Transient Phases of the Isometric Tetanus in Frog's Striated Muscle

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ABSTRACT   In an isometric tetanus in frog's sartorius muscle tension approaches the plateau exponentially with rate constant α. α depends on sarcomere length, s, and temperature, T, according to the Arrhenius equation

$$\alpha(s, T) = A(s) \exp\left[- \frac{E}{R}\left(\frac{1}{T} - 3.4 \times 10^{-3}\right)\right]$$

for temperatures between 1 and 20°C and for sarcomere lengths 2.0–2.8 μm. The energy of activation, E, does not vary significantly with s; $E = 13.9 \pm 2.4$ kcal/mole. $A(s)$ decreases monotonically with s; $A(2.1~\mu$m$)$ is about three times greater than $A(2.8~\mu$m$)$. Late in relaxation active tension approaches zero exponentially with rate constant r. r decreases exponentially with increasing duration of tetanus, D, from r_0 in a twitch to r_∞ for large D. The rate constant for decrease of r with D increases with s and with T. r_0 and r_∞ obey the Arrhenius equation and decrease with increasing s.

INTRODUCTION

The task of interpreting the behavior of a muscle fiber in terms of mechanical and chemical processes within the fiber remains uncompleted. The work of Jewell and Wilkie (1958) has demonstrated the inadequacy of the classical model of A. V. Hill (1938) for predicting the time course of tension in an isometric tetanus. The model developed by A. F. Huxley (1957) and recently extended by Julian (1969) describes some isometric and isotonic phenomena satisfactorily. However, this model only describes a fiber in which all sarcomeres have the same length and in which the number of cross-bridges in the region of overlap between thin and thick filaments remains constant throughout activity. The model should be extended to describe fibers in which sarcomeres near the ends are shorter than those near the center (Huxley and Peachey, 1961) and in which the extent of overlap changes during activity.

In order to develop these extensions we have made an experimental and theoretical study of the rise and fall of tension in an isometric tetanus. At an early stage in the inquiry we noted that the latter part of the rise of active tension, P, to the plateau value, P_o, was quite accurately fitted by an exponential:

$$\dot{P} = \alpha(P_o - P)$$

$$\dot{P} \equiv \frac{dP}{dt}.$$

During relaxation the fall in active tension is also exponential when P is near zero (Jewell and Wilkie, 1960):

$$\dot{P} = -rP.$$

We have investigated the dependence of the rate constants, α and r, on sarcomere length and on temperature. In this report we present data on α and r from isometric tetani of frog's sartorius muscle at various lengths, L, and temperatures, T. In a subsequent report[1] we present an extended Huxley-Julian model which allows partial interpretation of the experimental data.

METHODS

Biological Material and Dissection

A grass frog (*Rana pipiens*) was killed by decapitation but not pithed. The legs were cut from the body above the pelvic bone, skinned, mounted fully extended for dissection, and kept moist with cold curare glucose (CG) Ringer (see Solution). Length of the sartorius muscle *in situ*, L_o, was taken as the distance from the center of the pelvic bone to a loop of braided silk thread tied around the tibial tendon 0.2–0.3 mm distal to the tip of the muscle. An S-hook bent from 22 or 24 guage platinum wire was tied against this loop.

After dissection the muscle was placed in fresh CG Ringer and left at L_o and room temperature for 1 hr, then mounted in the chamber or left at 4°C. All experiments were begun within 6 hr of the end of dissection. All muscles used showed no parasites and few, if any, damaged fibers.

Stimulation

The muscle was mounted in a Lucite chamber, suspended between the pelvic bone and the S-hook, and lying against a multielectrode array which consisted of three platinum wires of one polarity alternating with two of the other. The train of stimulus pulses, generated with Tektronix Type 162 waveform generators, was coupled to the

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[1] Mittenthal, J. E., and F. D. Carlson. 1971. A theoretical analysis of the rise of tension in an isometric tetanus in frog's striated muscle. *J. Theor. Biol.* In press.

electrode array through a stimulus driver which fed a transformer. The pulses were square waves of 0.5 msec width. The polarity of pulses was constant during a tetanus but varied among tetani. At the end of experiments no muscle showed damage at the positions where it had touched the electrodes.

Pulses were approximately 1.5 times supramaximal. The frequency of pulses during a tetanus exceeded the fusion frequency. Preliminary experiments showed that the following frequencies always resulted in a fused tetanus: 20 sec^{-1} at 1 or 2°C; 40 sec^{-1} at 7 or 10°C; 63 sec^{-1} at 13°C; 100 sec^{-1} at 19 or 20°C. At each temperature every muscle was tetanized at the corresponding frequency. The fusion frequency for individual muscles was not ascertained in the experiments reported here, nor was the dependence of α or r on frequency investigated.

The duration of stimulation, D, was made sufficient to yield a well-defined plateau of tension, during which tension increased negligibly. At lengths above L_o the tension continued to increase slowly for a time after the plateau would have occurred at

TABLE I

DURATION OF STIMULUS AS A
FUNCTION OF LENGTH AND TEMPERATURE OF
MUSCLE; DURATION IN SECONDS

Temperature (°C)	1	7	13	19
Length				
(mm)				
$\leq L_o$	1.3	0.8	0.4	0.32
$L_o + 3$	2.5	1.0	0.63	0.5
$L_o + 6$	3.2	1.6	0.8	0.8

$L \leq L_o$; correspondingly, $D(L)$ was larger for $L > L_o$ than for $L \leq L_o$. The dependence of D on L and T is shown in Table I.

Control and Measurement of Temperature

Coolant circulating in the walls of the chamber kept the temperature of the muscle constant to within 0.4°C during a period at 1°C and to within 0.1°C for temperatures above 10°C. Temperature of the muscle was measured with a thermistor probe in a bridge circuit with 0.1°C precision just before each tetanus. The probe touched the muscle during measurement of temperature but not during the tetanus.

Control and Measurement of Length of Muscle

The tibial hook was attached to an 11.5 cm 20 gauge stainless steel tube which connected the muscle to an isotonic lever of the kind used by Jewell and Wilkie (1958). The lever and tube had an effective mass of 0.4 g, as measured by the force transducer. The lever had stiffness approximately 2×10^7 dyne/cm (calculated). Carlson, Hardy, and Wilkie (1963) found that a sartorius muscle from $R.$ $pipiens$, of size comparable to ours, had resting stiffness 1.6×10^6 dyne/cm.

The lever moved between stops and was pivoted on ball bearings. A Collins DC

linear motion transducer (model SS103) flexibly linked to the lever near its fulcrum provided a record of transient changes in L. The output from this transducer was recorded with a chart recorder (Hewlett-Packard model 320).

The weight of the lever and tube always loaded the muscle. During tetani at $L < L_o$ the muscle shortened actively against this load from L_o to L; during relaxation the load reextended the muscle to L_o. In tetani at $L \geq L_o$ the muscle remained at L throughout the tetanus.

Measurement of Sarcomere Length

At the end of each experiment the muscle was removed from the chamber and mounted in a Petri dish in CG Ringer. The beam of a laser (Spectra-Physics [Mountain View, Calif.] model 132) passed through the central part of an edge of the muscle to produce a diffraction pattern on graph paper 20 cm above the muscle. The distance between first-order lines in the pattern was measured and used to compute the sarcomere length, s, when the muscle was at five lengths ($L = L_o$ to $L_o + 12$ mm, intervals of 3 mm). The data were plotted on a graph of s vs. L, and a straight line was drawn by eye through the points and the origin. Data of Sandberg and Carlson (1966) confirm that a graph of s vs. L is a linear, to a good approximation.

Characteristically sarcomeres in fibers on the lateral edge of the muscle were longer than those on the medial edge; the ratio of lateral to medial sarcomere length was 1.07 ± 0.015 (mean $\pm$ sd; four muscles). Hence the s corresponding to a given L was read from the line having a slope midway between the slopes for medial and lateral fibers on the plot of s vs. L.

The mean sarcomere length corresponding to L_o was 2.33 ± 0.08 μm (six muscles).

Measurement of Force

A clamp made from $\frac{1}{16}$ inch stainless steel rod connected the pelvic bone in the chamber to the lower surface of the free end of a cantilever outside it. A Collins DC linear motion transducer (model SS203) measured displacement of the clamp. The cantilever, with clamp and transducer core attached, had resonant frequency 625 Hz and stiffness 1.4×10^8 dyne/cm. The sensitivity of the transducer was 0.6 mv/g. The output of the transducer was low-pass filtered twice (both roll-off frequencies 1 kHz) and amplified 300–600 times. The signal was recorded on a chart recorder (Hewlett-Packard 320). The signal and its negative were also input to the multiplexer of a PDP8 computer through 100 ft of Belden 8641 cable and added after inverting the negative signal. The resulting signal was sampled by an analogue-to-digital converter and the sample values were stored on magnetic tape. The frequency of sampling depended on the temperature of the muscle; 50 sec^{-1} or 100 sec^{-1} for 2°C, 100 sec^{-1} for 6–11°C, 200 sec^{-1} for 11–20°C.

In order to test the fidelity of the recording systems we used a low-pass filtered square wave as input to the amplifier. A least squares fit of an exponential to data recorded with the PDP8 or with the chart recorder gave slopes within the nominal accuracy of the filter rate constant for rate constants in the range 1 sec^{-1} to 50 sec^{-1}, a range including all data on α recorded in these experiments. By the same test the PDP8 gave accurate records to 100 sec^{-1} but the recorder did not; the PDP8 was used

to record the isometric twitches for which r is reported. The coefficients of variation (standard deviation:mean) for all test slopes obtained from PDP8 and chart recorder data were less than 0.2 and 1 %, respectively.

Solution

Curare glucose Ringer solution, used in all experiments, contained the following concentrations of solutes (mM): NaCl, 114; KCl, 2.5; CaCl$_2$, 1.8; NaH$_2$PO$_4$, 1.44; Na$_2$HPO$_4$, 3.58; glucose, 5.6; tubocurarine chloride (Squibb), 5 mg/liter. The pH was 7.1.

Solution in the reservoir above the chamber was bubbled vigorously with 100 % O$_2$. In part of one experiment N$_2$ replaced the O$_2$ for several tetani at 20°C. In tetani at sarcomere length 2.01 μm, α and r were the same in N$_2$ as in O$_2$. At 2.42 μm, α was the same in either gas, but r increased slightly in N$_2$ over its value in O$_2$.

RESULTS

Calculation of α and r

Fig. 1 shows the time course of force, $P(t)$, in isometric tetani at L_o − 6 mm, L_o, and L_o + 6 mm at four temperatures. Solid symbols indicate the interval used to evaluate α in each tetanus. Data in and around that interval are plotted as $\log(P_o - P(t_k))$ vs. t_k in Fig. 2 for the tetani of Fig. 1. The rate constant α, for each tetanus was first estimated as the slope of a straight line drawn by eye through the longest linear portion (solid symbols, Fig. 2) of the latter plot. t_k is the time at which the kth sample was taken; the value of this sample was $P(t_k)$. P_o was estimated by averaging at least five points near the largest value of P occurring in the tetanus.

The estimate of P_o obtained by this method was sometimes less than the true plateau value because of fatigue during the tetanus. (In all experiments analyzed, the decline of P from its peak value during the period of stimulation was slight.) Hence all differences $y_k = P_o - P(t_k)$ computed with the estimated P_o were smaller than the correct differences when fatigue occurred. This source of error caused points to fall below the straight line passing through points on the plot of $\log y_k$ vs. t_k only at small y_k.

In general 20 or more points lying on or near this line were used to determine it. At 20°C the sampling frequency was so low (200 sec^{-1}) that occasionally as few as eight points were used.

In Fig. 2 a period in which the rate of decrease of $\log y(t)$ is greater than α precedes the interval over which $y(t)$ declines exponentially with rate constant α. This was the case in all tetani. Exponential rise of P to P_o with slope α always occurred over at least the last 7% of the rise from $P = 0$ to P_o, and at 20°C the exponential portion was greater than 20% of P_o.

The straight lines drawn by eye through the linear regions of plots such as those in Fig. 2 were used as initial estimates in a least squares fit of an exponential to the data for $y(t)$ in these regions, using the method of Deming

(1943). The ratio of α obtained in the fit to the initial estimate of α was 1.004 ± 0.031; $N = 59$ (mean $\pm$ SD; $N =$ number of data). Since this ratio differs negligibly from one, the initial estimates were used for further calculations.

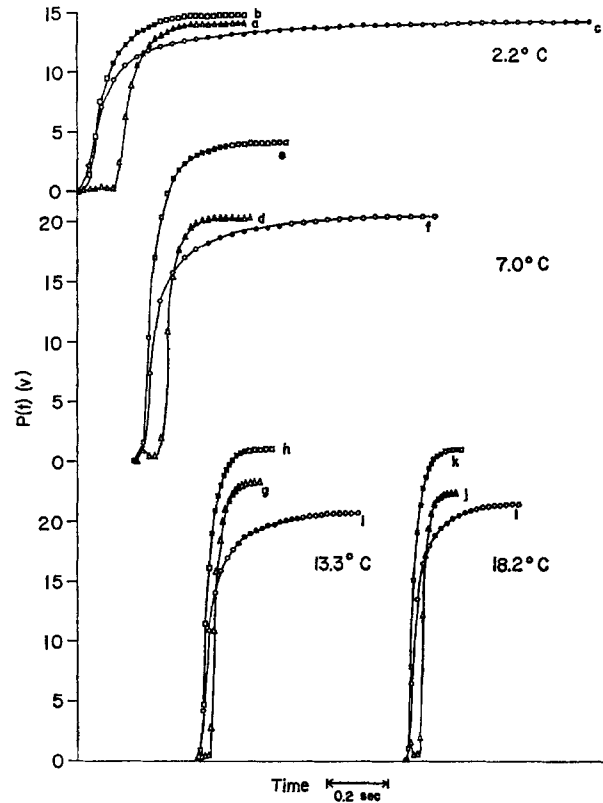For each fitted tetanus the fitting gave α and a standard deviation for

FIGURE 1.   Plot of $P(t)$ vs. t for 12 tetani from experiment of 10/1/69. $L_o = 39.2$ mm; mass of muscle 0.111 g. P in v; calibration 3.23 g/v. The three tetani at each temperature have the same origin, which corresponds to onset of stimulation. Origins for different temperatures are displaced by arbitrary interval on P and t scales. Sarcomere lengths: open triangles, 1.99 μm; open squares, 2.34 μm; open circles, 2.70 μm. Filled symbols (triangles, squares, and circles) indicate range of data used to evaluate α in each tetnaus. Temperatures, curves a, b, c, 2.2°C; d, e, f, 7.0°C; g, h, i, 13.3°C; j, k, l, 18°C. Curves were drawn by eye.

α, s_α. In a plot of log (s_α) vs. log α for 108 tetani, points lay near the line $s_\alpha = 0.0076 \, \alpha^{1.25}$. That is, the coefficient of variation s_α/α increased with α, from 1% at $\alpha = 3 \, \mathrm{sec}^{-1}$ to 2% at $\alpha = 50 \, \mathrm{sec}^{-1}$. Apparently $s_\alpha(\alpha)$ was greater for muscles than for exponentials used to test the apparatus (see Methods) because the variance of data about the least squares exponential was greater for real tetani than for the test data.

Rate constants, r, for the latter part of relaxation were estimated by eye from graphs of $\log (P(t_k))$ vs. t_k for this period. In order to obtain $P(t_k)$ it was necessary to subtract the resting tension from the record of total tension. The resting tension did not decrease significantly during brief tetani. During relaxation if $P(t)$ is sigmoidal (Aubert, 1956) the slope $d(\log P)/dt$ should
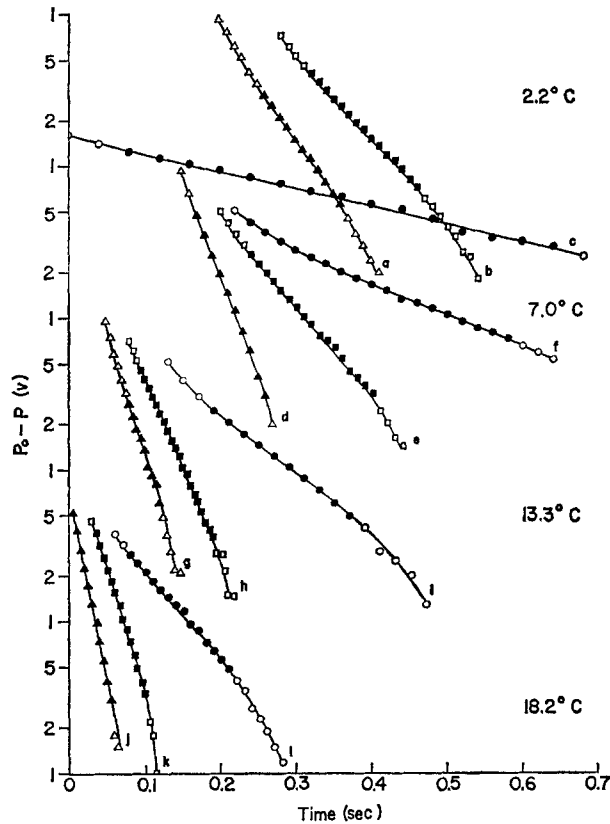

FIGURE 2.   Plot of $\log (P_o - P(t_k))$ vs. t_k for tetani of Fig. 1. $P_o - P$ in v, range from 0.1 to 10 v for each tetanus. Time interval between onset of stimulation and first point on each curve is arbitrary. Symbols as in Fig. 1. Curves were drawn by eye.

increase monotonically toward r as P approaches zero. This was the case in all experiments but one (10/21/69) in which the slope decreased toward r late in relaxation at $s = 2.86\ \mu$m, at 11 and 20°C.

Characteristics of α

DEPENDENCE OF α ON COURSE OF EXPERIMENT

During preliminary experiments the muscle was tetanized every 4 min; in later experiments in which the PDP8 was used, the interval between tetani

was 10 min. After each tetanus L was changed, as will be described below. Changing the temperature of the muscle after a series of tetani at fixed T required half an hour, during which the muscle was not stimulated. Each muscle performed 40–60 tetani of maximum duration 4 sec.

In general, minor changes in $\alpha(L_o, 1°C)$ occurred during an experiment Change was usually progressive in time, with fluctuations about a mean rate
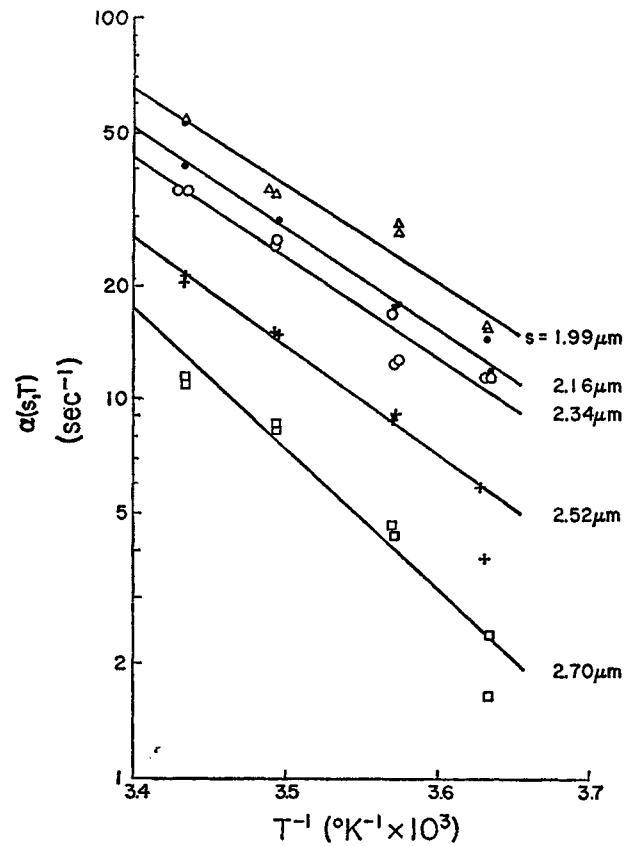

FIGURE 3.   log $\alpha(s, T)$ vs. T^{-1} for experiment of 10/1/69. Lines fitted by eye.

of increase or decrease in a plot of $\alpha(L_o, 1°C)$ vs. time of tetanus. In three experiments the average change in $\alpha(L_o, 1°C)$ per tetanus was about 0.8% of the value of α at the beginning of the experiment. This average rate of change of α persisted for 20–50 tetani.

We were not able to correlate the direction and extent of change in α with features of the history of stimulation of the muscle. Although correlations of these changes with stretch of the muscle to $L > L_o$ or with tetanization at $L \neq L_o$ seemed to exist in some sequences of tetani, contrary correlations appeared in others. Aljure and Borrero (1968) have observed that in the

sartorius of *Bufo marinus*, at 21–25°C, the rate of decline of maximum isometric tetanic tension depends on the length to which the muscle was set in prior tetani. If changes in α during an experiment reflect a similar history dependence, the variation in sequence of lengths and in duration of successive tetani was too great in these experiments to show the nature of the dependence.

Gordon, Huxley, and Julian (1966 *a*) found that passive stretch and release of a single fiber between tetani improved the uniformity of the fiber's response in successive tetani. Therefore, in our experiments we stretched the muscle to $L_o + 6$ mm and then shortened it to $L_o - 3$ mm three times in the interval between every two tetani. Stretch and shortening were passive. The muscle remained 15 sec at each length. The length of the muscle was set to L if $L \geq L_o$, or to L_o if $L < L_o$, 1 min before each tetanus. This procedure, with randomization of the sequence of lengths at which the muscle was tetanized, yielded good duplicate determinations of $\alpha(s, T)$, as Fig. 3 shows.

DEPENDENCE OF α ON SARCOMERE LENGTH AND TEMPERATURE

With four muscles we obtained data on $\alpha(s, T)$ by measuring α at three to five lengths in the interval, $L_o - 6 \leq L \leq L_o + 6$ mm, while holding T fixed. Each set of three to five tetani was performed twice at each temperature. In general the two sets were recorded in sequence, but occasionally we interposed sets at other temperatures between the two. The sequence of lengths in each set was random. The sequence of three or four temperatures (2, 10, 20 or 2, 7, 13, 20°C) was also random except that the 20°C sets were taken last to maintain condition of the muscle.

Fig. 3 is a plot of log α vs. $1/T$ from one of the experiments. At each L the data fit a straight line moderately well. The lines have similar slopes which seem to increase slightly with s. The intercepts at $T^{-1} = 3.4 \times 10^{-3}$ °K^{-1} decrease monotonically with increasing s. That is, the function

$$\alpha(s, T) = A(s) \exp\left[-\frac{E(s)}{R}\left(\frac{1}{T} - 3.4 \times 10^{-3}\right)\right]$$

for the ranges 2.0 μm $\leq s \leq$ 2.9 μm, 3.4 $\times$ 10^{-3} °K^{-1} $\leq \frac{1}{T} \leq$ 3.65 $\times$ 10^{-3}

°K^{-1}, approximately describes the data for $\alpha(s, T)$. R is the gas constant. Of 17 sets of data on the variation of α with T at fixed s, one set showed too much scatter to fit with an Arrhenius equation. The remaining intercepts, A and slopes E, are plotted against s in Figs. 4 and 5, respectively. Although E seems to increase with s, the dependence is not significant at the 95% level ($r = 0.35$; $N = 16$). At all sarcomere lengths $E = 13.9 \pm 2.4$ kcal/mole. The decrease of $A(s)$ with s is significant at the 99% level ($r = -0.81$; $N =$
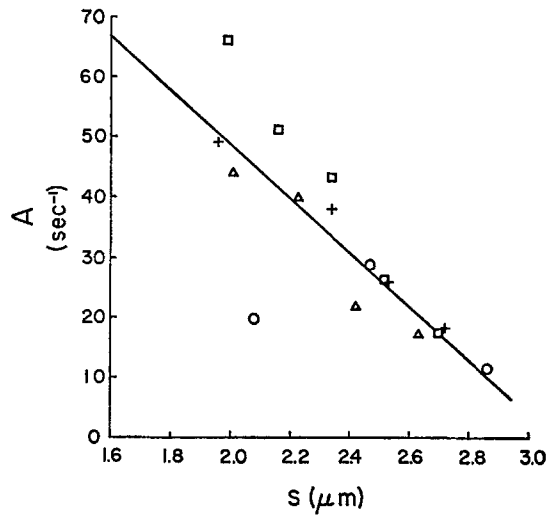
FIGURE 4.   Intercept A, from plots of log α vs. $1/T$, against s. Plus signs, 6/19/69; open square, 10/1/69; open circles, 10/21/69; open triangles, 11/25/69. Line is regression line of A on s.
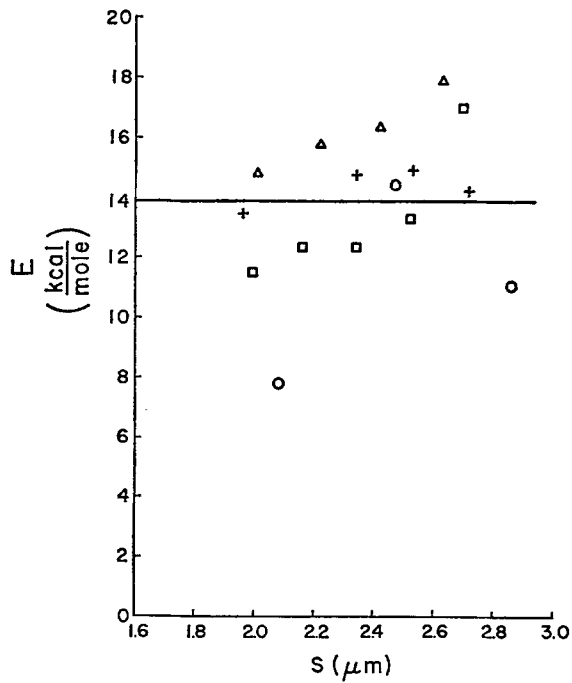

FIGURE 5.   Energy of activation, E, from plots of log α vs. $1/T$ against s. Symbols as in Fig. 3. Line is mean value of E.

16). A regression line for the data of Fig. 4 gives

$$A(s) = A_1 s + A_2$$

with $A_1 = -44.6 \pm 14.7 \ \mu m^{-1} \ sec^{-1}$ and $A_2 = 138.2 \pm 35.1 \ sec^{-1}$. This regression line is merely a convenient way of summarizing data for $A(s)$. We have no reason to believe that $A(s)$ is a linear function.

Fig. 4 shows that variation in $\alpha(s)$ among muscles decreases with increasing s. The diffraction pattern obtained from an edge of a muscle also becomes sharper with increasing s. This correlation of heterogeneity of length among sarcomeres with variability of α at a given modal sarcomere length may reflect the dependence of α on s.

The magnitude of the resting tension is not correlated with α at $L_o + 6$ mm and 2°C. In one experiment the resting tension declined to 52% of its initial value, in another to 64%, without change (other than fluctuation) in $\alpha(L_o + 6, 2°C)$ in either case. In the former experiment 24 tetani at various lengths occurred during the decline, and in the latter, 22 tetani.

CHARACTERISTICS OF r

The rate constant for relaxation, r, depends on the duration of stimulation during the tetanus, D, as well as on L and T. The results of Abbott (1951) and Aubert (1956) suggest that the dependence of r and D may be represented by the equation

$$r = (r_o - r_\infty)e^{-\phi D} + r_\infty .$$

In a twitch, when $D \doteq 0$, $r = r_o$. When D is sufficiently large, $r = r_\infty$. The rate constant, ϕ, characterizes the approach of r to r_∞ as D increases.

The parameters, r_o, r_∞, and ϕ, depend on L and T. In the experiment of 11/5/69 we measured r for duplicate twitches at 2, 10, and 20°C, at $s =$, 2.37 μm and 2.80 μm. The Arrhenius equation

$$r_o = B_o \exp\left[-\frac{F_o}{R}\left(\frac{1}{T} - 3.4 \times 10^{-3}\right)\right]$$

with $3.4 \times 10^{-3} \ °K^{-1} \leq \dfrac{1}{T} \leq 3.65 \times 10^{-3} \ °K^{-1}$ fitted these data satisfactorily, with the following values: for $s = 2.37 \ \mu m$, $B_o = 90 \ sec^{-1}$, $F_o = 16.6 \ kcal/mole$; for $s = 2.80 \ \mu m$, $B_o = 44 \ sec^{-1}$, $F_o = 19.0 \ kcal/mole$.

Fig. 6 is a plot of r vs. D for this experiment. r_o and r_∞ decrease but ϕ increases as sarcomere length increases. The difference $r_o - r_\infty$ is 7.5 sec^{-1} for $s = 1.93$ and 2.37 μm, but $r_o - r_\infty = 2.8 \ sec^{-1}$ for $s = 2.80 \ \mu m$. The rate constant, ϕ, is about three times greater for $s = 2.80 \ \mu m$—$\phi = 1.06 \ sec^{-1}$—

than for $s = 1.93$ or $2.37\,\mu$m, for which $\phi = 0.32$ sec^{-1} and 0.35 sec^{-1}, respectively. The relaxation rate constants, r_∞, for long tetani vary over a smaller range with sarcomere length than do the twitch constants, r_0.

An experiment to provide information about the temperature dependence of ϕ and of r_∞ agreed qualitatively with the above results. In this experiment duplicate tetani were performed at 27 combinations of the parameters D, L, and T. At $s = 2.08$ and $2.47\,\mu$m, r did not reach r_∞ in the range of durations used. At $s = 2.86\,\mu$m, r_∞ depended on temperature according to the equation

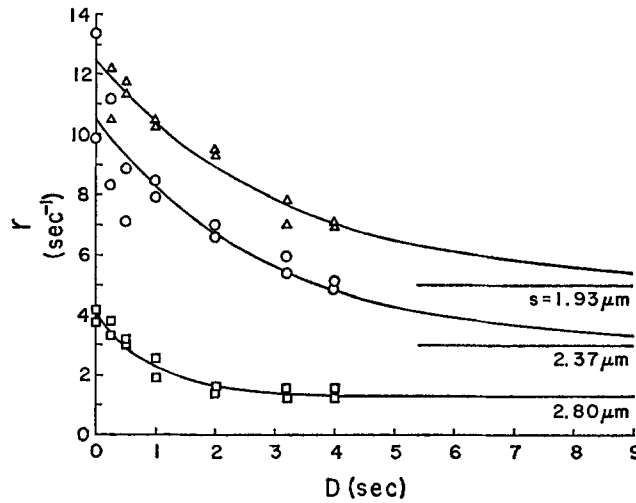$$r_\infty = B_\infty \exp\left[-\frac{F_\infty}{R}\left(\frac{1}{T} - 3.4 \times 10^{-3}\right)\right]$$

FIGURE 6. r vs. D for three sarcomere lengths, 2°C, 11/5/69. Curves are least square fits of exponential to data for each s. Horizontal lines at right are values of r_∞.

in the range 3.4×10^{-3} °K$^{-1} \leq \dfrac{1}{T} \leq 3.65 \times 10^{-3}$ °K^{-1}, with $B_\infty = 4.05$ sec^{-1} and $F_\infty = 10.6$ kcal/mole. The data were not sufficient in number or precision to estimate $\phi(s, T)$, although ϕ clearly increased with temperature at all sarcomere lengths.

DISCUSSION

The final portion of the rise of tension to P_o, the maximum tension, in an isometric tetanus is exponential with rate constant α. α varies with sarcomere length, s, and temperature, T. Sandow (1947) estimated $\alpha(L_o, 25°\mathrm{C}) = 45$ sec^{-1}. From our data $\alpha(s = 2.3\,\mu$m, 25°C) $= 48.1$ sec^{-1}. The data of Jewell and Wilkie (1958, Fig. 7) for the rise, plotted as $\dot{P}$ vs. P, terminated in a linear portion which intersected the P axis ($\dot{P} = 0$), after extrapolation, at the

measured value of P_o. These data gave $\alpha(L_o, 2°C) = 16.2$ sec^{-1}, in reasonable agreement with our measurements. Results of Hill (1953, Fig. 6 D) on sartorius of *R. temporaria* give $\alpha(L_o, 0°C) = 12.4$ sec^{-1}, $\alpha(1.31 L_o, 0°C) = 0.18$ sec^{-1}. If L_o corresponds to 2.3 μm, our data yield $\alpha(2.3 \mu$m, 0°C$) = 5.84$ sec^{-1}, $\alpha(3.0 \mu$m, 0°C$) = 0.57$ sec^{-1}.

From the data of Ramsey and Street (1940, Fig. 8) for an isolated semitendinosus fiber we estimate $\alpha(L_o, 12.5°C) = 26.5$ sec^{-1}, $\alpha(1.36 L_o, 12.5°C) = 6.8$ sec^{-1}. If we assume that L_o corresponds to $s = 2.1 \mu$m, the center of the range of s in which P_o is maximum, our data give $\alpha(2.1 \mu$m, 12.5°C$) = 22.2$ sec^{-1}, $\alpha(2.85 \mu$m, 12.5°C$) = 5.6$ sec^{-1}. Estimation from the data of Gordon, Huxley, and Julian (1966 *b*, Fig. 4 A, B) for an isometric region of uniform sarcomere length in an isolated semitendinosus fiber gives $\alpha(2.08 \mu$m, 4°C$) = 8.1$ sec^{-1}, $\alpha(2.57 \mu$m, 4°C$) = 5.9$ sec^{-1}. On whole sartorius we found $\alpha(2.08 \mu$m, 4°C$) = 11.3$ sec^{-1}, $\alpha(2.57 \mu$m, 4°C$) = 5.9$ sec^{-1}. Thus there seems to be no marked discrepancy in values for $\alpha(s, T)$ obtained from a uniform region of a single fiber and from a whole muscle.

α decreases as L increases, both below and above the length at which resting tension appears. Therefore the "parallel elastic element" does not produce the decrease of α with L, although it may affect α. The force vs. length characteristic for the series elastic element, which does affect α (see below), is independent of L over the range of L we have studied (Jewell and Wilkie, 1958). Apparent decrease in the stiffness of this element for $L > L_o$ results from a contribution by the parallel elastic element.

Since α depends on the time course of events during a cycle of activity at a myosin cross-bridge, it is not surprising that α has a Q_{10} of 2.4, a figure in the range characteristic of many enzyme-catalyzed reactions.

A theory for the dynamics of whole muscle should interpret the dependence of α on L and T which we report. The theory developed by Huxley (1957) and Julian (1969) accounts for many dynamical properties of single sarcomeres. Such a theory, together with information on the organization of whole muscle, must be integrated into a dynamical theory for whole muscle. Perhaps knowledge of whole muscle is insufficient for a synthesis at present. Sarcomeres near the motor end plate or near tendons might have special properties that would modify the time course of changes in force and length in fibers containing them. The behavior of a whole muscle in which adjacent fibers interact mechanically along their entire lengths, through connective tissue, might differ appreciably from that of a muscle in which fibers interact only through attachment to a common tendon.

If simpler assumptions provide a reasonable approximation, the path from a theory for a single sarcomere to a theory for whole muscle is straightforward, and the latter theory provides an interpretation for the dependence of α on mechanical and chemical aspects of the muscle. In a separate analysis[1] we have extended the Huxley-Julian model to describe a muscle in which fibers

are joined only at tendons. All sarcomeres of the same length in a myofibril are equivalent, though the myofibril may contain sarcomeres of various lengths. The model relates α to the rates of cross-bridge attachment to and detachment from thin filaments, the stiffness of attached bridges and of the series elastic element, the separation between thin and thick filaments in each sarcomere, and the distribution of sarcomere lengths in a fiber. While the analysis does not fully explain the dependence of α on s, it predicts a value for $\alpha(2.2 \, \mu m, 1 \, ^\circ C)$ in good agreement with the value reported here. This theory also suggests that neither variation in s within a fiber nor change in s during development of tension will suffice to account for the decrease in α with s if the rate constants for chemical processes in the fiber do not depend on s.

The same structures and processes are likely to govern the decline of tension after an isometric tetanus as determined the course of its prior increase. However, during the exponential rise of tension to P_o the fiber is probably fully active: Calcium released from lateral sacs has saturated troponin sites on thin filaments, allowing interaction of every cross-bridge with the thin filament apposed to it. During relaxation the number of bridges able to link to a thin filament decreases as the sarcoplasmic reticulum removes calcium from the region of overlap between thick and thin filaments (Weber, 1966; Winegrad, 1968).

It is unclear whether the rate of removal of calcium or the rate constants for interaction of bridges with thin filaments primarily govern the decline of tension during relaxation. Calcium released after the action potential may remain in the overlap region after the reticulum has withdrawn such calcium from the rest of the sarcoplasm. Although a muscle produces heat as long as it develops force in a twitch (Hill, 1949; Fraser, personal communication), evidence on the extent of hydrolysis of ATP during relaxation is contradictory (Wilkie, personal communication).

Since the biochemical state of the muscle during relaxation is not clearly established, we have not attempted to account for the dependence of r, the rate constant for the exponential decay of tension late in relaxation, on temperature or sarcomere length. Our result that α has a lower Q_{10} than does r agrees with observations of Hartree and Hill (1921) on the isometric twitch. Jewell and Wilkie (1960) found, as did we, that relaxation is slower in a twitch, the greater the muscle length.

Any explanation for the dependence of r on duration of stimulation, D, should interpret the results presented here together with two other observations. First, a decline in the rate of heat production, $\dot{Q}$, during the course of an isometric tetanus roughly parallels a decline with increasing D in an index for the rate of relaxation (Abbott, 1951; Aubert, 1956). Second, in the rectus femoris of the tortoise neither $\dot{Q}$ nor the time course of relaxation changes during a prolonged isometric tetanus (Woledge, 1968).

We suggest that the decline of r and of $\dot{Q}$ with D may be correlated with an increase of potassium ion concentration in the transverse tubular system, $[K_t]$, associated with an action potential (Gage and Eisenberg, 1969). During prolonged repetitive stimulation $[K_t]$ will increase to a steady-state value which should be attained more rapidly, the higher the frequency of stimulation. Abbott (1951) found that Q decreased to a steady value more rapidly at a higher frequency; the steady value was slightly affected by the increase in frequency.

After stimulation stops $[K_t]$ will decrease to the level characteristic of the resting fiber. The time interval required for this return will be longer, the nearer $[K_t]$ is to its steady-state value just after the last stimulus. If a higher $[K_t]$ reduces the rate of relaxation, our observation of a decline in r with D in frog sartorius muscle might be explained. In the rectus femoris of the tortoise the decrease of r to its steady value, r_∞, may be complete by the time isometric force attains the plateau value, P_o, and time-varying heat production associated with increase of force to P_o may mask a decline in $\dot{Q}$ such as that which occurs in the frog muscle.

This hypothesis does not explain our observation that r approaches r_∞ more rapidly at muscle lengths greater than L_o than at L_o. Clearly the relations among action potentials, muscle length, r, and $\dot{Q}$ deserve further investigation.

Although evaluation of r is more laborious than the methods Hartree and Hill (1921) and Abbott (1951) used to quantify relaxation, and more laborious than measurement of the half-time of tension fall (Jewell and Wilkie, 1960), r has two merits as an index of relaxation. r may be measured in both twitches and tetani, providing a means for comparing relaxation in these processes. Also, r is independent of the maximum tension obtaining in a twitch or tetanus, so that one may study the dependence of relaxation on s, T, and D without confusing interpretation through the dependence of maximum tension on these parameters.

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