Force and Velocity of Sarcomere Shortening in Trabeculae From Rat Heart

Effects of Temperature

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The effect of temperature on the force-sarcomere velocity relation (20°, 25°, and 30° C) and maximum velocity of sarcomere shortening (V0; range 15°–35° C) was studied in trabeculae from rat heart. Sarcomere length and V0 were measured by laser diffraction techniques. Sarcomere length and sarcomere velocity, determined from each of the first-order diffraction lines, differed by less than 4%. Slack sarcomere length in the trabeculae appeared to be 1.9 μm. Isovelocity release techniques were used to obtain sarcomere velocity and V0 directly. Sarcomere velocity was measured at SL=1.9–2.0 μm for elimination of contributions of parallel elastic force and restoring force to the external load of the sarcomeres. Peak twitch force development (F0) was maximal (F0=max) at 25° C at [Ca2+]o=1.5 mM. Lowering of the temperature below 25° C led to development of spontaneous sarcomere activity and depression of F0; both responses could be prevented by the addition of 0.5 mM procaine. Increase of temperature above 25° C reduced twitch duration and F0. Hill’s rectangular hyperbola fitted the force-velocity data if the load during shortening was less than 70% of F0. V0 appeared to be independent of the level of activation at all temperatures when F0 was maintained above 90% of F0=max, either by an increase of [Ca2+]o (to 3.0 mM) or by paired pulse stimulation. V0 increased with increasing temperature; the parameter a, calculated from force-velocity relations measured at 20°, 25°, and 30° C, decreased with increasing temperature. The Arrhenius plot of V0 was studied in detail over a wider temperature range (15°–35° C) and in smaller temperature increments. The relation was linear between 18° and 33° C; the observed Q10 defined as the ratio of V0 measured at temperature (T) over V0 at T–10° C, was 4.6. A Q10 of 4.6 for V0 is consistent with the reported temperature dependence of rat cardiac actin-activated myosin ATPase, which suggests that the same reaction step may limit the activity of the enzyme in vitro and during shortening of the cardiac sarcomeres at zero external load. (Circulation Research 1990;66:1239–1254)

In a classical study, Barany1 has shown a close correlation between the unloaded velocity of shortening (V0) and myosin ATPase activity of muscle over more than a 200-fold variation in V0. It is generally believed that the myosin ATPase reaction in vitro resembles the in vivo ATPase reaction when the mechanical load is zero. Barany’s results are consistent with the cross-bridge theory, which states that V0 is predominantly controlled by the detachment rate of cycling cross-bridges.2 Therefore, one would predict that the temperature dependence of the rate-limiting step for ATP hydrolysis by the actomyosin complex and for V0 is equal, provided that the mechanical load on the contractile proteins during measurement of V0 is negligible. The temperature dependence of V0 has been studied in both skeletal3–5 and cardiac muscle,3,6–8 as has that of myosin ATPase activity.3,8–10 The specific activity and temperature dependence of myosin ATPase depend on the specific enzyme under scrutiny; that is, these properties of calcium-activated myosin ATPase1,8 differ from those of actin-activated myosin ATPase,1,10 which again differ from those of myofibrillar myosin ATPase.9 This may indicate that different reaction steps are rate limiting for these ATPases in vitro. Even though actin-activated myosin ATPase clearly reflects the physiological mode of action of the enzyme in vivo,11 the question of which reaction step limits the rate of shortening is still unanswered. A

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Supported by grants from the Canadian Medical Research Council and the Alberta Heritage Foundation for Medical Research (AHFMR). P.P. de T. held a studentship from the AHFMR; H.E.D.J. ter K. is a Medical Scientist of the AHFMR.

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Received August 3, 1988; accepted December 7, 1989.
quantitative comparison of the temperature dependence of myocardial myosin ATPase reaction kinetics in vitro and \( V_o \) in cardiac muscle could provide insight into the above question. At present, however, such a comparison is not possible for cardiac muscle, because there is considerable variation in the reported temperature dependence of \( V_o \).

There are several reasons for the variability of the temperature dependence of \( V_o \). We will present evidence that it may be due to the effect of temperature on the level of activation of the contractile proteins by calcium ions, as shown by the effect of temperature on isometric force development. Another reason is the practice of measuring \( V_o \) by extrapolation of the force-velocity relation to zero load; this method is severely limited because the relation is very steep at low loads, especially at high temperatures. Furthermore, the effect of temperature on the force-velocity relation when sarcomere length rather than muscle length is measured has not been reported. Measurements of sarcomere shortening from muscle length changes during twitches are uncertain when there are inhomogeneities in cardiac muscle preparations.\(^{12-14}\) We have previously observed that isolated cardiac muscle preparations consist of assumedly normal sarcomeres in series with sarcomeres that contract only partially near the damaged and compliant ends of the muscle.\(^{14}\) It is probable that the partially contracting sarcomeres shorten during isotonic contractions at different velocities than do the normal central sarcomeres.\(^{14}\) This action negates the prediction that the velocity of isotonic muscle shortening is linearly proportional to the velocity of the sarcomeres in the presumably normal (central) region, even though the length of the passive series elastic element remains constant at a constant load. For these reasons, we considered it essential to measure sarcomere length. In addition, unambiguous measurement of \( V_o \) requires that the measurements be made at, or slightly above, the length at which the sarcomeres are completely unloaded, that is, the slack length (1.9 \( \mu m \)).\(^{14}\) Therefore, in the present study in rat cardiac muscle, we used a direct laser diffraction technique to measure the effect of temperature variation on the maximum velocity of sarcomere shortening in rat cardiac muscle.

To test whether measurement of sarcomere length by laser diffraction in our preparations is influenced by Bragg angle artifacts,\(^{15,16}\) we have evaluated the technique in the present study by the use of a dual first-order detection system. The results show that Bragg angle reflection, in homogeneous preparations that are thinner than 80 \( \mu m \), plays an insignificant role among the factors that determine the median position of the spatial distribution of the first-order diffraction band.

A preliminary report of this work has appeared.\(^{17}\)

### Materials and Methods

**Muscle Preparation**

Two- to four-month-old Sprague-Dawley rats of either sex, fed ad libitum, were anesthetized with diethyl ether. The heart was rapidly excised, perfused retrogradely via the proximal aorta with a modified Krebs-Henseleit solution, and placed in a dissection dish beneath a binocular microscope (model SMZ-1, Nikon, Tokyo, Japan; magnification \( \times 7-30 \)). Spontaneous beating of the heart was prevented by raising the potassium concentration in the perfusion medium to 15 mM, a concentration sufficient to stop pacemaker activity.

The free wall of the right ventricle was gently separated from the ventricular septum, and the right atrium was removed from the atrioventricular ring. Thin, unbranched, uniform trabeculae, running between the free wall of the right ventricle and the atrioventricular ring, were selected. Next, the selected trabeculae were carefully dissected by cutting through the atrioventricular ring on one end and removing a portion of the right ventricular wall on the other end. The 36 trabeculae used in this study were selected based on uniformity for prevention of potential artifacts associated with the measurement of sarcomere length; the trabeculae measured 50–200 \( \mu m \) in width, 2–5 mm in length, and 40–80 \( \mu m \) in thickness.

An image of the section of the muscle preparation that was (also) illuminated by the laser beam was observed by use of an inverted microscope (model Diaphot-TMD, Nikon, final magnification \( \times 100 \), long working distance objective) and a television system (model TR930 monitor, model WV1500 camera, Panasonic, Saito, Japan). Sarcomere length was measured in all preparations in a region of the muscle close to the stationary ventricular end, which was mounted in the platinum cradle of the force transducer (Figure 1). If translation during the contractions or ramp stretches or releases exceeded 25 \( \mu m \), the preparation was repositioned or remounted. Muscle preparations were discarded if this criterion could not be met.

After mounting, the muscles were stretched to a sarcomere length of 2.1 \( \mu m \) and left to equilibrate for 1 hour at 25°C in the perfusion medium (1.5 mM \( [Ca^{2+}]_o \)) while being stimulated at 0.5 Hz. Passive force development at this sarcomere length is negligible (Figure 3A). After mounting, the end regions (approximately 200 \( \mu m \) in length) exhibited contracture.\(^{14}\) During the equilibration period, sarcomere length both at rest and during the twitches decreased as a result of relaxation of the damaged ends. If peak twitch force development diminished during equilibration to less than 70% of the force directly after mounting and adjustment of sarcomere length to 2.1 \( \mu m \), the preparation was discarded. After this 1-hour period, the muscle was restretched to 2.1 \( \mu m \) passive sarcomere length, at which time force development was, on average, 125% of the force at the start of the experiment. The average success rate in obtaining a suitable preparation was about 25%.

### Solutions

The standard solution used was a modified Krebs-Henseleit solution with the following composition:
(mM): Na⁺ 140.5, K⁺ 5.0, Cl⁻ 127.5, Mg²⁺ 1.2, H₂PO₄⁻ 2.0, SO₄²⁻ 1.2, HCO₃⁻ 19, d-glucose 10.0, and [Ca²⁺]₀ as indicated. All chemicals were of the highest purity available (Analar grade, BDH, Toronto, Canada). In some instances, the solution also contained 0.5 mM procaine hydrochloride (Sigma Chemical, St. Louis). The solutions were equilibrated with a 95% O₂/5% CO₂ gas mixture, resulting in a pH ranging from 7.35 to 7.45 at a temperature range of 20°-30°C, respectively. The pH was measured by a glass electrode (model 13-639-3, Fisher Scientific, Pittsburgh, Pennsylvania) and double junction reference electrode (model 900200, Orion Research, Boston), connected to a digital pH meter (model 825MP, Fisher Scientific). The reference electrode was filled with 140 mM NaCl, the major component of the Krebs-Henseleit solution. This procedure excluded the large errors associated with the standard combination pH electrode, caused by junction potentials.

Under microscopic control, the muscles were positioned horizontally in an experimental chamber milled from plastic (Figure 1). The trough was 25 mm long, 2 mm wide, and 5 mm deep. The bottom of the trough was made from a microscope coverslip sufficiently thin to prevent parallax errors with the diffractometer. After mounting of the preparation, the top of the trough was covered with a piece of a microscope slide. The perfusion rate was adjusted to approximately 2.5
ml/min, just under the rate at which turbulence would develop. The temperature in the perfusion chamber was controlled (±0.1°C) by use of a glass heat exchanger at the inflow line and a circulating water bath (model F3, Haake, Karlsruhe, FRG).

**Electrical Stimulation**

Electrical stimulation of the preparation was achieved via platinum electrodes (0.4 mm diameter) positioned in the wall of the muscle bath along either side of the muscle preparation. Rectangular stimulating pulses were delivered by an isolated pulse generator (model DS2, Digitimer, Welwyn Garden City, UK). Pulse duration was 2 msec. The stimulation frequency was 0.5 Hz at all times. Stimulus strength was adjusted to 50% above the stimulus threshold of the preparation. The stimulus threshold increased in the presence of procaine, as expected from the known effects of this drug on sodium channel function. The stimulator was triggered by a timing device (model D4030, Digitimer) that also controlled the timing of the stretch and release ramps applied to the muscle.

**Force Measurement**

Twitch force was measured with a modified silicon strain gauge (model AE-801, SenSonor, Horten, Norway; see Figure 1) attached to a micromanipulator. To increase the frequency response of the transducer, the original silicon beam of 5-mm length was ground down to about 2 mm on a slowly rotating diamond disk. A lightweight basket (0.5 mm wide) made from platinum wire (100 μm diameter) was glued onto the tip of the silicon beam with epoxy. Care was taken to use a minimum amount of glue and not to cover the resistors on the silicon beam. Next, the silicon beam was covered with silicone glue mixed with carbon powder and toluene, the latter being used for smooth application of the glue. This procedure ensured both watertightness and lighttightness of the transducer. The platinum basket provided a stable mounting cradle in which the ventricular end of the trabecula was positioned (Figure 1B). The resonant frequency of the transducer was about 10 kHz with a muscle preparation attached and submersed in the perfusion medium. The transducer showed no signs of creep or hysteresis, the response was linear to at least 500 mg, compliance was 0.75 μm/g, noise was 0.5 mg peak to peak, drift was 2.5 mg/°C, and gain was constant in the range of 15°–35°C.

**Muscle Length Control**

The remnant of the tricuspid valve served as an attachment point to a servo-controlled motor (model 300S Dual Mode Servo, Cambridge Technology, Watertown, Massachusetts) via a stainless steel hook (100 μm diameter). The hook was glued onto a lightweight titanium motor arm. The servo-control system had been modified (Cambridge Technology) to increase the step response of the motor system to about 300 μsec. During the experiment, muscle length was controlled by three function generators, the outputs of which were fed into the position command of the servo-control system. The function generators were activated by a timing control system (model D4030, Digitimer). Thus, we were able to construct a complex stretch and release ramp function that we synchronized with the stimulus pulses and applied to the muscle preparation.

**Sarcomere Length Measurement**

Sarcomere length was measured by laser diffraction techniques that have been described in detail elsewhere. For sarcomere length (Figure 3). In short, the striations of cardiac muscle act as an optical grating to incident light, in this case the output of a 15-mW helium-neon laser (model 106-2, Spectra-Physics, Eugene, Oregon). The original diameter of the laser beam was reduced to 300 μm by a 50-cm focal-length lens placed in front of the laser. The trabecula diffractions the laser beam into a zero-order band and multiple, spatially symmetrical, higher order band pairs. The angle between the zero-order band and the first-order diffraction band is proportional to sarcomere length and the wavelength of the laser light (632.8 nm). For measurement of this angle, the left first-order diffraction band was projected onto a scanning, 512-element photodiode array (model RC 105, Reticon, Sunnyvale, California). In four muscles the left and right first-order diffraction bands were monitored simultaneously. The photodiode arrays were scanned every 0.5 msec. The median position of the first-order intensity distribution (determined by analog circuitry after correction for the contribution of the zero-order diffraction band and scatter) was converted into a voltage proportional to sarcomere length by means of a nonlinear amplifier. The transfer function of this amplifier was adjusted by placement of standard glass calibration gratings in the same position as the muscle (Figure 1D). The spatial resolution of the diffractometer was 10 nm. The highest V of that could be measured reliably was about 50 μm/°C, because the scanning rate of the photodiode array was 2 kHz and at least four samples of the sarcomere length signal were used, during a length change of 0.1 μm, for calculation of V.

**Isovelocity Release Technique**

V of was measured by the isovelocity release technique (Figure 2). Sarcomere length was kept constant at 2.0 μm by stretching of the muscle from the valvular end. Passive force development at this sarcomere length is negligible (Figure 3). When about 70% of isometric twitch force (F) was obtained, the muscle was quickly released to a new load level. Between 20° and 30° C, the time at which this release occurred ranged from 60 to 130 msec. This time was chosen because the maximum V is stable and maximal during a substantial period after this moment (Figure 3A). Next, a controlled release ramp was imposed. The speed and amplitude of this release ramp were adjusted such that force was...
maintained at a constant level. During the first 10–50 msec, average force and the $V_o$ were calculated by linear regression of the digitized force and sarcomere values (Figure 2); the window during which the velocity was calculated was inversely proportional to the velocity. The latter calculation also yielded mean sarcomere length during the period of shortening. Care was taken to ensure that minimum sarcomere length was 1.9 $\mu$m and that sarcomere shortening during the measurement was limited to 0.2 $\mu$m.

Isovelocity releases used to obtain $V_o$ are illustrated in Figure 3. $V_o$ was measured by imposition of release ramps onto the muscle so that force was zero. Starting at the lowest velocity at which force was zero, the velocity of the ramp was gradually increased until the preparation visibly buckled, at which time the deflection pattern disappeared. At ramp velocities between the latter two values, several observations were striking: 1) The $V_o$ in the area of observation was constant, 2) the muscle itself did not buckle, and 3) excess shortening was completely taken up by buckling of the valve.\(^{21}\) Thus, measurements were performed at a velocity of the motor arm halfway between the value at which force was just zero and the velocity at which buckling of the muscle started to occur. Hence, the trabecula proved to be completely unloaded during the measurement of $V_o$.

Data Acquisition

Force, sarcomere length, and muscle length were displayed on an electrostatic recorder (model ES1000, Gould, Cleveland, Ohio) equipped with digital storage amplifiers (bandwidth=100 kHz). Force and sarcomere length signals were also displayed on a storage oscilloscope (model V134, Hitachi, Tokyo, Japan) and sampled via an analog-to-digital (A/D) converter (model 2801A, Data-translation, Marlboro, Massachusetts) installed in an IBM PC-AT. The A/D converter collected 900 samples per signal; the sample rate was adjusted to the acquisition time. The recorder, oscilloscope, and A/D converter were all triggered by the timing device, which also controlled the muscle stimulator and the timing of the stretch and release ramp function generators.

The computer program for data analysis, written in FORTRAN, allowed the force and sarcomere length of each twitch to be displayed on a graphics monitor (Color Graphics Adapter video board, Personal Computer Color Display, IBM). By cursor control, the user could select a time window indicated on the screen in which $V_o$, mean sarcomere length, and force were to be calculated (Figure 2). The velocity, thus measured, was displayed separately as a function of force on the monitor. This procedure ensured that sufficient data were collected for each force-velocity relation. The force and sarcomere length recordings and the force-velocity coordinates were stored on hard disk for later processing, or plotted with a laser plotter (model LN03, Digital Equipment, Maynard, California).

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Method to measure force-sarcomere velocity relation. Sarcomere length (middle row) was kept constant by stretching of valvular end of trabecula (muscle length, top row). Peak twitch force (bottom row) was measured at sarcomere length of 2.0 $\mu$m ($F_o$, left panel) when no shortening step was imposed. Middle panel shows a release to 20% of peak twitch force. Velocity of sarcomere shortening ($V_o$, right panel) was calculated by linear regression of digitized sarcomere length values during initial phase of shortening, when force was stable, as indicated by dashed vertical lines. In addition, mean sarcomere length was calculated from sarcomere lengths during latter period. A release to zero force is shown in right panel, which allows direct determination of maximum, unloaded $V_o$, [Ca$^{2+}$]$_o$=1.5 mM; 0.5 mM procaine; 20° C; time calibration (dots) 20 msec. Arrow points indicate time at which muscle was stimulated.
Curve Fitting and Statistical Analysis

The force-velocity relations were fitted to the Hill\textsuperscript{22} equation by means of a nonlinear least-squares fitting procedure of the Marquardt type.\textsuperscript{23} The Hill equation was rearranged into a function in which the isometric twitch tension was substituted by a function for the unloaded velocity of shortening ($V_o$) as

$$V = [(a \times V_o) - (b \times F)] / (F + a)$$

In this relation, F stands for force or load and V for velocity of shortening at that force; a and b are identical to the parameters of the original Hill equation.

This modification deserves further comment. The force-velocity relations obtained in this study devi-
ated from a rectangular hyperbola at forces above 70% of peak twitch tension, as has been observed previously in skeletal,24 smooth,25 and cardiac21 muscle preparations. The measured value for isometric peak force, therefore, was substantially lower than the value predicted from the Hill equation. Since the protocol used in this study makes possible the direct measurement of \( V_o \), the above modification allows fitting of the data to the Hill equation at forces below 70% (see “Results”).

One-way analysis of variance or Student’s t test was employed for testing of statistical differences where indicated26; \( p < 0.05 \) was considered significant.

Results

General Properties of Sarcomere Shortening in Rat Cardiac Trabeculae

We examined the contribution of asymmetry of the diffraction pattern due to Bragg angle reflections to errors in the measured median of the sarcomere length distribution with the aid of two diffractometers, which sampled the opposite first orders of the diffraction pattern. Figure 1D shows typical diffraction band distributions from the left and right diffraction patterns during rest, just before release during a sarcomere length clamp, and during \( V_o \) after about 0.15 \( \mu m \) shortening. We monitored both first-order bands of the diffraction pattern and determined sarcomere length and \( V_o \) simultaneously in four muscles. Figure 4A shows the determination of \( F_o \) and \( V_o \) at 20\(^\circ\), 25\(^\circ\), and 30\(^\circ\) C with the dual diffractometer system. It is clear that sarcomere length and \( V_o \) obtained from the opposite first orders of the diffraction patterns differed by less than 4% (the maximal difference in the four muscles tested).

The effect of inhomogeneity of the trabeculae on the recorded velocity of shortening was also examined (Figure 5). The muscle was translated such that the center of the incident laser beam was at the location indicated in the figure, and \( V_o \) was subsequently measured. The valve was usually more compliant than the right ventricular end of these preparations. Therefore, regions of the muscle that are closer to the valvular attachment are expected to move more with respect to a stationary point, such as the laser beam, than those close to the force transducer. \( V_o \) measured along the muscle was found to be independent of location, except at positions very close to the valve, where lateral translation was largest. A location close to the ventricular end of the muscle was chosen in all further experiments. Apparently, slight lateral translation in a homogeneous muscle brought only a new population of functionally identical sarcomeres into the field of observation, as has been observed previously.14

We have ensured that the \( V_o \) was always measured at or slightly above slack sarcomere length (1.8 to 1.9 \( \mu m \); see Figure 3B), thus removing velocity artifacts that might have been produced by the contribution of passive elastic elements in the muscle preparation (Figure 3). Stretch of the muscle resulted in the development of passive force, but this force was less than 3% of \( F_o \) at sarcomere lengths below 2.1 \( \mu m \) (Figure 3B). At sarcomere lengths above 2.2 \( \mu m \), passive force development rose substantially. Figure 3C illustrates \( V_o \) measured in a trabecula plotted as a function of sarcomere length. It is clear that \( V_o \) was nearly independent of sarcomere length in the range of 1.9–2.2 \( \mu m \), as expected from the small passive forces that are present at these sarcomere lengths.

The contribution of viscosity of the muscle, leading to an internal load for the shortening muscle, was estimated by measurement of the force induced in an unstimulated muscle during stretches of sarcomere length between 1.9 and 2.0 \( \mu m \) at a velocity equal to \( V_o \). Passive viscous force was found to be 1.5% of peak twitch force (\( F_o \)) at 25\(^\circ\) C and at a stretch velocity of about 13 \( \mu m/sec \), 0.3% at 18\(^\circ\) C and 2 \( \mu m/sec \), and 0.8% at 32\(^\circ\) C and 40 \( \mu m/sec \).

Effect of [Ca\(^{2+}\)]\(_o\) on Force Development at Varying Temperatures

Previously, we have observed that both \( V_o \) and \( F_o \) increase with increasing [Ca\(^{2+}\)]\(_o\) at 25\(^\circ\) C.21,28 In these studies, \( V_o \) reached a maximum at [Ca\(^{2+}\)]\(_o\) = 1.0 mM while force development increased up to [Ca\(^{2+}\)]\(_o\) = 2.5 mM. To ensure that the effect of temperature on \( V_o \) would be measured independently of its effect on the level of activation, as reflected by force development, we first determined the \( F_o - [Ca^{2+}]_o \) relation at 20\(^\circ\), 25\(^\circ\), and 30\(^\circ\) C. Figure 6A shows that half-saturating levels of [Ca\(^{2+}\)]\(_o\) with respect to \( F_o \) increased with increasing temperature. This increase was accompanied by a decrease of twitch duration (Figure 4A).

Microscopic observation of the muscles suggested that depression of force development could have been due to substantial spontaneous sarcomere motion at 20\(^\circ\) C at [Ca\(^{2+}\)]\(_o\) above 0.5 mM, a phenomenon that has also been reported by Kort et al.29,30 Therefore, we added procaine to the medium. Procaine has been reported to inhibit calcium release by the sarcoplasmic reticulum.31–33 In preliminary experiments we observed that 1 mM of procaine depressed twitch force, while 0.5 mM did not. Procaine at 0.5 mM caused a small acceleration of relaxation (3–12%) and abolished spontaneous sarcomere motion at 20\(^\circ\) and 25\(^\circ\) C with [Ca\(^{2+}\)]\(_o\) below 2.0 mM.

Figure 6B shows the relation between \( F_o \) at constant sarcomere length and [Ca\(^{2+}\)]\(_o\) at 20\(^\circ\), 25\(^\circ\), and 30\(^\circ\) C in the presence of procaine. On the average, saturating \( F_o \) levels were observed at a lower [Ca\(^{2+}\)]\(_o\) at 20\(^\circ\) C than in the absence of the drug. However, \( F_o \) did decline slightly at [Ca\(^{2+}\)]\(_o\) levels greater than 2.0 mM at 20\(^\circ\) C, consistent with the persistence of spontaneous sarcomere motion at these [Ca\(^{2+}\)]\(_o\) even in the presence of 0.5 mM procaine. At 30\(^\circ\) C and [Ca\(^{2+}\)]\(_o\) = 1.5 mM, force development was substan-
Figure 4. Sarcomere length and velocity of sarcomere shortening ($V_o$) measured simultaneously with two diffractometers. Sarcomere length was measured from both diffraction bands simultaneously, as illustrated in Figure 1, at 20°, 25°, and 30° C. $[Ca^{2+}]_o$ was 1.5 mM at 20° and 25° C and 3.0 mM at 30° C. Procaine (0.5 mM) was present at all temperatures. Panel A shows twitch force development and sarcomere length (output of both diffractometers) at the three temperatures. Calibrations: sarcomere length 0.2 μm, force 100 mg, time 200 msec. Average sarcomere length was 2.0 μm. Panel B shows measurement of $V_o$ with two diffractometers. Computed values for $V_o$ calculated from left and right diffraction band are indicated in each panel. Calibrations: sarcomere length 0.2 μm, force 100 mg, time 20 msec. Sarcomere length prior to release was about 2.1 μm.

Effect of Temperature on Force Velocity Relation

The effect of temperature on the force-sarcomere velocity relation in a representative muscle is shown in Figure 7. Twitch force, at constant sarcomere length, was nearly independent of temperature, and $V_o$ was 5.5, 13.5, and 23.0 μm/sec at 20°, 25°, and 30° C, respectively. The fitted parameters for five to eight trabeculae at 20°, 25°, and 30° C are shown in Table 2. As can be seen, of the parameters $a$, $b$, and $V_o$, only $V_o$ showed a clear temperature dependence ($Q_{10}$, defined as the ratio of $V_o$ measured at a given temperature over $V_o$ measured at a temperature 10°
lower, ranged between 3.3 and 4.3). On average, parameter a decreased with increasing temperature, but the variability of this parameter was large and changes were inconsistent between experiments. A relative measure of the steepness of the force-velocity relation at small loads is given by the ratio of $V_0$ and $V$ at the 10% force level ($V_{10}$); this value is also shown in Table 2. The steepness of force-velocity relation in this range explains why extrapolation of the force-velocity relation (obtained at forces above 3%) to zero force led, in our study, to a lower estimated value of $V_0$ (20±7% at 20°C; 29±1% at 30°C) than the measured $V_0$. The force-velocity relations were steeper in the low-load range at higher temperatures. Parameter b, the horizontal asymptote of the Hill relation, showed no temperature dependence. Note, however, that this parameter pertains to a region of the force-velocity relation that cannot be described by the Hill equation.

**Effect of Temperature on $V_0$**

The effect of temperature on $V_0$ and isometric twitch force development was further examined in eight muscles over a temperature range of 16°C–35°C. In the absence of procaine and at a fixed [Ca$^{2+}$]$_0$ at all temperatures, isometric twitch force was maximal at about 22°C (Figure 8B). The Arrhenius plot of $V_0$ was linear above 25°C, and the $Q_{10}$ was 2.6 (Figure 8A). The effect of lowered force development on $V_0$ was further evaluated in a separate series of muscles in which [Ca$^{2+}$]$_0$=1.5 mM was employed below 25°C and at 25°C, and [Ca$^{2+}$]$_0$=3.0 mM above 25°C in the presence of 0.5 mM procaine at all temperatures. The results are shown in Figures 8C and 8D. Isometric twitch force was nearly constant under these circumstances at all but the highest (>30°C) and lowest (<20°C) temperatures. The Arrhenius plot was linear above 25°C, and the $Q_{10}$ was 3.3. Note that $V_0$ measured at temperatures above 25°C was higher under these conditions with [Ca$^{2+}$]$_0$=3.0 mM as compared with [Ca$^{2+}$]$_0$=1.5 mM. This result is consistent with the low isometric force levels at these temperatures and at [Ca$^{2+}$]$_0$=1.5 mM.

We also studied isometric force development and $V_0$ at [Ca$^{2+}$]$_0$=1.5 mM during paired pulse stimula-
tion in the presence of 0.5 mM procaine 1) to evaluate whether $V_0$ was still limited because isometric twitch force above 30° C was 84% of maximal twitch force at 25° C (Table 2 and Figure 8) and 2) to exclude the possible confounding factor of changing $[\text{Ca}^{2+}]_0$ at temperatures above 25° C. The results of this experiment are shown in Figure 9. The dashed lines in this figure are reproduced from Figure 8. The paired pulse stimulation protocol was adjusted such that the extrasystole was delivered at the time of 50% relaxation at all temperatures (range: 650 msec at 20° C; 100 msec at 35° C), which resulted in maximum increase of force development. Paired pulse stimulation at temperatures below 20° C did not result in increased force development. In some experiments paired pulse stimulation resulted in a decrease of force development; this decrease was always accompanied by spontaneous sarcomere motion. In the latter experiments this stimulus protocol was not employed. Maximal postextrasystolic potentiation (25 stimuli at 5 Hz, followed by a 30-second pause) resulted in a further increase of twitch force development of less than 10% up to 30° C and of less than 20% at temperatures above 32° C. Average twitch force development varied less than 20% while temperature was reduced to about 17° C (Figure 9A). Paired pulse stimulation caused a slight increase of $V_0$ at the higher temperatures but especially at the lowest temperatures compared with the results obtained by adjustment of $[\text{Ca}^{2+}]_0$. The Arrhenius plot of $V_0$ (Figure 9B) was linear under these conditions above 17° C, with an apparent $Q_10$ of 4.6.

**Discussion**

We investigated the effects of varying temperature on force development and on the velocity of sarcomere shortening in rat cardiac trabeculae. Sarcomere length was measured with laser diffraction techniques, which made accurate determination of the $V_0$ possible. The relation between twitch force and

**TABLE 1. Effect of Procaine on Force-Sarcomere Velocity Relation**

<table>
<thead>
<tr>
<th></th>
<th>$a$ (%)</th>
<th>$b$ (µm/sec)</th>
<th>$V_0$ (µm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.95±1.29</td>
<td>-1.11±0.54</td>
<td>12.13±0.43</td>
</tr>
<tr>
<td>Procaine (0.5 mM)</td>
<td>9.38±2.69</td>
<td>-1.23±0.32</td>
<td>12.43±0.67</td>
</tr>
</tbody>
</table>

Results of three trabeculae, expressed as mean±SEM. Force-velocity relations were measured as described in text and fitted to a modified Hill equation (see "Materials and Methods"), which resulted in parameters $a$ and $b$. Velocity of sarcomere shortening ($V_0$) was measured directly. $[\text{Ca}^{2+}]_0$ was 1.5 mM. Temperature was 25° C. Peak isometric twitch tension has been normalized to 100% in presence and absence of 0.5 mM procaine for comparison of parameters. Paired Student’s $t$ test revealed no significant differences between control and procaine groups (parameter $a$, $p=0.91$; parameter $b$, $p=0.90$; $V_0$, $p=0.64$).

**FIGURE 6.** Effect of temperature on force-[Ca$^{2+}$]$_0$ relation. Peak twitch force was measured at 20° C (circles), 25° C (triangles), and 30° C (squares) at 1.9 µm sarcomere length while [Ca$^{2+}$]$_0$ was varied. All values are normalized to value found at 25° C, 3 mM [Ca$^{2+}$]$_0$ in absence of procaine. Mean value of trabeculae is shown; error bars indicate SEM when larger than symbol. Peak twitch force development as a function of [Ca$^{2+}$]$_0$ was fitted to a modified Hill equation as described previously, which allowed calculation of $K_m$ values (the [Ca$^{2+}$]$_0$ at which force development is half-maximal). Panel A: Control without procaine. Calculated $K_m$ values were 20° C, 0.66±0.04; 25° C, 0.87±0.05; 30° C, 1.73±0.27. Panel B: Results with 0.5 mM procaine added to bathing medium. Calculated $K_m$ values were 20° C, 0.32±0.07; 25° C, 0.61±0.10; 30° C 1.69±0.34. Unpaired Student’s $t$ test on calculated $K_m$ values between control and procaine groups at each temperature revealed that at 20° C, p=0.012; 25° C, p=0.07; 30° C, p=0.94.
[Ca\textsuperscript{2+}]{\textsubscript{o}} appeared to depend on temperature, but maximal force was independent of temperature. At all temperatures, \(V_o\) appeared to be independent of the level of activation when \(F_o\) was maintained above 90\% of maximal \(F_o\) (\(F_o\textsubscript{max}\)), either by an increase of [Ca\textsuperscript{2+}]\textsubscript{o} (to 3.0 mM) or paired pulse stimulation, after spontaneous sarcomere motion had been suppressed by procaine. These procedures counteracted the effects of temperature on isometric force development. \(V_o\) increased with increasing temperature, which the parameter a, calculated from force-velocity relations measured at 20°, 25°, and 30° C, decreased with increasing temperature. The Arrhenius plot of \(V_o\) was studied in detail over a wider temperature range.

![Graphs showing force-velocity relation at different temperatures.](http://circres.ahajournals.org/)  

**Figure 7.** Effect of temperature on force-sarcomere velocity relation. Force-sarcomere velocity relations were determined at 20° C (panel A), 25° C (panel B), and 30° C (panel C), as described in text. Force is normalized to peak value found at 25° C. Lines shown are fitted through data points by Hill equation as described in text for forces below 70\% of isometric twitch force. Dotted line is drawn by eye. Bathing medium contained 0.5 mM procaine at all three temperatures. [Ca\textsuperscript{2+}]\textsubscript{o} was 1.5 mM at 20° and 25° C and 3.0 mM at 30° C. Average fit parameters of five to eight trabeculae are shown in Table 2. Note that 1) data points at zero force were obtained by direct measurement of \(V_o\) and 2) data points at zero velocity (\(F_o\)) are measured under sarcomere length clamp conditions (Figure 2A).

### Table 2. Effect of Temperature on Force-Sarcomere Velocity Relation in Presence of 0.5 mM Procaine

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>a (%)</th>
<th>b (µm/sec)</th>
<th>(V_o) (µm/sec)</th>
<th>(F_o) (%)</th>
<th>(V_o/V_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>15.1±4.24</td>
<td>-0.3±0.32</td>
<td>6.13±0.24</td>
<td>109±5.6</td>
<td>1.88±0.16</td>
</tr>
<tr>
<td>25</td>
<td>9.1±1.21</td>
<td>-0.8±0.31</td>
<td>12.68±0.52</td>
<td>100</td>
<td>2.05±0.11</td>
</tr>
<tr>
<td>30</td>
<td>5.3±0.41</td>
<td>-1.0±0.54</td>
<td>23.44±0.97</td>
<td>84±3.2</td>
<td>2.74±0.18</td>
</tr>
</tbody>
</table>

Results of five to eight trabeculae in presence of 0.5 mM procaine at all temperatures. Data are expressed as mean±SEM. \(n=26–97\); \(R=0.868–0.991\). Force-sarcomere velocity relation was measured as described in text and fitted to Hill equation (see “Materials and Methods”), which resulted in parameters a and b. Velocity of sarcomere shortening (\(V_o\)) was measured directly. Note that peak twitch force development (\(F_o\)) differed from the load calculated from Hill equation for a velocity of shortening equal to zero, as a result of nonhyperbolic behaviour of data at \(F>70\%\) \(F_o\) (see Figure 7). Parameter \(V_o/V_{10}\) is the ratio of \(V_o\) and velocity at 10\% force level, which is a measure of relative curvature of force-velocity relation at low loads. [Ca\textsuperscript{2+}]\textsubscript{o} was 1.5 mM (20°, 25° C), 3 mM (30° C). Solutions contained 0.5 mM procaine at all temperatures. \(F_o\) was normalized to value found at 25° C. 1.5 mM [Ca\textsuperscript{2+}]\textsubscript{o}. One-way analysis of variance of Hill-fit parameters, normalized for value at 25° C, revealed the following significances for effect of temperature: parameter a, \(p=0.20\); parameter b, \(p=0.18\; \(V_o\), \(p=0.0002\).
FIGURE 8. Arrhenius plot of length of sarcomere shortening ($V_0$) at varied and fixed $[Ca^{2+}]_0$. Isometric twitch force and $V_0$ were measured as described in text. Panels A and B: $[Ca^{2+}]_0=1.5 \text{mM}$ at all temperatures, no procaine added, five trabecula. Panel A shows regression line, fitted through data $>25^\circ \text{C}$ (open circles). Slope revealed an activation energy of $71.4\pm6.8 \text{kJ/mol}$, equivalent to a $Q_{10}$ of 2.6 (95% confidence limit, 2.2–3.2). Force-temperature relation in panel B was fitted by a third-order polynomial. Panels C and D: Bathing medium contained 0.5 mM procaine at all temperatures. $[Ca^{2+}]_0$ was 1.5 mM at temperatures $\leq 25^\circ \text{C}$; $[Ca^{2+}]_0$ was 3.0 mM at temperatures above $25^\circ \text{C}$. Panel C shows regression line fitted through data above $25^\circ \text{C}$ of six muscles (open circles). Activation energy derived from slope of this relation was $88.2\pm7.9 \text{kJ/mol}$ ($r=0.952$), equivalent to a $Q_{10}$ of 3.3 (95% confidence limit, 3.0–3.7). Panel D shows force-temperature relation of five muscles, fitted to a second-order polynomial.

(15°–35° C) and in smaller temperature increments. The relation was linear between 18° and 33° C; the observed $Q_{10}$ for $V_0$ was 4.6. A $Q_{10}$ of 4.6 for $V_0$ is consistent with the reported temperature dependence of rat cardiac actin-activated myosin ATPase, which suggests that the same reaction step limits the activity of the enzyme in vitro and during shortening of the cardiac sarcomeres at zero external load.

**Measurement of Sarcomere Length and Velocity of Sarcomere Shortening**

Before evaluating the effects of varied temperature on $V_0$, we will consider potential artifacts involved in the assessment of sarcomere length and $V_0$, particularly on the maximal $V_0$. Potential artifacts that are associated with the use of laser diffraction techniques for measurement of sarcomere length are caused by 1) Bragg angle reflection, 2) muscle inhomogeneity, and 3) translation of the specimen during shortening.

A population of sarcomeres that is oriented under an angle with respect to the longitudinal axis of a muscle will generate a diffraction pattern with asymmetric first orders. The asymmetry will manifest itself as differences in intensity and position of the first orders if the population is tilted in the plane of the incident laser beam. These so-called Bragg angle reflections will cause preferential sampling of the tilted population by the corresponding diffractometer. The sampled population could potentially change during the twitch, which would give rise to erroneous sarcomere length readings. Inhomogeneity of the muscle preparation will also result in a change of the sampled population, in this case due to translation of the muscle with respect to a stationary laser beam. Our measurement of sarcomere length and $V_0$ with two systems, which simultaneously sampled the two first-order lines of the diffraction pattern, is new and revealed that the error due to asymmetry of the diffraction pattern was small (less than 4%; see Figure 4).

The effect of nonuniformity of sarcomere shortening along the muscle also could be excluded as a significant cause of an artifact in the measurement of the $V_0$. $V_0$ measured along the muscle was found to
be independent of location, except at positions very close to the valve where lateral translation was largest (see Figure 5). Apparently, slight lateral translation in a homogeneous muscle brought only a new population of functionally identical sarcomeres into the field of observation, as has been observed previously.14

Measurement of $V_0$

For comparison of the temperature dependence of $V_0$ with the temperature dependence of actomyosin ATPase in vitro, it was essential to eliminate any load on the actomyosin complex during shortening. The total load of the muscle evidently depends on the sum of the external load and the internal load. The results obtained here show that the external load to the contractile system could be made zero with the isovelocity release technique (see Figure 3D).21 The internal load, during shortening, depends on inertia, elasticity, and viscosity of the muscle. The inertial term is probably small during shortening at constant velocity. The elastic force, exerted on the sarcomeres, depends on sarcomere length and is illustrated in Figure 3. If no force was exerted on the unstimulated trabeculae, the sarcomeres were slack at a length of 1.85 to 1.9 µm, as has been described before.14 It is evident that stretch induced a small parallel elastic force (less than 2% of $F_{c,max}$), provided that sarcomere length was less than 2.1 µm. After a twitch, sarcomeres returned spontaneously to slack length. This suggests the presence of restoring forces within the trabecula at lengths shorter than 1.9 µm. Apparently, the restoring force and the parallel elastic forces balance at slack length, and the sarcomeres in the unstimulated muscle are completely unloaded.

To minimize the elastic term, we have measured $V_0$ while ensuring that sarcomere length was at, or slightly above, slack length, that is, under conditions in which elastic forces were negligible (Figure 3).

We have also estimated the contribution of viscosity of the muscle by measuring the force induced in an unstimulated muscle during stretches between sarcomere length 1.9 and 2.0 µm at a velocity equal to $V_0$.27 The viscous force that resisted shortening in the passive muscle was less than 1.5% under all conditions, and approximately equaled the parallel elastic force at the sarcomere length at which we studied $V_0$. Although we do not know whether the structures that give rise to the elastic and viscous properties of the relaxed muscle change with activation, we feel that measurement of $V_0$ at a sarcomere length of 1.9 µm ensures that the contractile system is unloaded.

$V_0$ depends on the level of activation of force development both in cardiac muscle21,27,28 (Figure 8) and in skeletal muscle (for review see Podolin and Ford34). This evidently may confound the interpretation of the effects of temperature variation as shown in this study, because both the level of activation and $V_0$ appeared to change in response to a temperature change. In previous studies, we have observed that $V_0$ increases with increasing $[Ca^{2+}]_0$, up to a maximum and then saturates.21,27

The mechanism of the effect of activation on $V_0$ is not exactly known, although several mechanisms have been considered.35 This study was not aimed at the analysis of the underlying mechanisms; therefore, we have chosen to use varied $[Ca^{2+}]_0$, or paired pulse stimulation to allow maximal force development and maximal $V_0$ at all temperatures studied.

Effect of Temperature on Force-$[Ca^{2+}]_0$ Relation

The prominent effect of temperature variation on isometric force development at constant $[Ca^{2+}]_0$ (Figure 3) observed in this study is in agreement with previous observations.3,6,7 Changes in temperature apparently cause a shift in sensitivity to $[Ca^{2+}]_0$. In the presence of procaine at saturating calcium concentrations, $F_0$ is only slightly temperature dependent. These results are consistent with findings in skinned skeletal36 and cardiac37 muscle preparations. The shift in $[Ca^{2+}]_0$ sensitivity upon an increase in temperature could be due to 1) a
shift in calcium sensitivity of the contractile proteins or 2) a change of the cytosolic calcium transient. The latter would be affected by the magnitude of calcium influx into the cell, the amount of calcium released from the sarcoplasmic reticulum, the rate of calcium reuptake into the sarcoplasmic reticulum, and the rate of calcium extrusion from the cell through the sarcolemma.

In skinned cardiac fibers, an increase in calcium sensitivity has been found when temperature is increased from 20° to 25° C.37,38 This shift in sensitivity is opposite to the shift observed in the present study. Furthermore, calcium influx via the slow inward current39 and calcium release by the sarcoplasmic reticulum40 have been shown to increase with increasing temperature.

Hence, the decrease in sensitivity to [Ca++], of intact cardiac muscle with increasing temperature is probably due to a dominant effect of acceleration of the calcium reuptake by the sarcoplasmic reticulum,41 and to accelerated calcium extrusion via the sarcolemmal Na+-Ca++ exchanger.32,43

Lowering of the temperature consistently induced spontaneous sarcomere motion. This observation is in agreement with previous reports on isolated rat myocytes and papillary muscle39 and in rat cardiac trabeculae,44,45 and can be explained by the induction of spontaneous calcium release from the sarcoplasmic reticulum.46 This would account for diminished twitch force at 20° C, since the amount of releasable calcium would be lower during the electrically stimulated twitches. In addition, nonuniformity of the preparation could result from this process since some active sarcomeres are stretching nonactive sarcomeres. Procaine increased the sensitivity of twitch force development to [Ca++], at 20° C (p=0.012; see Figure 3 legend) and probably at 25° C (p=0.07), but not at 30° C. This increased sensitivity was most likely the result of suppression of spontaneous activity in these preparations, which is consistent with the reported ability of procaine to inhibit calcium release from the sarcoplasmic reticulum.31–33 Both positive47 and negative48 inotropic effects of procaine on cardiac muscle have been reported. It is likely that these differences may have been due to differences in experimental conditions, such as extracellular calcium concentration and temperature.

The combined effects of an increase in the apparent [Ca++], sensitivity of twitch force development with decreased temperature in cardiac muscle, together with an increased tendency to spontaneous uncoordinated calcium release,29,30,44–46 may explain why twitch force measured while temperature is varied (but at constant [Ca++],) reveals a maximum at 25° C (see Bennett4 for review).

Effect of Temperature on Force-Sarcomere Velocity Relation

Temperature has marked effects on the force-velocity relation, as shown in Figure 4. The effects are most pronounced with regard to the maximum velocity of sarcomere shortening, and to a lesser degree with regard to the parameter a, as has been reported previously both in skeletal4,5 and cardiac muscle.6–8 No attempt has been made in previous studies on cardiac muscle to keep the level of activation constant. In our studies, partial activation of the contractile proteins at high temperatures resulted in a diminished maximum V0. This effect has also been described by Edman et al.,7 who recalculated his force-velocity relations, assuming that the Hill parameters would not change at the different temperatures, to obtain the “true” effect of temperature on V0. Parameter a varied significantly with temperature in the range from 20° to 30° C, and, therefore, we do not feel that it is justified to assume that a is constant. Because of the variability of a, we will not attempt to interpret the relation between temperature and this variable.

Parameter b showed no temperature dependence over the range of temperatures studied. Even so, the value of this parameter is questionable since the forces and velocities measured at high loads obviously cannot be described adequately by the Hill relation. For production of a better fit to the measured data points, the addition of a linear component to the Hill equation at the higher load levels has been proposed.19 However, the force-velocity relations presented in this study showed considerable deviation at high loads and could not be fitted to such a function. Further studies of the effect of temperature on the force-velocity relation with measurements of sarcomere velocity, both at forces above isometric twitch force (stretch velocities) and velocities at forces below zero load, are needed to describe the complete force-velocity relation of cardiac muscle. Forces below zero can be achieved with the method of Edman,50 in which the passive force, stored in the parallel elastic element, is employed to impose a compressive (i.e., negative) force on the cross-bridges during shortening.

Effect of Temperature on V0

To our knowledge, this is the first study of the temperature dependence of the cardiac force-velocity relation in which the effects of temperature on excitation-contraction coupling have been eliminated. This conclusion is based on the observation that maximal postextrasystolic potentiation resulted in only a slight (less than 10%) increase of force development at temperatures up to 32° C, above the force during paired pulse stimulation. A similar level of force development was obtained by raising [Ca++], to 3 mM, while a further increase of [Ca++], increased neither twitch force nor V0. In contrast, twitch force could still be increased by maximal postextrasystolic potentiation by about 20% above the force during paired pulse stimulation at temperatures above 32° C. Similarly, at temperatures below 17° C, force was submaximal under all conditions. Hence, data from experiments at a temperature below 17° C were not included in the calculation of the Q10 of V0.
(Figure 9). Omission of the data obtained at temperatures above 32°C did not affect the calculated $Q_{10}$. The observation that both increase of $[Ca^{2+}]_o$ and paired pulse stimulation increased $V_o$ to a maximal value at temperatures above 25°C and at $[Ca^{2+}]_o=1.5$ mM is consistent with the hypothesis that $V_o$ is a function of the level of activation of the contractile filaments by $Ca^{2+}$ ions. In fact, paired pulse stimulation appeared to ensure near maximal twitch force development and maximal $V_o$ over a substantial temperature range ($17°$–$32°$ C). Therefore, the $Q_{10}$ of 4.6 obtained with paired pulse stimulation probably correctly reflects the temperature dependence of $V_o$ in rat myocardium.

As we have pointed out above, a small but finite contribution of viscosity of the muscle is expected to impose an internal load on the contractile apparatus. This load would be velocity dependent and would decrease with increasing temperature. Correction for such a viscous load, based on our measurements in passive trabeculae, would slightly increase the $Q_{10}$ of truly unloaded shortening velocity (to 4.9).

The $Q_{10}$ value of 4.6 found in this study in the temperature range of $17°$–$35°$ C (paired pulse stimulation) is higher than the values reported previously for both cardiac6–8 and skeletal muscle.4,5 This difference deserves further comment. From the review by Woledge et al,5 it is clear that the $Q_{10}$ of $V_o$ depends on muscle type and species. Two aspects of the analysis of temperature effects in studies on mammalian cardiac muscle probably have resulted in the underestimation of $V_o$ at higher temperatures and, thus, have led to reduction of the calculated $Q_{10}$ value.6–8 1) In our study, extrapolation of the force-velocity relation to $V_o$ led to lower values of $V_o$ than the actual measured $V_o$. 2) We also found that it was necessary to maintain force development at nearly maximal level, despite variations of temperature, to ensure that $V_o$ was maximal and independent of the level of activation. Failure to ensure maximum activation levels led to a lower $Q_{10}$ (Figure 9).

The temperature dependence of actin-activated myosin ATPase activity has been determined to be $Q_{10}=4$ for skeletal1 and $Q_{10}=5$ for rat cardiac muscle.10 The latter value corresponds with the $Q_{10}$ value (4.6) of $V_o$ in this study and is nearly identical to the $Q_{10}$ of the maximal $V_o$ after correction for the viscous internal load (see above). This close correspondence suggests that the rate-limiting steps of the actomyosin interaction in vitro and during shortening at zero load are identical. However, further experiments in which the temperature dependence of actomyosin ATPase activity and $V_o$ is measured in the same hearts, perhaps with varied isomyosin composition and in different species, are needed to confirm this conclusion.

Acknowledgments

We thank Dr. Lincoln Ford for valuable comments during the preparation of the manuscript. We are grateful to Drs. Christine Lamont and Naomi Anderson for reading the manuscript.

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**Key Words** • cardiac muscle • force-velocity relation • temperature • sarcomere length • laser diffraction techniques
Force and velocity of sarcomere shortening in trabeculae from rat heart. Effects of temperature.

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doi: 10.1161/01.RES.66.5.1239

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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