A Reexamination of the Thermoelastic Effect in Active Striated Muscle

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ABSTRACT   Isolated *Rana pipiens* sartorius muscles at $0°C$ were stimulated via their nerves and small stretches or releases applied during the plateau of the isometric tetanus at ℓ_o. Extra heat above the isometric maintenance heat was produced during the drop in tension caused by release and, for very small releases ($\Delta \leq$ $0.5\% \ \ell_o$), was completely reabsorbed during tension recovery. The extra heat was always directly proportional to the tension change. Heat absorption proportional to the tension change was also observed during the increase in tension produced by small stretches in the range $0.5\% \ \ell_o \leq \Delta\ell \leq 3.0\% \ \ell_o$. The mean heat:tension ratio R in seven experiments was -0.0084, which is within the range of values reported previously by Woledge. In addition, it was found that during tension recovery after small releases of $1.0\% \ \ell_o \leq \Delta\ell \leq 3.0\% \ \ell_o$ the "contractile" component seems able to shorten about $1\% \ \ell_o$ without producing shortening heat.

INTRODUCTION

After Hill's (1953) and Woledge's (1961, 1963) studies of the thermoelastic phenomenon in active muscle the thermoelastic heat has been routinely allowed for in drawing up the energetic balance sheet for any part of the cycle of muscle contraction and relaxation (e.g. Dickinson and Woledge, 1973; Homsher et al. 1973; Homsher and Rall, 1973; Aubert and Lebacq, 1971; cf. Woledge, 1971). Hill's study showed that when an active muscle is released to produce drop in tension there is a contemporaneous heat production proportional to the change in tension. It was inferred that an increase of tension must be accompanied by a corresponding heat absorption, but this phenomenon was impossible to demonstrate directly since any substantial stretch applied to the muscle to increase the tension is accompanied by heat production due to unspecified processes sufficient to overwhelm any thermoelastic heat absorption in the amount expected. Woledge used an indirect method and demonstrated that heat is indeed absorbed during tension increase both in the early part of contraction (1963) and during recovery of tension after a release (1961). More specifically, what these latter experiments showed was that heat production during tension rise falls below the expected value by an amount comparable to the thermoelastic heat. Hitherto there is no recorded case in which unambiguous thermoelastic heat absorption has been demonstrated by actual cooling of the muscle during tension increase. The thermoelastic correction, though small, would be more satis-

factory if such a condition could be found. With this in mind the present study was made to explore the thermal changes associated with very small changes in length and tension. From the standpoint of what is now known of the ultra-structure of muscle (cf. Huxley, 1969), it is reasonable to suppose that the use of smaller length changes would be less likely to upset steady-state cross-bridge interactions than would the larger changes used by earlier investigators. Such experiments would also provide a good opportunity to determine if any correlation exists between the mechanical responses to small length changes observed in single fibers and small fiber bundles observed by others (e.g. Huxley and Simmons, 1971; Sugi, 1972; Heinl et al., 1974) and thermal changes in whole muscle.

The use of very small length changes ($0.5\% \ \ell_o \leq \Delta\ell \leq 3.0\% \ \ell_o$) allowed us to observe unambiguously a heat absorption during tension increase, both during recovery after a very small release ($\Delta\ell \leq 0.5\% \ \ell_o$) and during a small stretch. Furthermore, the extra heat produced during tension recovery after releases in the range ($1.0\% \ \ell_o \leq \Delta\ell \leq 3.0\% \ \ell_o$) was less than the amount of shortening heat expected, indicating that some shortening of the muscle, about $1\% \ \ell_o$, can occur without the production of shortening heat.

METHODS

Rana pipiens of both sexes, $2^{1}/_{2}$–3 inches (5–7.5 cm) long, were obtained from Carolina Biological Supply Co., Burlington, N.C. The frogs were kept in large tanks at room temperature with a few inches of water in the bottom and platforms out of the water for them to sit upon. They were fed mealworms once a day, and only the most vigorous and fleshy frogs were used. Animals with parasites were also rejected.

The frogs were killed by decapitation and both sartorius muscles and their nerves dissected out, leaving the proximal ends of the muscles attached to the pelvic bone and tying each of the distal tendons to a silver chain link. The muscles were mounted on either side of a thermopile by means of a pelvis yoke fixed to the thermopile frame, and the links at the tendon ends were tied to a small silver chain brought out through the hollow neck of the thermopile frame and attached by a hook to a tension transducer. The thermopile assembly was equipped with one set of platinum electrodes for stimulating the nerves and another set for direct muscle stimulation. Nerve stimulation was used in all experiments reported here to eliminate the stimulus artifact heat associated with direct muscle stimulation. The muscles were subjected to a series of test stimuli at fixed length and inspected to ensure that no movement of the muscles over the active part of the thermopile occurred during stimulation. The thermopile assembly was then submersed in a muscle chamber containing oxygenated Ringer solution at 0°C, pH 7.0, which contained (mM): 111 NaCl, 2.5 KCl, 2.5 Na_2HPO_4, 0.69 NaH_2PO_4, and 1.8 $CaCl_2$. The solution was removed from the chamber during muscle stimulation. The muscles were bathed with the solution for 1–$1^{1}/_{2}$ h before proceeding with the experiment.

After another series of test stimulations to determine stimulus parameters and the length at which maximal isometric tension was developed (ℓ_o, about the same as the maximum *in situ* length of the muscle), the muscles were again allowed to rest. In the earlier experiments with releases, 15–20-min rest periods were allowed after each tetanus. For most of the later experiments in which both stretches and releases were applied, data were taken by tetanizing the muscles twice, the tetani of a pair of such stimulations being about 3 min apart, and then allowing the muscles to rest in oxygenated Ringer solution

for 15–20 min before the next pair of stimulations. The tension developed and heat produced in the second tetanus of a pair were always smaller than in the first, the difference in tension being about 5%, but no similar effect was observed on the change in tension on stretch or release or the thermal response to the change in tension.

Small stretches and releases at about 30–40 mm/s were made in the muscles with a pneumatic ergometer operating at constant velocity. The stretch or release stroke was initiated by the activation of a solenoid from the stimulation assembly, which reversed the direction of air flow in the ergometer. Thus the length changes could be time-locked to the stimulation of the muscles and were usually made to occur about halfway through the tetanus.

Tension was recorded by means of an RCA5734 force transducer (RCA Corp., Camden, N.J.) mounted on the end of the ergometer lever. The 5734 was linear in the range from 0 to 1.7 N and the transducer and connections had a compliance of 0.40 μm/mN.

Heat was measured by means of a Hill-Downing-type thermopile (cf. Hill, 1965) connected to a galvanometer whose output was amplified photoelectrically. Thermopile N, which was used for most of the experiments, had 67 AuPd-PtIr thermal junctions arranged in two banks. The top bank of 34 junctions had a resistance of 28 Ω and a sensitivity of 1.33 mV/°C. Thermopile 1, which was used in a few experiments, had four banks of constantan-chromel junctions. Its total resistance was 134 Ω and its sensitivity 2.27 mV/°C. The recorded temperature changes were converted to millijoules per gram of muscle by multiplying by the specific heat of muscle, 0.211 mJ/g/K (from Hill, 1965). The thermopile calibration and the validity of the conversion from temperature to heat were confirmed by electrically heating a pair of dead muscles with a known amount of energy and measuring the thermopile output.

To record the data, tension and heat signals were fed into two oscilloscopes and a computer of average transients (CAT). The first oscilloscope was programmed for a slow sweep time and used to visually monitor muscle tension and heat production for the entire stimulation period. The CAT, also with a slow sweep time, digitally recorded the tension and heat voltage signals for the entire stimulation period. This information was retrieved with an XY plotter and used to assess the general behavior of the muscle preparation. The second oscilloscope was programmed for a 1-s sweep and triggered approximately 100 ms before activation of the length change mechanism. A camera mounted on its face recorded tension and heat only during the 1-s period in which the length change occurred, thus providing a clear, magnified record of the muscles' thermal and mechanical responses during that time. Examples of CAT-XY plotter and photographic records are shown in Fig. 2 A and B, respectively.

For the muscles used in these experiments, the rate constant for heat loss for thermopile N was about 5%/s and about 3%/s for thermopile 1. The time intervals of interest were on the order of 300 ms, so that correcting for heat loss was unnecessary.

Correction for heating lag was somewhat difficult for the short time intervals under consideration. The time resolution of the system was quite good, in that its output in response to two 10-ms heating signals that occurred 10 ms apart could be easily distinguished from the output in response to one 20-ms signal (Fig. 1). Since it was not possible to analyze the records for lag at intervals as small as 10 ms, the lag for steady-state responses (i.e. shortening heat) was taken into account by shifting the heat trace backwards in time about 30 ms for thermopile N and 40 ms for thermopile 1, their respective time constants. The time constants were determined for each thermopile from the slope of a semilog plot of $(1 = (h/h_\infty))$ versus time from curves similar to Fig. 1a.

Previous work on the thermoelastic property of active muscle suggests that negative changes in tension are accompanied by production of extra heat and vice versa. There-

fore any transient tension change should be accompanied by a transient change of the opposite sign in temperature. If the temperature transient is rapid in comparison with the response time of the temperature-measuring system, then the system lag will not only cause a delay between the tension change and the recorded temperature change but will also cause the temperature signal to be attenuated. The amount of attenuation expected was determined for several sizes of thermoelastic response in the following way. Each tension response was approximated as a series of ramp functions of various durations. Temperature ramps of corresponding durations were applied to a pair of dead muscles and results recorded. These records were normalized for the relative slope of each ramp and combined to give a synthesized output which was then compared with the input to give the amount of signal attenuation due to heating lag. This procedure was carried out for each thermopile used and the thermoelastic heat ΔQ corrected for lag by multiplying by input/output. This method does not account for the small difference between the time required for heat to flow from the contractile apparatus through the connective tissue to
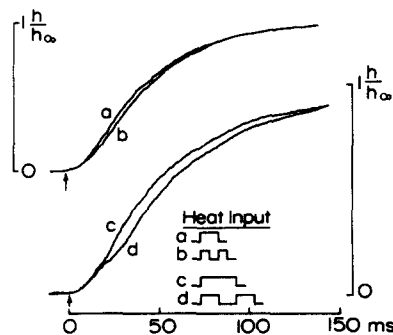
FIGURE 1. Time resolution of the heat-measuring system. Dead muscles were heated electrically by series of pulses with duration of 50 μs, amplitude of 80 V, and frequency of 1 kHz gated as shown under "heat input" in the figure. The system is able to distinguish somewhat between two 5-ms bursts separated by 5 ms *(b)* and one 10-ms burst *(a)*. The distinction is quite clear between two 10-ms bursts separated by 10 ms *(d)* and one 20-ms burst *(c)*.

the thermal junctions when the muscle contracts and the time required for heat flow from the connective tissue and the contractile apparatus to the junctions when the muscles are heated electrically. This would result in our attenuation figures and the thermoelastic ratio (i.e. $\Delta Q/e_{\Delta P}$) being slight underestimates of the true values.

The most serious source of error in experiments of this kind arises from the movement of the muscles over the thermopile during a length change. It was expected that the time course of a true thermal effect originating from the muscles would be independent of whichever bank of the thermopile was used to measure it, whereas the time course of a movement artifact would change as different banks were used. This expectation was confirmed by control experiments in which muscles were deliberately mounted in such a way that they moved visibly when stimulated. Data which could not be duplicated from different banks of the thermopile were rejected. Despite the fact that the thermopiles used had different geometries, the effects observed were indistinguishable once heating lag was taken into account.

A potentially serious artifact is the thermoelastic property of the thermopile frame itself (cf. Hill, 1965). The muscles are connected at one end to the frame, thus any force

changes in the muscles are also experienced by the frame and could conceivably be associated with thermal changes arising from the thermoelastic characteristics of the frame material. These thermal changes would then be sensed by the "cold junctions" of the thermopile, which are mounted upon the frame itself, and would be recorded as though they arose from the muscles. An experiment similar to the one described by Hill (1965) was made to test for the existence of such an effect. Fresh dead muscles were mounted on the thermopile and fixed in a sufficiently slack condition that the length changes used caused no force changes or thermal changes in the muscles. Stout thread was then attached to the apparatus in parallel with the muscles and connected to the force transducer and ergometer. Length changes were applied to the thread of a size such that the resulting force changes were similar to the largest of the force changes encountered in the experiments. No thermal changes were observed, thus the thermopile frame was eliminated as a potential source of aberrant effects.

RESULTS

The Thermoelastic Effect

Hill (1953) and Woledge (1961) have shown that quick releases in active muscle cause an extra production of heat that is directly proportional to the amount of tension change. Woledge's observations in which the length change was imposed during the tension plateau of an isometric tetanus were complicated by the production of shortening heat during the subsequent tension recovery, and the observation of heat absorption during a quick stretch was precluded by the large amount of heat produced from the energy of the stretch itself. The present study involved the use of somewhat smaller length changes, to see if the production of such large quantities of extra heat could be avoided. The results of a stretch and a release of 0.30 mm in a 37-mm muscle pair are shown in Fig. 2. In the enlarged responses in Fig. 2 B, the contrast between the thermal effects of stretch and release is immediately obvious. Stretch (+) results in a transient heat absorption, while release (−) causes a largely transient production of extra heat.

The magnitude of the initial transient phase of the thermal response to length changes was found to be proportional to the magnitude of the tension change. Two families of heat and tension responses to several amounts of length change are shown in Fig. 3.

Fig. 3 A shows tension and heat production in response to small stretches in the range of 0.4% ℓ_0 ≤ $\Delta\ell$ ≤ 2.4% ℓ_0. The thermal response consists of a transient absorption of heat during the rise in tension, followed by a production of extra heat as tension falls and the energy from the stretch is dissipated. The magnitude of the heat absorption is proportional to the magnitude of the tension change.

Fig. 3 B shows composites of heat and tension records during releases in the range 0.5% ℓ_0 ≤ $\Delta\ell$ ≤ 3.0% ℓ_0. The thermal response to the smallest release (a) is a transient production of heat during the fall in tension which is completely reabsorbed as tension is redeveloped. This thermal response is rather small but was a consistent finding in these experiments. Its time course also follows satisfactorily that predicted for a thermoelastic response, after the lag of the temperature-measuring system is taken into account. Larger releases (1% ℓ_0 ≤

$\Delta\ell \leq 3\%$ ℓ_o) produce a somewhat more complicated thermal response $(b\text{-}e)$. The initial phase of extra heat is still present and is proportional to the tension change. It is followed by a momentary decline in the rate at which the extra heat is produced before shortening heat evolves during tension recovery. The cause of this decline in heat rate is puzzling and will be examined in detail below. Here it is treated operationally as a convenient point in time for measuring the
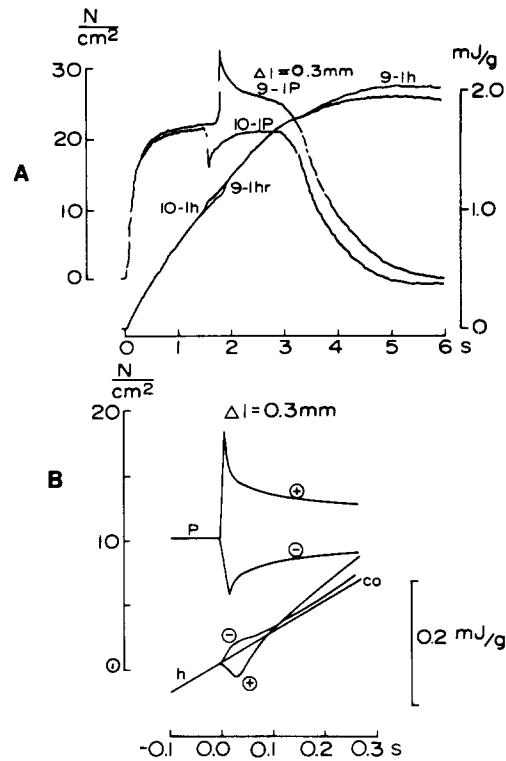

FIGURE 2. Tension (P) and heat production (h) during stretch and release. $\ell_o = 31$ mm, $m = 176$ mg, stimulus via nerve of 80 V, 200 μs, 10/s for 3 s. Summer frog. (A) CAT-XY plotter traces. Numbers refer to trace numbers. (B) Composite traces from photographic records of duplicate responses to stretch and release of same amount in same muscle pair as in A. $\oplus$, stretch, $\ominus$, release.

magnitude of the thermoelastic heat ΔQ, thus avoiding the tedious procedure of calculating the expected shortening heat and subtracting it from the observed extra heat for each individual record (Woledge, 1961). ΔQ for releases was therefore taken as the maximum value of extra heat attained during the first phase of extra heat production. These data were consistent with that obtained from stretches.

The proportionality between ΔQ and the tension change ΔP for stretches and releases is shown in Fig. 4. The effect of attenuation of ΔQ due to heating lag was taken into account as described in Methods. The data are tabulated fully by

experiment in Table I and are listed in the order in which they were recorded in each experiment. R does not appear to be dependent upon the order of data acquisition or upon $\Delta \ell$. As seen by the dates of the experiments, the first four were performed with muscles from winter frogs, while summer frogs were used in the last three. Since the difference between mean R's for summer and winter frogs was not statistically significant ($P > 0.05$), the data were pooled for linear regression analysis (solid line in Fig. 4). The slope of the line corresponds to $R = -0.0084 \pm 0.0002$, with a correlation coefficient of 0.986.
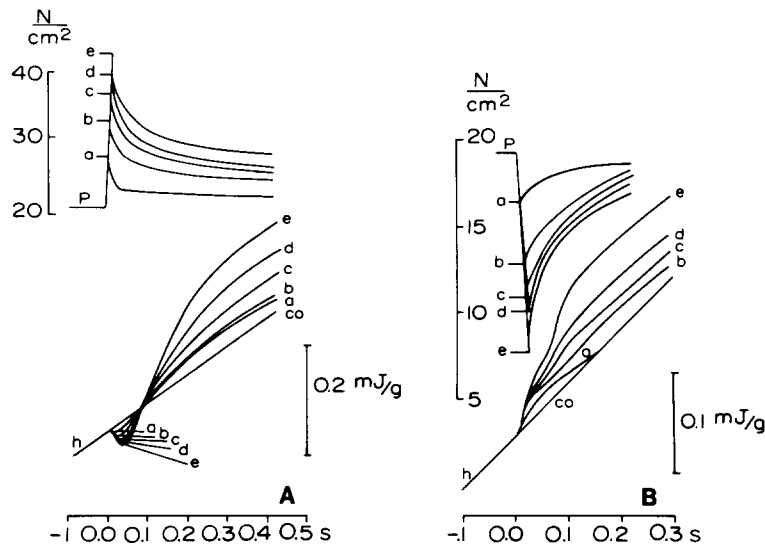

Figure 3. Thermal and mechanical responses to various amounts of stretch (A) and release (B). Curves traced from composites of photographic records of responses to duplicate length changes. Amounts of release in millimeters were: a, 0.15; b, 0.30; c, 0.45; d, 0.60; e, 0.90. (A) Results of experiment 8/24/74, summer frog. Same preparation as in Fig. 2. (B) Results of experiment 1/20/74, winter frog. h records shifted -30 ms to allow for heating lag. $\ell_o = 28$ mm, $m = 70$ mg.

Shortening Heat during Tension Redevelopment

The problem of interest here is the shortening heat (h_s) produced during tension redevelopment after a small release. As seen in heat trace a of Fig. 3 B, tension recovery without shortening heat after a sufficiently small release suggests that even in larger releases some early tension recovery occurs without h_s being produced. To examine these phenomena more closely, a comparison was made between the time course of recorded h_s (h_{sr}) and its expected time course (h_{sc}) calculated from Hill's shortening heat coefficient α (Hill, 1964). Four experiments were analyzed in this way, one of which is shown in Fig. 5.

The calculation of h_{sc} as a function of time was based on the assumption that tension recovery is accomplished by shortening of a contractile component against a series elastic component (SEC), with the production of shortening heat (Hill, 1938). The shortening heat is given by $h_s = \alpha x$, in which x is the distance

shortened read from the curve of ΔP versus $\Delta \ell$ (the length-tension curve of the SEC) determined in that experiment. α is given by Hill's relation $\alpha = (0.16 P_o + 0.18 P)$. Thus h_{sc} was calculated from P at 5-ms intervals, since x and α were both determined from P, and summed to give the time course of h_{sc} during tension recovery.

Comparison of this result with h_{sr} required that the time course of the second phase of the extra heat produced in response to release be separated from the
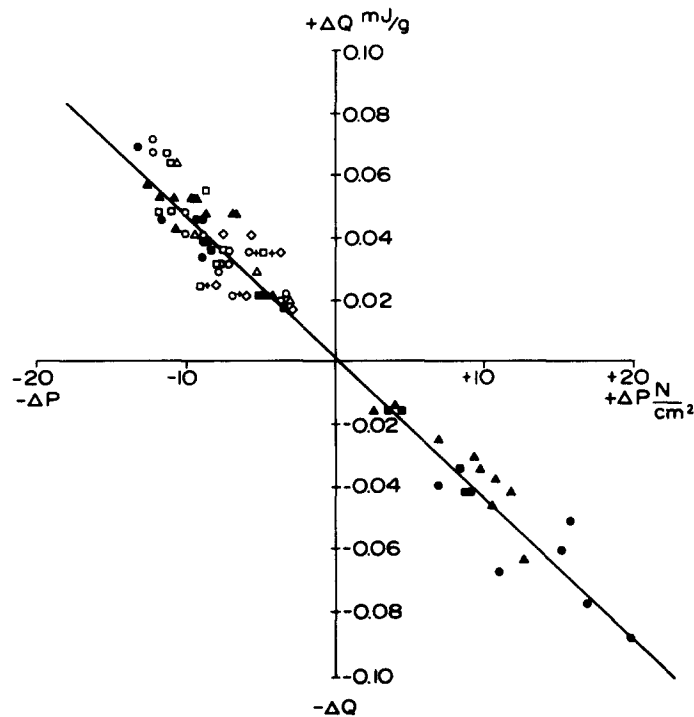

FIGURE 4. Linear relation between ΔP, the change in tension, and ΔQ, the first-phase extra heat in response to small length changes. ΔQ has been corrected for attenuation due to heating lag as described in Methods. Open symbols, winter frogs, closed symbols, summer frogs. Each symbol represents a separate experiment. The straight line was computed by a linear regression analysis of the data. The slope is equivalent to $R = -8.4 \times 10^{-3}$, with a correlation coefficient of 0.986.

time course of ΔQ, the thermoelastic heat. The time course of ΔQ was synthesized from the time course of the tension change for each amount if release and the lag characteristics of the thermopile-galvanometer system as described in Methods. Then ΔQ at 5-ms intervals was subtracted from the total extra heat recorded, leaving as the remainder h_{sr}, the recorded shortening heat. The total extra heat from release (eh), h_{sc} and h_{sr} for several amounts of release are shown in Fig. 5. For the smallest release of 0.15 mm, no shortening heat was recorded. (This recorded extra heat is virtually the same as the synthesized ΔQ for this amount of release.) After the 0.30-mm release (b of Fig. 5), tension recovery

proceeded for about 50 ms with no shortening heat, followed by a period of about 50 ms in which shortening heat was produced. Subsequent tension recovery proceeded with no further shortening heat. Responses to greater releases were similar, except that recorded shortening heat seemed to begin earlier with

TABLE I

ΔP AND ΔQ IN RESPONSE TO VARIOUS AMOUNTS OF RELEASE IN ISOMETRIC TETANI

Experiment	$\Delta\ell$	ΔQ	ΔP	$-R \times 10^{-3}$	Experiment	$\Delta\ell$	ΔQ	ΔP	$-R \times 10^{-3}$
	mm	mJ/g	N/cm²			mm	mJ/g	N/cm²	
12-29-73	-0.45	+0.036	-7.3	8.6	7-13-74	-0.15	+0.022	-5.1	7.6
	-0.60	+0.036	-7.7	8.2		-0.15	+0.022	-5.1	7.6
	-0.75	+0.048	-11.2	7.5		-0.30	+0.036	-8.5	7.4
	-1.05	+0.067	-11.4	10.3		+0.30	-0.043	+8.5	8.9
	-1.20	+0.048	-12.1	7.0	7-18-74	-0.075	-0.017	+2.4	12.4
	-1.20	+0.065	-11.2	10.2		+0.15	-0.014	+4.1	6.0
	-0.90	+0.055	-8.8	11.0		-0.15	+0.022	-4.3	9.0
	-8.60	+0.031	-8.0	6.8					
	-0.60	+0.031	-7.8	7.0		-0.30	+0.048	-7.1	11.8
1-19-74	-0.45	+0.041	-7.6	9.5		-0.30	+0.048	-6.9	12.2
	-0.30	+0.041	-5.8	12.4		+0.30	-0.026	+7.0	6.5
	-0.45	+0.036	-7.2	8.8		+0.30	-0.026	+6.8	6.7
	-0.45	+0.031	-7.3	7.4		+0.45	-0.031	+9.2	5.9
	-0.15	+0.017	-3.0	9.9		+0.45	-0.036	+9.6	6.6
	-0.60	+0.031	-7.9	6.9		-0.45	+0.053	-9.4	9.9
	-0.60	+0.029	-7.9	6.4		-0.45	+0.048	-8.8	9.6
1-20-74	-0.30	+0.036	-7.3	8.6		-0.60	+0.053	-11.0	8.4
	-0.30	+0.031	-7.3	7.4		-0.60	+0.043	-10.8	7.0
	-0.15	+0.019	-3.7	9.0		+0.60	-0.038	+10.6	6.3
	-0.15	+0.022	-3.4	11.3		+0.60	-0.048	+10.4	8.1
	-0.45	+0.041	-9.0	8.0		+0.90	-0.043	+11.7	6.4
	-0.45	+0.041	-9.0	8.0		+0.90	-0.065	+12.5	9.1
	-0.60	+0.048	-10.2	8.2		-0.90	+0.057	-12.7	7.9
	-0.60	+0.041	-10.2	7.0		-0.90	+0.053	-12.0	7.7
	-0.90	+0.072	-12.4	10.2	8-24-7	-0.30	+0.045	-9.0	8.8
	-0.90	+0.067	-12.4	9.5		-0.30	+0.045	-9.4	8.4
1-23-74	-0.30	+0.029	-5.3	9.6		-0.30	+0.038	-9.0	7.4
	-0.15	+0.019	-3.1	10.7		-0.45	+0.045	-11.8	6.7
	-1.20	+0.041	-9.6	7.5		+0.45	-0.062	+15.1	7.2
	-1.20	+0.065	-10.8	10.5		+0.45	-0.069	+15.7	7.7
	-1.05	+0.053	-9.6	9.7		+0.30	-0.053	+11.8	7.9
	-0.90	+0.053	-9.8	9.5		+0.30	-0.069	+10.8	11.2
7-13-74	-0.075	+0.017	-3.5	8.5		-0.30	+0.033	-9.0	6.4
	+0.075	-0.017	+3.5	8.5		-0.30	+0.038	-8.6	7.7
	+0.075	-0.017	+4.3	6.9		+0.15	-0.041	+6.8	10.6
	+0.075	-0.017	+3.5	8.5		+0.60	-0.079	+16.7	8.3
	+0.15	-0.036	+8.1	7.8		-0.60	+0.069	-13.3	9.1
	+0.15	-0.043	+8.9	8.5		+0.90	-0.091	+19.8	8.1

$\Delta\ell$, amount of release; ΔQ thermoelastic heat after correction for heating lag; ΔP, tension change; R, the thermoelastic heat:tension ratio.

increasing amounts of release. The same kind of analysis of other experiments produced similar results, indicating that active muscles regaining tension after a quick release are able to do so by two thermally distinguishable processes, one accompanied by the production of extra heat over and above isometric maintenance heat, the other not accompanied by such heat.

From this analysis it is possible to calculate further the amount of shortening that is *not* accompanied by shortening heat. The total amount of shortening

necessary to restore the tension to the level attained at the end of each tension record in Fig. 5 is simply the difference between the total amount of release applied by the ergometer and change of length corresponding to that tension on the length-tension curve of the SEC for that muscle pair. The amount of shortening accompanied by h_s is obtained for each 5-ms interval by dividing the increment of h_{sr} produced in that interval by α for the same time interval and summing over the time period of interest. The results of such a procedure are shown in Table II. The amount of shortening that occurs without shortening heat seems to approach 1% ℓ_o, or about 100 Å/half sarcomere.
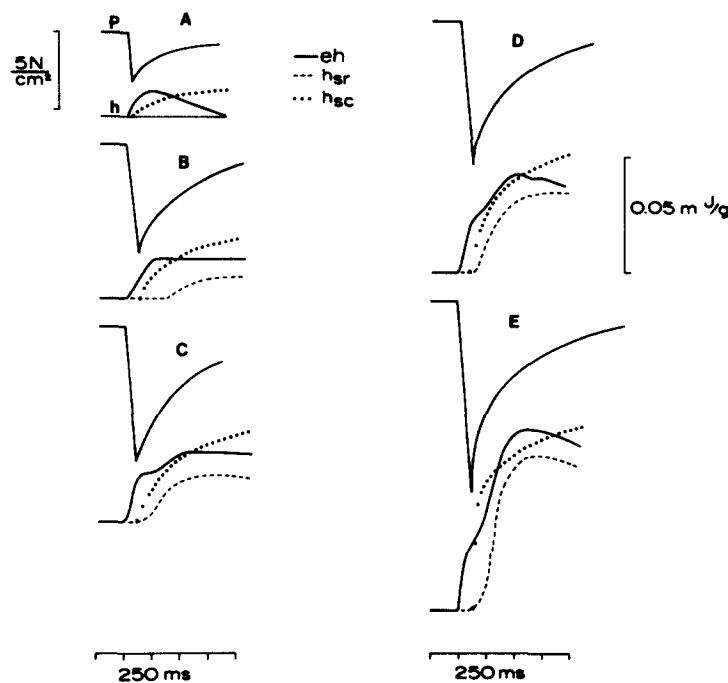

FIGURE 5. Comparison between calculated shortening heat (h_{sc}) and second phase of extra heat recorded during tension recovery after several amounts of release (h_{sr}). Analysis of data shown in Fig. 3 B. eh is the total extra heat resulting from release. The amount of release is given in millimeters in each tension record.

TABLE II

COMPARISON BETWEEN SHORTENING ACCOMPANIED BY SHORTENING HEAT AND TOTAL SHORTENING NECESSARY FOR TENSION REDEVELOPMENT

$\Delta\ell$	x_{tot}	x_h	x_{nh}	$x_{nh}, \% \ell_o$
0.15	0.10	0	0.10	0.4
0.30	0.26	0.07	0.19	0.7
0.45	0.38	0.16	0.22	0.8
0.60	0.53	0.33	0.20	0.7
0.90	0.81	0.55	0.26	0.9

$\Delta\ell$, amount of release; x_{nh}, amount of shortening not accompanied by shortening heat, calculated as described in text; $\% \ell_o - x_{nh}$ normalized to maximum in situ length of muscle.

DISCUSSION

The Thermoelastic Effect

The data presented above indicate that there is a tension-dependent, time-independent thermal change in fully active muscles during small length changes. Small stretches are accompanied by an increase in tension and absorption of heat, an effect which has not been previously observed directly. Small releases are accompanied by a decrease in tension and production of extra heat above isometric maintenance heat. The amount of heat produced or absorbed is proportional to the amount of tension change, and its time course follows that of the tension change. The heat:tension ratio R in our experiments is -0.0084, which is within the range of values reported previously (Woledge, 1961, 1963, 1971). Thus our data lead to the same conclusion reached by the earlier investigators; active muscle does possess normal, rather than rubberlike, thermoelastic behavior.

Alternative Interpretation of Thermoelastic Behavior

Huxley and Simmons (1972) raised the question of whether the "thermoelastic" effect in active muscle might in fact be heat from enthalpy changes associated with "stepping" of the myosin "heads" from one to another of several stable configurations in their attachments to actin. They suggested an experiment to distinguish between thermoelastic heat and stepping heat. From their mechanical experiments on single fibers, they had obtained two curves of tension versus length change $\Delta\ell$. The T_1 curve is approximately the length-tension curve of the instantaneous elasticity within the muscle fibers and is nearly linear in the range $-0.1\% \ \ell_0 \leq \Delta\ell \leq 0.6\% \ \ell_0$. T_2 is the tension level attained at the end of the very rapid tension recovery caused by the stepping reaction and changes very little from the isometric tetanic tension in the length range $-0.4\% \ \ell_0 \leq \Delta\ell \leq +0.4\% \ \ell_0$ (see Huxley and Simmons, 1971, figure 3). They suggested that heat production should be measured while the muscle is released slowly from the tetanic tension plateau along the T_2 curve. If the extra heat observed is of thermoelastic origin, then it should be proportional to the tension change and will be very small or even zero for small length changes and substantially larger for larger releases. If the heat comes from the stepping reaction, however, it should be proportional to the extent of stepping, which is the horizontal distance between the T_1 and T_2 curves or the amount of length change. This heat would be present even for small releases.

While the velocity of release employed in these experiments was certainly slow in comparison with the experiments of Huxley and Simmons, the tension changes observed do not appear to lie along their T_2 curve. Vibrations in our length-changing apparatus prevented our observing the force transients seen by Huxley and Simmons and others, but comparison of our muscle "stiffness" (ratio of $\Delta P/P_0$ to $\Delta\ell/\ell_0$ for a particular amount of release) with theirs suggest that our tension changes lie closer to their T_1 curve. Similar observations were made in whole toad muscles by Bressler and Clinch (1974) using a more precisely controlled and vibration-free mechanical system with lower compliance. They showed that the series elastic component length-tension curve departs from

linearity with slow releases only for releases of $\Delta\ell \leq 1\%\ \ell_o$ (their figure 6 a). Thus it seems that the behavior of whole muscle precludes the kind of experiment Huxley and Simmons suggested. Perhaps the ambiguity could be somewhat alleviated by examining the extra heat produced upon release as a function of release velocity: slower releases would be accompanied by more stepping and more heat of stepping, whereas faster releases would be accompanied by greater tension changes and therefore greater thermoelastic heat.

Our experiments do suggest that ΔQ is proportional to ΔP rather than to $\Delta\ell$, however. Examination of the data in Table I shows that R does not vary in a consistent way with $\Delta\ell$, whereas if ΔQ was proportional to $\Delta\ell$, R would be expected to increase with $\Delta\ell$ for the larger length changes where our ΔP versus $\Delta\ell$ curves become flat. While our results do not rule out the possibility that the first phase of extra heat on release is associated with the hypothetical stepping reaction, they do argue against that conclusion, insofar as the stepping model of Huxley and Simmons is presently able to predict the nature of such an association. Further examination of both the model and thermal responses of this sort is indicated.

Shortening Heat during Tension Recovery

The results reported above indicate that shortening heat is produced with a time course that is different from what is expected. In calculating the expected shortening heat we have used Hill's (1964) value for the shortening heat coefficient α. Homsher et al. (1973) have pointed out the importance of the thermal base line in calculating this h_s. In the present experiments our base-line conditions were effectively stabilized by using only tetani, and employing very small length changes in which there was no shift along the length-tension curve. Furthermore, no simple change in this coefficient could account for the somewhat complicated time course of the observed shortening heat. One may ask why observations of this sort have not been made before. The only experiments strictly comparable to those reported here are those of Woledge (1961). Close examination of his figure 4, specifically lines 3 and 3 a, indicates that he slightly "overcorrected" his records for shortening heat after a release of about 1% ℓ_o. If this sort of discrepancy was a consistent finding in his experiments, which it seemed to be in ours, then it supports our findings.

Residual uncertainty surrounding the comparison of observed and calculated shortening heat lies in the heating lag correction applied to the records. Analysis of the records for the short time intervals required produces oscillatory solutions and is therefore invalid, thus less than ideal substitute methods were used. It is useful here to examine the sort of errors that would result from errors in lag correction. In generating our h_{sr} curves in Fig. 5, we have accounted for lag in two ways as explained in Methods: (a) the ΔQ curves were synthesized as functions of time from the tension time course and the lag characteristics of the system; (b) the recorded extra heat curves were shifted backwards in time by one time constant. Synthesis does yield a ΔQ time course satisfactorily close to the observed ΔQ for small releases in which no h_s is produced (a of Fig. 5), and the rising phases of the extra heat responses to larger releases are also fitted by

similar syntheses from the appropriate tension time curves. The shift method is not suitable for very fine time discrimination early in the temperature rise, and the early transient changes are blurred for this reason. This kind of distortion may contribute to the early difference between h_{sc} and h_{sr} but cannot account for the fact that the distortion is greater after small releases than large ones (compare curves b and e of Fig. 5). Neither can it explain why the production of h_s ceases while tension redevelopment and shortening still continue late in the tension recovery process.

It remains to attempt to relate the present findings to contemporary views on the mechanics of muscle contraction. Why, following these small releases, does tension recovery proceed first without the production of h_s, later with h_s and finally without h_s again? This observation certainly suggests that following a small release the cross bridges detach in a way that is different from the way they detach during isotonic shortening and probably during the isometric tension plateau. Several reasonable explanations come to mind in this regard. Perhaps shortening heat is only produced when bridges break under negative tension, which they would do during isotonic shortening but not after small length changes. Perhaps shortening heat is obscured by a decrease in isometric maintenance heat resulting from bridges breaking under a lower tension after a quick release. Perhaps isotonic shortening necessitates conformational changes in the cross bridges that are associated with heat production, whereas tension recovery after a small release requires less drastic cross-bridge adjustments. It seems likely that further examination of recent mechanical models (e.g. Julian and Sollins, 1974, 1975) could be productive along these lines.

On less solid ground, it is tempting to try to relate the thermal changes observed to the "stepping hypothesis" of Huxley and Simmons. Perhaps stepping is thermally neutral with respect to isometric maintenance heat, while cross-bridge relocation produces heat. The thermal changes we observed are much slower than the mechanical transients underlying the stepping hypothesis. However, it is possible that nonuniformities among fibers of a whole muscle have a "spreading" effect, so that the response to release is sluggish. Certainly the abovementioned observations of Bressler and Clinch on whole muscle suggest that all of the stepping is not over by the end of even a slow release of sufficiently small size. The only way this speculation can be tested is by measuring heat during experiments made with better mechanical apparatus to produce data more directly comparable to that from single fiber experiments.

Certainly more careful and precise studies of the kind presented here will be useful in ultimately understanding the details of energy conversion in muscle contraction. More than a century after the first attempts to measure heat production in active muscle, the full extent of the technique's usefulness in giving a clue to the shape of the final set of answers about muscle contraction is still not fully appreciated.

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