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EFFECTS OF TEMPERATURE ON TENSION, TENSION-DEPENDENT HEAT, AND ACTIVATION HEAT IN TWITCHES OF FROG SKELETAL MUSCLE

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SUMMARY

1. The effects of temperature on muscle energy liberation (heat plus work production) and isometric twitch force production were measured at rest length (l_0) and at long muscle lengths (approx. 1·35 l_0) where twitch force was greatly depressed.

2. Force production and energy liberation at l_0 declined progressively as muscle temperature was elevated from 0 to 20 °C. Force production decreased to a greater extent than did energy liberation. A plot of energy liberation *vs.* twitch force suggested that a fraction of the l_0 energy liberation was produced independent of temperature.

3. The energy liberated at long muscle lengths, the activation heat, was independent of muscle temperature. The activation heat is interpreted as reflecting the energy dissipated during Ca^{2+} cycling and thus suggests that, under the conditions of these experiments, the amount of Ca^{2+} released with stimulation is independent of muscle temperature and subsequent muscle force production.

4. Analysis of the results also supports the conclusions that skeletal muscle energy liberation is dependent on muscle force production and that the energy liberation associated with Ca^{2+} cycling is essentially independent of muscle length in the range of l_0–1·35 l_0.

INTRODUCTION

The mechanical force produced by an isolated frog skeletal muscle during an isometric twitch is profoundly affected by temperature. The kinetics of the response are accelerated with an increase in temperature from 0 to 20 °C and many authors (e.g. Close, 1972; Hill, 1951) have observed a decline in twitch amplitude over the same temperature range. One possible explanation for the decreased twitch amplitude is that the amount of Ca^{2+} released with a single maximal stimulus decreases at higher temperatures. It has been shown (Homsher, Mommaerts, Ricchiuti & Wallner, 1972; Smith, 1972) that the energy liberated (measured as heat plus work produced) during an isometric twitch at long muscle lengths where twitch force is near zero appears to be proportional to the ATP split by the muscle to reaccumulate Ca^{2+} released during contraction. This energy liberation has been called the activation heat. Thus the amplitude of the activation heat might be expected to be proportional

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to the amount of Ca^{2+} released upon stimulation. Measurement of the activation heat was employed to test whether the decline of tension with increased temperature was due to a decline in muscle activation.

METHODS

Rana pipiens of both sexes were obtained from the Mogul Corporation (Oshkosh, Wis.) and kept unfed in tanks with continuously running cold water (approx. 5 °C) at room temperature (approx. 20 °C). Experiments were performed during the months of November to February. Generally on the day of, but occasionally on the day before, an experiment an animal was killed by decapitation and the dorsal heads of a pair of semitendinosus muscles were dissected. The muscle pair, still attached to the pelvic bone, was mounted on a thermopile and aerated in 95% O_2, 5% CO_2 at 0 °C in a Ringer solution containing (mM): 95·0 NaCl, 20 $NaHCO_3$, 2·5 KCl, 1·0 $MgCl_2$, 1·0 $CaCl_2$ and 11 dextrose. When the dissection was performed on the day before an experiment the muscle pair was aerated overnight at approximately 1 °C and mounted on the thermopile on the day of the experiment. The muscle length at which the isometric twitch force was maximal, l_0, ranged from 17 to 22·5 mm and the blotted weight of the muscle pairs (m) ranged from 0·046 to 0·109 g. The blotted weight of the muscle pairs averaged 91 ± 0·4% of the drained weight. For purposes of calculating the energy liberation the dried weight was assumed to be 20% of the blotted weight. At 0 °C the peak twitch tension per cross-sectional area ($P_{0t}l_0/m$) averaged 158 ± 3 mN/mm² (mean ± S.E. of mean, n = 12) and the accompanying energy liberation, E, averaged 9·57 ± 0·37 mJ/g.

Mechanical and myothermal measurements. Force measurements were made with capacitance transducers having a resonant frequency of 3 kHz and a compliance of 0·02 μm/mN. Energy liberation during contraction was measured as heat plus work produced. Since all contractions were isometric and since the internal work done by the muscle is quantitatively returned to the muscle as heat, energy liberation was measured by monitoring muscle heat production. Heat production was measured by employing three thermopiles consisting of constantan-silver plated constantan thermocouples (Ricchiuti & Mommaerts, 1965; Homsher *et al.* 1972). These thermopiles were 16 mm (P2), 23 mm (P3), and 12·3 mm (P4) long with thicknesses of approximately 75 μm and sensitivities of 7·93, 12·36, and 6·02 μV/m °C respectively. The majority of the experiments were done on P3 (nine of the twelve experiments). The lengths of the recording regions were 13 mm (P2), 18·5 mm (P3), and 9 mm (P4). The remaining length of each thermopile consisted of protective junctions and platinum electrodes. These stimulating electrodes were oriented in the plane of the thermopile at each end. The output of the thermopiles was amplified by an Astrodata 120 nV amplifier. The output of each thermopile, in μV, was divided by its sensitivity, multiplied by the muscle heat capacity and divided by the muscle blotted weight to obtain the energy liberation in millijoules per gram. Myothermal records were corrected electronically for an exponential heat loss. For those heat records that were corrected for the time lag of the thermopile system, an equivalent half thickness for the thermopiles of 20·1 μm was calculated from the thermopile constituents and their heat capacities (Hill, 1949). The time course analysis was performed by the method of factors (Hill, 1965).

The temperature in the muscle chamber was controlled by immersing it into a 40L Tamson Model TEV 40 thermostatic bath connected to a Naslab model RTE 4 refrigerated bath circulator. With this combination it was possible to maintain constant the bath temperature, monitored continuously just outside the muscle chamber, within 0·01 °C for over a 20–30 min experimental series. At the beginning of the experiment the system temperature was brought to 0 °C by the use of a Neslab model PBC-2 portable bath cooler.

General experimental protocol. One muscle of a pair was mounted on each face of a thermopile and the tendons of the pair attached with as little thread as possible to a hollow piece of aluminum tubing connected to a force transducer. The thermopile and muscle were immersed in a Ringer filled glass chamber and aerated. This system was submerged in the 40L thermostatic bath at 0 °C for a 45 min equilibration period. During an experimental series of 20–30 min the Ringer was drained by gravity from the chamber and the muscles were stimulated at 90 sec intervals. After the muscles were re-equilibrated in Ringer for 20–30 min, another series began. Typically during the first series optimum stimulus parameters and l_0 were determined. Maximal isometric

twitch responses were obtained when the muscles were stimulated with square-wave pulses of 3 msec duration of 12·5–20 V at all temperatures. All experiments started at 0 °C where energy and force were recorded at l_0 and at stretched muscle lengths where twitch force was decreased by at least 93 % of the value at l_0, P_0. After the muscle length was returned to l_0, the temperature was elevated to 5, 10, 15, or 20 °C and after a 30 min equilibration period the experiment was repeated. For any given muscle pair two or three different temperatures were investigated. The stimulus parameters determined at 0 °C were found to give also the maximum twitch response at elevated temperatures. The rest and long muscle lengths at elevated temperatures were the lengths derived from the 0 °C series. Thus in these experiments the data at the elevated temperature is compared to 0 °C data derived from the same muscle pair. The significance of the differences between sample means was tested with the t test. A 5% level of significance ($P < 0·05$) was accepted throughout.

RESULTS

Energy liberation and force production at l_0 as a function of muscle temperature

In Fig. 1 the force generation during an isometric twitch and the accompanying energy liberation is displayed for four muscle pairs. For each pair a 0 °C control value is shown plus a value at 5, 10, 15, or 20 °C. At all elevated temperatures the twitch force amplitude at l_0, P_{0t}, is decreased and the kinetic parameters of the mechanical response are accelerated. The energy liberated, E, during the isometric twitch is decreased but to a lesser degree than is force. Average results for all experiments are displayed in Table 1. From 0 to 20 °C the average twitch force amplitude declines to 36% of the 0 °C value whereas the average energy liberation decreases to 59% of the 0 °C value. The relationship between energy liberation and twitch force as the temperature is elevated is seen in Fig. 2 for all the experiments at all temperatures. Force per cross-sectional area, $P_{0t}l_0/m$, is plotted against energy liberation, E, as fitted symbols in this Figure. It is apparent that the energy liberation does not vary in direct proportion to the force. A fraction of the energy liberation during muscle contraction appears to be independent of muscle force production. From linear regression analysis, the magnitude of the tension independent energy liberation, E, as filled symbols in this Figure. It is apparent that the energy liberation does not decline to the same extent that muscle twitch force does as the temperature is elevated.

Energy liberation and force production at long muscle lengths as a function of temperature

In Fig. 3 force generation during an isometric twitch at long muscle lengths and the accompanying energy liberation is shown at muscle temperatures from 0 to 20 °C. The same four preparations are displayed in Fig. 3 as shown in Fig. 1. It is apparent that the twitch force amplitude decreases while the kinetic parameters of the twitch are accelerated. Despite this fact the long length energy liberation, termed the activation heat, appears to be independent of temperature whereas its kinetics are clearly temperature sensitive. On the average the muscles were stretched to a length of $1·35 \pm 0·02$ l_0 and the twitch force was reduced to a value of $0·04 \pm 0·004$ P_{0t}. Further average results are shown in Table 1. Despite the fall in energy and force at l_0 with increasing temperature, the amplitude of the activation heat, A, is insensitive to muscle temperature.

An interesting observation can be derived from the open symbols of Fig. 2. Here

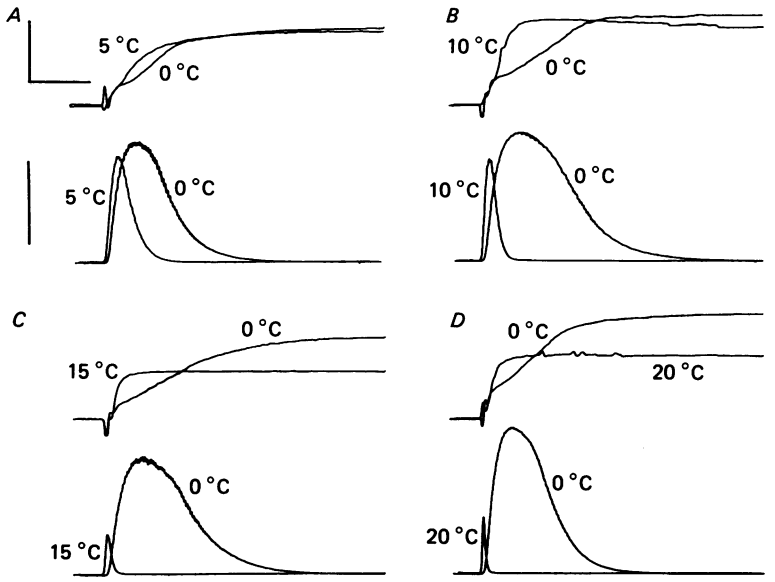

Fig. 1. Energy liberation and force production at l_0 as a function of muscle temperature. In four different muscle preparations the mechanical and energetic responses from isometric twitches were determined first at 0 °C and then at the following elevated temperatures: *A*, 5 °C; *B*, 10 °C; *C*, 15 °C; or *D*, 20 °C. In each quadrant: upper records, heat production and lower records, force. Initial deflexions at the moment of stimulation on the heat records are stimulus artifacts. Horizontal calibration line: 400 msec; vertical calibration lines: heat records, 6 mJ/g; force records, 0·3 N.

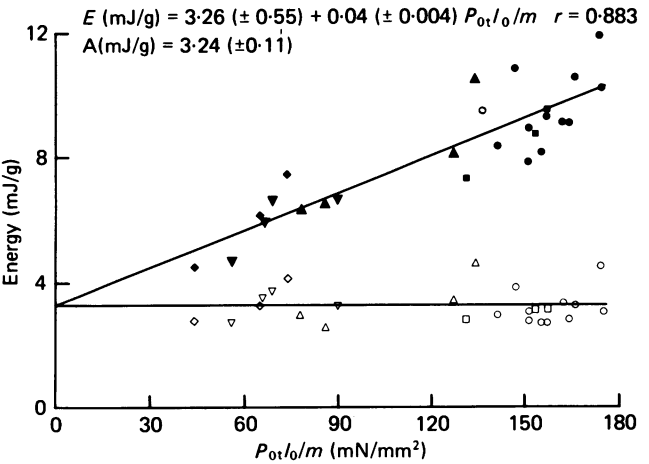

Fig. 2. Plot of energy liberation at l_0, E, and at long muscle lengths, A, as a function of the maximum force production at l_0, $P_{0t}l_0/m$. Force production was varied by elevating muscle temperature from 0 to 20 °C. Data is taken from twelve different preparations. At each value of $P_{0t}l_0/m$ there is a value for E (filled symbols) and A (open symbols). Mean value for A is the numerical average of all values of A. Least-squares linear regression analysis was employed to obtain the relation of E *vs.* $P_{0t}l_0/m$ shown. The correlation coefficient is given by r. Symbols: ● and ○, 0 °C; ■ and □, 5 °C; ▲ and △, 10 °C; ▼ and ▽, 15 °C; ◆ and ◇, 20 °C.

TABLE 1. Effect of temperature on mechanical and energetic properties of muscle contraction*

Temperature (°C)	n	$\frac{(P_{0t}l_0/m)_{\Delta T}}{(P_{0t}l_0/m)_0}$†	$\frac{(E)_{\Delta T}}{(E)_0}$†	$\frac{(A)_{\Delta T}}{(A)_0}$†	$\frac{A}{E}$
0‡	12	1·0	1·0	1·0	0·34 ± 0·01
5	3	0·93 ± 0·05§	0·95 ± 0·02	1·03 ± 0·02	0·36 ± 0·02
10	6	0·78 ± 0·06	0·88 ± 0·03	1·00 ± 0·01	0·44 ± 0·02
15	4	0·45 ± 0·06	0·63 ± 0·05	1·06 ± 0·05	0·56 ± 0·03
20	4	0·36 ± 0·06	0·59 ± 0·04	0·95 ± 0·03	0·56 ± 0·02

* Symbols: $P_{0t}l_0/m$, peak isometric twitch force per cross-sectional area; E, energy liberated during an isometric twitch at l_0; A, activation heat, energy liberated at long muscle lengths during an isometric twitch; 0, results obtained at 0 °C; ΔT, results obtained at 5, 10, 15 or 20 °C.

† Ratio of the value at the elevated temperature to the value at 0 °C.

‡ Average 0 °C values: $P_{0t}l_0/m$ = 158 ± 3 mN/mm²; E = 9·57 ± 0·37 mJ/g; A = 3·26 ± 0·18 mJ/g.

§ Values given as mean ± S.E. of mean.

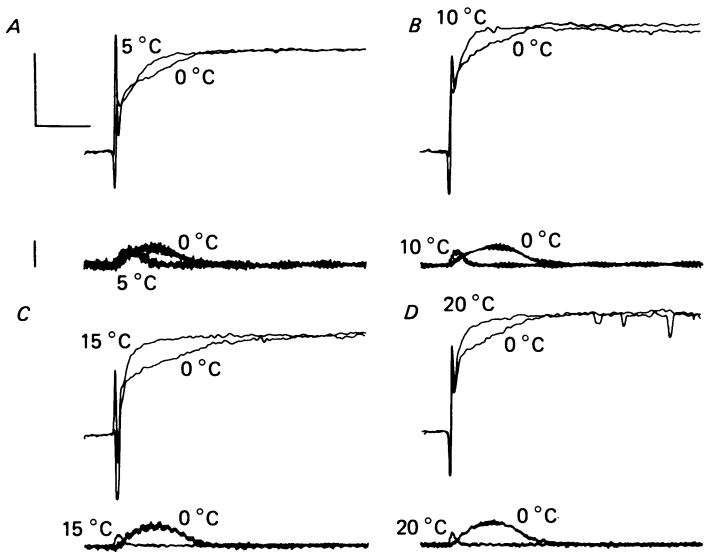

Fig. 3. Energy liberation and force production at long muscle lengths as a function of temperature. Same four preparations as shown in Fig. 1. Energy liberation and force production during an isometric twitch were first determined at l_0, then at a long muscle length at 0 °C and the muscles were returned to l_0 and the temperature elevated to 5 °C in *A*, 10 °C in *B*, 15 °C in *C*, or 20 °C in *D* and the procedure repeated. In each quandrant: upper records, heat production and lower records force. Long length, resulting force and A/E at 0 °C are: *A*, 1·32 l_0, 0·04 P_{0t}, 0·35; *B*, 1·33 l_0, 0·03 P_{0t}, 0·37; *C*, 1·48 l_0, 0·05 P_{0t}, 0·33; *D*, 1·31 l_0, 0·05 P_{0t}, 0·31. Horizontal calibration line: 500 msec; vertical calibration lines: heat records, 2 mJ/g; force records, 3 mN.

the values of A are plotted against the maximum force generated at l_0, expressed as $P_{0t}l_0/m$, at a particular temperature. Thus for each value of $P_{0t}l_0/m$ there is a corresponding energy liberation value E at l_0 and A at stretched lengths. Taken together this graph shows that despite the decline in the energy liberation at l_0 with the decline in force due to elevated temperature the activation heat remains constant.

The average value for A is $3{\cdot}24 \pm 0{\cdot}11$ mJ/g and is similar to the energy liberation at l_0 that appears to be tension independent. Presumably they are both estimates of the amount of energy liberation associated with Ca^{2+} cycling. It is noteworthy that one value was derived from experiments at l_0 and the other from experiments at long muscle lengths.

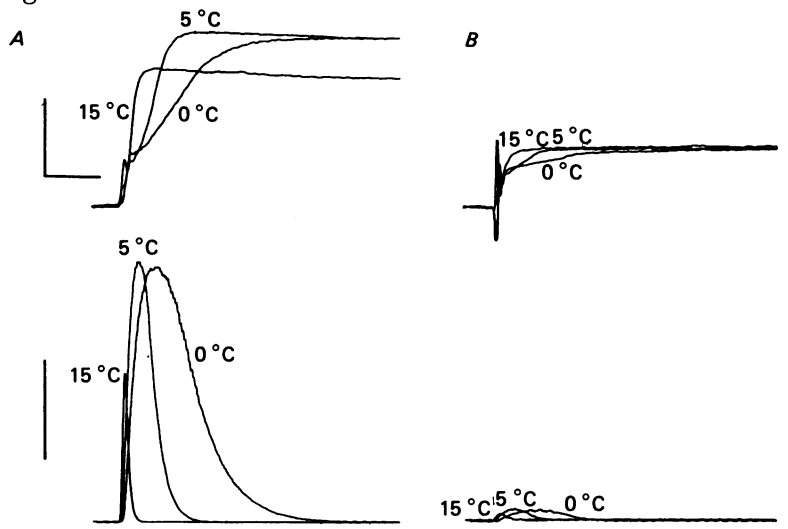

Fig. 4. Comparison of energy liberation and force production at l_0 (A) and at $1{\cdot}31\ l_0$ (B) as a function of muscle temperature. In this example data was derived at 0, 5, and 15 °C from the same muscle preparation. Upper records, heat and lower records, force. Horizontal calibration line: 500 msec; vertical calibration lines: heat records, 4 mJ/g; force records, 0·2 N.

Because of the temperature invariance of the activation heat and the decline in the energy liberation associated with twitch force, the ratio A/E increases as the temperature is elevated as shown in Table 1. The ratio A/E goes from a value of $0{\cdot}34 \pm 0{\cdot}01$ to a value of $0{\cdot}56 \pm 0{\cdot}02$ as the temperature is elevated from 0 to 20 °C. All of these results are summarized together in the example shown in Fig. 4 where the energy liberation and force production were measured at l_0 and $1{\cdot}31\ l_0$ and at 0, 5, and 15 °C in the same muscle preparation.

Kinetics of mechanical and energetic properties as a function of muscle temperature

In Fig. 5 the temperature dependence of selected mechanical and energetic properties of the isometric twitch are summarized. The time for one half relaxation from peak force, $T_{\frac{1}{2}r}$, during an isometric twitch is more sensitive to temperature than the maximum rate of rise of force development $(dp/dt)_{max}$. The same result is obtained if the temperature sensitivity of the time constant for the exponential phase of isometric relaxation is substituted for $T_{\frac{1}{2}r}$. The change in either of these parameters, $T_{\frac{1}{2}r}$ or $(dp/dt)_{max.}$, with temperature can not be described by a single Q_{10} value over the total range of 0–20 °C. The absolute values of these parameters at 0 °C are: $T_{\frac{1}{2}r}$ equal 402 ± 21 msec, $(dp/dt)_{max.}$ equal $4{\cdot}27 \pm 0{\cdot}24$ mN/msec. To compare with the mechanical data the temperature sensitivity of the slow exponential phase of the activation heat was determined. Homsher *et al.* (1972) have suggested

that this slow phase reflects the rate of ATP splitting by the sarcoplasmic reticulum to reaccumulate Ca^{2+}. The slow phase is observed clearly after the stimulus artifact in the records of Figs. 3 and 4. That this phase is sensitive to temperature is shown clearly in Figs. 3 and 4. To determine the time constant for this phase the energy records must be corrected for the time lag of the thermopile system. The time constant,

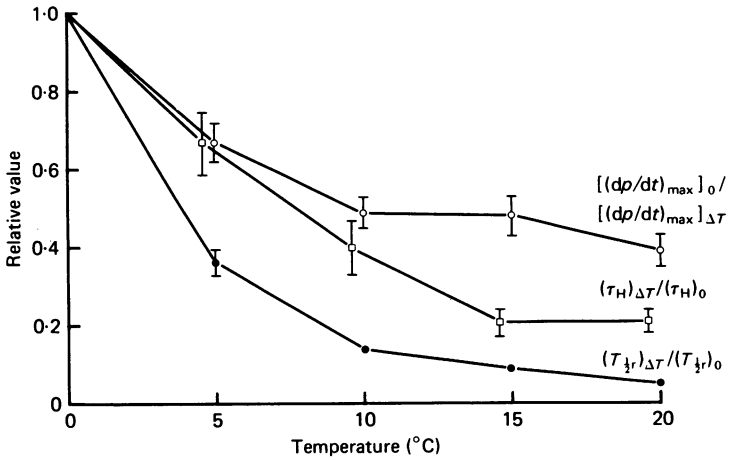

Fig. 5. Plot of mechanical and energetic properties of the isometric twitch as a function of muscle temperature. Plotted parameters: mechanical properties-time for one half relaxation from peak twitch force expressed relative to the value at 0 °C, filled circles, $(T_{\frac{1}{2}r})_{\Delta T}/(T_{\frac{1}{2}r})_0$, and maximum rate of rise of isometric twitch force production (elevated temperature value divided by 0 °C value), open circles, $[(dp/dt)_{max.}]_0/[(dp/dt)_{max.}]_{\Delta T}$; and energetic property-exponential time constant of slow phase of the activation heat relative to the value at 0 °C, open squares, $(\tau_H)_{\Delta T}/(\tau_H)_0$. Results are expressed as mean ± S.E. of mean (standard errors of $(T_{\frac{1}{2}r})_{\Delta T}/(T_{\frac{1}{2}r})_0$ at 10 through 20 °C are within the size of the circles). Number of muscle preparations constituting mechanical results: 5 °C, 3; 10 °C, 6; 15 °C, 4, and 20 °C, 4. Number of muscle preparations constituting energetic data: 5 °C, 2; 10 °C, 4; 51 °C, 4; and 20 °C, 3.

τ_H, at 0 °C is 328 ± 47 msec and the slow phase constitutes 39 ± 3% of the total value of A at 0 °C. The temperature sensitivity of the slow phase is shown in Fig. 5 as $(\tau_H)_{\Delta T}/(\tau_H)_0$. Despite the fact that the results are quite variable, it seems that the temperature sensitivity of the slow phase of the activation heat does not match the temperature sensitivity of either the contraction or relaxation phases of the isometric twitch.

DISCUSSION

These experimental results clearly show that the decline in isometric twitch tension observed in frog skeletal muscle as temperature is elevated from 0 to 20 °C can not be attributed to a decline in muscle activation. When the temperature is changed from 0 °C to 5, 10, 15, or 20 °C the average isometric twitch force decreases to 0·93 ± 0·05, 0·78 ± 0·06, 0·45 ± 0·06, or 0·36 ± 0·06 respectively of the 0 °C values (Table 1). Despite this decrease in twitch force the activation heat or Ca^{2+} cycling energy liberation under these same conditions is 1·03 ± 0·02, 1·00 ± 0·01, 1·06 ± 0·05, and 0·95 ± 0·03 respectively of the 0 °C values (Table 1). These results show that the

Ca^{2+} cycling energy liberation is not different from the value obtained at 0 °C for any of the temperatures studied. Whereas Homsher *et al.* (1972) found that A was not significantly different at 20 °C from the value observed at 0 °C their results could not, because of the scatter of the measurements, rule out the possibility that A may have increased by 20% or more (the ratio of A at 20 °C to A at 0 °C was $1{\cdot}22 \pm 0{\cdot}13$). The experiments summarized in Table 1 demonstrate with greater certainty the temperature independence of the amount of Ca^{2+} cycling energy liberation under the conditions of these experiments. These results are consistent with the interpretation that the amount of Ca^{2+} released during a maximal stimulation is not dependent on muscle temperature and not correlated, under these conditions, with the subsequent force development. This conclusion assumes that the molar enthalpy changes of the reactions associated with Ca^{2+} cycling and the stoichiometry of the ATP driven Ca^{2+} pump of the sarcoplasmic reticulum are temperature insensitive over the range of 0–20 °C. In support of this assumption, the molar enthalpy change for ATP splitting in solution at pH 7 and pMg 2·5 is altered by less than 10% as the temperature is elevated from 0 to 25 °C (Alberty, 1972). The net effect of this temperature independence is that under the conditions of these experiments greater than 50% of the energy produced by a muscle generating an isometric twitch at 20 °C is utilized to reaccumulate Ca^{2+} (Table 1). Thus the usual reported values of the fraction of energy liberation associated with Ca^{2+} cycling of 18–33% as summarized by Homsher & Kean (1978) must be interpreted in light of the experimental conditions under which they were measured. Another point worth noting is that it is sometimes stated that a muscle may produce less twitch force at a higher temperature because it is not as fully *activated* as at a lower temperature. It is clear that the degree of *activation* in this sense may not reflect the amount of Ca^{2+} released because the amount of Ca^{2+} released may be the same at both temperatures.

If one accepts the conclusion that the amount of Ca^{2+} released with a maximal stimulus does not change with muscle temperature, then other explanations must be sought for the decrease in twitch force reported in these experiments. Fig. 5 shows that muscle relaxation is more sensitive to an increase in temperature than is the rate of rise of force production. These results are consistent with the results of others (e.g. Hartree & Hill, 1921). It is possible that the twitch force falls because of a differential effect of temperature on the actomyosin interaction producing force and the Ca^{2+} pump leading to relaxation. A similar idea has been advanced before (Homsher, Mommaerts & Ricchiuti, 1973; and Gibbs & Chapman, 1974) to explain the observed decrease in the maximum external work done by a frog muscle when the temperature is elevated. Also undoubtedly the compliance of the force measuring device and connexions would have an effect on the twitch force as described by Jewell & Wilkie (1958). One would expect that the decline in twitch force with elevated temperature would be exaggerated in more compliant muscle preparations. This phenomenon could also be explained by a differential effect of temperature on the actomyosin system and Ca^{2+} pump.

These mechanical and energetic results can be compared to other data in the literature. Generally when temperature is increased from 0 to 20 °C the isometric twitch tension of frog skeletal muscle decreases. In frog sartorius muscle Hill (1951) observed a 50% decrease in twitch tension and in frog semitendinosus muscle Close

(1972) found that the twitch force at 20 °C was 38% of the 0 °C value. These results are similar to the value of $36 \pm 6\%$ found for a temperature change of 0–20 °C as reported in Table 1. Jewell & Wilkie (1958) on the contrary observed that the amplitude of the isometric twitch force was nearly temperature independent (over a range of approximately 2–20 °C). Concerning the energetic results at 0 °C, Table 1 shows that the activation heat, A, is $34 \pm 1\%$ of the energy liberation at l_0. This value is slightly higher than the extrapolated zero force values of 26–30% reported by Homsher *et al.* (1972) and Smith (1972). This higher value probably can be attributed to the fact that at the stretched muscle lengths employed in this study the muscle still produced a small amount of force which obligates some extra energy liberation. The absolute value of A at 0 °C is $3{\cdot}26 \pm 0{\cdot}18$ mJ/g which is similar to the values of 2·6–4·2 mJ/g reported by Homsher *et al.* (1972) and Smith (1972). The time constant of the slow phase of A, 328 ± 47 msec, and the total amount of the slow phase, $39 \pm 3\%$ of A, are similar to the values of 331 msec and 42% respectively in the literature (Homsher *et al.* 1972).

In an effort to gather more information about the effect of temperature on force production and Ca^{2+} cycling, the temperature dependence of the slow phase of the stretched length energy liberation was compared with the mechanical properties of the twitch as shown in Fig. 5. If the slow phase of the Ca^{2+} cycling energy liberation reflects that rate of ATP splitting by the Ca^{2+} pump of the sarcoplasmic reticulum as suggested by Homsher *et al.* (1972) and if the muscle relaxation from an isometric twitch reflects Ca^{2+} removal from the sarcoplasm, one might expect both processes to have a similar temperature dependency. It is clear from Fig. 5 that these processes, $T_{\frac{1}{2}r}$, and τ_H, appear to have different temperature sensitivities. Thus one or both of the above simple assumptions seems unlikely as stated. The complexity of the situation is emphasized by the fact that none of the kinetic parameters of the isometric twitch displayed in Fig. 5 can be reasonably assigned a single Q_{10} value for the 0–20 °C temperature range.

A number of other pertinent observations from this work should be discussed. First, the results of Fig. 2 are consistent with the interpretation that peak muscle force production is a determinant of energy liberation irrespective of muscle temperature and thus irrespective of the magnitude of the time integral of muscle tension. This conclusion is similar to the one recently reached by Rall (1968) for experiments done at a constant temperature. If this conclusion is valid one would predict that the slopes of muscle energy liberation versus force production in an isometric twitch should be the same if the force is changed by altering muscle temperature (Fig. 2) or by altering muscle length. The slope of Fig. 2 is $0{\cdot}040 \pm 0{\cdot}005$ mJ/g per mN/mm^2. The slopes derived from the 0 °C stretch experiments with *Rana pipiens* semitendinosus muscles are: $0{\cdot}043 \pm 0{\cdot}002$ (Homsher *et al.* 1972) and $0{\cdot}048 \pm 0{\cdot}002$ mJ/g per mN/mm^2 (Rall, 1978). This agreement re-emphasizes the importance of peak force development as a determinant of muscle energy liberation. Interestingly Smith (1972) has observed a considerably higher slope of 0·074 mJ/g per mN/mm^2 for *Rana temporaria* semitendinosus muscles.

Results from this study provide information on the length dependence of Ca^{2+} release with stimulation over the range of l_0 to $1{\cdot}35\, l_0$. There is evidence in the literature of a length dependent decrease in Ca^{2+} release with stimulation (Blinks *et*

al. 1978; Frank & Winegrad, 1975). However Homsher & Kean (1978) have argued that the decrease in Ca^{2+} release measured by Blinks, Rudel & Taylor (1978) and Frank & Winegrad (1976) is likely to be less than 5% of the total Ca^{2+} released from the sarcoplasmic reticulum. The results of Fig. 2 are consistent with this argument. In Fig. 2 the estimated value of the activation heat is the same as the tension independent energy liberation obtained by changing force by altering muscle temperature at l_0. The precision of the experiments is not great, i.e. the intercept of the regression line of Fig. 2 has a standard error of $\pm 17\%$. Nonetheless the agreement between the values obtained for A, $3{\cdot}24 \pm 0{\cdot}11$, and for the intercept of Fig. 2, $3{\cdot}26 \pm 0{\cdot}55$, is striking. It should be noted that A as determined in these experiments is slightly over-estimated (by less than 10%) because twitch force was not completely eliminated. It seems likely that both techniques provide estimates of the same process(es), i.e. the energy liberation associated with Ca^{2+} cycling. The important implication of these results is that the magnitude of the Ca^{2+} cycling energy liberation is insensitive to muscle length in the range of $1{\cdot}0$–$1{\cdot}35\ l_0$ under the conditions of these experiments. Within the precision of these data these results suggest that the amount of Ca^{2+} released with stimulation in these experiments was unaltered by stretching the muscle from l_0 to $1{\cdot}35\ l_0$.

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