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THE HEAT PRODUCED BY FROG MUSCLE IN A SERIES OF CONTRACTIONS WITH SHORTENING

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SUMMARY

1. Heat production has been measured in muscles undergoing rapid shortening 1 sec after the start of stimulation in a 2 sec tetanus. In a series of three such tetani where the periods of shortening were separated by 5 sec the shortening heats in the second and third tetani were 111 (±4) % and 116 (±6) % respectively of that in the first tetanus (mean ± S.E. of mean, nine experiments).

2. When a similar series was carried out after an interval of 3 min the shortening heat in the first tetanus of the second series was 127 (±3) % of that in the first tetanus of the first series (mean ± S.E. of mean, three experiments).

3. The results are not in agreement with those of Dickinson & Woledge (1974). The discrepancy is explained by an artifact which, with certain arrangements of the apparatus, affects the measurements of shortening heat in a series of contractions. We have measured this artifact by two independent methods; the shortening heat values quoted above have been corrected accordingly.

4. The increase in the shortening heat in repeated contractions is not a specific consequence of previous shortening; an increase of the same magnitude is observed following isometric tetani.

5. During the time when the muscle is shortening, the total rate of heat production, which is initially high, falls to a steady value. This value is not significantly different in the three tetani of the series.

INTRODUCTION

When a stimulated muscle is allowed to shorten it produces heat at a faster rate than in an isometric contraction (Hill, 1938). The total amount of heat produced in excess of that in the isometric case is known as the shortening heat. This heat presumably is produced by extra chemical reactions occurring in a shortening muscle; we will refer to the reaction or set of reactions which produce this heat as the 'shortening heat reaction'. It has been shown by Rall, Homsher, Wallner & Mommaerts (1976) that, at least under some conditions, the shortening heat is not produced by ATP or phosphorylcreatine splitting. They therefore proposed that the shortening heat originates in an exothermic reaction involving the actomyosin ATPase system. Their results suggest that this reaction may be partially reversed a

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few seconds after shortening by coupling to ATP splitting, but the time course of the reversal process is not well established. The shortening heat is still apparent as a net excess heat production at the end of relaxation from a tetanus (Aubert & Lebacq, 1971; Dickinson & Woledge, 1973) but the latter authors found evidence of an endothermic reaction during relaxation after shortening and suggested that this was the partial, or perhaps complete, reversal of the shortening heat reaction. If, as the evidence suggests, the shortening heat reaction is not completely reversed within a few seconds, it might be possible to detect the depletion of its substrate by measuring the heat in another shortening soon after the first one. Substrate depletion might be apparent as a smaller shortening heat in the second shortening than the first. We have studied shortening heats in a closely-spaced series of tetani to test this possibility.

Preliminary experiments of this type have been performed by Dickinson & Woledge (1974). These authors claimed that in two tetani with periods of shortening separated by 5 sec, the shortening heat in the second tetanus was about 75 % of that in the first. This reduction was specifically dependent on previous shortening, and was not produced by a previous isometric contraction. In our experiments on a similar plan we have paid particular attention to an artifact which may be seen in this type of experiment. This artifact can occur when a muscle, heated by previous activity, shortens and moves over the thermopile.

METHODS

Sartorius muscles of frogs (*Rana temporaria*) were used. They were dissected along with 3–4 cm of nerve and then left in Ringer solution at 0 °C for at least 2 hr. The Ringer solution contained (m-mole/l.) NaCl, 115·0; KCl, 2·5; $CaCl_2$, 1·8; $MgSO_4$, 1·0; NaH_2PO_4, 1·0; Na_2HPO_4, 2·0 and was bubbled with O_2. All experiments were performed at 0 °C; the pH of the Ringer solution at this temperature was 7·0. When making heat measurements the muscles were maximally stimulated at 10 Hz via their nerves. All muscles were also stimulated directly to check that nerve stimulation activated all fibres.

The muscles were connected via length and tension transducers to a Ling 100 series vibration generator. The length transducer was of the type described by Jewell, Kretzschmar & Woledge (1967). Muscle length was made to follow a control signal by a feed-back network driving the vibration generator. The tension transducer was an aluminium ring 2 cm in diameter to which strain gauges had been bonded. The stiffness of the transducers and feed-back system was 11×10^3 N.m^{-1}. This is more than ten times stiffer than the series compliance of the muscle (Jewell & Wilkie, 1958).

Heat measurements were made using a thermopile of the Hill-Downing type which was 28 mm long with 28 flattened chromel-constantan thermocouples each about 0·4 mm in width. Connexions were made to the thermopile at a number of points along its length, so that temperature measurement could be made from various regions of the muscle. The couples were electrically insulated with kapton-teflon laminar film (Dupont) on either side, fused together in the gaps between the thermocouples by heating under pressure. The thermopile was thin ('equivalent half-thickness' 17 μm; Hill (1965)) and of small thermal capacity, so for the purposes of these experiments no correction was necessary for lag in heat conduction between muscle and thermopile. The use of nerve rather than direct stimulation eliminated any artifact on the heat records due to the stimulus. The electrical output of the thermopile was measured by a Kipp A80 galvanometer with photoelectric amplification (Hill, 1965) or an Ancom DC3a chopper amplifier. In some experiments these were used simultaneously to record the output of two banks of thermocouples. The sensitivity (μV/deg) for the various banks of thermocouples was measured by the Peltier method (Kretzschmar & Wilkie, 1972, 1975). Heat loss from the muscle was exponential with rate constants in the range 0·51–3·33 min^{-1} and was corrected for by the method of Hill (1965). Heat capacity of muscles and thermopile was determined by the method of Hill & Woledge (1962). A pair of copper-constantan thermocouples was glued into small holes in the thermopile frame and measured the longitudinal temperature gradient in it. Their output was amplified by a Keithley nanovoltmeter.

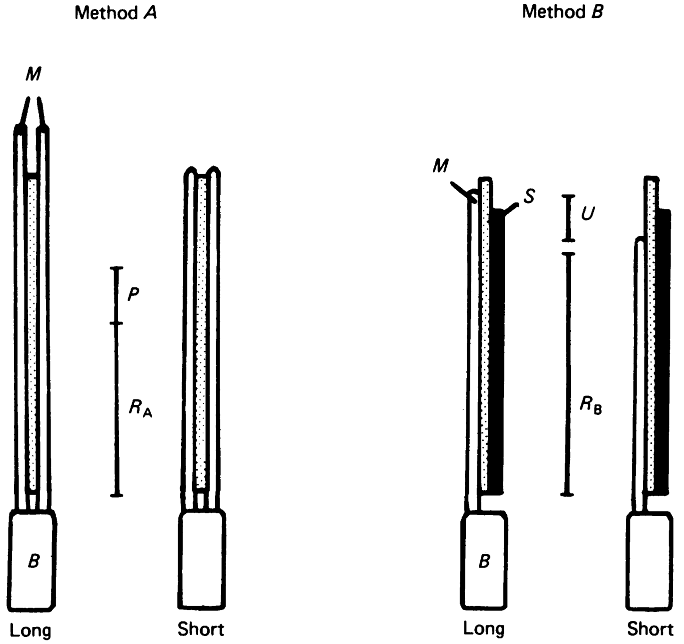

Fig. 1. Diagram of experimental arrangement of muscles (*M*), silver (*S*) and thermopile (shaded), showing muscles in the positions they occupied before and after shortening. Thickness of the components has been exaggerated with respect to length. Method *A* used two muscles attached to the pelvic bone (*B*), and after shortening the muscles still covered the entire length of the thermopile. Separate temperature measurements were made simultaneously from regions R_A and *P*. Method *B* used only one muscle which was always shorter than the thermopile; temperature measurement was made in region R_B. Region *U* becomes uncovered when the muscle shortens.

The two different arrangements of muscle and thermopile used are shown in Fig. 1. Muscles were selected according to size for use in one of these. In method *A* a pair of muscles which produced maximum tension over the range of muscle length 31–36 mm was mounted on the thermopile so that all the thermocouples were covered by muscle even after the shortening. Temperature measurements were made from the pelvic 20 mm of the muscles. Method *B* used single muscles which produced maximum tension over the length range 26–28 mm; some of the thermocouples at the top of the thermopile became uncovered when the muscle shortened. Temperature measurements were made from either 20 or 24 mm of muscle measured from the pelvic end. A piece of silver (26 x 3 x 0·03 mm) was attached to the reverse face of the thermopile. In both methods each series of contractions consisted of three 2 sec tetani with intervals of 5 sec. The third tetanus of the series always had 4 mm of shortening; the first two either had 4 mm shortening or were isometric at the long or short length. When the muscles were at the short length before a tetanus with shortening, they were stretched to the long length in 0·15 sec, the stretch being complete 0·2 sec before the start of the next tetanus. Between sets of observations the muscles were immersed in Ringer solution for 10 min (method *A*) or 20 min (method *B*).

RESULTS

Measurement of the shortening heat in a single tetanus

Shortening heat is defined as the heat produced by a muscle when it is shortening, in excess of that produced under isometric conditions at the same muscle length. Thus the heat production in isometric contractions at two or more muscle lengths must be measured in order to estimate the base line heat (i.e. that which would have been produced in an isometric contraction) in the length range over which the shortening occurs. To reduce the number of control observations, the isometric heat was not measured in separate contractions, but in a single tetanus, before and after a period of shortening.

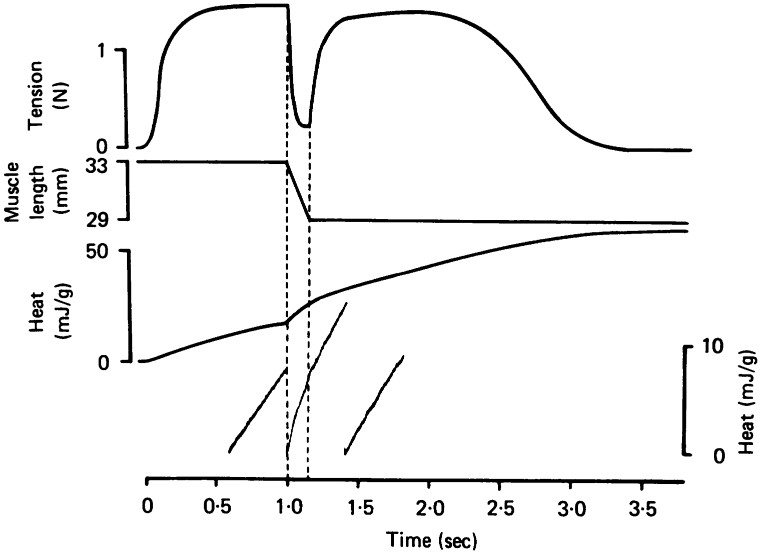

Fig. 2. Tension, muscle length and heat production in a 2 sec tetanus. The muscle pair contracted isometrically for 1·0 sec at the long length, then shortened a distance of 4 mm in 0·15 sec at a constant velocity of 26·7 mm/sec. Stimulation continued for a further 0·85 sec with the muscle pair isometric at the short length. The top three records have been replotted; the lower trace shows part of the original heat record on a larger scale (right hand scale). The record was automatically returned to base line at 0·4 sec intervals. Method *A*, blotted weight of muscle pair = 205 mg, length producing maximum isometric tension = 31 mm.

A typical observation is shown in Fig. 2. After 1·0 sec of isometric contraction at the long length the muscle shortens 4 mm at constant velocity for 0·15 sec then contracts isometrically for a further 0·85 sec at the short length. The starting length was chosen so that the isometric tension developed was about the same in tetani at both the long and short lengths. The heat record was measured at 0·6, 1·0, 1·4 and 1·8 sec after the first stimulus and, after correcting for heat loss, the corresponding heat values $h_{0\cdot 6}$ mJ etc. were obtained. The isometric heat rate at the long length ($\dot{h}_L$) mJ.sec^{-1} was calculated as $(h_{1.0}-h_{0.6})/0\cdot 4$ mJ.sec^{-1} and the isometric heat rate at the short length ($\dot{h}_S$) mJ.sec^{-1}, from the period after tension redevelopment, as

$(h_{1\cdot 8}-h_{1\cdot 4})/0{\cdot}4$ mJ.sec^{-1}. Although the muscle length stops changing at the end of the 0·15 sec release, sarcomere shortening continues as tension redevelops and series elastic elements are stretched. Tension redevelopment is virtually complete within 0·4 sec of the start of the release, so shortening heat is obtained from this central period (consisting of 0·15 sec release and 0·25 sec tension redevelopment). Since the net tension change in this period is very small, the contribution from the thermoelastic heat (Woledge, 1961) is negligible. The isometric control heat in this period (h_c) mJ. is calculated as a suitably weighted average of the isometric heats at the long and short lengths. Thus

$$h_c = \tfrac{1}{2}(\dot{h}_L+\dot{h}_S)\times 0{\cdot}15+\dot{h}_S\times 0{\cdot}25 \text{ mJ.}$$

The shortening heat is therefore given by the expression

$$(h_{1.4}-h_{1.0})-h_c \text{ mJ.}$$

It is assumed in the above calculation that heat is produced at a constant rate during isometric contraction. In fact this is not quite correct (Aubert, 1956). The magnitude of the resultant error in shortening heat is however negligible (see Discussion).

Experiments with pairs of muscles, longer than the thermopile

In method A (see Methods) the muscles were longer than the thermopile, even after shortening, and heat was recorded from about half the muscle length at the pelvic end. Two series of three tetani were given; the first (series 1) occurred 2 min after draining the Ringer solution and the second (series 2) followed after a further 3 min. Within a series stimulation periods started at 5 sec intervals. The shortening heats in these tetani, calculated as described above, are shown in Fig. 3 (circles) for four experiments of this design. The quantity plotted is expressed as a percentage of the shortening heat in the first contraction of series 1. The actual value of the shortening heat in this contraction was 5·08 (± 0·37) mJ.g^{-1} (mean ± S.E. of mean, twenty-three observations, four muscle pairs) and the dimensionless quantity α/P_0 (shortening heat per unit shortening)/(isometric tension) as defined by Hill (1938) was 0·190 (± 0·006). As can be seen from the Figure, the observed shortening heat was found to *increase* in a series of contractions. It was also greater in series 2 than in series 1.

There is a clear discrepancy between these results and those of Dickinson & Woledge (1974). They found that, in a similar series of three contractions with rapid shortening, the shortening heat in the third tetanus of a single series was about 64 % of that in the first. They used a single muscle which was shorter than the thermopile and recorded from nearly the whole length of the muscle as in our Method *B*. Using this method we obtained a result similar to theirs (see below). The discrepancy between the results from the two methods suggests that there is an artifactual contribution to the observed shortening heat in one or both of them. We have therefore estimated the magnitude of this artifact in our experiments.

A possible artifact in shortening heat measurements

Part of the muscle, which is not initially in the region of the thermopile from which temperature is being recorded, will enter it when the muscle shortens. Before the shortening commences, this part of the muscle lies on part of the thermopile known as the 'protecting region' (Hill, 1937). Suppose that for some reason this part of the muscle is at a different temperature from the part already in the recording region; if so, an extra temperature change will be recorded during shortening and an error in shortening heat measurement will be produced. We will refer to this hypothetical error as the movement artifact. It may be particularly important when shortening heat is measured in a closely spaced series of contractions, when the muscle does not have time to cool off in the interval. We have devised two methods of measuring this possible artifact. The 'stretch method' is based on the idea that an equal and opposite artifact should be seen when the muscle is stretched out in the relaxed state between tetani. The 'dual recording method' depends on measuring the temperature in the protecting region as well as in the normal recording region.

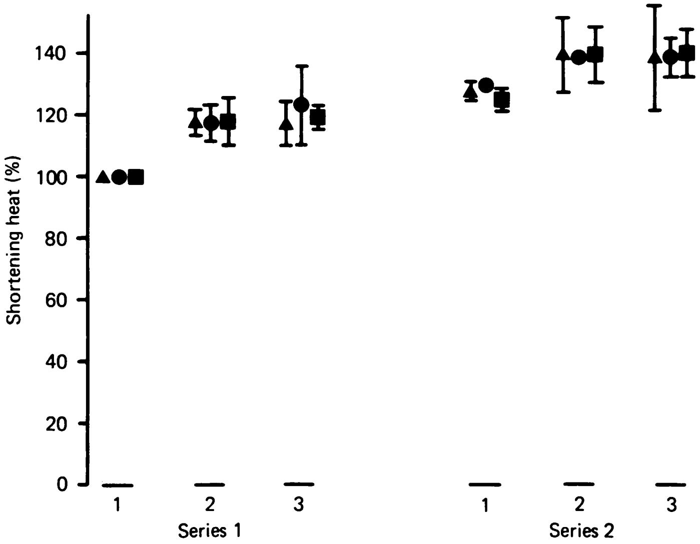

Fig. 3. Shortening heats in two series of three tetani with shortening observed by method *A*. Within a series, the time between the first stimulus in each tetanus was 5 sec; there was an interval of 3 min between the series. All shortening heat values are expressed as a percentage of the value for the first tetanus in series 1. Filled circles: observed shortening heat; triangles: shortening heat corrected by the stretch method; squares: shortening heat corrected by the dual recording method. Symbols show the mean and bars show ± S.E. of mean ($n = 4$ for observed shortening heat and shortening heat corrected by stretch method in series 1; $n = 3$ for all other points). In the first tetanus of series 1 the actual values of the observed and corrected shortening heats differed by less than 5%. This difference was not significant ($P > 0{\cdot}25$, paired t test).

The stretch method. The movement artifact described above will produce an artifactual temperature change (ΔT_A) in the recording region when the muscle shortens. The observed temperature change will be the sum of ΔT_A and that (ΔT_S)

corresponding to the heat actually produced by the muscle when it shortens. ΔT_A can be measured by observing the temperature change in the recording region if the muscle can be moved while heat production is zero. When the muscle is stretched back to its original length in the resting state between contractions of a series, the thermo-elastic heat produced (Hill, 1952) is negligible in comparison with the shortening heat. The observed temperature change in the recording region due to the stretch is therefore $(-\Delta T_A)$, since equal and opposite artifacts will be seen on stretching and shortening the muscle over a given length range. The temperature changes observed during the passive stretches between contractions can therefore be used to measure the movement artifact. There is one further complication, however. The temperature change in a passive stretch is found to be greater if the stretch occurs in an interval later in the series. This is presumably because longitudinal temperature gradients are related to the over-all temperature of the muscle, which increases during the series. In order to obtain ΔT_A at the time of the shortening by interpolation, it was assumed that ΔT_A was proportional to the temperature of the muscle in the recording region.

The movement artifact obtained in this way was subtracted from the observed shortening heat to obtain corrected shortening heat values. These are shown in Fig. 3 (triangles). In no case did the corrected and observed values differ by more than 6 % of the shortening heat in the first tetanus of series 1. The average difference is small compared with the variability between different muscles. It is clear therefore that there is no significant error in these experiments due to the movement artifact as measured by the stretch method. However, the possibility remained that a small heat production occurred during the passive stretches and cancelled out the movement artifact. To investigate this possibility the movement artifact was measured by an independent method.

The dual recording method. Simultaneous recordings of temperature were made from two regions of muscle in three out of the four experiments described above. Temperature measurements were made from the parts of the thermopile 0–16 mm and 16–20 mm from the end where the pelvic tendon of the muscle was fixed (regions R_A and P respectively in Fig. 1). When the muscle shortens, about 2 mm of muscle originally in region P moves into region R_A, so the movement artifact can be calculated from the difference in temperature, measured immediately before the shortening, between region R_A and this 2 mm of muscle. The best available estimate of the temperature of the latter is the mean temperature of region P just before the shortening and this was used in calculation of the movement artifact. This method therefore provides an approximate measurement of the movement artifact, but one which is independent of heat production and made at the time of shortening. It was necessary to check one further point, however. The thermopile measures temperature difference between the central thermo-junctions, where the muscles lie, and the thick aluminium frame. The temperature gradient in the frame must be measured before it is possible to calculate the temperature gradient in the muscle from the difference of output of two banks of thermocouples. The temperature gradient in the frame over the relevant region was therefore determined during these experiments; it was found to be negligibly small.

The values of shortening heat corrected by the dual recording method are shown in

Fig. 3 (squares). Again there is no significant difference between these and the observed shortening heat values. Two conclusions can be drawn from the results in Fig. 3. First, there is no significant difference between measurements of movement artifact by the stretch method and the dual recording method. Secondly, in experiments with method A, the movement artifact had a negligible effect on shortening heat measurements.

Heat and tension changes in a series of tetani

In Table 1, the observed and corrected shortening heats in these experiments are shown for comparison with tension developed and the total heat rate during the various phases of the tetanus. In repeated contractions with shortening (columns C_1, C_2, C_3) the shortening heat increases but the isometric heat rates ($\dot{h}_L$, $\dot{h}_S$) show a large decrease. The latter observation is consistent with the idea that there is a smaller proportion of labile heat in the later tetani of a series (Aubert, 1956; Aubert & Maréchal, 1963). Isometric tension also shows a decline in a series of contractions. Seen against this background, the increase in shortening heat is even more striking.

To test whether this change in the shortening heat is a specific consequence of shortening, control observations were included in the experiments in which the first two tetani in a series of three were isometric at either the short or long length. The shortening heat in the third tetanus of such a series is shown as columns D_3 and E_3 of the table respectively. Comparison of columns C_3, D_3 and E_3 shows that the shortening heat in the third tetanus of a series is the same whether it was preceded by two isometric tetani or by two tetani with shortening. Thus the change in shortening heat in these experiments is *not* a consequence of previous shortening, but merely of previous contractile activity.

The total steady heat rate during shortening in each type of contraction is also shown in Table 1. This was measured as the gradient of the heat record during the second half of the shortening, when tension is approximately constant and heat is produced at a steady rate (Fig. 2). Since the mean value of the movement artifact in these experiments was not significantly different from zero no correction was made to the values of total steady heat rate during shortening. This quantity does not show a significant change in a series of three contractions, but is higher in the first tetanus of series 2 than in that of series 1, by 16·6 (± 3·0) % (mean ± S.E. of mean, four muscle pairs). This is a significant increase ($P < 0{\cdot}02$, t test).

Experiments with single muscles, shorter than the thermopile

In view of the striking difference between our results and those of Dickinson & Woledge (1974), we have copied the design of their experiments as closely as possible (Fig. 1, method B) and measured the movement artifact under these conditions. Single muscles were used and heat was measured from a region which was shorter than the muscle at its short length by less than 4 mm. A piece of silver was attached to the reverse face of the thermopile. The observed shortening heat in five experiments of this type is shown in Fig. 4 (circles). The value of this quantity in the third tetanus of a series is 50 (± 17) % (mean ± S.E. of mean, five experiments) of that in the first tetanus. This is not significantly different from the decrease found by Dickinson

TABLE 1. Experiments with method A. C_1, C_2 and C_3 are a series of three tetani each with shortening. D_3 and E_3 are tetani with shortening which have been preceded by two isometric tetani at either the short or long length, respectively. All tetani were of 2 sec duration and repeated at 5 sec intervals within a series. There was a 3 min interval between the series. The data under D_3 and E_3 in series 1 and for all of series 2 is compiled from three experiments (i.e. three muscle pairs); data under C_1, C_2 and C_3 in series 1 is from four experiments with the exception of shortening heat corrected by the dual recording method, which is from three. There were usually six observations of each type in the experiment on each muscle pair. All quantities are expressed as percentages of the value in the first tetanus of series 1 (C_1); this value is also given in absolute units (per blotted weight for heats, per cross-sectional area for tensions) under C_1

	Series 1					Series 2				
	C_1	C_2	C_3	D_3	E_3	C_1	C_2	C_3	D_3	E_3
Observed shortening heat	100 (5·08 mJ . g^{-1})	117·1	122·9	117·4	104·6	128·9	138·2	138·2	147·8	129·5
S.E. of mean		6·1	12·8	8·0	11·5	1·1	0·4	6·4	8·2	6·5
Shortening heat corrected by stretch method	100 (4·96 mJ . g^{-1})	116·9	116·7	123·7	118·3	127·2	138·9	138·1	143·8	141·2
S.E. of mean		4·1	6·9	5·0	5·3	3·1	12·2	17·1	13·5	2·2
Shortening heat corrected by dual recording method	100 (4·80 mJ . g^{-1})	117·5	118·8	118·1	117·0	124·5	139·0	139·9	141·5	140·8
S.E. of mean		7·5	3·5	2·8	2·0	3·6	9·1	7·2	5·1	5·0
Total steady heat rate during shortening	100 (47·2 mW . g^{-1})	103·4	103·0	100·2	91·1	116·6	116·3	109·8	121·2	109·3
S.E. of mean		2·1	7·7	4·0	7·8	3·0	0·6	5·0	3·7	4·8
Isometric heat rate at long length ($\dot{h}_L$)	100 (24·4 mW . g^{-1})	78·3	70·2	69·2	69·4	91·7	75·1	66·4	69·1	69·4
S.E. of mean		2·3	2·4	3·8	4·3	0·7	2·3	2·5	3·3	5·9
Isometric heat rate at short length ($\dot{h}_S$)	100 (23·2 mW . g^{-1})	91·1	81·1	83·4	80·5	95·2	88·6	76·5	78·9	80·3
S.E. of mean		2·7	2·5	3·4	3·3	1·1	0·8	2·4	3·9	4·1
Isometric tension preceding shortening	100 (230 mN . mm^{-2})	93·8	89·4	91·6	89·8	88·9	85·7	83·4	85·9	85·0
S.E. of mean		0·8	0·7	2·3	1·3	1·0	1·3	1·3	1·9	1·3

& Woledge, which was 64 (± 4) % (mean ± S.E. of mean, eighteen experiments), so it appears that we have been able to duplicate the conditions of their experiments. The shortening heat is still greater in series 2 than in series 1 in experiments with this method. The changes in isometric heat rates ($\dot{h}_L$ and $\dot{h}_S$) and in the tension were also similar to the corresponding values shown in Table 1.

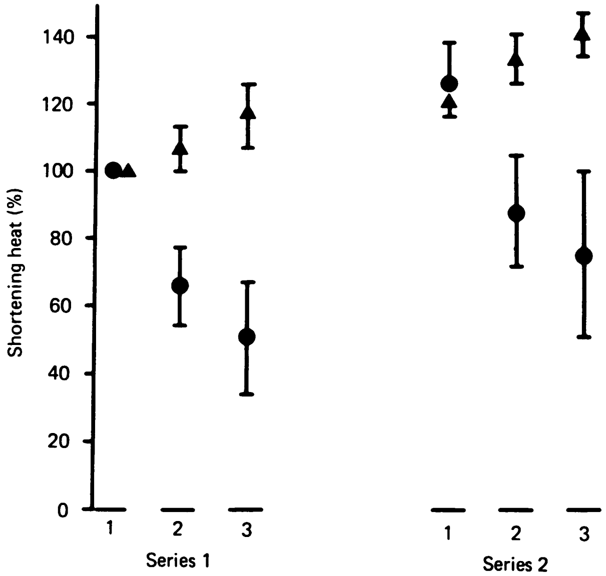

Fig. 4. Method *B*. Shortening heats in two series of three tetani with shortening. Within a series the time between the first stimulus in each tetanus was 5 sec. Series 1 was always performed 2 min after draining the Ringer solution, series 2 followed after a pause of 3, 6 or 9 min and the results from each experiment were the means of observations with at least two at each of these times. Shortening heat values are expressed as a percentage of the respective value in the first tetanus of series 1; in this tetanus observed and corrected shortening heats were 1·83 ± 0·15 and 2·28 ± 0·14 mJ.g^{-1} respectively (mean ± S.E. of mean, five experiments). Circles: observed shortening heat; triangles: shortening heat corrected by stretch method. Points are mean of five experiments; bars show ± S.E. of mean.

The movement artifact was measured in these experiments by the stretch method and the corrected shortening heats obtained are shown in Fig. 4 (triangles). In these experiments there is a very large correction due to the movement artifact and the corrected values *increase* in a series of tetani with shortening. It is also apparent that the corrected values show less variability than the observed ones. This fact can be expressed statistically by the correlation coefficient (r) between the observed shortening heat and the movement artifact. Pooling the data for the six tetani with shortening in five experiments gives $r = 0{\cdot}84$ for $n = 30$, $P < 0{\cdot}001$. This highly significant correlation means that part of the variability in the observed shortening heat values is due to the movement artifact. The corrected shortening heat values are in no case significantly different from those seen in our experiments with method A, in which the movement artifact was negligible. We conclude that in the method used by Dickinson & Woledge there is an error in the shortening heat measurements caused by the movement artifact; when a correction is made for this, the results from the two methods are the same.

DISCUSSION

The movement artifact

We have demonstrated the existence of an artifact which contributes to shortening heat measurements under some experimental conditions. Hill (1938, 1964) and most subsequent investigators of shortening heat used two muscles mounted on a thermopile which was shorter than the muscles (as in our method *A*). There was a region of thermocouples (the protecting region) between the region from which temperature was measured and the end of the muscle. We find that under these circumstances, the movement artifact makes no significant contribution to the shortening heat, in either the first tetanus or subsequent tetani of a series.

In order to record from a larger region of the muscle, a different set of experimental conditions (like our method *B*) was used by Dickinson & Woledge (1974). They attempted to remove any movement artifact by attaching a thin piece of silver to the back of the thermopile to reduce temperature gradients along the muscle. They used only one muscle, shorter than the thermopile as in Fig. 1, method *B*. In these conditions we find that there is a large movement artifact; the part of the muscle in the protecting region becomes cooler than that in the lower recording region during a series of tetani. Dickinson & Woledge showed that the apparent decrease in shortening heat in a series of contractions depended on previous active shortening of the muscle. We propose the following mechanism for the production of this effect. In the first tetanus the muscle shortens normally and the correct shortening heat is recorded. It remains at the short length for at least 3 sec and, for about half of this time, the muscle is still producing heat at a rapid rate. However, the part of the thermopile which was uncovered when the muscle shortened cools off quickly during this time. When the muscle is stretched before the next tetanus, it comes into contact with this cold part of the thermopile and is cooled by it. It is now cooler than the muscle below it in the recording region so that when the muscle next shortens the cooled part enters the recording region and the apparent shortening heat is reduced. These effects are not eliminated by placing on the back of the thermopile a piece of silver of similar dimensions to that used by Dickinson & Woledge.

The magnitude of the apparent shortening heat change produced by the above mechanism is calculated in the Appendix; it is shown there that the mechanism can quantitatively account for the decrease in shortening heat in a series of contractions observed by Dickinson & Woledge and in our method *B*.

An error involved in measurement of shortening heat in a single tetanus

Our method of measuring the shortening heat in a single tetanus involves a linear interpolation of the isometric heat from the periods before and after shortening to estimate the heat base line during shortening. In fact, the isometric heat rate in a tetanus is not constant but, as shown by Aubert (1956), is given by

$$0{\cdot}025\, P_0(1{\cdot}23 \exp(-1{\cdot}0t)+1)\ \mathrm{mJ\,.\,sec^{-1}} \quad (t \text{ is in sec}),$$

where P_0 is the maximum tension (in g wt.) exerted by the muscle. The variation in isometric heat rate in these experiments is fitted reasonably well by this expression. The first term in the expression is the labile heat, and has been found to be much reduced when a tetanus is repeated within a few minutes of an earlier one (Aubert & Maréchal, 1963; Aubert, 1968). One can calculate from the above expression the error introduced by our linear interpolation in the first tetanus in a series. The heat base line during the shortening period is underestimated by about 4 % and this leads to an overestimate of the shortening heat in the first tetanus by about 5 %. Subsequent tetani have much less labile heat so the error is smaller. Our method of measuring shortening heat will therefore produce an apparent decrease of about 5 % during a series of contractions, most of this occurring between the first and second tetani. Our observation of the *increase* in shortening heat in a series cannot therefore be due to the influence of the labile heat on the measurements.

Variation of the shortening heat in a series of contractions

For the three tetani with shortening in series 1 one can combine the values of shortening heat, corrected by the stretch method, for all the experiments reported here. The values, relative to that in the first tetanus (100 %), are 110·9 (± 4·3) % in the second tetanus, and 116·4 (± 5·7) % in the third tetanus (means ± S.E. of mean, nine experiments). The shortening heat in the second and third tetani are thus significantly different from that in the first ($P < 0{\cdot}05$) but not from one another.

The shortening heat in the first tetanus of series 2 relative to that in series 1 has a similar value in experiments using method *A* (Fig. 3, Table 1) and method *B* (Fig. 4). Even though the interval between series was different with the two methods it seems justifiable to combine the observations from both, giving a value of 125·2 (± 3·6) % (mean ± S.E. of mean, nine experiments). It may be that the within-series and between-series changes represent the same effect of previous activity on shortening heat, although the possibility remains that the between-series change is merely a function of time since the Ringer solution was drained from the muscle.

Whatever its origin, the increase in shortening heat in a series of contractions is not a specific consequence of shortening. It ought, however, to be considered in the design of shortening heat experiments. Hill (1938) was aware of the possible effects of previous contractions: 'before records are begun, several contractions should be allowed, to enable the muscle to come into regular and uniform mechanical and thermal contact with the thermopile'. Thus his measurements were similar to the later contractions of a series in our design.

The base line for the shortening heat

Although the shortening heat increases in repeated tetani it was shown in Table 1 that the total steady rate of heat production during shortening did not change in a series of three tetani with shortening. This reveals that the increase in shortening heat can be completely accounted for by the decrease in the base line heat in a series of contractions and calls into question the validity of using such a base line in order to define the shortening heat. The original idea behind the subtraction of the isometric base line heat was that by doing so one would eliminate sources of heat production which were identical in isometric and shortening muscle. The quantity resulting from the subtraction (the shortening heat) was independent of the time at which the shortening occured after the start of stimulation in a tetanus (Hill, 1938) even though the isometric heat did depend on this parameter. This result was regarded as a justification of the base line subtraction and led to the idea of the shortening heat as the energetic consequence of a separate process occurring only in shortening muscle. The results of the present investigations show that the shortening heat as defined by Hill *is* affected by previous stimulation of the muscle, if that stimulation occurred in a separate tetanus. In these circumstances the base line subtraction results in a quantity which is no more universal than the directly observed heat production in a shortening muscle; both depend on all the parameters defining the state of the muscle. More direct evidence exists which is relevant to this question: in some conditions the rate of ATP hydrolysis (and the reactions which resynthesise it) is less in a contraction with rapid shortening than in an isometric contraction (Kushmerick, Larson & Davies, 1969; Rall *et al.* 1976). This reaction must be responsible for much of the heat production of isometrically contracting muscle, so this part of the isometric base line heat at least cannot be considered to continue to the same extent during shortening. In spite of these limitations, the shortening heat remains a useful quantity if it is considered as the net energetic consequence of shortening, obtained as it is by comparison of muscle contractions at the same sarcomere length and therefore with the same degree of potential actin-myosin interaction.

In these experiments the total heat production during shortening did not vary in a series of tetani, whereas the heat rate during isometric contraction did vary, largely because the first tetanus in a series contains a large proportion of labile heat in its isometric phase. We can therefore put forward two extreme hypotheses about the interaction of shortening and labile heat. First, the labile heat could be switched off completely during shortening, and the processes producing heat during shortening could occur to the same extent in a series of three tetani. Secondly, the labile heat could continue unchanged during shortening and the remaining reactions could be potentiated to the extent that the total heat during shortening remained constant in a series of tetani. Experiments by Hill (1938) and Aubert (1956) suggest that the classical shortening heat has the same value whether the shortening occurs at the beginning of a tetanus or after a few seconds of isometric contraction. The labile heat in isometric contraction declines by a large amount in this time, so the total heat during shortening must do likewise. The simplest interpretation of their results is that the labile heat continues unchanged during shortening, corresponding to our second hypothesis above. This question requires further investigation.

The chemical equivalent of the shortening heat

Irrespective of whether one considers the classical shortening heat or total heat production during shortening it is clear that there is no significant decrease in heat production during shortening as a consequence of previous shortening with an interval of 5 sec between the shortenings. We conclude that the reactions which occur to produce the shortening heat occur to a similar extent when the shortening is repeated. Either the reactions have been reversed within 5 sec, or substrate depletion due to 4 mm of shortening is not sufficient to decrease the extent of reactions in subsequent shortening. Further experiments are required to distinguish between these possibilities.

APPENDIX

The magnitude of the movement artifact in closely spaced contractions with shortening

In certain experimental conditions, such as those used by Dickinson & Woledge (1974) and those in our method B, an artifactual decrease in shortening heat is seen in a series of contractions with shortening. A mechanism which would produce such a decrease was proposed above. It is possible to calculate the magnitude of the effect produced by this mechanism for the conditions of method B (see Fig. 1).

Let C_M = heat capacity per unit length of muscle,
C_T = heat capacity per unit length of thermopile,
C_S = heat capacity per unit length of silver,
K_M = thermal diffusivity of muscle,
K_S = thermal diffusivity of silver,
T_S = temperature rise in the muscle due to 4 mm shortening,
T_T = temperature rise in the muscle due to a 2 sec tetanus with 4 mm shortening.

Initially we will neglect the effect of the silver on the back of the thermopile. In the first tetanus with shortening in a series the correct shortening heat is recorded but, after the muscle has shortened, a 4 mm region (region U, Fig. 1) of thermopile becomes uncovered. The time constant for heat loss along the thermocouple wires is directly proportional to the thermal capacity loading the central thermojunctions, so the uncovered region cools off very quickly ($t_{\frac{1}{2}} \simeq 1$ sec) to the reference temperature (that of the thermo-junctions in contact with the thermopile frame). At the end of relaxation from the first tetanus, the temperature difference between the muscle and region U of the thermopile is simply T_T. At this time the muscle is re-stretched to the original length and the top 4 mm of muscle will be cooled by an amount

$$\frac{C_T}{C_M + C_T} \times T_T$$

on contact with region U. Since longitudinal conduction of heat in the muscle is very slow ($K_M = 1{\cdot}2 \times 10^{-3}\,\text{cm}^2.\text{sec}^{-1}$), this temperature difference, between the top 4 mm of muscle and the remainder, will still exist when the next shortening occurs in the second tetanus. Because the recording region R_B is on average 2 mm shorter than the muscle at its short length, the average length of cooled muscle which will enter R_B during this second shortening is 2 mm. The average length of R_B is 21 mm, so the artifactual temperature change observed when the muscle shortens will be

$$\left(\frac{2}{2+21}\right) \times \left(\frac{C_T}{C_T + C_M}\right) T_T.$$

This is a fraction

$$\left(\frac{2}{2+21}\right) \times \left(\frac{C_T}{C_T + C_M}\right) \frac{T_T}{T_S}$$

of the total temperature rise produced by the shortening.

For the muscles used in method B, $C_T/C_M = 0{\cdot}16$ and $T_T/T_S = 18{\cdot}0$, so the predicted fractional decrease in shortening heat in the second tetanus with shortening in a series is

$$(2/23)\times\left(\frac{0{\cdot}16}{1{\cdot}16}\right)\times 18{\cdot}0 = 0{\cdot}22.$$

This may be compared with the decrease actually observed of 0·34 ± 0·12 (mean ± S.E. of mean, $n = 5$) in experiments using method *B*.

Using data given by Dickinson (1975) it is possible to calculate that in the experiments of Dickinson & Woledge (1974) $C_T/C_M = 0{\cdot}10$, $T_T/T_S = 15{\cdot}0$, mean length of recording region = 25·5 mm, mean length of cooled muscle entering recording region = 3 mm. Repeating the above calculation for these values, the predicted decrease in shortening heat in the second tetanus of a series as a fraction of that in the first is 0·14. The experimentally observed decrease was 0·27 ± 0·05 (mean ± S.E. of mean, $n = 18$).

So far we have not considered the effect of the silver on the back of the thermopile. Silver has very high thermal diffusivity ($K_S = 1{\cdot}6\ \text{cm}^2.\text{sec}^{-1}$) and was originally employed by Dickinson & Woledge in order to reduce longitudinal temperature gradients in the muscle. The description of heat flow in the muscle/thermopile/silver composite is a complicated problem in two dimensions and we will not attempt a complete solution. We can however deduce in a qualitative way the effect of the silver on the magnitude of the movement artifact by making some simplifying assumptions. First, since the composite is very long compared to its thickness, we will assume that there are no transverse temperature gradients, i.e. we only need consider longitudinal heat conduction. Secondly, since the thermal diffusivity of the silver is much greater than that of the muscle or thermopile we will assume that all longitudinal heat flow occurs through the silver. The effective longitudinal thermal diffusivity (K_E) is then given by $K_S C_S/(C_X + C_S)$ where C_X = heat capacity per unit length of components which do not conduct longitudinally.

In the region of thermopile covered by muscle, therefore, $K_E = K_S C_S/(C_M + C_T + C_S)$. In the conditions of method *B*: $C_S/C_M = 0{\cdot}08$, $C_T/C_M = 0{\cdot}16$ so $K_E = 0{\cdot}10\ \text{cm}^2.\text{sec}^{-1}$. We may interpret this result in a simple and approximate way using the expression $K_E \simeq (\Delta x)^2/\Delta t$ where Δt is the characteristic response time to temperature gradients existing over a distance Δx. Considering $\Delta x = 2{\cdot}6$ cm, the typical length of the muscle in method *B*, one obtains $\Delta t \simeq 70$ sec. From this order of magnitude calculation it is apparent that the silver is so thin compared to the muscle that it cannot effectively remove temperature gradients along the muscle in the time between adjacent contractions. The situation is rather different in the end part of the silver which is not covered by muscle: here $K_e = K_S C_S/(C_S + C_T) = 0{\cdot}53\ \text{cm}^2.\text{sec}^{-1}$. Typically there is 2 mm of silver uncovered when the muscle shortens and temperature gradients in this region would be removed with a characteristic time of $(0{\cdot}2)^2/0{\cdot}53$ sec = 0·07 sec. Similar values for these two characteristic times apply for the conditions of Dickinson & Woledge.

We are now in a position to consider the effect of the silver on the movement artifact. After the first shortening part of the uncovered region (*U*) of the thermopile has silver on the back and this will rapidly conduct heat out of the top few mm of the muscle. On reaching the bare part of the thermopile this heat will be quickly conducted away by heat loss along the wires of the thermopile. The effect of the silver is therefore to produce an additional cooling of the top of the muscle compared with the rest, and there is not sufficient time between contractions for the temperature gradient produced in the muscle to be removed by the silver. The silver strip will therefore *increase* the size of the movement artifact produced by the mechanism described above, and our estimates of its magnitude are therefore underestimated by some unknown amount. It seems likely, however, that the additional effect of the silver could account for the discrepancy between the theoretical and observed decreases in the apparent shortening heat in a closely spaced series of contractions with shortening.

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REFERENCES

AUBERT, X. (1956). *Le Couplage Energetique de la Contraction Musculaire*. Brussels: Editions Arscia.

AUBERT, X. (1968). In *Symposium on Muscle*, ed. ERNST, E. & STRAUB, F. B., pp. 187–190. Budapest: Akademisi Kiado.

AUBERT, X. & LEBACQ, J. (1971). The heat of shortening during the plateau of tetanic contraction and at the end of relaxation. *J. Physiol.* **216**, 181–200.

AUBERT, X. & MARÉCHAL, G. (1963). La fraction labile de la thermogenèse associée au maintien de la contraction isometrique. *Archs int. Physiol. Biochim.* **71**, 282–283.

DICKINSON, V. A. (1975). An investigation of shortening heat in frog skeletal muscle. Ph.D. thesis, University of London.

DICKINSON, V. A. & WOLEDGE, R. C. (1973). The thermal effects of shortening in tetanic contractions of frog muscle. *J. Physiol.* **233**, 659–671.

DICKINSON, V. A. & WOLEDGE, R. C. (1974). Reduction of shortening heat during a series of tetanic contractions in frog sartorius muscle. *J. Physiol.* **242**, 98–99*P*.

HILL, A. V. (1937). Methods of analysing the heat production of muscle. *Proc. R. Soc.* B **124**, 114–136.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc.* B **126**, 136–195.

HILL, A. V. (1952). A discussion on the thermodynamics of elasticity in biological tissues. *Proc. R. Soc.* B **139**, 464–527.

HILL, A. V. (1964). The effect of load on the heat of shortening of muscle. *Proc. R. Soc.* B **159**, 297–318.

HILL, A. V. (1965). *Trials and Trails in Physiology*. London: Arnold.

HILL, A. V. & WOLEDGE, R. C. (1962). An examination of absolute values in myothermic measurements. *J. Physiol.* **162**, 311–333.

JEWELL, B. R., KRETZSCHMAR, K. M. & WOLEDGE, R. C. (1967). Length and tension transducers. *J. Physiol.* **191**, 10–12*P*.

JEWELL, B. R. & WILKIE, D. R. (1958). An analysis of the mechanical components in frog's striated muscle. *J. Physiol.* **143**, 515–540.

KRETZSCHMAR, K. M. & WILKIE, D. R. (1972). A new method for absolute heat measurement, utilizing the Peltier effect. *J. Physiol.* **224**, 18–21*P*.

KRETZSCHMAR, K. M. & WILKIE, D. R. (1975). The use of the Peltier effect for simple and accurate calibration of thermoelectric devices. *Proc. R. Soc.* B **190**, 315–321.

KUSHMERICK, M. J., LARSON, R. E. & DAVIES, R. E. (1969). The chemical energetics of muscle contraction I. Activation heat, heat of shortening and ATP utilization for activation-relaxation processes. *Proc. R. Soc.* B **174**, 293–313.

RALL, J. A., HOMSHER, E., WALLNER, A. & MOMMAERTS, W. F. H. M. (1976). A temporal dissociation of energy liberation and high energy phosphate splitting during shortening in frog skeletal muscles. *J. gen. Physiol.* **68**, 13–27.

WOLEDGE, R. C. (1961). The thermoelastic effect of change of tension in active muscle. *J. Physiol.* **155**, 187–207.