presented as the ratio of the value in the test contraction to the average of the values in the bracketing control contractions for each fiber. Data are reported as mean values ± SEM. Statistical significance was calculated by Student’s t-test, with P < 0.05 taken as indicative of a significant difference.

Results

General Observations

After development of isometric force, imposition of a rapid decrease in muscle length of 10–30% of the initial muscle length (l₁) results in a rapid drop to zero force. This unloaded state is maintained for a period of time, dependent on the amplitude of the decrease, before redevelopment of force can be observed. The duration of time at zero force (tₑ) can be used to estimate the unloaded shortening velocity (Vₑ₀) of the muscle fiber (cf. Edman, 1979). After attainment of a stable isometric force, various step decreases in length were imposed on the fiber. In a control set of experiments, to be discussed below, no changes in velocity with time during the maintenance of isometric forces could be detected. The time course of isometric force after imposition of various step decreases in length and the dependence of tₑ on the magnitude of the imposed step shortening is shown in Figure 1. A least squares linear fit yielded correlation coefficients greater than 0.98, indicating reasonable linearity. The extrapolated values of the length change at the origin of the time axis corresponds to a very rapid initial shortening, which is presumably due to the recoil of series elastic
elements (cf. Pfitzer et al., 1982). The magnitude of this recoil was consistently greater in carotid artery, 10.7 ± 0.5% \( l_t \) (\( n = 6 \)), than that observed in taenia coli, 8.0 ± 0.4% \( l_t \) (\( n = 5 \)). The velocity of unloaded shortening, obtained from the slope of the linear portion of the length change vs. time curve, was substantially higher for taenia coli than the carotid artery at the same temperature.

The temperature dependence of \( V_{us} \) is shown in Figure 2. A substantial dependence was found with an approximate \( Q_{10} \) of 3.6 for taenia coli and 3.7 for carotid artery. The differences in \( V_{us} \) between these smooth muscles was observed at all temperatures. Though not studied in detail, steady state isometric force was relatively independent of temperature.
and differences in the immediate elastic recoil measured at different temperatures were small, and no consistent effect of temperature was observed.

Effects of Ca**

The dependence of $t_{el}$ for a taenia coli fiber on length steps of 10 and 15% at various Ca** concentrations is shown in Figure 3. As shown in Figure 4, both $V_u$ and isometric force ($F_0$) are strongly dependent on the Ca** concentration. The changes in $V_u$ and $F_0$ with Ca** were approximately proportional over a 4-fold range, as indicated by a least squares correlation coefficient of 0.98 (see below). It is of interest to note that—under these conditions, when both force and velocity show substantial changes—the initial elastic recoil was constant, averaging 8.7 ± 0.1% 1, (for five Ca** concentrations). Similar results were observed for carotid artery; i.e., the elastic recoil was independent of the level of isometric force.

The dependence of $V_u$ on Ca** suggested by this experiment was verified by means of a different protocol. To control for variability due to the order of contraction or fiber-to-fiber differences, a protocol was used in which measurements at a high Ca** concentration (12.5 μM) were preceded and followed by contractions at a lower Ca** concentration (0.2 μM). To ensure reproducibility, these measurements were made in the presence of constant calmodulin concentrations (Sparrow et al., 1981; Ruegg and Paul, 1982): 1 μM in the experiments with taenia coli and 4 μM for carotid artery. The results, presented in Table 1, indicate a consistent increase of about 2-fold in $V_u$ at the higher Ca** concentration for both

### Table 1

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<tr>
<th></th>
<th>0.2 μM Ca**</th>
<th>12.5 μM Ca**</th>
<th>0.2 μM Ca**</th>
<th>b/0.5(a + c)</th>
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<tr>
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### Table 1

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Columns a, b, and c: values given are the mean ± SEM for n fibers. Column d: ratios of the test contraction, b, to the average of the control contractions, 0.5/(a + c), computed for individual fibers then averaged.

**FIGURE 4.** Relation between isometric force ($F_0$, X's), unloaded shortening velocity ($V_u$, O's), and the Ca** concentration (M). Data from experiment on guinea pig taenia coli fiber are presented in Figure 3.
taenia coli and carotid artery. The relative change in $F_o$ was similar in magnitude, but consistently smaller than that observed for $V_{us}$.

**Effects of Calmodulin**

In previous work (Sparrow et al., 1981; Rueegg and Paul, 1982), we reported that the Ca$^{++}$ sensitivity of isometric force could be altered by calmodulin. At a fixed Ca$^{++}$ concentration, both isometric force and the rate of force development were increased in the presence of added calmodulin. An experimental series analogous to the above Ca$^{++}$ experiments was undertaken in which measurements at 1.8 μM Ca$^{++}$ and high calmodulin levels was bracketed by measurements during contractions at the same Ca$^{++}$ concentration but lower calmodulin levels (0.01 μM). The results are presented in Table 1. For taenia coli, a contraction in the presence of 1 μM calmodulin, developed over twice the force and shortening velocity when compared with a contraction in the presence of 0.01 μM calmodulin. The results for carotid artery differed in that—whereas, in the presence of 4 μM calmodulin, $V_{us}$ was doubled—$F_o$ was increased only marginally compared with that at 0.01 μM calmodulin.

**Dissociation of the Ca$^{++}$-Calmodulin Sensitivity of $F_o$ and $V_{us}$**

Up to this point, approximately proportional effects of $F_o$ and $V_{us}$ were elicited by changes in the Ca$^{++}$-calmodulin concentration. However, there were indications that the effects on $F_o$ could be saturated before that of $V_{us}$ (cf. Fig. 4 and Table 1). This hypothesis was tested by comparing contractions in the presence of 1 μM calmodulin, developed over twice the force and shortening velocity when compared with a contraction in the presence of 0.01 μM calmodulin. The results for carotid artery differed in that—whereas, in the presence of 4 μM calmodulin, $V_{us}$ was doubled—$F_o$ was increased only marginally compared with that at 0.01 μM calmodulin.

**Effects of ATP-γS on $F_o$ and $V_{us}$**

To elucidate further the role of myosin light chain phosphorylation, we studied the effects of the ATP analogue, ATP-γS, on $F_o$ and $V_{us}$. ATP-γS is a substrate for myosin kinase, but thiophosphorylated myosin is only a poor substrate for the phosphatase (Sherry et al., 1978), leading to essentially irreversible phosphorylation of myosin, which is thereafter independent of Ca$^{++}$. In the presence of ATP-γS, the level of thiophosphorylated myosin light chains in skinned fibers is reported to be substantially higher than the levels of phosphorylation achieved at saturating Ca$^{++}$ concentrations (Cassidy et al., 1979). Both skinned taenia coli (Sparrow et al., 1981) and carotid artery (Peterson, 1980) develop maximal isometric force which is independent of Ca$^{++}$ after exposure to ATP-γS. Thiophosphorylating conditions were obtained by rinsing the fibers for 5 minutes in an ATP-free solution, then incubating for 10 minutes in 2 mM ATP-γS in the presence of 21.5 μM Ca$^{++}$ and 1 μM calmodulin, followed by rinsing free of ATP-γS in the initial ATP-free solution for 2 minutes (cf. Sparrow et al., 1981). The velocity of unloaded shortening in the presence of 21.5 μM Ca$^{++}$ and 1 μM calmodulin under thiophosphorylated conditions was not different from the maximal velocity observed in the absence of thiophosphorylation. The velocity ratio observed under these conditions was 1.02 ± 0.08 ($n=6$). After exposure to ATP-γS, a small but significant dependence of $V_{us}$ on the Ca$^{++}$ concentration was observed. The ratio of $F_o$ in a contracture in the presence of 21.5 μM Ca$^{++}$ and 1 μM calmodulin to that in bracketing contractions in the absence of Ca$^{++}$ (Ca$^{++}$ concentrations less than 10$^{-8}$ μM) was 0.96 ± 0.03, whereas that for $V_{us}$ was 1.28 ± 0.04 ($n=6$).

**Dependence of $V_{us}$ on the Duration of Contraction**

In living hog carotid artery, the time course for development of isometric force and shortening velocity is dependent on the mode of stimulus. The development of shortening velocity generally precedes the development of $F_o$, with the maximum in shortening velocity occurring when approximately 70% of the maximal $F_o$ is attained (Dillon and Murphy, 1982). Shortening velocity then declines, reaching a stable value of approximately one-third of the maximum observed between 8 and 12 minutes after stimulation.

The time course of isometric force in skinned
carotid arteries at 30°C in a contraction induced by 21.5 μM Ca++ in the presence of 4 μM calmodulin was studied. The rate of force development was similar to that observed in living carotid artery at 37°C with electrical stimulation (Dillon and Murphy, 1982). In both cases, the peak of force occurs between 1 and 2 minutes from the initiation of contraction. In an attempt to isolate the greatest difference, we measured \( V_{us} \) in skinned carotid arteries after 10 minutes of contraction, bracketed by measurements at the peak of the development of isometric force. In contrast to the living artery, no significant difference in \( V_{us} \) measured at the peak of contraction and at 10 minutes was observed in the skinned hog carotid artery. The ratio of \( V_{us} \) at 10 minutes to the average of \( V_{us} \) at the peak force in bracketing contractions was 1.06 ± 0.17 (n = 5) in the presence of 4 μM calmodulin and 1.12 ± 0.17 (n = 3) in the absence of calmodulin. The ratio of \( F_0 \) for these eight fibers was 1.03 ± 0.03.

Similar results were obtained for taenia coli, as can be seen in Figure 5. In this experimental series, \( V_{us} \) was measured at various time points in multiple contractions in a randomized fashion. The rate of development of \( V_{us} \) can be seen to precede the development of force. This is more clearly seen in the contraction elicited at a lower Ca++ concentration (0.34 μM, Fig. 5A) in which the rate of force development was slower than that at higher Ca++ concentration (12.5 μM, Fig. 5B). \( V_{us} \) was found to attain a stable value somewhat before the peak of isometric force, which remained constant for contraction durations up to 18 minutes. As seen in Figure 5, there was a trend indicative of a somewhat higher value for \( V_{us} \) early in the contraction. This was investigated in a systematic fashion, using bracketing contractions to control for variability due to fiber or order of contraction differences. The results are presented in Table 2. For taenia coli, \( V_{us} \) at 15 seconds, a time at which 66% of the isometric force had been developed, was 1.5 times the steady state value of \( V_{us} \) measured at 300 seconds. A smaller difference could also be detected between 15 and 60 seconds. For carotid artery, the overall mean values of \( V_{us} \) indicated a tendency for somewhat higher values at earlier times during contraction. However, a more restrictive comparison of the velocity ratios for individual fibers indicated that the differences between \( V_{us} \) at 120 or 300 seconds and \( V_{us} \) in the bracketing contractions at 35 seconds were small (<20%) and the ratios were not statistically different from 1. Differences in \( V_{us} \) in the bracketing contractions of shorter duration were negligible.

### Discussion

Experiments with chemically skinned muscle are unique, in that the myofibrilar Ca++ and calmodulin concentrations can be controlled. In previous work (Sparrow et al., 1981; Rueegg and Paul, 1982), we have shown that the level of isometric force is dependent on both the Ca++ and calmodulin concentrations. These results suggested that the number of activated cross-bridges are dependent on the activity of myosin light chain kinase, a Ca++-calmodulin-dependent enzyme. In this work, the dependence of unloaded shortening velocity, a mechanical parameter associated with the rate of cross-bridge cycling (Rueegg, 1971) and actomyosin ATPase (Barany, 1967), was measured. \( V_{us} \) measured by the technique of rapid imposition of relatively large (~20%) step decreases in length, has been reported to yield values for \( V_{us} \) that are in good agreement with the values of the maximal velocity estimated from fitting isotonic velocities and loads to the Hill equation in experiments on tracheal smooth muscle (Mitchell and Stephens, 1982). In our work, the linearity of the duration of time under unloaded conditions as a function of the amplitude of the step releases, indicates that the amplitude of the changes in muscle length used in these experiments did not have a significant effect on the measured \( V_{us} \) (Fig. 1). For taenia coli, our measured values for \( V_{us} \) of 0.1 l/sec at 20°C and 0.35 l/sec at 30°C are in good agreement with the value of \( V_{us} \) of 0.14 l/sec at 23°C determined from the force-velocity measurements on both chemically skinned and intact taenia coli recently reported by Arner.
(1982). The technique for measurement of \( V_u \) in our experiments has the advantage of permitting shortening velocity to be determined precisely, with relatively few contractions, compared with that required for extrapolation from force-velocity experiments.

\( V_u \) measured in skinned hog carotid artery at 30°C under optimal conditions ranged between 0.05 and 0.1 l/sec. This is similar to that reported for living carotid artery at 37°C (Dillon and Murphy, 1982). The velocity for skinned fibers at 37°C would be about 2.5-fold higher, based on the measured temperature dependence (Fig. 2). Because of the strong dependence of \( V_{\text{max}} \) in living arteries on the duration of contraction and on the mode of stimulation, exact comparison is difficult. However, \( V_u \) in the skinned arteries is at least as great as, if not greater than, that reported for the living preparation.

For both skinned taenia coli and carotid artery, \( V_u \) was found to be dependent on the Ca ++ concentration. For the living preparations, there are considerable differences between muscles as to the role of the external Ca ++ concentration on shortening velocity. For intact guinea pig taenia coli, \( V_{\text{max}} \) is reported to vary with extracellular Ca ++ ( Mashima and Handa, 1969; Arner, 1982). In contrast, Dillon and Murphy (1982) reported that \( V_{\text{max}} \) was unaffected by external Ca ++ in K+-depolarized contractions in living hog carotid artery. Similarly, Arner (1983) reported a Ca ++ dependence of \( V_{\text{max}} \) in skinned portal vein, whereas, in the living preparation, no such dependence has been reported (Hellsstrand et al., 1972; Peiper et al., 1976). The basis for these differences is unclear; however, the strong dependence of shortening velocity on the duration of contraction in living smooth muscle may in part play a role.

For both preparations, \( V_u \) was also found to be dependent on the calmodulin concentration. At submaximal Ca ++ concentrations, both isometric force and unloaded shortening velocity could be increased by the addition of calmodulin (Table 1). Even at high Ca ++ concentrations (21.5 \( \mu \)M), for which the isometric force response was maximal, a small but statistically significant increase in \( V_u \) was elicited by the addition of calmodulin. These findings suggest that \( V_u \) is related to the level of myosin light chain phosphorylation, mediated by the Ca ++-calmodulin dependency of the myosin light chain kinase. This hypothesis is supported by the observation of Cassidy et al. (1981) that the degree of myosin light chain phosphorylation in skinned chicken gizzard fibers can be increased by addition of calmodulin at optimal Ca ++ concentrations. However, additional effects of calmodulin on \( V_u \) not related to myosin phosphorylation cannot be excluded on the basis of our observations.

Although both \( V_u \) and \( F_0 \) are strongly dependent on the Ca ++-calmodulin concentration, the immediate recoil of series elastic elements did not exhibit any significant dependence (Fig. 3) on Ca ++-calmodulin and, hence, on \( F_0 \). The lack of a dependence of the elastic recoil on force is compatible with a model in which the series elastic element is located within the cross-bridge (Huxley and Simmons, 1971). However, in view of structural constraints,
is unlikely that an element of the magnitude measured in these experiments (~10% l) could be completely localized in the cross-bridge. Pfitter et al (1982) have recently reported, for both living and skinned hog carotid artery, that a component of the elastic recoil of approximately 1-2% l, could be attributed to cross-bridges. However, the elastic recoil of both hog carotid artery (Pfitter et al., 1982) and taenia coli (Armer, 1982) are characterized by a substantial region of high compliance in the low force range. The technique of large length changes used in these experiments would yield values for the elastic recoil which would include this region of high compliance. The force and/or Ca++-calmodulin dependence of this highly compliant region, although likely to be small, cannot be ascertained with precision with the techniques used for Vus determinations in these experiments.

Although both F0 and Vus were dependent on the Ca++-calmodulin concentration, the dependencies were not identical. In the presence of 1 μM calmodulin, F0 reached its maximum value in skinned taenia coli at a Ca++ concentration of 0.8 μM. However, increases in either Ca++ or calmodulin elicited a further increase in Vus of 1.5- to 2-fold. Similar results were obtained for carotid artery; however, F0 appeared to be saturated at a somewhat lower Ca++ concentration. These results may provide a mechanism underlying the decline in shortening velocity during the maintenance of constant and maximal isometric force observed in living smooth muscle. There are several reports which indicate that the activity of phosphorylase, which is dependent on an enzyme cascade with an absolute Ca++ requirement, also declines with the duration of contraction in living smooth muscle (Galvas et al., 1981; Paul, 1983; Silver and Stull, 1982). These observations suggest that intracellular Ca++ may also decline. Our results indicate that there is a range of Ca++ concentrations over which velocity can vary in spite of the maintenance of constant force.

After incubation of the fibers in the presence of ATP-γS, a procedure which would be expected to increase the level of myosin light chain phosphorylation above that observed in the presence of saturating Ca++-calmodulin (Cassidy et al., 1979), no further increase in Vus was found. Thus, if Vus is related to myosin light chain phosphorylation, it is likely to be maximized at phosphorylation levels less than the maximum. The basis for the small but statistically significant Ca++ dependence of Vus after exposure to ATP-γS is unknown. Although thio-phosphorylated myosin is a poor substrate for phosphate, the phosphorylation state may slowly decay with the duration of time after exposure to ATP-γS. This could account for the slight Ca++ dependence of Vus seen after exposure to ATP-γS. Resolution of these questions is dependent of measurement of myosin phosphorylation in these fibers.

In agreement with the time course observed in living tissues, the development of Vus preceded that of F0 in skinned smooth muscle. The shortening velocity in skinned fibers appears to be relatively stable compared with the decline in velocity with contraction duration reported for living smooth muscle (cf. Murphy et al., 1983). In living carotid artery, Vus, calculated from Hill force-velocity curves declines during the stable phase of force maintenance, and even greater differences in Vus are suggested, compared with earlier times during contraction (Dillon and Murphy, 1982). In skinned carotid artery, no difference in Vus between times corresponding to the peak of contraction and at 10 minutes of contraction were observed. Compared with earlier times—when, in relation to isometric force development in living carotid artery, a difference in Vus of minimally 2-fold with respect to the longer durations would be expected—no statistically significant differences were found (Table 2). Similar experiments with skinned taenia coli, little change in Vus was seen during the maintenance of stable isometric force. However, a relatively small increase in Vus of approximately 1.5-fold could be detected early in the contraction, when 66% of the maximal isometric force had been developed, compared with the stable values observed at 5 minutes. Thus, whereas Vus in the presence of constant levels of Ca++ and calmodulin is relatively stable compared with that observed in living tissues, it remains possible that the changes observed in living tissue may not be ascribable entirely to changes in intracellular Ca++.

Our results indicate that both F0 and Vus are dependent on the Ca++-calmodulin concentration. The dependence of Vus reported here is novel, and suggests that cross-bridge cycle rate, as well as the number of bridges, is regulated by Ca++-calmodulin. Three general classes of mechanism can be evoked to explain the dependence of Vus on Ca++-calmodulin. The first class involves cooperation interaction upon phosphorylation between myosin heads or myosin cross-bridges such that the cross-bridge cycle rate itself is modulated. Such cooperative interaction has been suggested on the basis of the dependence of smooth muscle actomyosin ATPase on the myosin phosphorylation state (Persechini and Hartshorne, 1981). The second class does not directly require alteration in the individual cross-bridge cycle, but involves an internal load concept to change the overall velocity of the fiber. One example would be an interaction between actin and nonphosphorylated myosin, the "latch" bridges suggested by Murphy et al. (1983). Slowly or non-cycling "latch" bridge, in parallel with rapidly cycling, phosphorylated bridges, would in effect provide an internal load resulting in a Ca++-calmodulin-independent shortening velocity. A third class involves a role for Ca++-calmodulin in the regulation of shortening velocity which is independent of its effect on myosin phosphorylation. Such an addi-
tional mechanism may not be unreasonable in light or the recent work of Cole et al. (1980).

Our data, indicating that the dependencies of $F_o$ and $V_{as}$ on Ca$^{2+}$-calmodulin are not identical, rule out mechanisms involving a constant internal load. Moreover, the evidence showing that incubation in ATP-$\gamma$S [which is reported to increase the levels of myosin phosphorylation beyond that observed at optimal levels of Ca$^{2+}$-calmodulin (Cassidy et al., 1979)] does not effect $V_{as}$, suggest that $V_{as}$ is not related to myosin phosphorylation in a simple fashion. However, no conclusions can be drawn in the absence of data concerning the phosphorylation state of myosin in these fibers, which, at the present time, cannot be resolved at the same level of precision as can the mechanical parameters measured here.

Whereas, the underlying mechanism is unclear, shortening velocity is strongly dependent on the Ca$^{2+}$-calmodulin concentration in these smooth muscles. In taenia coli, the ATPase activity of skinned fibers has recently been reported (Guth and Junge, 1982) to show a dependence on Ca$^{2+}$ similar to that of $V_{as}$. Similarly, in living carotid artery, the tension cost of force maintenance is reported to decrease with contraction duration (Krisanda and Paul, 1982) in a manner similar to that reported for shortening velocity (Dillon and Murphy, 1982). In conjunction with the present work, it thus appears that smooth muscle has the ability to "shift gears" so that, while maintaining constant force, both shortening velocity and energy utilization are decreased.

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The dependence of unloaded shortening velocity on Ca++, calmodulin, and duration of contraction in "chemically skinned" smooth muscle.

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