

## THE KINETICS OF HEAT PRODUCTION IN RESPONSE TO ACTIVE SHORTENING IN FROG SKELETAL MUSCLE

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### SUMMARY

1. Tension and heat production were measured at 0 °C in sartorius muscles from *Rana temporaria* in response to two extents of shortening at five velocities. Shortening was from approximately 2.4 to 2.2  $\mu\text{m}$ , 2.4 to 2.3  $\mu\text{m}$  and 2.3 to 2.2  $\mu\text{m}$  at velocities per half-sarcomere from 0.2 to 1.56  $\mu\text{m s}^{-1}$ .

2. Tension became approximately constant at all velocities. Records of heat rate, obtained by differentiating traces from which thermoelastic heat had been subtracted became negative early in shortening and then rose. Heat rate became constant during shortening only at the lowest velocity and was still rising at the end of shortening at higher velocities. The highest heat rate occurred at the end of shortening at the two highest velocities. At the end of shortening at all velocities heat rate gradually approached the isometric level measured at the short length, the half-time for decline being largest following the slowest larger shortening.

3. Heat produced as a consequence of shortening but not associated with tension recovery was determined by subtracting shortening heat measured in response to two extents of shortening to the same muscle length. The differences in shortening heat continued to increase after shortening ended, and more of the extra heat produced in response to shortening appeared after the end of rapid shortening than during shortening itself.

4. Shortening heat coefficients calculated in different ways were similar to coefficients determined in previous studies. Coefficients calculated from measurements that excluded heat produced by tension recovery and allowed for continued production of heat by processes initiated by shortening were found to increase linearly with the force maintained during shortening.

5. The results show that the kinetics of heat production during and after shortening are very sensitive to the speed of shortening and that steady rates of energy liberation are not attained during shortening of  $\leq 10\%$  of muscle length at velocities  $\geq 12\%$  of maximum velocity.

### INTRODUCTION

Characterization of the energetics of transient and steady states in actively shortening muscles is required for the development of a comprehensive theory of the mechanism of energy conversion. It has been known since the studies of Fenn (1923,

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1924) and Hill (1938) that muscles liberate more energy as both heat and work during steady shortening than when they maintain force isometrically. In developing his cross-bridge theory of muscle contraction, A. F. Huxley (1957) used Hill's measurements of velocity and heat production during shortening under different loads to estimate rate constants for attachment and detachment of cross-bridges. A modification to the theory postulating that attachment occurred in two steps (Huxley, 1973) accounted for Hill's subsequent discovery that the shortening heat coefficient decreased with increasing velocity (Hill, 1964).

Both of Hill's formulations of shortening heat imply that muscles produce heat and work at a steady rate throughout shortening. In recent studies (e.g. Irving & Woledge, 1981*a, b*; Homsher, Irving & Wallner, 1981; Homsher, Yamada, Wallner & Tsai, 1984), shortening heat has been determined as the difference in heat production measured at the beginning and end of an interval of contraction which included shortening. The experiments were not designed to distinguish between energy liberation associated with steady states and energy produced by transitions between steady states. Where small extents of rapid shortening are involved, much of the energy could be associated with the transitions.

Observations from energy-balance studies by Homsher and his colleagues (Homsher *et al.* 1981, 1984) have called attention to the need for a more careful characterization of the kinetics of energy liberation by shortening muscles. The time during shortening at which balance was achieved between energy liberation and substrate utilization was sensitive to velocity. During shortening at  $V_{\max}$  too little phosphocreatine was hydrolysed to account for the energy being liberated simultaneously, while a few hundred milliseconds after shortening ended energy balance had been restored (Homsher *et al.* 1981). The extent of phosphocreatine hydrolysis was sufficient to account for the energy liberated during shortening at half  $V_{\max}$  (Homsher *et al.* 1984). The results show that transient energetic states can persist for several tenths of a second after shortening begins, which is substantially longer than the duration of mechanical transients (Podolsky, 1960; Huxley & Simmons, 1971). Furthermore, both studies showed that both the heat and the work produced as the result of shortening were adequately explained by the amount of phosphocreatine hydrolysed. It is therefore unlikely that either heat or work results from unidentified biochemical reactions, and measurements of the kinetics of heat production can provide information pertinent to the kinetics of energy conversion not available from other kinds of experiments.

The purpose of the experiments reported here was 2-fold: first, to measure the kinetics of heat production during shortening at several velocities; secondly, to characterize the heat produced as a direct consequence of shortening, exclusive of processes involved in tension recovery at the end of shortening, by determining the difference in heat produced in response to two extents of shortening in which tension recovery was the same.

#### METHODS

##### *Preparation and experimental procedure*

Pairs of sartorius muscles from *Rana temporaria* were used. Frogs were stored in a refrigerator at 4 °C for 1–4 weeks prior to the experiments. After dissection the muscles were mounted one on

each side of a thermopile. The pelvis was held in a clamp attached to a photo-electric force transducer mounted on the thermopile frame. The tibial tendons were looped through links from a platinum chain and tied very close to the insertions of the muscles. The links were tied together and caught by a hook at one end of a length of stainless-steel tubing. The other end of the tubing passed through the neck of the thermopile frame and was attached to a hook on a strain-gauge force transducer mounted on the moving element of the servo-motor used to make length changes.

Striation spacing was measured by laser diffraction in the relaxed muscles at each of the muscle lengths to be used, both before and after the experiment, to verify that the correct spacing had been maintained. The thermopile frame was screwed into an aluminum chamber-reservoir assembly and clamped to an adjustable stand and the chamber-reservoir submerged in a large Dewar flask containing a mixture of ice and water. Ringer solution was forced into the chamber from the reservoir by a flow of oxygen and drained from the chamber when the direction of oxygen flow was changed. The direction of oxygen flow was controlled by solenoid valves activated through switches by signals from a computer, which controlled the timing of the experiment as described below. The composition of the Ringer solution was (mM): NaCl, 113.5; KCl, 2.0; CaCl<sub>2</sub>, 1.8; phosphate buffer, 2.0. Its pH at room temperature was 7.0. The experiments were performed at 0 °C.

The muscles were bathed in oxygenated Ringer solution for at least 1 h prior to the experiment. During the experiment they were stimulated for 2.5 s by suprathreshold pulses (duration 0.5–0.8 ms, frequency about 11 s<sup>-1</sup>) generated by a WPI isolated stimulator (W-P Instruments, New Haven, CT) and applied to pairs of platinum electrodes at each end of the thermopile. No heat from the stimuli appeared during recording intervals as long as 10 s in a preparation inactivated by exposure to an isotonic solution of sucrose and KCl (see Gilbert, 1978). Solution was forced into the chamber for 3 min between contractions and drained from it for 2 min before stimulation. The stability of the thermopile signal for at least 30 s prior to stimulation indicated that the 2 min draining interval was sufficient for thermal stability to be achieved.

All the experiments in the study were identical except for the order in which the different types of contractions were made. Each experiment consisted of at least a dozen preliminary contractions followed by eighteen types of contractions from which the data were analysed. Three types of shortening were applied, after 1.4 s of isometric contraction, at each of five velocities, from long (columns labelled 1 in Table 1) to intermediate length (2), from intermediate to short length (3) and from long to short length. The smaller extent of shortening was made to occur through both halves of the length range of the larger extent to assess the effects of the difference in muscle length on mechanical performance and heat production, as described in more detail under the heading Control observations. Measurements were also made during fully isometric contractions at each of the three lengths. The sequence of contraction types was assigned randomly in each experiment and repeated in reverse order to compensate for the decline in isometric force during the experiment by averaging the two records. The average decrease in force was 11 % for the seven experiments. The extents of shortening were 1.5 and 3.0 mm (about 58.5 and 117 nm half-sarcomere<sup>-1</sup>) and the velocities were 5–40 mm s<sup>-1</sup> (about 0.2–1.56  $\mu\text{m s}^{-1}$  per half-sarcomere).

The timing and other aspects of the experiments were controlled by a Nova 4/S minicomputer (Data General Corporation, Westboro, MA), which also recorded the data. Digital output signals triggered the stimulator and two switches, one to select the gain of the heat signal and a second to control the direction of oxygen flow to fill and drain the chamber. The command signal to the length changer was delivered from one of the digital-to-analog channels of the computer, and the heat signal was reset to zero by the output from another digital-to-analog channel.

### *Thermopile*

A single thermopile (H1M) was used for all experiments. It consisted of sixty active thermal junctions (constantan-chromel) arranged in three banks each 4 mm long and each containing twenty junctions, with protecting regions of 2 mm at the top and 1 mm at the bottom of the instrument. The sensitivity was 50  $\mu\text{V deg C}^{-1}$  per junction. The total length of the thermopile and electrodes was 22 mm. In the first two experiments of the study heat production was recorded separately from the bottom and centre banks of the thermopile. Heat production measured from the two regions was the same, and subsequent experiments were made with the two banks connected in series. Signals were amplified by an Ancom 15C-3a chopper-stabilized amplifier (Ancom Ltd., Cheltenham), whose output was delivered to a second-stage amplifier and to a digitally triggered

T switch. The T switch allowed the output of either the first- or second-stage amplifier to be delivered to the 'heat' channel of the analog-to-digital converter of the computer.

Characteristics of the two transducers and of the length changer have been described previously (Gilbert & Ford, 1986). The total compliance of the two transducers and connexions to the muscles (exclusive of tendon compliance) was  $114 \mu\text{m N}^{-1}$ .

#### Data acquisition and analysis

Analog signals of length, force from each transducer and heat production measured from one or two sections of the thermopile were digitized by analog-to-digital conversion channels of the computer and stored on disk. A record from a single experiment is shown in Fig. 1. The identity

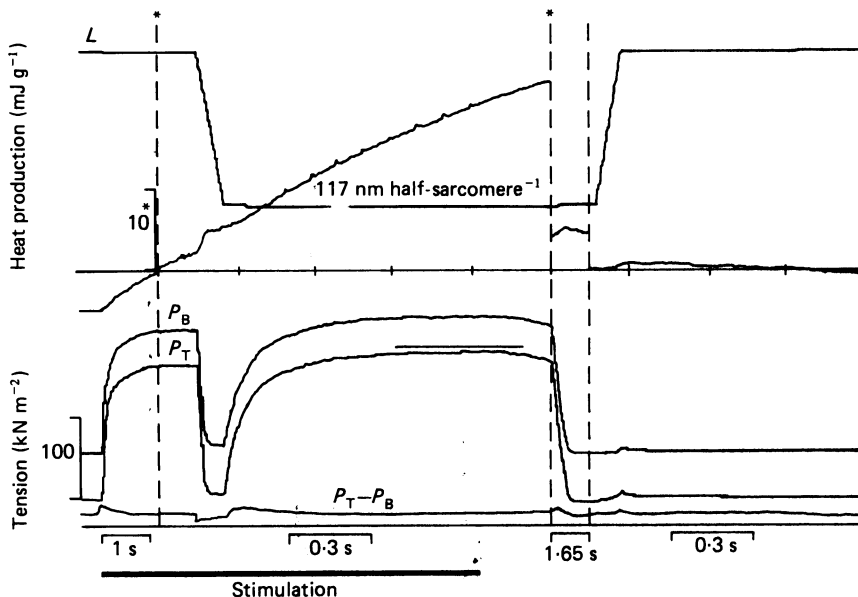


Fig. 1. Digitized records of length ( $L$ ), heat production and tension recorded from the pelvic ( $P_B$ ) and tibial ( $P_T$ ) ends of the muscle. The tension difference across the muscle is shown by the lowermost record ( $P_T - P_B$ ). Data were sampled by four channels of the analog-to-digital converter in four time sections (vertical dashed lines), each with different sampling frequencies and gains for the heat records. The heat calibration bar (\*) pertains only to the second and fourth time sections. Time calibrations and the period of stimulation are shown beneath the records. The velocity of shortening per half-sarcomere was  $1.17 \mu\text{m s}^{-1}$ . Experiment 4, blotted muscle mass 178 mg; see Table 1 for other details.

of each signal is specified in the legend. Each record consisted of four time sections, indicated by the vertical dashed lines, in which both conversion frequency for all signals and amplification of the heat signal were changed, to magnify selected portions of the record. Sampling intervals were 16.9, 3.0, 33.0 and 3.0 ms per point, respectively. The amplification of the heat signal was increased at the beginning of the second and fourth sections of the record by a T switch controlled by a pulse from the digital output module of the computer that selected between the first- and second-stage amplifier signals passing through the switch into the heat channel of the analog-to-digital converter. During rapid sampling the amplification of the heat signal was about eight times its value during slow sampling. The heat base line was reset to zero by making the input to the second heat amplifier the difference between the last point recorded by the computer in the previous time section, which was delivered from one of its digital-to-analog channels, and the output of the first heat amplifier. Programs for analysing data could sample any combination of records, analog-to-digital conversion channels and time sections.

The command signal for the length changer was delivered through a digital-to-analog channel whose conversion interval was the same as that of the analog-to-digital converter. The muscles were arbitrarily restretched at the same velocity at which they had shortened. Four time sections were used in the records to avoid the choice between sampling many points of unused data at a high frequency during relaxation and applying a series of voltage steps to the length changer by having the muscles restretched when the analog-to-digital and digital-to-analog converters were operating at a low frequency. (The heat change apparent during restretch in the fourth time section of Fig. 1 is a movement artifact which probably resulted from uneven relaxation along the muscle, since it varied in size and direction from one contraction to another and was also different in records made simultaneously from two banks of the thermopile in the first two experiments.)

The first step in analysis was to average each of the four or five signals for pairs of like contractions in the experiment. All subsequent operations were performed on the averaged records. Isometric tension developed before shortening was determined from the first time section. All other measurements were made from the second time section.

*Correction for heat conduction lag.* The thermopile signal  $h(t)$  at time  $t$  in response to a sudden production of heat by the muscle  $h_0$  is given by Hill's solution of the diffusion equation (Hill, 1965):

$$h(t) = h_0 [1 - \operatorname{erf}(z/t)^{\frac{1}{2}}]. \quad (1)$$

The value of  $z$  was calculated from the thicknesses and thermal capacities of the layers of insulation, solution and tissue interposed between the myofibrils and the thermal junctions, as described in more detail in the following section.

#### *Control observations*

The following control observations were made to assess the effects of several possible artifacts.

#### *Mechanical measurements*

*Resistance to movement over the thermopile.* Tension was measured at both ends of the muscle in order to estimate the heat produced by a mechanical resistance to movement of the preparation over the thermopile. Gilbert & Ford (1986) found that a substantial quantity of the heat measured when length changes were applied to rigor muscles was characteristic of heat produced by such a resistance. As shown in Fig. 1 (lowermost trace), the tension difference in these experiments was very small. The 'movement' heat calculated from it was less than 5% of the shortening heat and was ignored, since it was within the standard errors of the measurements. The difference between these observations and those of Gilbert & Ford (1986) was shown by separate experiments to result from insulating this thermopile with mylar instead of kapton, which had been used on the older instruments.

*Effects of differences in muscle length.* Isometric tension, heat rates and striation spacings at three muscle lengths are summarized in Table 1 for seven preparations. Striation spacings were measured in the relaxed muscles before and after each experiment. Quick releases of 0.5–0.6 mm were sufficient to drop the isometric force to zero, indicating that the total compliance of the tendons and apparatus was about 2% of muscle length. The striation spacings at the beginning of shortening were therefore about 2.4, 2.3 and 2.2  $\mu\text{m}$  at muscle lengths 1, 2 and 3. Heat rates were measured from 1.27 to 2.5 s of stimulation and tension at 1.27 s. All experiments were made in the length range in which heat production during shortening is proportional to filament overlap, and records of heat production made during length changes applied to unstimulated muscles showed that no heat was produced by the thermoelastic properties of the passive muscle (Homsher, Irving & Lebacqz, 1983; Gilbert, 1986).

Heat produced as the result of shortening from 2.3 to 2.2  $\mu\text{m}$  was to be subtracted from that produced by shortening from 2.4 to 2.2  $\mu\text{m}$ . Differences in filament overlap could produce differences in tension changes at the beginning and end of shortening at the two initial sarcomere lengths, which would contribute to differences in heat produced in response to two extents of shortening because of thermoelastic effects (see Gilbert & Ford, 1986) and differences in the extents of thermally significant processes responsible for tension recovery.

To assess the effect of muscle length on the drop in tension at the beginning of shortening, changes in tension resulting from the smaller extent of shortening through the two length ranges were measured at all velocities. Differences between the tension changes measured in the two length ranges were calculated separately in each experiment and averaged at each velocity. None of the

mean differences was statistically significant by Student's *t* test for paired observations, indicating that the drop in tension at the beginning of shortening was the same at 2.4 and 2.3  $\mu\text{m}$ . Therefore unequal thermoelastic effects at the beginning of shortening did not contribute to differences in shortening heat produced by two extents of shortening.

TABLE 1. Striation spacing, isometric tension and heat rate in seven preparations

Experiment	Striation spacing ( $\mu\text{m}$ )			Isometric tension ( $\text{kN m}^{-2}$ )			Heat rate ( $\text{mW g}^{-1}$ )			$L_0$ (mm)	$L_m$ (mm)
	1	2	3	1	2	3	1	2	3		
1	2.47	2.36	2.25	194	210	214	12.8	14.3	15.4	30.5	31.0
2	2.41	2.30	2.19	168	164	162	11.0	10.9	10.6	30.0	28.5
3	2.53	2.40	2.27	184	192	205	12.2	15.0	15.0	26.1	29.5
4	2.54	2.42	2.30	160	177	180	12.6	13.3	14.9	28.0	30.0
5	2.47	2.35	2.23	145	132	136	9.0	9.4	10.0	28.0	30.0
6	2.47	2.36	2.25	202	216	238	13.1	13.9	15.7	28.8	28.5
7	2.48	2.35	2.22	139	158	173	13.7	14.9	16.6	25.8	27.0
Average	2.48	2.36	2.24	170	178	187	12.1	13.1	13.9	28.2	29.2
S.E. of mean	0.02	0.01	0.01	9	11	13	0.6	0.8	0.9	0.7	0.5

1, 2 and 3 indicate long, intermediate and short lengths, respectively. Striation spacings were measured in resting muscles.  $L_0$  - length of muscles at striation spacing of 2.2  $\mu\text{m}$ ;  $L_m$  - maximum *in situ* length of muscles. The average blotted mass of the muscles was  $153 \pm 10$  mg.

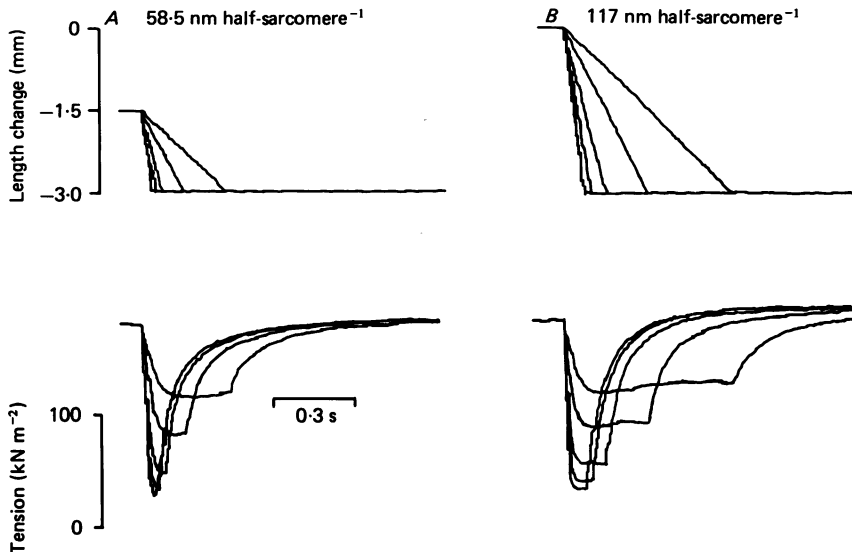


Fig. 2. Tension responses to two sizes of shortening at five velocities. Averages of records from seven experiments. Velocities per half-sarcomere were 0.2, 0.39, 0.78, 1.17 and 1.56  $\mu\text{m s}^{-1}$ . Both extents of shortening ended at a striation spacing of about 2.2  $\mu\text{m}$ .

*Effects of velocity and extent of shortening on tension recovery.* Fig. 2 shows records of tension during and after shortening from intermediate to short length (A) and from long to short length (B). Both the extent and the speed of tension recovery were affected by the extent and the speed of the preceding shortening. The records in Fig. 2B show that the extent of tension recovery diminished

as velocity decreased, as characterized in detail by Marechal & Plaghki (1979). Tension recovery following both extents and all velocities of shortening was shown by regression analysis to fit the equation:

$$P(t) = P_f - [P_f - P_s][1 - \exp(-kt)], \quad (2)$$

in which  $P_f$  is tension at the end of stimulation,  $P_s$  the tension at the end of shortening,  $P(t)$  the tension redeveloped at time  $t$  after the end of shortening and  $k$  the rate constant for tension recovery. The equation describes the time course of tension recovery normalized to the total amount of tension redeveloped. Regression analyses of the linear form of the equation were made separately for each shortening velocity in each experiment. All regression coefficients were greater than 0.97.

Muscle length had no effect on the rate constants. Mean differences in  $k$  for the two types of small shortening at all velocities in each experiment were calculated and their significance tested by the Student's  $t$  statistic for paired observations. None of the differences was significant ( $P > 0.05$ ).

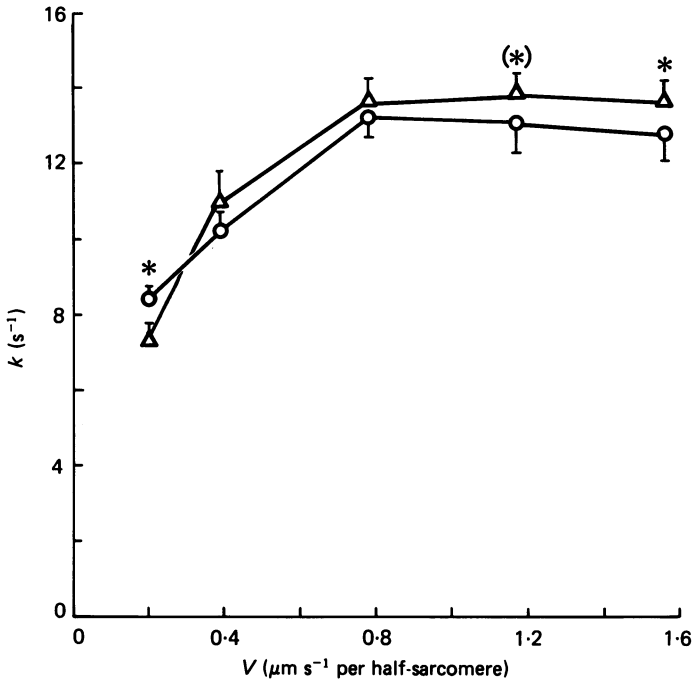


Fig. 3. Effect of velocity on rate constants of tension recovery following two extents of shortening. Means and standard errors (vertical bars) of seven experiments. Extents of shortening in nm half-sarcomere $^{-1}$  were 117 (O) and 58.5 ( $\Delta$ ; averages for shortening through two ranges of muscle length). Significances of differences were determined by Student's  $t$  tests for paired observations: \*,  $0.001 < P < 0.01$ ; (\*\*),  $0.05 < P < 0.1$ ; other differences not significant ( $P > 0.10$ ).

The effect of velocity on the rate constants is shown in Fig. 3. Significant differences in the rate constants for the two extents of shortening are indicated by the asterisks. Although the differences were not substantial, recovery was slower following the smaller than the larger shortening at the lowest velocity and higher following the fastest shortening. Thus differences in heat production obtained by subtraction of records from two extents of shortening could contain small contributions from differences in the kinetics of tension recovery. These contributions were minimized by determining differences in shortening heat measured at various time constants ( $\tau = 1/k$ ) of tension recovery, when the fractional extents of tension recovery were the same following the two extents of shortening.

### Heat measurements

*Correction for heat conduction lag.* The correction was applied to the second time section of records from which the heat produced during isometric contractions at the initial muscle length had been subtracted. Analysis of simulated records showed that correcting the total heat for lag and then subtracting the isometric heat from the result was equivalent to making the correction on the subtracted record. Simulated records were produced by convolving combinations of different speeds of ramps with eqn. (1). For full simulated records, results were independent of the order of subtraction and deconvolution. For partial records, like the second time section analysed here, subtraction of isometric heat prior to deconvolution was necessary to avoid a small error at the beginning of the time section.

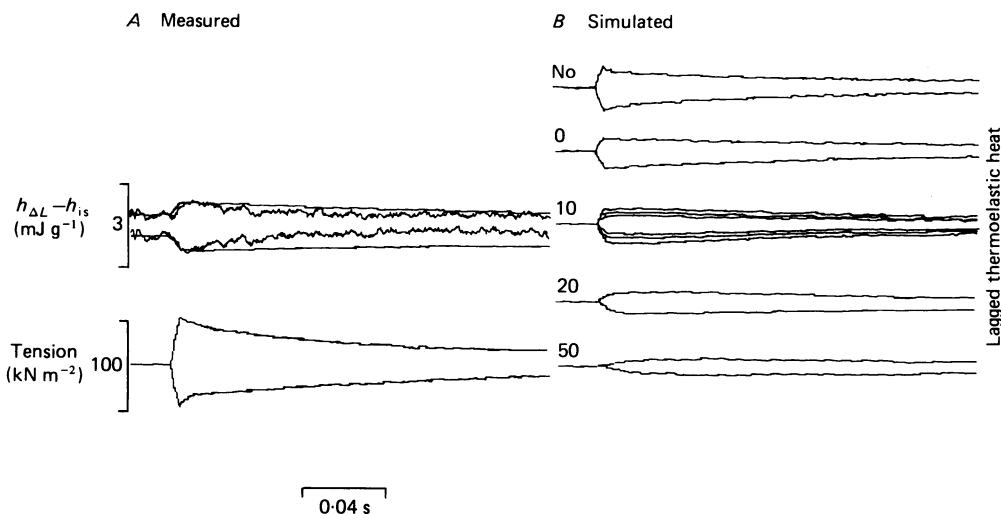


Fig. 4. *A*, tension and heat production in response to a 0.1 mm stretch and release. Traces are from the second time section of records similar to that shown in Fig. 2 except that the sampling interval was  $0.4 \text{ ms point}^{-1}$ . Isometric heat ( $h_{is}$ ) at the initial length was subtracted from the heat records ( $h_{\Delta L}$ ). *B*, simulated time courses of thermoelastic heat expected from tension changes in *A*. The traces labelled No were obtained by multiplying tension records in *A* by  $-0.0128$ , the mean thermoelastic heat/tension ratio measured in rigor muscles. The others were obtained by convolving the first pair with eqn. (1) with values of  $z$  calculated from the indicated thicknesses of inert layer (0, 10, 20 and  $50 \mu\text{m}$ ). Traces labelled 0 correspond to the thermoelastic heat that would be measured by the thermopile if the only barrier to heat conduction were the thermopile insulation. The three pairs of traces labelled 10 were calculated for the mean (centre) and range ( $-0.009$  to  $-0.018$ ) of thermoelastic coefficients measured in rigor muscles. The centre pair are superimposed on the measured records shown in *A* for comparison. Experiment 5, blotted muscle mass 194 mg; see Table 1.

The size of the lag introduced into heat records by the time required for conduction of heat from the myofibrils to the thermal junctions depends on the thicknesses and thermal capacities of the intervening layers of connective tissue, solution and thermopile insulation, which determine the value of the constant  $z$  in eqn. (1). The thickness of the insulation was measured after the thermopile was destroyed at the end of the series of experiments. The thickness of layers of tissue and solution (the 'inert' layer) could not be measured directly but was estimated as follows.

The estimate was based on the assumption that the first 4 ms of thermal responses to very small rapid length changes ( $\leq 0.7\%$  of muscle length complete in 4 ms) consisted of thermoelastic effects superimposed on isometric heat production. Measurements of heat produced by active muscles in response to such length changes were then compared with traces of thermoelastic heat simulated by convolving with eqn. (1) the time course of the tension change scaled by the thermoelastic



coefficient measured in rigor muscles (Gilbert & Ford, 1986). Fig. 4 shows actual (*A*) and simulated (*B*) records from one preparation. The responses recorded during stretches and releases of the active muscles were symmetrical and opposite in sign from the tension changes. Their time courses were compared with the series of simulated records in Fig. 4*B* generated by convolving the scaled tension changes with eqn. (1) in which  $z$  was assigned several values calculated from thicknesses of tissue and solution indicated by the numbers 0 to 50 (in  $\mu\text{m}$ ). The best fit between the initial phase of the simulation and the recorded responses was obtained with a value of  $z$  corresponding to an inert

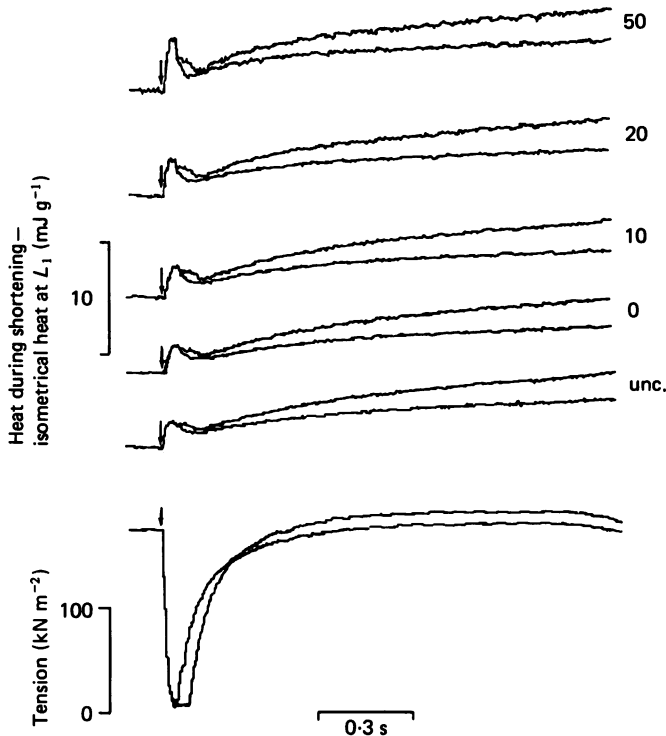


Fig. 5. Tension and heat production in response to two extents of shortening ( $58.5$  and  $117$  nm half-sarcomere $^{-1}$ ) at a velocity per half-sarcomere of  $1.56 \mu\text{m s}^{-1}$ . Isometric heat at the initial length ( $L_1$ ) was subtracted to obtain the pair of records labelled unc. Other records were obtained by deconvolving the first pair with eqn. (1) assuming values of  $z$  corresponding to layers of solution and tissue  $0$ ,  $10$ ,  $20$  and  $50 \mu\text{m}$  thick in addition to the thermopile insulation. Experiment 4; see Table 1.

layer  $10 \mu\text{m}$  thick, as shown by the superposition of the recorded and simulated responses in Fig. 4*A*. The range of thicknesses of inert layer in the seven experiments was  $3$ – $15 \mu\text{m}$ , with a mean of  $10 \mu\text{m}$ . The use of lower values of  $z$ , corresponding to thinner inert layers, produced traces that rose more quickly and to higher levels than the recorded thermal responses (traces labelled 0 in Fig. 4*B*), while higher values of  $z$  had the opposite effect (traces labelled 20 and 50). Records from each experiment were corrected for lag separately and the average compared with records obtained by first averaging traces from separate experiments and then correcting for lag assuming an inert layer  $10 \mu\text{m}$  thick. The results were the same. In one experiment responses to stretches and releases complete in  $1.2$  and  $4$  ms were compared. The ratios of heat to tension changes measured at the end of the length change were the same, strengthening the assumption that the thermal responses recorded during the length changes were largely thermoelastic. The 'equivalent half-thickness' of the thermopile and intervening layers, i.e. the thickness of a layer of muscle having the same thermal capacity as these layers (see Hill, 1965), is about  $18 \mu\text{m}$  when the inert layer is  $10 \mu\text{m}$  thick.

Fig. 5 shows the effect of applying lag corrections for several thicknesses of inert layer to heat

records obtained in response to two extents of shortening at a velocity per half-sarcomere of  $1.56 \mu\text{m s}^{-1}$ . The correction was made by deconvolving the pair of traces labelled unc. with eqn. (1), to produce traces labelled with the thicknesses of inert layer indicated beside the records. The principal effect of the lag correction was on the kinetics of heat production at the beginning and end of shortening when the largest changes in heat rate occurred. Later parts of the records were less affected, particularly the divergence of the records following the two extents of shortening in each pair. Isometric heat produced at the initial length was added back to the records before 'shortening heat' was calculated, as explained below.

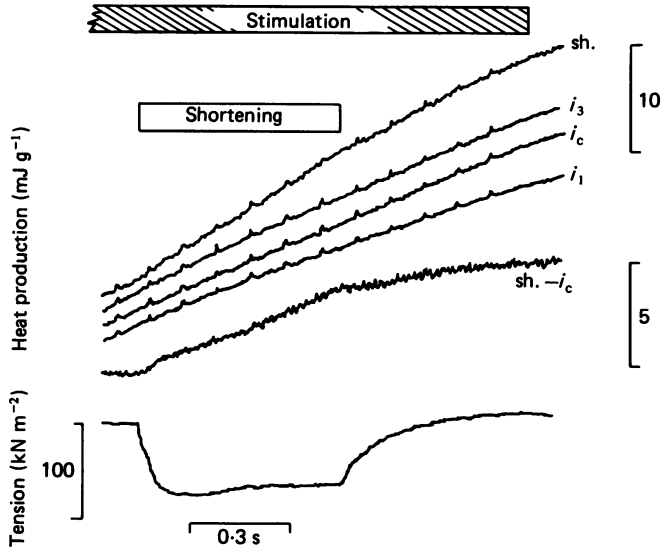


Fig. 6. Records from a single experiment showing method of calculating shortening heat. The extent of shortening was  $117 \text{ nm half-sarcomere}^{-1}$  and the velocity per half-sarcomere  $0.2 \mu\text{m s}^{-1}$ .  $i_1$ , and  $i_3$ , heat produced during isometric contractions at the initial (1) and final (3) lengths.  $i_c$ , composite isometric heat production calculated from  $i_1$  and  $i_3$  as described in the text. The lowermost heat record is the shortening heat, obtained by the subtraction  $\text{sh.} - i_c$ . Experiment 6, blotted muscle mass 131 mg. See Table 1.

*Definition of shortening heat.* The kinetics of the production of shortening heat as usually defined was determined as illustrated in Fig. 6. The records are from a single experiment and show tension and total heat production during shortening (labelled sh.) and in isometric contractions at the initial ( $i_1$ ) and final ( $i_3$ ) lengths. Heat production in response to shortening was corrected for lag as described above and isometric heat at the initial length added to the corrected record. The isometric heat  $i_c$  is the time integral of the weighted average of the instantaneous heat rates at the initial and final lengths, in which the weighting factor of each point during shortening was determined by the muscle length at that point, using a linear interpolation between the two isometric rates. It is identical to  $i_1$  until the onset of shortening and has a rate identical to that of  $i_3$  after the end of shortening. The record labelled 'sh. -  $i_c$ ' shows shortening heat, defined as the total heat liberated in response to shortening minus the isometric heat produced at the same instantaneous muscle length. A similar definition was used by Homsher *et al.* (1983) in experiments in which the dependence of shortening heat on filament overlap was characterized.

## RESULTS

*Heat production during shortening*

Shortening heat produced at three velocities is shown in Fig. 7*A*. The arrows in the records indicate the beginning of shortening, the time at which tension became approximately steady (after shortening of about 39 nm half-sarcomere<sup>-1</sup>) and the

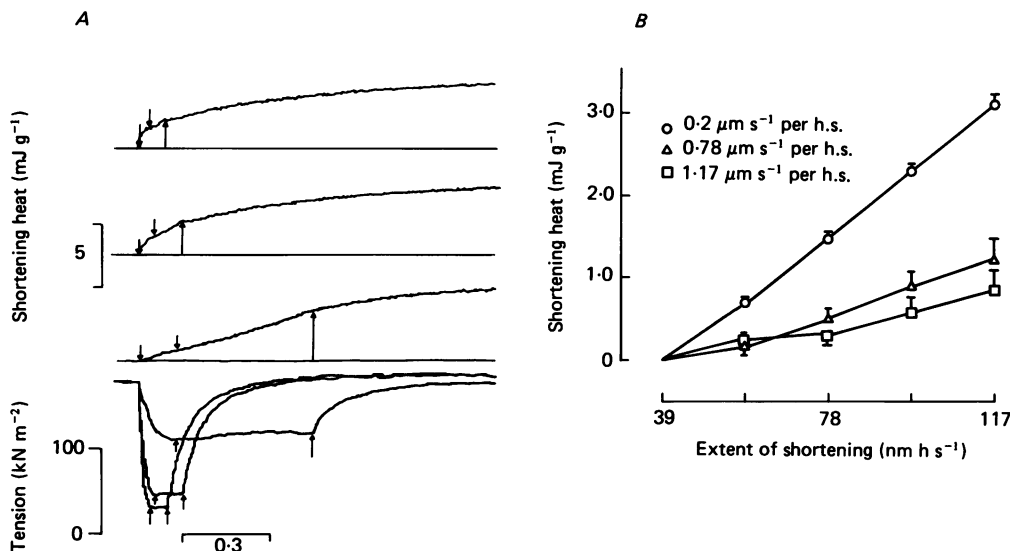


Fig. 7. Effects of velocity and extent of shortening on shortening heat. *A*, records of tension and shortening heat. These and all subsequent records are averages from seven experiments. Arrows indicate the beginning of shortening, the time at which tension became approximately constant and the end of shortening. The height of the last arrow shows the amount of shortening heat produced during shortening. The extent of shortening was 117 nm half-sarcomere<sup>-1</sup> and the velocities per half-sarcomere (h.s.) were 0.2, 0.78 and 1.17  $\mu\text{m s}^{-1}$ . *B*, extra heat produced during shortening plotted against the extent of shortening. Each point in this and all subsequent graphs is the mean of separate measurements of records from each experiment, with standard errors indicated by vertical bars. Shortening heat produced during the early part of shortening (to the second arrow in *A*) was subtracted from subsequent points.

end of shortening. The burst of heat associated with the transition from isometric contraction to constant power production was largest at the highest velocity. Heat produced during the entire period of shortening was greatest at the lowest velocity, as indicated by the height of the third arrow in each trace. Tension recovery at the end of shortening was accompanied by an inflexion in the heat records, which was most pronounced after the fastest shortening.

The relation between shortening heat and the extent of shortening at constant power is shown in Fig. 7*B*. Shortening heat produced during the first 39 nm half-sarcomere<sup>-1</sup> of shortening was subtracted from subsequent points. The relation between shortening heat and the extent of shortening at 0.2  $\mu\text{m s}^{-1}$  per half-sarcomere was approximately linear throughout the last 78 nm half-sarcomere<sup>-1</sup> of shortening

but was linear during only the last 39 nm per half-sarcomere at the two higher velocities. There is no evidence that less heat was produced by a given extent of shortening the longer shortening continued. Results obtained at the other two velocities, 0.39 and 1.56  $\mu\text{m s}^{-1}$  per half-sarcomere, are not shown but would lie above the triangles and on top of the squares, respectively.

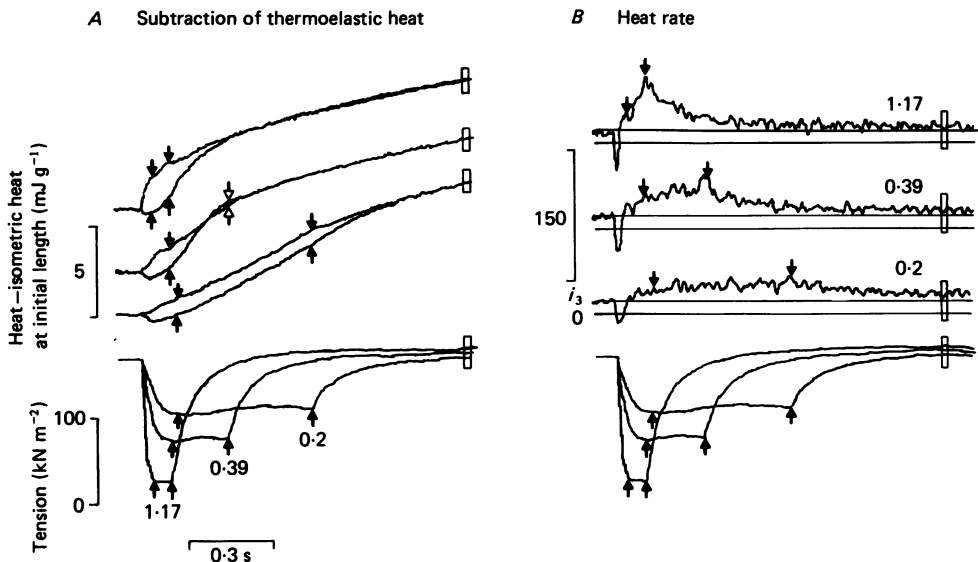


Fig. 8 Kinetics of heat production at three velocities of shortening. Velocities per half-sarcomere in  $\mu\text{m s}^{-1}$  are indicated beside the records in *B*. Isometric heat at the initial length was subtracted from the records. Arrows indicate the beginning of shortening, the time at which power became constant and the end of shortening. The end of stimulation is indicated by the bars. *A*, heat production before (left) and after (right) subtraction of thermoelastic heat, calculated as described in the text. The open arrows indicate that the right-hand record lies above the left-hand one. *B*, rate of heat production determined by numerical differentiation of the right-hand traces in *A* at 3 ms intervals. After differentiation the records were smoothed by averaging for the  $i$ th point the points  $i-1$ ,  $i$  and  $i+1$  and repeating the procedure three times. Smoothing reduced noise substantially but attenuated the maximum heat rate at the end of the fastest shortening by less than 10%.

*The rate of heat production during shortening.* Records of heat rate were obtained as illustrated in Fig. 8. Heat records at each velocity were corrected for heat conduction lag and averaged for the seven experiments. Pairs of records at three velocities are shown in Fig. 8*A* before (left) and after (right) subtraction of thermoelastic heat. (The isometric base line subtracted from these records was that recorded at the initial length, which appeared at a constant rate, instead of the 'composite' isometric heat, shown by trace  $i_c$  in Fig. 6 and used as the base line for the shortening heat, whose rate was made to vary during shortening. This base line was used to obtain absolute heat rates upon differentiation, rather than shortening-heat rates. The records in Fig. 8*A* are therefore not comparable to the records of shortening heat shown in Fig. 7*A*.) Thermoelastic heat was calculated by multiplying

the tension records by the thermoelastic coefficient measured in rigor muscles ( $-0.0128$ ; Gilbert & Ford, 1986). The pairs of records show that subtraction of thermoelastic heat removed the inflexions at the beginning and end of shortening.

Heat rates shown in Fig. 8*B* were calculated at 3 ms intervals from the right-hand records in Fig. 8*A*. The horizontal lines show the zero base line and the isometric heat rate at the final length. The first 50 ms of the records shows the heat rate at the initial length, which was slightly less than the isometric rate at the final length (see Table 1). Heat rate became negative at the beginning of shortening and then exceeded the isometric rate. It was still rising when force became steady (first arrow) and became constant during shortening only at the lowest velocity ( $0.2 \mu\text{m s}^{-1}$ ). It seemed to be approaching a constant value at  $0.39 \mu\text{m s}^{-1}$  (middle trace). After shortening ended, heat rate fell gradually and reached the isometric level only after the fastest shortening shown ( $1.17 \mu\text{m s}^{-1}$ ). Maximum heat rates measured at the five velocities of the larger shortening were approximately 40, 55, 65, 70 and  $70 \text{ mW g}^{-1}$  (slowest to fastest). Heat rate fell more rapidly following the smaller than the larger shortening at all velocities and declined most slowly following the slowest larger shortening. The approximate half-times for its decline were 130, 80, 95, 75 and 85 ms and 100, 70, 75, 70 and 85 ms following shortening of the larger and smaller extents, respectively (slowest to fastest shortening). At the end of stimulation following the slowest shortening, heat rate was still slightly higher than the isometric rate at the short length (bottom record in Fig. 8*B*), while it was equal to the isometric rate at that time following the smaller extent of shortening at the same speed.

#### *Difference in heat produced by two extents of shortening*

The records in Fig. 8 show that more of the extra heat produced by shortening appeared during tension recovery following rapid than slow shortening (Fig. 8*A*), while the decline in heat rate during tension recovery was more rapid after faster shortening (Fig. 8*B*). The possible sources of the heat are processes that produced tension recovery and others initiated by shortening that continued during the recovery. Since the increase in tension was greater following faster shortening, more heat would evolve following rapid than slow shortening if the reactions responsible for it produce heat.

To determine whether any of the extra heat that appeared during tension recovery resulted from processes not associated with tension recovery, differences in tension and shortening heat produced in response to two extents of shortening were determined, as shown in Fig. 9. Tension and heat records for the smaller shortening were shifted to the right to superimpose the traces at the end of shortening. The tension records were also shifted vertically to superimpose the last tension points recorded during shortening. Difference records calculated from the end of shortening at all five velocities are shown in Fig. 9*C*. Differences in tension were small or non-existent, while the differences in shortening heat amounted to almost 50% of the shortening heat produced during the larger shortening. Thus a substantial quantity of the extra heat that appeared after shortening resulted from processes initiated by shortening that continued during tension recovery but were independent of it. More of this heat appeared after rapid than slow shortening, and it was also produced at a higher rate.

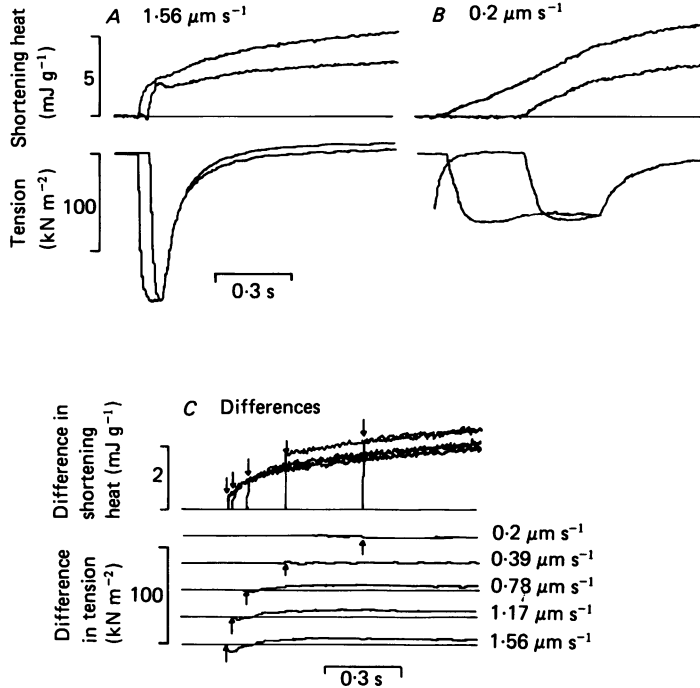


Fig. 9. Comparison of tension and shortening heat in response to two extents of shortening ending at the same muscle length. The extents were 117 and 58.5 nm half-sarcomere<sup>-1</sup>. In *A* and *B* tension and heat records for the smaller shortening were shifted to superimpose the end of shortening on that of the larger extent. Differences obtained from similar records at five velocities are shown in *C*. The arrows indicate the end of shortening, and velocities per half-sarcomere in  $\mu\text{m s}^{-1}$  are shown beside the records.

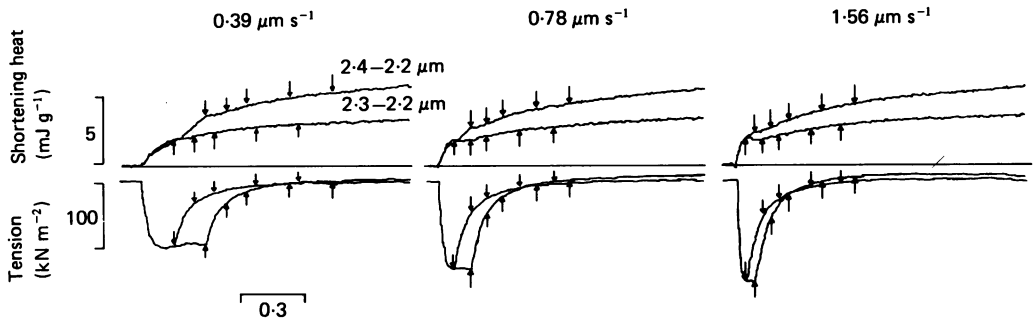


Fig. 10. Records of tension and shortening heat produced by two extents of shortening at three velocities ending at 2.2  $\mu\text{m}$ . The extents of shortening were 58.5 and 117 nm half-sarcomere<sup>-1</sup>. The arrows indicate (from left to right) the end of shortening and one, two, four and six times the time constant ( $\tau = 1/k$  from Fig. 3) for tension recovery.

Differences in shortening heat were also determined from measurements made in individual experiments at the end of shortening and at several subsequent intervals. The intervals were one, two, four and six time constants ( $\tau = k^{-1}$ ; see Fig. 3) of tension recovery, to ensure that the relative extents of the reactions responsible for tension recovery were as nearly equal as possible at the times at which the differences were calculated. The method is illustrated in Fig. 10 by three sets of records for two extents

of shortening ending at the same length. The tension records were shifted vertically so that they superimposed at the time shortening began and show that the drop in tension at the beginning of the two extents of shortening was the same. The initial bursts of heat produced at the beginning of shortening were also the same. The arrows indicate the times at which measurements were made.

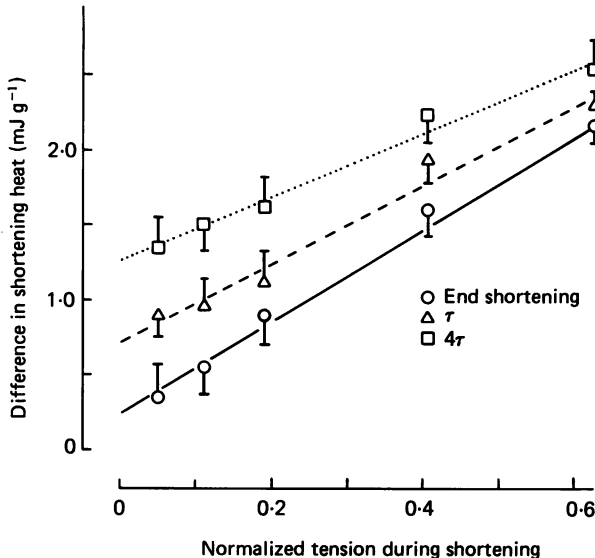


Fig. 11. Difference in heat produced by two extents of shortening plotted against normalized tension maintained during shortening. Differences were calculated from measurements made in each experiment of records similar to those in Fig. 10 at the times indicated by the first, second and fourth arrows. Slopes and intercepts were obtained for each interval in each experiment and averaged for the seven experiments to produce the lines shown on the graph. Average values of the intercepts (in  $\text{mJ g}^{-1}$ ) and slopes (in  $\text{mJ g}^{-1}$  per unit of  $P/P_0$ ) for all five intervals were, respectively: end of shortening,  $0.25 \pm 0.19$  and  $3.19 \pm 0.21$ ;  $\tau$ ,  $0.73 \pm 0.18$  and  $2.62 \pm 0.18$ ;  $2\tau$ ,  $1.04 \pm 0.15$  and  $2.25 \pm 0.20$ ;  $4\tau$ ,  $1.26 \pm 0.18$  and  $2.14 \pm 0.12$ ;  $6\tau$ ,  $1.56 \pm 0.23$  and  $1.69 \pm 0.32$ . The parameters at  $6\tau$  were determined from four points instead of five, because the time  $6\tau$  following the slowest larger shortening occurred after stimulation ended. Intercepts determined at different intervals were all significantly different ( $P < 0.05$  by Student's  $t$  tests for paired observations). The slope at the end of shortening was significantly higher than the other slopes ( $P < 0.05$  by Student's  $t$  tests for paired observations). The negative correlation between time of measurement and slope was statistically significant in five of the seven experiments (values of correlation coefficients less than  $-0.8$ ). In the other two experiments the correlation coefficients were  $0.059$  and  $0.274$ .

The relation between the tension maintained during shortening and the difference in shortening heat measured at three of the intervals (end of shortening,  $\tau$  and  $4\tau$ ) are shown in Fig. 11. Tension on the abscissa is directly proportional to the extra work done by the larger shortening. Parameters of lines obtained at all five measurement intervals are given in the Figure legend. The intercepts increased progressively as the difference in shortening heat was determined later after shortening ended, and the increase was statistically significant in all cases ( $P < 0.05$ ), indicating that more heat was produced after the end of rapid than slow shortening.

The slope was greater at the end of shortening than at all subsequent intervals ( $P < 0.05$ ). Slopes determined from measurements at subsequent intervals showed a tendency to decrease with time, but none of the differences were statistically significant ( $P > 0.05$ ). The approximately linear relation between tension and the difference in shortening heat throughout tension recovery indicates that shortening heat increased with the extra work produced by the larger shortening.

The measurements summarized in Fig. 11 were made from records from which thermoelastic heat had not been subtracted, as shown in Fig. 10. Measurements were also made from shortening heat records from which thermoelastic heat had been subtracted, with identical results, and from shortening heat records which had not been corrected for heat-conduction lag, which produced very similar results.

*Shortening heat 'coefficients'*. Shortening heat 'coefficients' were calculated to provide a direct comparison between these measurements and results from other studies. The shortening heat coefficient  $\alpha$  is defined as the ratio of shortening heat to the extent of shortening, normalized to the isometric tension measured at a striation spacing of 2.2  $\mu\text{m}$  (Hill, 1938, 1964). The relation between  $\alpha$  and the force maintained during shortening was assessed by linear regression analyses of the equation:

$$\alpha = \alpha_1 + \alpha_2(P/P_0), \quad (3)$$

in which  $\alpha_1$  is the intercept,  $\alpha_2$  the slope and  $P/P_0$  the normalized force maintained during shortening. Coefficients calculated from shortening heat measured over several intervals of contraction are shown in Table 2. All except one of the measurement intervals began with the onset of shortening.

The general characteristics of the coefficients are consistent with the kinetics of heat rate shown in Fig. 8B. Coefficients in part A of Table 2 were determined from measurements made at the beginning and end of intervals of contraction which included tension recovery and any associated heat production. The first three coefficients were determined from shortening heat produced from the beginning of shortening to the end of stimulation, which occurred 0.54 s after the end of the slowest larger shortening and 1.1 s after the end of the fastest smaller one. The coefficients are larger for small than large shortening and show little if any variation with force. The next three coefficients were determined 0.54 s after both extents and all velocities of shortening. The durations of the intervals ranged from 0.58 s for the fastest smaller shortening to 1.14 s for the slowest larger one. All three coefficients increased with force, but the force dependence was somewhat weaker with the smaller shortening. The third set of coefficients were determined from measurements made at the same relative extents of tension recovery. The intervals ranged from 111 to 718 ms. These coefficients are more strongly dependent on force than the previous ones, and the force dependence is greatest for the larger shortening.

The coefficients in part B of Table 2 were determined from measurements that excluded heat produced by tension recovery. Shortening heat used to determine the first coefficient was measured at the time at which power became constant (after shortening of about 40 nm half-sarcomere<sup>-1</sup>; see Fig. 8) and at the end of shortening. The last five coefficients were calculated from the fitted parameters given in the legend of Fig. 11, obtained by linear regression of the force maintained during



shortening and the difference in shortening heat produced in response to two extents of shortening measured at the same relative extents of tension recovery. The force dependence of these coefficients decreased as the measurement intervals became longer, but all except the last slope are significantly higher than those in part A of Table 2.

TABLE 2. Shortening heat coefficients obtained from measurements made at different intervals

	Intercept	Slope
A Including tension recovery		
1.14 s after beginning of shortening (end of stimulation)		
2.4 to 2.2 $\mu\text{m}$	0.29 $\pm$ 0.04	0.07 $\pm$ 0.02
2.3 to 2.2	0.35 $\pm$ 0.04	0.04 $\pm$ 0.03
2.4 to 2.3	0.35 $\pm$ 0.06	0.03 $\pm$ 0.01
0.54 s after end of shortening		
2.4 to 2.2 $\mu\text{m}$	0.23 $\pm$ 0.03	0.13 $\pm$ 0.02
2.3 to 2.2	0.30 $\pm$ 0.03	0.09 $\pm$ 0.03
2.4 to 2.3	0.29 $\pm$ 0.04	0.07 $\pm$ 0.02
4 $\tau$ after end of shortening		
2.4 to 2.2 $\mu\text{m}$	0.19 $\pm$ 0.02	0.19 $\pm$ 0.02
2.3 to 2.2	0.25 $\pm$ 0.02	0.15 $\pm$ 0.04
2.4 to 2.3	0.24 $\pm$ 0.02	0.16 $\pm$ 0.05
B Excluding tension recovery		
End of shortening from 2.36 to 2.2 $\mu\text{m}$	0.04 $\pm$ 0.02	0.34 $\pm$ 0.04
Difference: (2.4 to 2.2 $\mu\text{m}$ ) - (2.3 to 2.2 $\mu\text{m}$ )		
end of shortening	0.03 $\pm$ 0.02	0.34 $\pm$ 0.04
$\tau$	0.07 $\pm$ 0.02	0.28 $\pm$ 0.04
2 $\tau$	0.11 $\pm$ 0.02	0.24 $\pm$ 0.03
4 $\tau$	0.13 $\pm$ 0.02	0.22 $\pm$ 0.02
6 $\tau$	0.17 $\pm$ 0.03	0.18 $\pm$ 0.04

Shortening heat was measured at the beginning and end of intervals that began with the onset of shortening, except for the first line in part B. Stimulation ended 1.14 s after shortening began in all cases and 0.54 s after the end of the largest extent of the slowest shortening. Slopes and intercepts are averages ( $\pm$  standard errors) of values obtained in seven experiments by separate linear regression analyses of the equation

$$\alpha = \alpha_1 + \alpha_2 P/P_0,$$

which describes the dependence of the shortening heat produced per unit of shortening ( $\alpha$ ) on the normalized force maintained during shortening ( $P/P_0$ ).

#### DISCUSSION

Two important properties of heat produced by shortening muscles were demonstrated by these experiments. First, at no velocity was heat rate constant throughout shortening. Secondly, a substantial quantity of the heat liberated by processes initiated during shortening appeared after shortening ended, and more heat was produced after rapid than slow shortening. The interpretation of measurements of shortening heat therefore depends on the times at which the measurements are made, as shown by the shortening heat coefficients presented in Table 2. While measurements of the kinetics of heat rate can be useful in understanding the process of energy conversion, measurements of total heat produced between the beginning and end of fixed intervals of contraction that include shortening can lead to confusing

and even contradictory conclusions about the effect of velocity on the rate of energy liberation during steady shortening.

#### *Comparison with previous studies*

The observation that shortening heat continued to be liberated after shortening ended is consistent with results reported by Homsher *et al.* (1983) and by Irving & Woledge (1981 *a*). In these experiments, heat produced as a consequence of shortening itself was separated from that produced by processes involved in tension recovery at the end of shortening, and its dependence on force maintained during shortening was quantified at several intervals.

The shortening heat coefficients presented in Table 2 are quantitatively similar to those reported by previous workers, despite differences in procedures (Hill, 1964; Irving & Woledge, 1981 *a, b*) and, in some cases, the muscles and species of frogs used (e.g. Homsher, Mommaerts & Ricchiuti, 1973). The behaviour of the muscles used in these experiments was therefore virtually identical to that of preparations used in the earlier experiments.

*Force dependence.* The apparent force dependence of the shortening heat coefficients in Table 2 varied considerably when shortening heat was measured at different intervals. Coefficients determined from measurements made at a fixed time after shortening began were independent of force; on the other hand, those determined at a fixed time after the end of shortening varied approximately linearly with force, as did those determined from measurements that excluded heat associated with tension recovery. This behaviour is expected from the records of heat rate shown in Fig. 8 *B* and the variation with time of the linear relation between shortening heat and force shown in Fig. 11. Most of the heat produced in response to rapid shortening appeared after shortening ended, while heat rate declined most slowly after the end of slow shortening. Measurements made at a fixed interval after shortening began would therefore over-estimate heat produced in response to rapid relative to slow shortening: the processes that produced the heat occurred at a higher rate and would have proceeded to a greater extent, because of both the higher rate and the longer interval between the end of shortening and the time of measurement.

#### *The kinetics of heat production by shortening muscles*

The most striking feature of the results obtained in these experiments is the dissociation of heat from work during shortening, which suggests that the processes producing heat and work are not always concurrent even when power output is constant. Before the interpretation and physiological implications of this observation are discussed, the effects of assumptions made in analysing the data on conclusions drawn from the results must be examined.

*Effects of corrections for lag and thermoelastic heat.* Thermoelastic effects scale with tension changes and are therefore largest at the beginning and end of rapid shortening. Heat-conduction lag has the greatest effect on the same parts of heat records, when changes in heat rate are largest. The thermal consequence of the transitions between isometric tension maintenance and shortening at constant power are therefore most susceptible to misinterpretation because of errors in these two

corrections. Heat produced after the end of shortening was characterized by subtracting shortening heat produced by two extents of shortening, rather than by subtracting thermoelastic heat itself, and the results were similar whether or not the records were corrected for heat-conduction lag. The only aspect of the results affected by errors in the analysis would therefore be the kinetics of heat rate at the beginning of shortening, when the rate became momentarily negative.

The use of the thermoelastic effect to estimate the thickness of the inert layer of tissue and solution between the myofibrils and the thermal junctions makes it likely that heat rate did become negative early in shortening. A low estimate of the thickness of the inert layer would be obtained if rapid tension recovery (i.e. phase 2; see Ford, Huxley & Simmons, 1977) associated with the quick stretch and release used to estimate the thickness of the inert layer produced thermal responses having opposite signs from the thermoelastic effect, i.e. if heat were produced in response to stretch and absorbed in response to release. To produce the thermal responses observed, however, the thermoelastic coefficient would have to be larger than was measured in rigor muscles. Undercorrection for lag would produce records with a smaller burst of heat at the beginning of shortening than the muscle actually produced, from which *too little* thermoelastic heat would be subtracted. Use of higher values for both the thickness of the inert layer and the thermoelastic coefficient would produce a larger burst of heat and a larger correction for thermoelastic heat, and a negative heat rate would still be seen early in shortening.

*Interpretation of the results.* In these experiments heat rate was changing while power was constant at a wide range of velocities. The thermal consequences of shortening most likely result from changes that occur in the actomyosin cycle in response to shortening: the dependence of shortening heat on filament overlap (Homsher *et al.* 1983) is similar to that of isometric tension (Gordon, Huxley & Julian, 1966) and stiffness (Ford, Huxley & Simmons, 1981), and the extent of phosphocreatine hydrolysis that occurs in response to shortening is sufficient to account for the total energy liberated (Homsher *et al.* 1981, 1984). The results are therefore discussed within the familiar conceptual framework of the cross-bridge cycle, in which the chemical transitions occurring are those that involve the interaction of myosin with actin during the course of which heat and work are produced and ATP is hydrolysed.

The total rate of energy liberation by a contracting muscle at a given time is determined by the sum for the reactions occurring of the products of three terms for each reaction: (1) its molar enthalpy change, times (2) its extent in moles of reactant (or product), times (3) its rate constant. Under conditions in which an energetic steady state can be demonstrated, the appropriate reaction to consider is the over-all actomyosin ATPase cycle. Then variations in the rate of energy liberation will occur as the result of changes in the number of moles of reactant participating and/or changes in the over-all cycling rate. Whenever an energetic steady state cannot be demonstrated, the enthalpy changes observed must come from transitions within the cycle. Under those conditions, changes in the rate of energy liberation with time can reflect a changing distribution of reactants among the states of the cycle. The cycle itself cannot be in a steady state so long as the rate of energy liberation is changing.

At the beginning of shortening, heat rate (Fig. 8*B*) dropped below what it had been

in the preceding isometric steady state, while power production increased. Since the muscle was shortening, the rate of cross-bridge detachment must have increased. The observed change in heat rate could have resulted from a redistribution of reactants among states around the cycle such that they accumulated in states preceding transitions that absorb heat. As shortening continued, power became steady, indicating that net fluxes through the transitions responsible for filament sliding and work production became constant. Heat rate, however, was rising and continued to increase throughout shortening at all velocities except the lowest. Under those conditions reactants were accumulating in states preceding transitions that produce heat. At velocities less than half  $V_{\max}$  (e.g. middle trace in Fig. 8B), heat rate was approaching a steady value and may have attained it had the extent of shortening been larger. Heat rate did not become steady at the lowest velocity, suggesting that a new stable distribution of reactants had been established. The failure to achieve a steady rate during faster shortening indicates that reactants were continuing to accumulate in states preceding heat-producing transitions. The decline in heat rate immediately after the end of shortening at all velocities shows that the net accumulation of reactants in those states was reversed by the cessation of shortening.

The negative heat rate at the beginning of shortening may be associated with the hydrolysis of ATP by cross-bridges that detached after completing their work stroke. Woledge & Kodama (1979) found that the transition from myosin ATP to the myosin-products complex is endothermic, with a molar enthalpy change of about 70 kJ mol<sup>-1</sup> in rabbit skeletal myosin at room temperature. If the enthalpy change were similar in intact frog muscle at 0 °C, a minimum of 6% of the myosin heads would have been required to participate in the transition to produce the negative heat rate observed at the beginning of the slowest shortening. The calculation is based on three additional assumptions: (1) that 25% of the maintenance heat rate (about 3 mW g<sup>-1</sup>; Table 1) is not associated with actomyosin reactions (Homsher, Mommaerts, Ricchiuti & Wallner, 1972), making the negative heat rate associated with actomyosin 12.5 instead of 9.5 mW g<sup>-1</sup>; (2) that the rate constant for the transition is 10 s<sup>-1</sup> (Ferenczi, Homsher, Simmons & Trentham, 1978); and (3) that the concentration of myosin heads is 0.28 μmol g<sup>-1</sup> (Ebashi, Endo & Ohtsuki, 1969). A similar calculation for shortening at 0.39 μm s<sup>-1</sup>, where the negative heat rate attributable to cross-bridge activity was 26 mW g<sup>-1</sup>, shows that at least 13% of the heads would have been required to participate.

The steady heat rate observed during the last half of the slowest shortening could have resulted entirely from the degradation of the free energy released by ATP hydrolysis, if the over-all cycle were in a steady state (see Wilkie, 1960; also Homsher *et al.* 1984). The heat rate was 40 mW g<sup>-1</sup>, the power output about 30 mW g<sup>-1</sup> (calculated from results in Table 1 and Fig. 2) and the ratio of power to energy rate about 40%, which is consistent with the value of thermodynamic efficiency obtained by Kushmerick & Davies (1969) from measurements of ATP hydrolysis and work production at a similar velocity. The agreement between these results indicates that the cycle was in a steady state. Its turnover rate would be equal to the rate of cross-bridge detachment (15 s<sup>-1</sup> = 200 nm s<sup>-1</sup>/13 nm stroke<sup>-1</sup>; see Huxley, 1980). The fraction of participating myosin heads would therefore be almost 35%, given a free-energy change for hydrolysis of about 50 kJ mol<sup>-1</sup> (Kushmerick & Davies,

1969, Appendix). Single fibres are about 80% as stiff during shortening at that velocity as in an isometric contraction (Ford, Huxley & Simmons, 1985), which suggests that at least 40% of the heads are attached during an isometric contraction.

Similar calculations cannot be made from rates of energy liberation during faster shortening, because steady states were not attained during these relatively small extents of shortening. The highest heat rates measured in these experiments at  $V_{\max}$  and half  $V_{\max}$ , the velocities used by Homsher and co-workers to measure energy balance with larger extents of shortening, are higher than the average heat rates calculated from their results (Homsher *et al.* 1981, 1984). Heat rate measured during larger shortening is therefore expected to reach a maximum and then decline, perhaps to a steady level (see Irving & Woledge, 1981*b*). The kinetics with which this occurred would provide information about the transitions responsible for the high rate of heat production during rapid shortening.

The results indicate that a complete kinetic scheme for energy transduction must incorporate features that allow constant flux through a transition that produces work and sliding in the presence of a varying flux through another part, possibly a parallel branch (Woledge, Curtin & Homsher, 1985, chapter 5), of the same cycle. The scheme must include transient fluxes that produce heat at very high rates late in rapid shortening and other transient fluxes that absorb heat early in shortening. More information is needed about the maximum heat rates attainable at all velocities and the kinetics of heat rate during transitions at the beginning and end of shortening before explicit schemes of this type can be generated and tested.

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