TEMPERATURE DEPENDENCE OF MAMMALIAN MUSCLE CONTRACTIONS AND ATPASE ACTIVITIES

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ABSTRACT Isolated rat and mouse extensor digitorum longus (EDL) and soleus muscles were studied under isometric and isotonic conditions at temperatures from ~8°–38°C. The rate constant for the exponential rise of tension during an isometric tetanus had a Q_{10} of ~2.5 for all muscles (corresponding to an enthalpy of activation, $\Delta H\ddagger = 66$ kJ/mol, if the rate was determined by a single chemical reaction). The half-contraction time, contraction time, and maximum rate of rise for tension in an isometric twitch and the maximum shortening velocity in an isotonic contraction all had a similar temperature dependence (i.e., $\Delta H\ddagger \sim 66$ kJ/mol). The Mg^{++} ATPase rates of myofibrils prepared from rat EDL and soleus muscles had a steeper temperature dependence ($\Delta H\ddagger = 130$ kJ/mol), but absolute rates at 20°C were lower than the rate of rise of tension. This suggests that the Mg^{++} ATPase cycle rate is not limiting for force generation. A substantial fraction of cross-bridges may exist in a resting state that converts to the force-producing state at a rate faster than required to complete the cycle and repopulate the resting state. The temperature dependence for the rate constant of the exponential decay of tension during an isometric twitch or short tetanus (and the half-fall time of a twitch) had a break point at ~20°C, with apparent enthalpy values of $\Delta H\ddagger = 117$ kJ/mol below 20°C and $\Delta H\ddagger = 70$ kJ/mol above 20°C. The break point and the values of $\Delta H\ddagger$ at high and low temperatures agree closely with published values for the $\Delta H\ddagger$ of the sarcoplasmic reticulum (SR) Ca^{++} ATPase. Thus, the temperature dependence for the relaxation rate of a twitch or a short tetanus is consistent with that for the reabsorption rate of Ca^{++} into the SR.

INTRODUCTION

The biochemical processes that limit and control muscular contraction and relaxation in vivo remain uncertain despite many years of study. In principle, any of the steps from the generation of a conducted action potential to the observed tension recorded with a transducer attached to a muscle tendon could limit the rate of rise of tension. These processes include (*a*) the release of Ca^{++} from the sarcoplasmic reticulum (Endo, 1977), (*b*) the diffusion and binding of Ca^{++} to troponin (Johnson et al., 1979, 1980), which is commonly thought to (*c*) shift the tropomyosin molecule (Squire, 1981) so that (*d*) the myosin heads can bind to actin (Huxley et al., 1981). Finally, (*e*) force generated by active elements within the muscle must be transmitted to the tendon and may be subject to viscoelastic lags (Hill, 1949).

From studies using the Ca^{++}-sensitive photoprotein aequorin injected into barnacle muscle fibres (Ashley and Ridgway, 1970) or frog muscle fibres (Blinks et al., 1978), we find that the release of Ca^{++} from the sarcoplasmic reticulum (SR) appears to be a rapid process. The maximum concentration of free Ca^{++} considerably precedes the maximum force (F) and is more nearly correlated with the maximum rate of rise of force (dF/dt). However, these measurements of free Ca^{++} do not necessarily reflect the concentration of Ca^{++} bound to troponin, which facilitates the interaction between actin and myosin via tropomyosin. If steps (*b*) or (*c*) above were rate limiting, initial development of force should be much slower than the redevelopment of force after breaking bonds during a tetanus, because the maximum amount of Ca^{++} would be bound to troponin during a tetanus, and the tropomyosin molecules would already have shifted. There is evidence that the initial development of force is much slower under conditions where Ca^{++} release is limited by the use of D_2O (Cecchi et al., 1981) or in skinned fibres with less than optimal Ca^{++} concentrations, but not under normal conditions (Griffiths et al., 1979).

Viscoelastic lags (*e* above) are also unlikely to be rate limiting inasmuch as recent studies with time-resolved x-ray diffraction techniques (Matsubara and Yagi, 1978; Huxley et al., 1981) have found no noticeable delays between cross-bridge attachment and force measured at the tendon in frog muscles. The formation of cross-bridges stiffens the muscle against small, rapid displacements (Huxley and Simmons, 1971; Ford et al. 1977, 1981) and the small-amplitude stiffness may even lag behind force production in mammalian muscles (Stein and Parmiggiani, 1979; Parmiggiani and Stein, 1981).

Thus, some process associated with myosin binding to

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actin is most likely to be rate limiting for force production. There is a correlation in a wide variety of skeletal muscles between the maximum shortening velocity and the rate of the actomyosin ATPase (Bárány, 1967; Bárány and Close, 1971). A correlation is also found between shortening velocity under isotonic conditions and the time to peak force (contraction time) during an isometric twitch in muscles whose contractile properties have been changed by altering the nervous innervation (Close, 1969). Although changes in contractile speed are correlated with changes in rate of myosin ATPase, transformation of biochemical properties does not always parallel the transformation of physiological parameters of contraction (Sréter et al., 1975). Muscles with quite different twitch contraction times may also have the same shortening velocity (Luff, 1981). Of course, contraction times will depend on relaxation rates as well as contraction rates.

Attempts have also been made to relate force generation to biochemical events in glycerinated fibres at low temperatures (e.g., Cooke and Bialek, 1979). However, rates are depressed in glycerinated fibres (Arata et al., 1977) and are much slower at low temperatures. In this paper, we have attempted to determine the rate-limiting steps in muscular contraction by studying the temperature dependence of various parameters of force generation in mammalian muscles and comparing them with the reaction rates of actomyosin ATPase of myofibrils isolated from the same muscles. Absolute rates of cross-bridge cycling determined biochemically were compared with the rates of physiological processes. Differences were observed both in terms of absolute rates and the temperature dependence of these rates, but these differences can be resolved if force production is limited by a different step in the actomyosin cycle than that which determines the overall cycle rate.

Decay of force is often interpreted in terms of the reabsorption of Ca^{++} by the SR (e.g., Sandow, 1965), but other possibilities have been suggested (see Discussion). To obtain further evidence about the limiting process, the temperature dependence of the decay rate for twitch and tetanic tension has been compared with the temperature dependence of the Ca^{++} ATPase isolated from the SR (Inesi et al. 1973). The close correspondence between the temperature dependence of tension decay and Ca^{++} reabsorption is consistent with Ca^{++} uptake by the SR being the rate-limiting step in muscle relaxation.

METHODS

Mice (strain C57) and rats (Sprague-Dawley) were anesthetized with Nembutal (30 mg/kg), and their soleus and extensor digitorum longus (EDL) muscles were dissected with their nerves. In vitro, the muscles were stimulated indirectly via the nerve, and the electrical activity (EMG) of the muscles was recorded with fine silver wires (75 μm) inserted in the belly of the muscles or around the tendon. Indirect stimulation was used and EMG recorded to determine if the muscle was continuously activated during repetitive stimulation. This check was particularly important at low temperatures where excitation and conduction of the muscle action potential becomes tenuous.

Force was measured using either a Devices Ltd. (Hertfordshire, Great Briton) UF1 strain gauge or the force output of a Cambridge Technology Inc. (Cambridge, MA) dual-mode servo system. The servo system could be switched from isometric to isotonic conditions to measure the velocity of shortening against various, constant loads. The twitch contraction to a single stimulus (Figure 1*A*) was often averaged on-line using a PDP 11-34 computer (Digital Equipment Corp., Marlboro, MA). All data were stored directly on computer disks and various parameters of the contractions were measured from the stored records as shown in Fig. 1:

(*a*) the latent period (*L*) from the time of stimulation to the onset of contraction;

(*b*) the peak force of the twitch with respect to the resting level;

(*c*) the maximum rate of rise of tension during a twitch (Fig. 1*B*);

(*d*) the half-contraction time (HCT) from the onset of contraction to a force level half that of the peak;

(*e*) the contraction time (CT) from the onset of the contraction to the peak force level;

(*f*) the half-fall time (HFT) from the peak force to a level half of the peak on the falling phase of the twitch;

(*g*) the decay rate constant obtained by fitting an exponential to a segment of the decay of the tetanus (Fig. 1*C*) or a twitch (Fig. 2.)

The rising phase of the tetanus (as well as the falling phase) could be well fitted by an exponential, as shown in Fig. 1*C*. The curve is of the form

$$y = \alpha + \beta e^{-\gamma t} \quad (1)$$

where α is the asymptotic level reached during a long tetanus, β is the magnitude of the exponential curve fitted, and γ is the rate constant of the

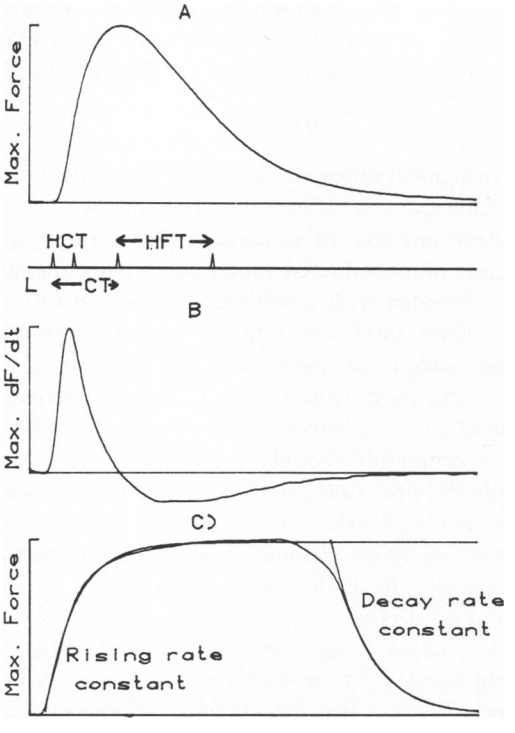

FIGURE 1 The following parameters were computed automatically from averaged records: *A*, of a twitch: peak force, latency (*L*), half-contraction time (HCT), contraction time (CT), half-fall time (HFT); *B*, of a differentiated twitch: maximum rate of rise of force; and *C*, of a tetanus: maximum (max.) tetanic force, rising rate constant and decay rate constant. The representative data shown are from a rat EDL muscle at 25°C. No scales are shown since the data are merely to illustrate the Methods, but the time scale in *C* is twice that in *A* and *B*.

exponential. A nonlinear curve-fitting algorithm was used (Hartley, 1961) that minimized the mean-square error of the data points from the fitted curve. This algorithm was implemented on the PDP 11-34 by Mr. Ted Milner, a graduate student at the University of Alberta, and could be applied to any selected portion of the data.

The timing of stimuli was controlled by digital circuitry, and at any preselected time, the Cambridge Technology servo could be switched from isometric to isotonic mode with a preset force level. Straight lines were fitted to the approximately linear, initial length changes to determine the shortening velocity using standard least-mean-squares regression techniques. The maximum shortening velocity is the value obtained when the force is suddenly changed to the resting value during a tetanus.

Myofibrils were isolated as described by Perry and Grey (1956). Twenty muscles of each type were homogenized at one time using full speed in a Sorvall Inc. (Newtown, CT) Omnimixer for 5 s. The homogenate was centrifuged for 10 min at 600 *g* and the supernatant discarded. The pelleted material was homogenized again for 2 min and then centrifuged for 10 min at 600 *g*. The upper fluffy layer was gently resuspended in borate-KCl buffer and homogenized using a Teflon-glass homogenizer. This was then centrifuged for 10 min at 600 *g*. The fluffy, whitish upper layer was resuspended and washed with buffer again 3–4 times. The purity of the preparation was monitored by phase-contrast microscopy and electron microscopy. The soleus myofibril preparation contained significantly more membranous material than the EDL, which had predominantly single myofibrils or bundles of a few (<5) myofibrils. These preparations were used within 24 h. After 4 d, the ATPase activity fell to half that initially seen. Muscles that had been glycerinated at 4°C overnight showed no change in activity, but glycerination at −20°C resulted in significantly different values. The experiments described here were with fresh myofibrils from unglycerinated muscles or muscles glycerinated above 0°C. Protein concentration was determined by the method of Lowry et al. (1951).

The Mg^{++} ATPase activity of the myofibrils was determined by rapidly adding a suspension of myofibrils (normally 4–8 mg/ml) to a rapidly stirring ATP-buffer solution in a water-jacketed, temperature-controlled cell. Aliquots were removed at specific times and quenched in 11.5% trichloroacetic acid and then analyzed by the method of Taussky and Schorr (1957) for orthophosphate. The ATP-buffer solution contained 50 mM PIPES, 0.1 M KCl, 5 mM ATP, 10 mM $MgCl_2$ and 5×10^{-5} M $CaCl_2$, pH 7. At high temperatures, aliquots were collected at 5 s intervals, and only the initial rate was used for analysis. Between 30 and 60 s after initiation of the reaction at high temperature with EDL myofibrils, there was an abrupt change in ATPase rate and an apparent coagulation of the reaction solution, presumably due to supercontraction. Myofibrillar ATPase rates are expressed as turnover rates using a molecular weight for myosin of 500,000. It was assumed that both heads are independently active and that 50% of the myofibrillar protein is myosin (Marston and Taylor, 1980).

RESULTS

Fig. 2*A* shows the response of a mouse muscle to one, two, and twenty impulses at intervals of 16 ms. The same data are displayed in *B* after a logarithmic transform of the force values. Note that there is an approximately linear decay of force (on a logarithmic scale) for all traces with similar slopes. The fitted straight lines in Fig. 2*B* correspond to the fitted exponentials in Fig. 2*A* and the similarity in slopes implies that the exponential rate constants are similar (between 18 and 20 s^{-1}). Thus, at least for the short trains used here (but not for longer trains which produce fatigue; Edwards et al., 1975), the force decays exponentially (after falling to a force value one-half

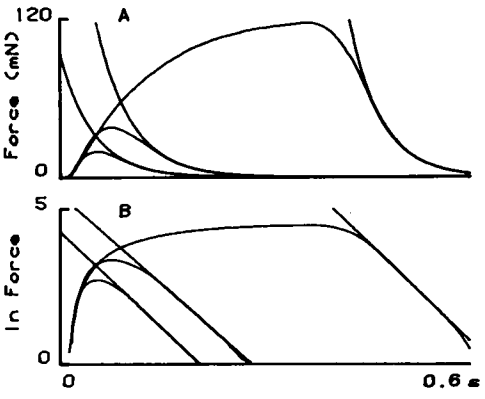

FIGURE 2 Force (*A*) and the natural logarithm of the force (*B*) generated by a mouse soleus muscle (26°C) in response to 1, 2, and 20 maximal stimuli at intervals of 16 ms, starting 10 ms after the beginning of the trace. The decay after the tension has fallen to between half and two-thirds of its maximal value has been fitted by straight lines on the semilogarithmic plots of *B* which correspond to the exponentials in *A*. Note that the rates of decay are similar for varying numbers of stimuli.

to two-thirds of the peak) with a rate constant which is independent of the number of stimuli.

Length

The rate constant of decay does depend, however, on muscle length, as shown in Fig. 3. As the muscle is lengthened out, the decay rate slows appreciably, both for a

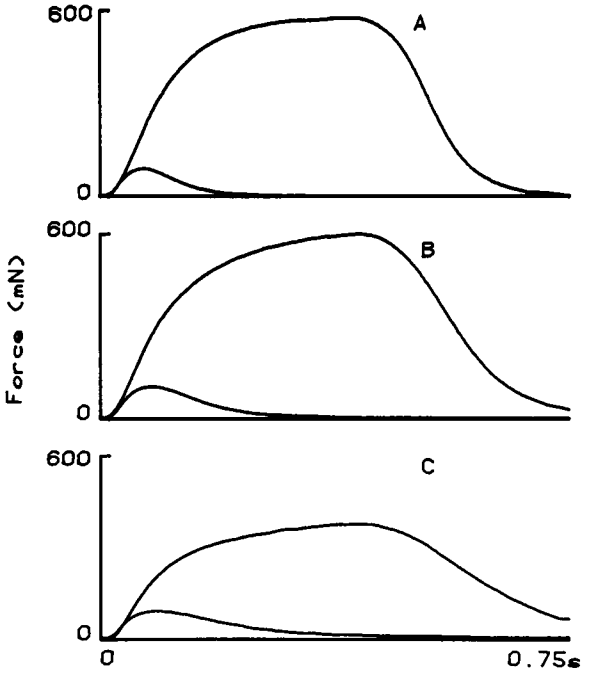

FIGURE 3 Twitches and tetani for a mouse soleus muscle held isometrically at (*A*) L_0 − 1 mm, (*B*) L_0, and (*C*) L_0 + 1 mm, where L_0 is the muscle length at which the twitch has its maximum value. Note that the decay rate slows down as the length is increased. Twenty stimuli were applied in each tetanus at an interval of 20 ms beginning 10 ms after the start of the trace. Temperature was 21°C.

twitch and a short tetanus. Length was measured relative to the length (L_0) at which a twitch produced the most force. This length could be determined readily without a risk of fatiguing the fast twitch muscles appreciably. Fig. 4*A* shows that both the twitch and tetanic force levels vary in the manner expected from the overlap between thick and thin filaments (Gordon et al. 1966), although the largest twitch occurs at longer lengths than the largest tetanus (Rack and Westbury, 1969). Fig. 4*B* shows the linear decline in the exponential rate constant for decay of tetanic force (□) with length over about a fourfold range. In contrast, the rate constant for the rising phase of the tetanus (computed as described in the Methods) hardly changed with length. The description of muscle contraction in terms of two rate constants that have a different dependence on length is consistent with previous work (Mannard and Stein, 1973; Bawa et al., 1976). All the subsequent measurements from whole muscles were made after adjusting the muscle to its optimal length for force generation during a twitch.

Temperature

Fig. 5 shows the large variation in the time course of twitch and tetanic contractions that results from 10°C changes in temperature (note the difference in time scales). The small muscles used equilibrated rapidly to a change in temperature of the bath and the values indicated on the figure were measured with a small thermistor (Yellow Springs Instrument Co., Yellow Springs, OH) placed near the muscle.

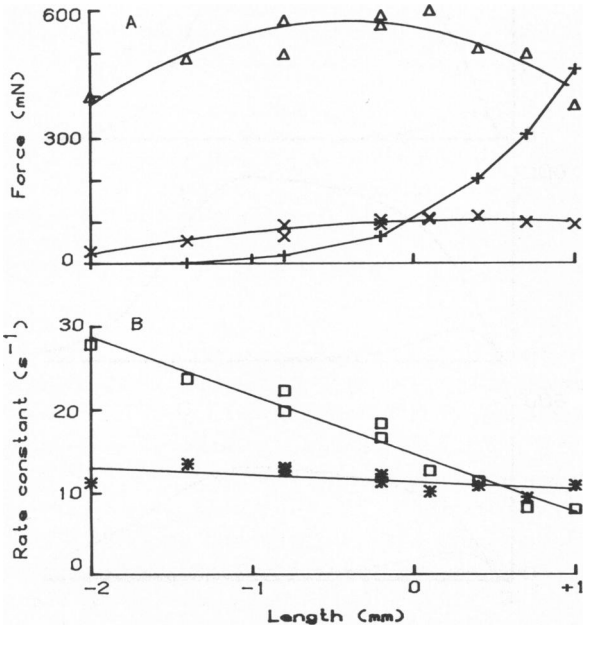

FIGURE 4 (*A*) Maximum tension during a tetanus (Δ) and a twitch (×) and the resting tension in the absence of stimulation (+) for the mouse soleus muscle shown in Fig. 3. (*B*) The decay rate constant (□) decreases markedly with length, whereas the rising rate constant (*) is little affected. Lengths are measured relative to the length at which the twitch was maximum.

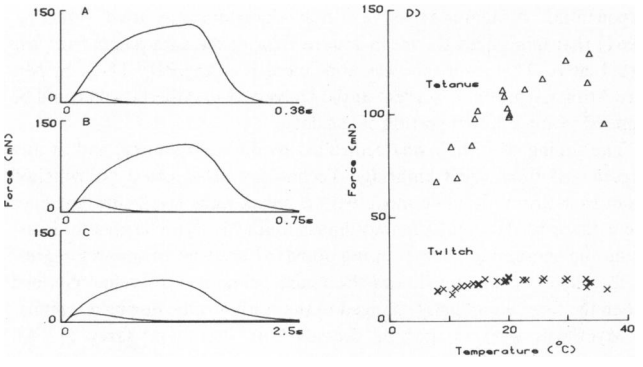

FIGURE 5 Twitch and tetanic tensions produced by maximal stimulation of a mouse soleus muscle at (*A*) 32°C, (*B*) 22°C, and (*C*) 12°C. For the tetanus, twenty stimuli were applied at intervals of (*A*) 10, (*B*) 18, and (*C*) 50 ms beginning (*A*) 10, (*B*) 20, and (*C*) 30 ms after the onset of the trace. The interval in each case was ~60% of the contraction time for the twitch. Note that as the muscle was cooled, the falling phase lengthened out more than the rising phase of the contractions. (*D*) The tetanic tension (Δ) increased steadily with increasing temperature up to 30°C, while the twitch tension (×) was relatively constant.

Note that the rising and falling phases of the twitch and tetanic contractions are not equally affected by the change in temperature (Hartree and Hill, 1921), so that the shape of the waveform changes. The tetanus is not well maintained at the lowest temperature because of a progressive block in muscle excitation during the period of stimulation, as measured by EMG electrodes (see Methods). Thus, the twitch is more constant down to very low temperatures, but it eventually blocks (Fig. 5*D*). At high temperatures, the twitch and tetanus decline as well, and above 40°C some of the decline is irreversible. The limiting factor is presumably the diffusion of O_2 for oxidative metabolism, because the muscles were isolated from their blood supply and maintained in a bath into which a mixture of 95% O_2 and 5% CO_2 was bubbled. Above 40°C, some denaturation of the muscle proteins can occur, which would account for the irreversible decay. Typically in these experiments, temperature was varied from ~8° to 38°C to minimize irreversible changes.

The rate constants for the rise of force during a tetanus are plotted in Fig. 6*A* for a wide range of temperatures. The data were often well fitted by a single straight line (on this semilogarithmic plot) and the slope corresponds to a 2.6-fold change for 10°C variation in temperature (Q_{10} = 2.6). For comparison, the rate constants for the exponential decay of force for 1, 2, 3, and 20 stimuli are shown in Fig. 6*D*. The values are similar for varying numbers of stimuli at any one temperature (see also Fig. 2) and cluster around two straight lines that intersect at a break point near 20°C. The Q_{10} for the fitted line is 6.3 for $T \leq 20$°C and 2.3 for $T \geq 20$°C. Thus, particularly at low temperatures, the decay phase changes much more rapidly than the rising phase of contraction, which accounts for the change in shape seen in Fig. 5.

Fig. 6 also shows the variation with temperature of the

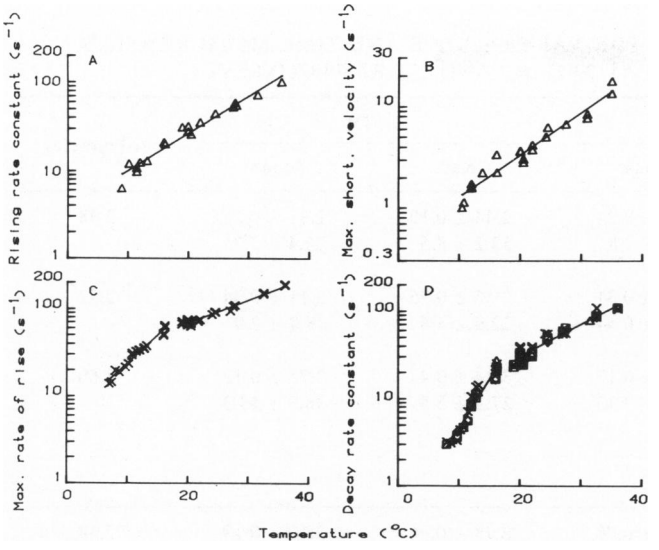

FIGURE 6 Rate constants were studied over a range of temperatures from 8°–38°C. The rate constant for the exponential rate of rise of tension of a rat EDL muscle during an isometric tetanus (*A*) and the maximum shortening (max. short.) velocity during an unloaded contraction (*B*) had similar slopes (on a semilogarithmic plot) and little or no curvature. The maximum (max.) rate of rise of tension during a twitch (*C*) also had a similar slope, but showed a break point near 20°C. The decay rate constants (*D*) for one (×), two (□), three (◇) or twenty (△) stimuli were similar and had a much sharper break at 20°C and a steeper slope below this temperature. Further details in the text.

maximum shortening velocity under isotonic conditions (Fig. 6*B*) and the maximum rate of rise in force under isometric conditions (Fig. 6*C*). The maximum shortening velocity in millimeters per second has been divided by the whole muscle length to give a value in muscle lengths per second, or simply in reciprocal seconds. The data for the rising rate constant and for the maximum shortening velocity generally showed little or no curvature. Furthermore, the slopes and Q_{10} values were always close, which suggests that these parameters of isometric and isotonic contractions are limited by the same process (see Discussion).

The maximum rate of rise for the twitch (Fig. 6*C*) was divided by the maximum force generated in each twitch to give a value with the dimensions of reciprocal seconds. The maximum rate of rise shows some degree of curvature, but not as much as the falling rate constant (Fig. 6*D*). Division by tetanic force, rather than twitch force would have given lower absolute rate constants, and a slightly different temperature dependence, because twitch and tetanic tensions vary with temperature in a different way (Fig. 5*D*). Thus, the shape of the curve in Fig. 6*D* must be interpreted with some caution.

Fig. 7 gives the variation with temperature of the half-contraction time (HCT), contraction time (CT), and half-fall time (HFT). These parameters are defined in Fig. 1 of the Methods. The slope is greater for the HFT than for the parameters of the rise in force during the twitch.

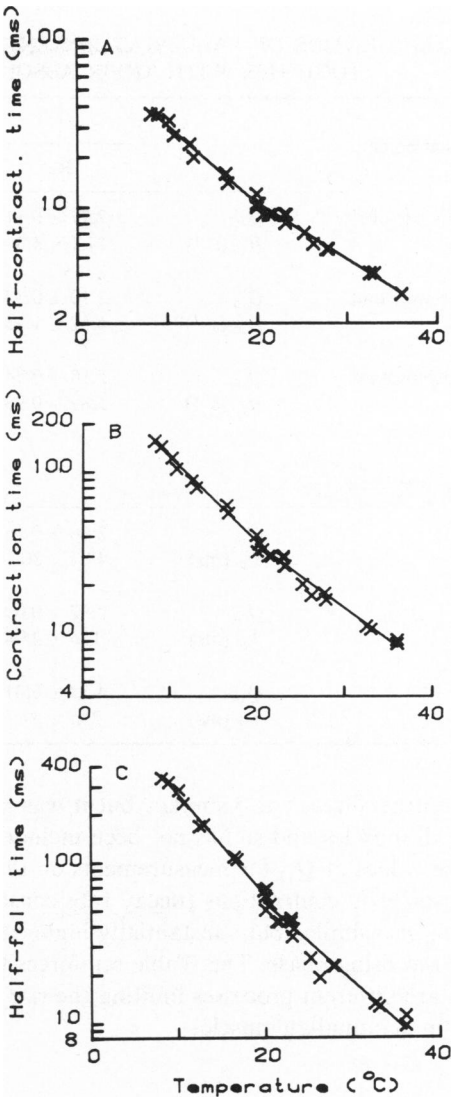

FIGURE 7 The times to reach half (*A*) and full (*B*) contractile force during a twitch, as well as the time for the force to fall to half its maximal value (*C*) have been plotted as a function of temperature on a semilogarithmic scale for a rat EDL muscle, as in Fig. 6. Note the steeper slope (and greater range) of values for the HFT than the parameters of the rising phase.

Table I summarizes the values of Q_{10} obtained for all measurements of slow (soleus) and fast (EDL) rat and mouse muscles. Each entry gives the mean and standard deviations of measurements over the entire temperature range for four muscles. The values of Q_{10} for the pure slow muscle (rat soleus) tend to be greater than those for the pure fast muscle (mouse EDL), but the changes are small compared with the large changes in rates (R_{20}) or times (T_{20}) measured at 20°C, which vary by a factor of 4–10. The averages for all 16 muscles are given on the right and the Q_{10} for all measurements on the rising phase of isometric contractions (maximum rate of rise, rising rate constant, half-rise time and contraction time) are similar. The Q_{10} for the maximum shortening velocity during an

TABLE I

AVERAGE RATIOS OF VALUES AT 10°C INTERVALS (Q_{10}) FOR VARIOUS RATE AND TIME MEASUREMENTS, TOGETHER WITH THEIR ABSOLUTE VALUES AT 20°C (R_{20} AND T_{20}, RESPECTIVELY)

Rate measurements		Soleus		EDL		All muscles
		Rat	Mouse	Rat	Mouse	
Maximum rate of rise	Q_{10}	2.77 ± 0.11	2.40 ± 0.24	2.44 ± 0.19	2.31 ± 0.25	2.48
	R_{20} (s^{-1})	17.1 ± 4.3	32.1 ± 3.8	53.2 ± 8.5	72.4 ± 7.3	
Rising rate constant	Q_{10}	3.10 ± 0.33	2.53 ± 0.31	2.35 ± 0.26	2.11 ± 0.34	2.52
	R_{20} (s^{-1})	4.52 ± 1.25	5.38 ± 0.54	22.9 ± 5.6	18.7 ± 2.9	
Decay rate constant	Q_{10}	5.10 ± 0.74	3.30 ± 0.17	3.24 ± 0.47	2.75 ± 0.12	3.60
	R_{20} (s^{-1})	3.85 ± 0.93	8.49 ± 2.13	27.2 ± 5.5	38.3 ± 13.3	
Time measurements						
HCT	Q_{10}	2.66 ± 0.16	2.27 ± 0.20	2.48 ± 0.26	2.10 ± 0.19	2.38
	T_{20} (ms)	47.4 ± 30.1	21.7 ± 4.7	13.4 ± 1.8	8.37 ± 1.30	
CT	Q_{10}	2.92 ± 0.09	2.55 ± 0.14	2.70 ± 0.21	2.33 ± 0.14	2.63
	T_{20} (ms)	126 ± 25.4	77.6 ± 10.1	40.8 ± 2.9	28.2 ± 1.7	
HFT	Q_{10}	4.33 ± 0.41	3.22 ± 0.06	3.29 ± 0.51	2.81 ± 0.09	3.41
	T_{20} (ms)	235 ± 51	118 ± 24	54.3 ± 6.9	38.2 ± 8.7	

isotonic contraction was also similar, but it was not measured for all muscles and so has not been included in the Table. The values of Q_{10} for measurements on the falling phase of isometric contractions (decay rate constant and HFT) were also similar but substantially higher than the values for the rising phase. This Table reinforces the view that there are different processes limiting the rise and fall of tension in mammalian muscles.

Activation Enthalpy

Alternatively, the rate constants can be displayed in an Arrhenius plot (Fig. 8) vs. inverse temperature (in degrees Kelvin). 20°C corresponds to 3.4×10^{-3} per degree Kelvin. The advantage of the Arrhenius plot is that the slope can be converted into an apparent activation enthalpy ($\Delta H\ddagger$) for the processes determining these rate constants, which can then be compared with similar measurements on biochemical reactions that can be studied in vitro. The slopes in Fig. 8 for the rising rate constants correspond to $\Delta H\ddagger$ = 71 kJ/mol, and for the decay constant to $\Delta H\ddagger$ = 126 kJ/mol for $T \leq 20$°C and $\Delta H\ddagger$ = 63 kJ/mol for $T \geq$ 20°C for the rat EDL muscle. The slopes are somewhat steeper and the values of $\Delta H\ddagger$ somewhat greater for the rat soleus muscle. Average values are given in Table II. The same trends are evident as in Table I. The values of maximum shortening velocity are only given for rat EDL muscles, because this is the only category in which a full set of measurements was available on all four muscles. Values for the decay rate constant are given separately for temperatures above and below 20°C. It is only for $T \leq 20$°C that the apparent enthalpies of activation are strikingly greater.

Isolated myofibrils

The active release of phosphate was measured from isolated rat myofibrils (see Methods). At higher temperatures, there was a noticeable decrease in the activity after 25 s (Fig. 9*A*). This correlated with a change in the appearance of the myofibrils and may represent the onset of supercontraction (Marston and Taylor, 1980). The reaction rates measured here are derived from the linear

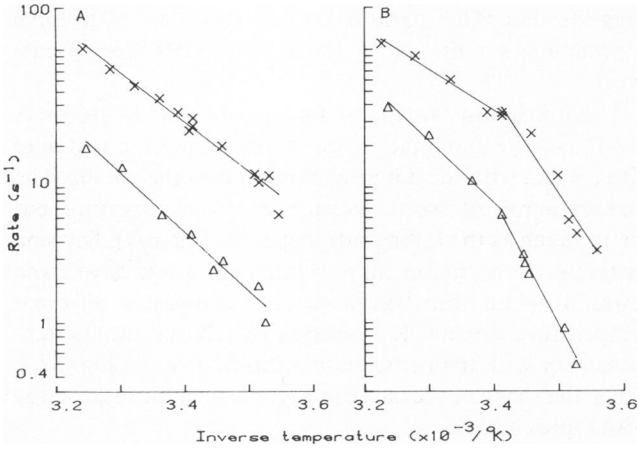

FIGURE 8 Arrhenius plots of rate constants for the exponential rise (*A*) and fall (*B*) of tension (on a logarithmic scale) as a function of inverse temperature (in degrees Kelvin) for a fast EDL (×) and a slow soleus (Δ) muscle in the rat. The decay rates at each temperature were the average of values measured from 1, 2, 3, or 20 stimuli (cf., Fig. 6*D*).

TABLE II

APPARENT ENTHALPY OF ACTIVATION (kJ/mol) FOR REACTIONS LIMITING VARIOUS PARAMETERS OF MUSCULAR CONTRACTION

Rate measurement	Soleus		EDL		All muscles
	Rat	Mouse	Rat	Mouse	
Maximum rate of rise all points	73 ± 3	62 ± 8	65 ± 6	60 ± 8	65
Rising rate constant all points	81 ± 8	66 ± 8	61 ± 10	55 ± 8	66
Maximum shortening velocity all points	—	—	69 ± 13	—	69
Decay rate constant					
≥20°C	83 ± 13	70 ± 7	64 ± 7	62 ± 5	70
≤20°C	155 ± 16	111 ± 16	114 ± 12	90 ± 8	117

All values are means of measurements plus or minus standard deviation for four individual muscles, except for the final column which is the average of all muscles studied. The standard errors of the mean would be half the standard deviation in each case.

rate of release of phosphate prior to the decrease. The rate of phosphate release is shown in Fig. 9*B* as a function of temperature in the form of Arrhenius plots for EDL and soleus muscles. At 20°C, the EDL and soleus myofibrillar ATPase rates were 3.0 s^{-1} and 0.7 s^{-1} per myosin head.

Over the measurable temperature range (1°–27°C), the Arrhenius plots of the myofibrillar ATPase activity were linear with apparent activation energies near 125 kJ/mol (Fig. 9*B*). The activity of the soleus was consistently less than EDL by a factor of 4 over the entire temperature range. We did not observe a nonlinear Arrhenius plot such as reported by Bárány (1967) for fresh myofibrils. A linear Arrhenius plot would not be obtained if the activity at high temperatures was derived from data obtained following the decrease in activity after 25 s mentioned above. (A nonlinear relation was also observed in one experiment in which soleus and EDL myofibrils were prepared from muscles that had been stored at −20°C in 50% glycerol for 2 wk. In this experiment, the curves were linear and parallel for EDL and soleus above 10°C, with an activation enthalpy of 84 kJ/mol. For EDL there was a noticeable break at 10°C, with an activation enthalpy of ~167 kJ/mol below 10°C).

DISCUSSION

One of the striking results of this study is the similarity in the temperature dependence for the contractile properties of fast and slow muscles from different species (rat and mouse). Although the absolute rates or times at 20°C differed by a factor of 4–10 from the slowest (rat soleus) to the fastest (mouse EDL) muscles, the Q_{10}'s generally varied only a few tenths of one unit and the apparent activation enthalpies by a few kilojoules per mole (see also Ranatunga, 1980 and his references to earlier work on the temperature dependence of mammalian muscle). A striking similarity is seen in the temperature dependence of all the parameters measured during the initial phases of isometric and isotonic contractions (Tables I and II; maximum rate of rise, rising rate constant, HCT, CT, maximum shortening velocity). The parameters measured later in the contraction are also similar to each other (decay rate constant, HFT) but differed from the earlier measurements. The actual values agree closely with those measured many years ago by Hartree and Hill (1921) for frog muscle. They found a Q_{10} = 2.5 for the maximum rate of rise and Q_{10} = 3.6 for the maximum rate of fall of tension. Hill (1938, 1951) found a somewhat lower value (Q_{10} = 2) for the maximum shortening velocity during an isotonic contraction, but more recently Edman (1979) reported a Q_{10} = 2.7 over the range 2°–12°C. Hill did not report a break point in the temperature dependence, but with frog muscles, the highest temperatures studied were 20° and 25°C, which would probably not be adequate to observe the break point near 20°C.

These data imply that there are two different processes that separately limit the rate of rise and the rate of fall in force. Furthermore, these same processes probably operate in a wide range of vertebrate muscles. We will now discuss their possible basis in light of the detailed biochemical data that are available on the reactions underlying muscular contractions.

Decay of Tension

As with the rise of tension (see Introduction) a number of steps could in principle limit the fall of tension: (*a*) the binding affinity of Ca^{++} for troponin, (*b*) the rate of reuptake of Ca^{++} into the SR, (*c*) the rate of rotation and/or breakage of bonds between actin and myosin, (*d*) viscoelastic delays.

Several authors have correlated the rate of uptake of Ca^{++} into isolated sarcoplasmic reticulum with the SR Ca^{++} ATPase activity. For example, Inesi et al. (1973) found activation energies of 28 and 17 kcal/mol (117 and 71 kJ/mol) below and above a break point of 20°C. The break point may be a property of the protein itself (Dean and Tanford, 1978) and of its interaction with its lipid environment (Dean and Suárez, 1981). Whatever the

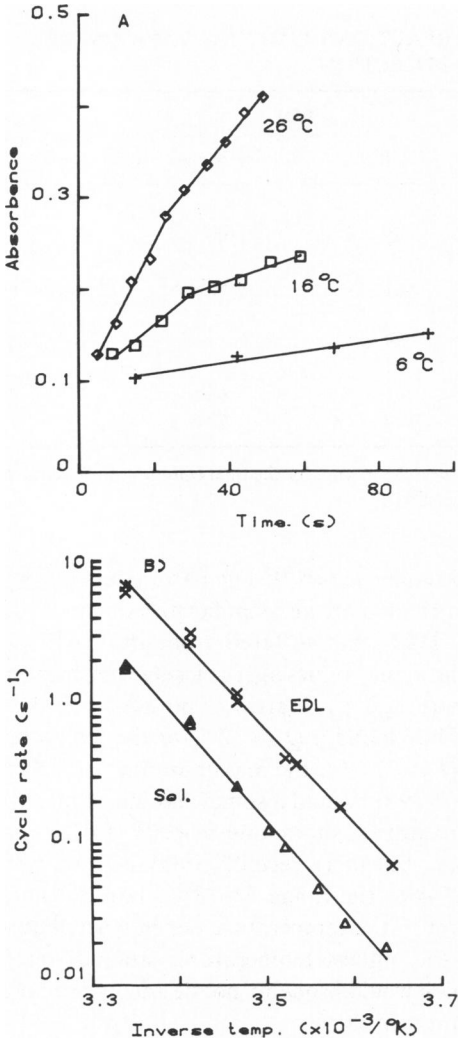

FIGURE 9 (*A*) The phosphate released (measured as increased absorbance) from myofibrils prepared from rat EDL increases at a steady rate for a period of time, but then the rate decreases (the decrease is not seen at low temperatures). The myofibril assay mixture consisted of ATP (3.5 mM), $MgCl_2$ (7.1 mM), $CaCl_2$ (3.5×10^{-5} M), PIPES buffer (pH 7; 36 mM), KCl (0.071 M), EDL myofibrillar protein (1.77 mg/ml). Aliquots containing 0.35 mg (26°C), 0.53 mg (15.5°C), and 0.89 mg (6°C) protein were used for assay of P_i. One absorbance unit represents 0.53 μmol of phosphate. The temperatures shown are 26°C (◇), 16°C (□) and 6°C (+). (*B*) The initial rates of phosphate release from rat EDL (×) and soleus (△) muscles are plotted as a function of inverse temperature in an Arrhenius plot. Data from two different preparations are superimposed.

molecular mechanism involved, the values agree remarkably well with the apparent enthalpies of activation measured here for the rate constant of tension decay (117 kJ/mol below the break point at 20°C and 70 kJ/mol above 20°C). This is consistent with the view held by Sandow for many years (Sandow, 1965) that relaxation is limited by the uptake of Ca^{++} into the SR (possibility *b* above). In fact, in a paper published shortly before Sandow's death, Sandow and Zeeman (1979) reported data similar to ours for relaxation rates, but over a much more limited range of conditions.

The correspondence in the temperature dependence is a necessary, but not a sufficient condition for assuming that relaxation is limited by the SR Ca^{++} ATPase, and several problems have been raised. For example, the rapid fall of free Ca^{++} levels in muscle has been taken as evidence against the SR Ca^{++} ATPase limiting the rate of relaxation (Gordon and Ridgway, 1978). However, Ca^{++} levels will inevitably fall rapidly as the ions bind to troponin and other Ca^{++} binding proteins (Robertson et al., 1981; Somlyo et al., 1981). There should also be a slow phase for decay of free Ca^{++}, in parallel with a reduction in tension, as Ca^{++} is released from troponin, and Ashley and Lignon (1981) recently reported such a slow phase in barnacle muscle. This slow phase will depend on the product of the reuptake rate of Ca^{++} into the SR and the equilibrium constant for Ca^{++} binding to troponin. To the extent that the equilibrium constant is relatively independent of temperature over the range of temperatures studied, the temperature dependence of the fall in tension will be that of Ca^{++} uptake into the SR.

One must also explain why the relaxation rate is less at long muscle lengths in frog muscle (Hartree and Hill, 1921; Edman and Kiessling, 1971), cat muscle (Rack and Westbury, 1969; Bawa et al., 1976), human muscle (Bawa and Stein, 1976), and rat and mouse muscles (See Figs. 3 and 4). In contrast the Ca^{++} ATPase of the SR appears to be independent of length (Applegate and Homsher, 1981). However, as stated above, the slow phase of free Ca^{++} decay (and tension decay) will depend as well on the equilibrium constant of Ca^{++} binding to troponin. Muscle length has been shown to influence the number of Ca^{++} binding sites (Fuchs, 1978), although the equilibrium constant may be greatly affected (Fuchs, 1977).

From his studies on the length and caffeine dependence of relaxation in frog muscle, Pagala (1980) suggested that the actomyosin ATPase might also play a role in determining the relaxation rate (possibility *c* above). Edwards et al. (1975) found a close correspondence between the fall in ATP levels and the slowing of relaxation during fatigue of mouse muscles, suggesting that the actomyosin ATPase was rate limiting for relaxation. However, with the more complete data possible from ^{31}P NMR studies of whole muscles, Dawson et al. (1980) concluded that the SR Ca^{++} ATPase and not the actomyosin ATPase determined the relaxation rate during fatigue.

Finally, it has been suggested that the exponential decay of tension is difficult to interpret because some cross-bridges are contracting and others lengthening. Internally, the muscle is no longer in an isometric state (Huxley and Simmons, 1970; Edman and Flitney, 1977). However, while the time to the onset of the exponential phase can be altered simply by changing the mechanical recording conditions (Huxley and Simmons, 1972), the rate constant for the exponential decay is quite independent of large length and load changes (Jewell and Wilkie, 1960). Thus, the smooth exponential decay observed for example in

Figs. 1 and 2 probably reflects the overall decline of free Ca^{++} levels, rather than the internal viscoelastic complexities of cross-bridge interaction in the slightly nonisometric conditions that will prevail.

On balance, the data available from this study and others support the view that the reabsorption rate of Ca^{++} into the SR determines the temperature dependence of relaxation in mammalian skeletal muscle, although changes in the equilibrium constant for Ca^{++} binding to troponin can effect the absolute rates.

Rise of Tension

The contrast to the close agreement of physiological and biochemical measurements on the rate of fall of tension, a clear difference was observed between the rate of rise of tension and actomyosin ATPase rates. The Arrhenius plot of the myofibrillar ATPase activity was linear over the temperature range from 1°–27°C. EDL activity was consistently higher than soleus by a factor of ~4, but the activation enthalpies for the two muscles were similar (near 125 kJ/mol). Experiments at 0.1 M KCl, pH 7.9, with rabbit acto–S-1 (actin plus myosin subfragment 1) gave an activation enthalpy of 138 kJ/mol from the maximum cycle rates for acto–S-1 at various temperatures (Shriver, unpublished observations). This compares favorably with the activation enthalpy determined by Barouch and Moos (1971), and Moos (1972).

In all the physiological measurements on the early phases of contraction, whether under isometric or isotonic conditions, apparent activation enthalpies of 65 to 70 kJ/mol were observed, which is significantly less than the activation enthalpy of the myofibrillar ATPase. There are also significant differences between the absolute rates. For example, the rise of tension in an isometric contraction is extremely well fitted by a single exponential (see Fig. 1*C*) with rate constants (at 20°C) of 23 and 4.5 s^{-1} for rat EDL and soleus muscles respectively. The rates of release of phosphate from ATP per myosin head are significantly less at 3 and 0.7 s^{-1} (20°C) for EDL and soleus respectively. Low actomyosin ATPase rates have been observed in many biochemical studies (e.g., Lymn and Taylor, 1971; Arata et al., 1977; Travers and Hillaire, 1979).

Our results appear to be roughly consistent with the correlation demonstrated by Bárány (1967) between actomyosin ATPase rate and maximal velocity of shortening. Bárány (1967) recognized a discrepancy between the temperature dependence of muscle contraction velocity and actin-activated myosin ATPase, and similarly found a larger temperature dependence of the ATPase rate than that of the muscle shortening speed. However, at low ionic strength, the myofibrillar ATPase activity had a lower temperature dependence and compared favorably with the temperature dependence of maximal velocity of shortening. The experiments at low ionic strength were felt to reflect more accurately the actin-activated ATPase activity, because affinity of actin and myosin are increased under conditions of low ionic strength and might resemble that found in vivo.

The temperature dependence of actomyosin ATPase activity at inifinite actin concentration gives $\Delta H\ddagger \sim 30$ kcal/mol (125 kJ/mol), both at high and low ionic strength (Barouch and Moos, 1971; Moos, 1972). This provides rather convincing evidence that even under conditions of maximal actin association, the activation enthalpy for actomyosin ATPase is significantly higher than that for the speed of shortening. Thus, we feel that the $\Delta H\ddagger$ determined for the myofibrillar ATPase in 0.1 M KCl at pH 7 (i.e., under conditions closely resembling the physiological situation) is an accurate representation of the enthalpy of activation of the ATPase in the muscle fibres used for our physiological experiments (especially under conditions of zero load contraction).

The discrepancies between activation enthalpies and the absolute rates determined for the ATPase reaction and force production can be resolved if even the simplest possible model of a cyclic process is considered (cf., Huxley, 1957). The binding of actin to myosin leading to force production must proceed at a relatively high rate (f in Huxley's [1957] notation), but with low activation enthalpy to account for the force production (Fig. 10). If the return reaction (g in Huxley's notation) proceeds with a lower rate, it will determine the overall cycle time and the enthalpy of activation measured from the maximum actin activated ATPase rate or myofibrillar ATPase rate (V_{max}) in vitro. Furthermore, the measurements of force production and phosphate splitting permit f and g to be determined as a function of temperature. Because of the different enthalpies of activation, the difference in rate between f and g decreases with increasing temperature. Extrapolating to body temperature (37°C) the rate of f and g become approximately equal (Stein, unpublished calculations). At the other extreme, Homsher et al. (1981) found that much of the phosphate splitting in frog muscle

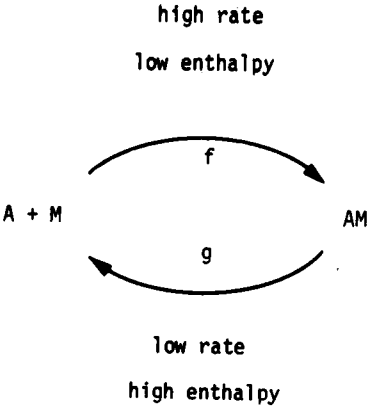

FIGURE 10 The discrepancies between the temperature dependence of force generation and phosphate release can be resolved using even the simplest model of acto-myosin interaction (Huxley, 1957), if the two rate constants f and g have different rates and activation enthalpies (further discussion in the text).

at 0°C occurred in the second after completion of the force and enthalpy production in a 3 s tetanus.

Huxley's (1957) model is obviously an oversimplification and more realistic models containing three or more states should be considered (Huxley and Simmons, 1971; Cooke and Bialek, 1979; Eisenberg and Hill, 1978; Eisenberg et al., 1980). However, the points we wish to make here are that the apparent discrepancy between rates of force generation and ATP splitting can be easily resolved with even a very simple model and that these discrepancies are only marked at lower than physiological temperatures for mammalian muscles. More detailed biochemical measurements can be used to further localize the reactions that determine f and g. In their study on four types of vertebrate muscle, Marston and Taylor (1980) concluded that the association and dissociation of cross-bridges are fast processes and that the release of products from actomyosin is the slowest step of the cycle. Furthermore, the temperature dependence for the release rate of ADP from myosin (Bagshaw and Trentham, 1974) is the same as the activation enthalpy measured here for the rate g (125 kJ/mol or more). Thus, the increase of force appears to be limited by a step between the association of actin with an activated myosin (M** ADP.P in the notation of Lymn and Taylor, 1971) and the release of products. The cycling of cross-bridges proceeds at a slower rate with a different enthalpy of activation which appears to be limited by the release of products.

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