

VARIATION IN THE ISOMETRIC MAINTENANCE HEAT RATE WITH MUSCLE LENGTH NEAR THAT OF MAXIMUM TENSION IN FROG STRIATED MUSCLE

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SUMMARY

1. Tension and heat rate were measured as a function of muscle length in the range 0.75 – $1.25 l_0$ in 10-sec isometric tetani in frog striated muscle at 0°C in seven experiments. l_0 was defined as the length at which maximal tension was developed.

2. The length at which the stable maintenance heat rate (\dot{h}_B) was maximal was 7 – 16% l_0 shorter than the length at which tension was maximal (P_{\max}).

3. The range of \dot{h}_B at the length at which tension was maximal was 0.82 – 0.97 times the maximum value of \dot{h}_B .

4. For equal values of tension of $0.9 P_{\max}$ on each side of l_0 , \dot{h}_B was almost 40% greater at the shorter muscle length.

5. The results show that \dot{h}_B varies considerably with muscle length near l_0 , where tension varies little, and imply that tension is not the sole determinant of energy liberation in this length region.

INTRODUCTION

Aubert (1956) showed that the stable isometric maintenance heat rate (\dot{h}_B) in tetanic contractions of frog striated muscle is a linear function of tension (P) on both sides of the tension–length diagram. However, he found a quantitative difference in the relations, the slope being greater and the intercept on the heat axis smaller for $l > l_0$ than for $l < l_0$. Homsher, Mommaerts, Ricchiuti & Wallner (1972) made a detailed study for tetani in the length range $l > l_0$, including very long lengths where tension is virtually zero. They showed that both \dot{h}_B and total heat bear a simple linear relation to tension, but the data on total heat show considerable variability at $P = P_{\max}$, near l_0 , where Aubert's results suggest the existence of a transition from one linear relation to the other.

It is important to know how the isometric maintenance heat behaves in this length region, since it affects the interpretation of many energetics studies made near l_0 . For example, various energy balance studies, where energy output as $(\dot{h} + w)$ and substrate utilization and restortation are compared, seem to have been made near

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l_0 (e.g. Gilbert, Kretzschmar, Wilkie & Woledge, 1971; Kushmerick & Paul, 1976*a, b*; Homsher, Rall, Wallner & Ricchiuti, 1975; see also recent reviews by Kushmerick, 1977; Curtin & Woledge, 1978; Homsher & Kean, 1978). Also, since shortening heat is classically defined in terms of the isometric maintenance heat at the same length (Hill, 1938, 1964), it seems to have been customary to measure it in this length region where isometric tension and therefore, it was presumed, isometric heat production do not vary substantially with changes in length. The purpose of this study was to examine in detail the behaviour of \dot{h}_B in this length region.

Isometric tension and heat production were measured in the length range 0.75–1.25 l_0 . Heat production was found to vary more with muscle length in this range than tension does, being almost 40% higher at the shorter than at the longer length at which tension was 90% P_{\max} . Preliminary reports of this work have been presented (Gilbert & Aubert, 1978, 1979).

METHODS

Paired sartorius muscles from *Rana temporaria* were used. Animals were housed at 4 °C without food, and all experiments were made at 0 °C. Muscle length *in situ* (see below) was 32–43 mm, muscle mass for pairs of muscles 188–336 mg. The range of P_{\max} at the ninth second of stimulation was 163–268 kN/m² and that of the corresponding heat rate 11.6–16.1 mW/g.

Tension was measured by a strain gauge and heat production by a thermopile of the Hill-Downing type. These signals were appropriately amplified and recorded by an SE U.V. Recorder, model 3006.

The thermopile was 10 mm long and had 40 constantan–chromel junctions, connected in three ‘banks’. Temperature changes were monitored from the centre (5 mm, 20 junctions) and bottom (2.5 mm, 10 junctions) banks connected in series. The geometry of the thermopile and stimulating electrodes was such that the lowermost junction of the bottom bank was 10–12 mm distal to the acetabulum. Thus the region of muscle used always excluded the tapered tendinous end. In some experiments heat rate was measured from the top bank (2.5 mm) of the thermopile during preliminary contractions and was found to be virtually superimposable upon records taken from the bottom bank alone. The sensitivity of the thermopile was 58 $\mu\text{V}/\text{deg C}$ per junction, and the resistance was about 2 Ω per junction. The signal was amplified by an Ancom 15C-3a chopper amplifier.

In parallel with the Ancom amplifier was a differentiator consisting of an Amplislot Galvanometer Amplifier with a 1-millifarad capacitor at the input in series with the thermopile. The output of the Amplislot passed through a 10 Hz filter. The use of the differentiator slowed the heat signal considerably, but since only steady-state rates of heat production were of interest, no correction for heating lag was made.

Heat loss was measured during or after each experiment at least once at each muscle length used and varied consistently with muscle length in only one experiment, although the exponential rate constant k was lowest at the shortest length in all but two experiments. The range of the average k for the seven experiments was 0.020–0.026 sec^{-1} and for the experiment showing consistent length dependence of k was 0.017 sec^{-1} for the shortest length and 0.021 sec^{-1} for the longest length. Heat rate at each length was corrected for heat loss using the k measured at that length.

Before the muscles were dissected, their *in situ* length was measured, from the pelvis to the distal tendon at the knee, with the thighs at right angles to the long axis of the body. In four of the seven experiments, sarcomere length at this muscle length was measured by laser diffraction and found to be 2.1–2.2 μm . The length at which tension was maximal was defined as l_0 for purposes of normalizing the data, and it was within 1–2 mm of the *in situ* length.

After dissection, the muscles were mounted on the thermopile and connected to the strain gauge by a fine rhodium chain. The thermopile was fitted into a chamber-reservoir assembly containing oxygenated Ringer solution, and this was immersed in a large container of ice-water

mixture for maintaining constant temperature. The composition of the Ringer was (mM): NaCl, 113.5; KCl, 2.0; CaCl_2 , 1.8; Na_2HPO_4 , 1.2; NaH_2PO_4 , 0.6. The pH was 7.5.

Stimulation was via two pairs of platinum electrodes, one pair at each end of the thermopile, and consisted of alternating condenser discharges (70 V, time constant about 1 ms) at 25 sec^{-1} for 10 sec. No stimulus heat was observed when the stimulus was applied to a pair of muscles made inexcitable with isotonic KCl. Whenever the muscles were not being stimulated, they were bathed in oxygenated Ringer.

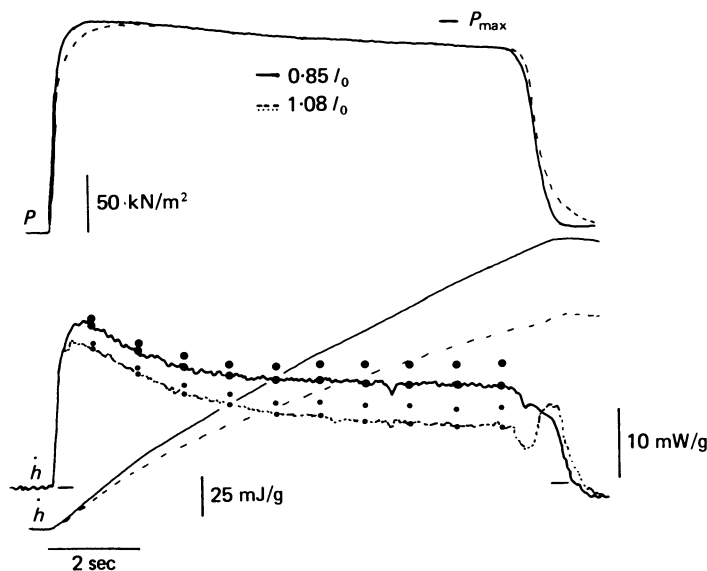


Fig. 1. Tension (P), heat rate (\dot{h}) and total heat (h) in isometric contractions at two muscle lengths. P_{\max} is tension at the ninth second of stimulation at l_0 . Curves traced from U.V. recorder records. The pairs of filled circles show data as read (in the differentiated heat trace) and after correction for heat loss (above the trace). Experiment 17 Jan. 1978, $l_0 = 34 \text{ mm}$, $m = 255 \text{ mg}$, $P_{\max} = 183 \text{ kN/m}^2$, rate constant for heat loss = 0.0205 sec^{-1} .

After an initial 30-min period of thermal equilibration in Ringer, the muscles were stimulated to produce isometric twitches at 0.5 min^{-1} so that stimulus parameters could be optimized and a length-tension curve determined to pinpoint the length range in which the experiment would be carried out. This usually required 15–25 twitches. Then tetanic stimulation was applied at 20-minute intervals. Data accumulation began only at the fourth or fifth tetanus of the experiment, since the first two were usually different from subsequent ones. The sequence of lengths used in each experiment was chosen randomly and the initial sequence repeated in a mirror pattern or randomized block design to remove any bias in the data due to progressive deterioration of the preparation. Tension dropped slightly during each contraction (6–13%), more at short than at long lengths. The average difference between tension in the first tetanus for which data was taken and the last tetanus at the same length was 6% for all the experiments.

RESULTS

Typical records of tension (P), heat rate (\dot{h}) and total heat (h) are shown in Figure 1 for two muscle lengths near l_0 . Tension is in the upper records. \dot{h} appears as recorded (noisy traces) and after correction for heat loss (dots above the records);

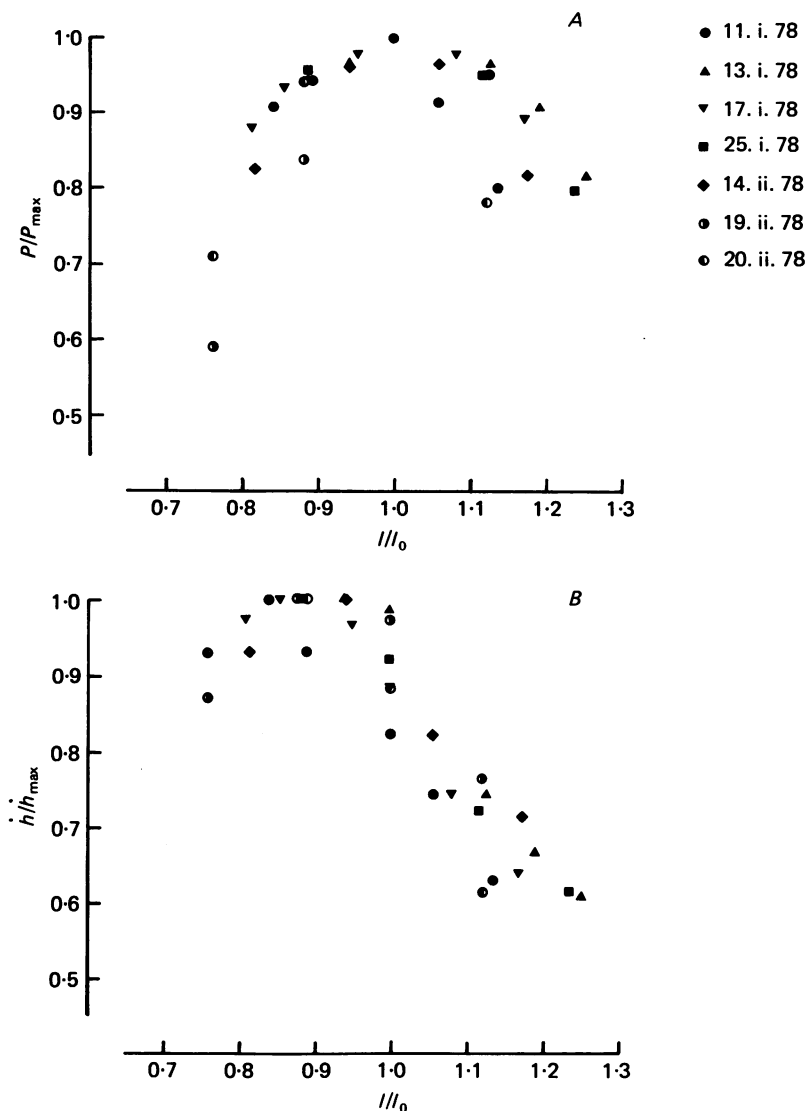


Fig. 2. Variation of tension (*A*) and heat rate (*B*) with muscle length. Results from seven experiments. l_0 was defined as the length at which isometric tension was maximal. Each point represents the normalized mean of tension (P/P_{max}) or heat rate (\dot{h}/\dot{h}_{max}) at the ninth second of stimulation in two to five contractions in a single experiment.

dots in the records show where the points were read). \dot{h} increases fairly rapidly during tension development (with the time constant of the differentiator) and declines gradually to a steady level after 5–6 sec. This steady level of \dot{h} is different at the two muscle lengths, being 35% less at the long than at the short length, although the active tension maintained is exactly the same.

Figure 2 shows normalized data from seven experiments. Tension (Fig. 2*a*) and \dot{h} (Fig. 2*b*) at the ninth second of stimulation were normalized to their respective

maximal values in each experiment. l_0 was defined as the length at which tension was maximal. Each point is the mean of two to five measurements in a single preparation. The maximal values of \dot{h} in all the experiments occur in the length range $0.84\text{--}0.93 l_0$. Thus in the range l_0 to $0.93 l_0$, \dot{h}/\dot{h}_{\max} actually increases as tension decreases. It can readily be seen that in any one experiment the heat rate for a given level of isometric tension is greater at the short length than at the long length.

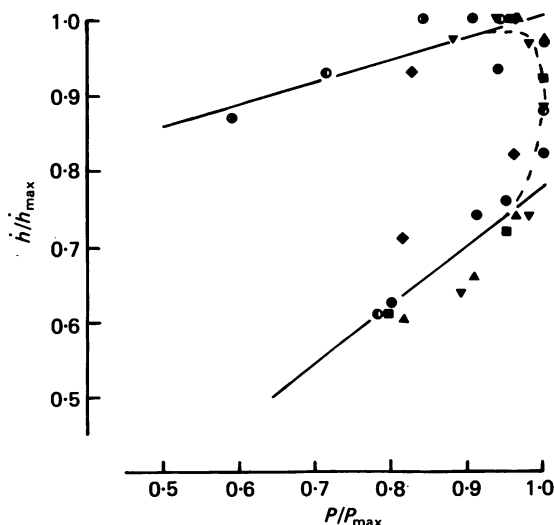


Fig. 3. Relation between heat rate and tension at different muscle lengths. Same data as shown in Fig. 2, with symbols corresponding to experiments as in Fig. 2. Solid lines from separate linear regression analyses on data at $l < l_0$ (upper line; intercept 0.72 ± 0.07 (s.e.), slope 0.29 ± 0.07 , $r = 0.791$) and $l > l_0$ (lower line; intercept 0.003 ± 0.131 , slope 0.78 ± 0.14 , $r = 0.823$). Points at $P/P_{\max} = 1$ were not included in the regressions. Dashed line obtained as described in the text.

The same results are shown in Figure 3 with \dot{h}/\dot{h}_{\max} plotted against P/P_{\max} . The variability in \dot{h}/\dot{h}_{\max} at $P/P_{\max} = 1$ is similar to that seen in the work of Homsher *et al.* (1972, their Fig. 3), where total heat was plotted against tension. The solid lines were drawn from separate linear regression analyses of the data at $l < l_0$ (upper line; intercept 0.72 ± 0.07 (s.e.), slope 0.29 ± 0.07 , $r = 0.791$) and $l > l_0$ (lower line; intercept 0.003 ± 0.131 , slope 0.78 ± 0.14 , $r = 0.823$) for all $P/P_{\max} < 1$. The lowest heat rate at $l < l_0$ near $P/P_{\max} = 1$ is $0.93 \dot{h}_{\max}$ ($P/P_{\max} = 0.94$). The highest heat rate at $l > l_0$ near $P/P_{\max} = 1$ is $0.82 \dot{h}_{\max}$ (for $P/P_{\max} = 0.96$). The lines show clearly the difference in tension dependence of \dot{h}/\dot{h}_{\max} on the two sides of l_0 .

The dashed line was calculated from the regression equations for the solid lines and is explained in detail in the Discussion.

DISCUSSION

The linear relation between \dot{h}/\dot{h}_{\max} and P/P_{\max} for $l > l_0$ is in general agreement with the results of Homsher *et al.* (1972). The rather small value of the intercept

cannot be taken seriously in view of its large standard error and the narrow range of tension in these experiments. Others have found that the $P = 0$ intercept of heat versus tension is a substantial fraction of the total maintenance heat in experiments where the full range of tension was examined (e.g. Smith, 1972, on striated muscle from *Rana temporaria* and *Bufo bufo*, and Matsumoto & McPhedran, 1977, on chick striated muscle).

The results for the whole length range $0.75-1.25 l_0$ are in general agreement with the observations of Aubert (1956), where the tension dependence of the isometric maintenance heat rate was found to obey two different linear relations on the two sides of l_0 . What the present study shows more clearly, however, is the transition near l_0 from the 'long muscle' to the 'short muscle' relation (lower and upper lines, respectively, in Figure 3). The transition is not abrupt but gradual, which could suggest that some exothermic reaction gradually increases its rate as the length at which the muscle is stimulated is reduced, and this could provide a clue to the identity of the reaction(s) responsible. It is possible, however, that the transition would be abrupt if it were possible to observe it in a single sarcomere. The dashed line in Fig. 3 shows the effect that could result from recording the average heat production of many sarcomeres, some of which may be shorter than others. Points on this line were calculated from the regression equations for the solid lines in the figure. First it was assumed that there are two populations of sarcomeres in the length range near l_0 , those whose heat rate-tension relation is like that for the 'short muscle' and those for which it is like that for the 'long muscle'. Next it was assumed that for any given tension level at some $l < l_0$ most of the sarcomeres would be 'short', while at the same tension level at $l > l_0$ most of the sarcomeres would be 'long'. Then a composite \dot{h}/\dot{h}_{\max} was calculated for the two lengths. For example, at $P/P_{\max} = 0.99$, \dot{h}/\dot{h}_{\max} is 1.00 for short sarcomeres and 0.78 for long sarcomeres. If at $l < l_0$ where $P/P_{\max} = 0.99$, 60% of the sarcomeres are short and 40% are long, $\dot{h}/\dot{h}_{\max} = 0.6(1.00) + 0.4(0.78) = 0.91$. At $l > l_0$, the ratio is reversed, and $\dot{h}/\dot{h}_{\max} = 0.87$. Other pairs of points were calculated for various values of P assuming ratios of 90% short, 10% long (and vice versa) for $0.98 P_{\max}$, 97.5% short, 2.5% long for $0.95 P_{\max}$, 99.5% short, 0.5% long for $0.925 P_{\max}$ and 99.9% short, 0.1% long for $0.90 P_{\max}$. These ratios were chosen so as to produce continuity between the solid lines and the dashed ones. The dashed line fits the data reasonably well, suggesting that even an abrupt transition to a higher heat rate for a given value of tension at the sarcomere level could result in a smoother change in the whole muscle if the sarcomeres are not all exactly the same length.

The results show that the rate of some exothermic reaction increases substantially with decreasing length near l_0 . There are several reactions that could be involved. Exothermic reactions responsible for the isometric maintenance heat are: (1) the hydrolysis of ATP by actomyosin ATPase, i.e. cross-bridge cycling; (2) the hydrolysis of ATP by the calcium-dependent ATPase of the sarcoplasmic reticulum, i.e. the 'calcium pump'; (3) changes of state of calcium such as binding to troponin and other proteins; (4) whatever unidentified reaction(s) are responsible for the unexplained energy liberation in contracting muscle. (See the recent reviews by Curtin & Woledge, 1978, and Homsher & Kean, 1978, for complete discussions of the 'energy gap' and the identity of heat-producing reactions in contracting muscle.) There

appears to be no evidence as to whether any of the first three reactions increase in rate with decreasing length near l_0 . In his discussion of the difference in tension-dependence of \dot{h} on the two sides of l_0 , Aubert (1956) suggested the existence of an internal force opposing the force developed by the contractile mechanism. Such a force could cause an increase in the rate of cross-bridge cycling, if, for example, the rate of cross-bridge detachment were to increase as force on the bridge decreases. However, Edman (1978) has recently measured V_{\max} as a function of sarcomere length in single muscle fibres and has found no length-dependence of V_{\max} in the sarcomere length range 1.65–2.60 μm , suggesting that there is no internal force in this length range. The sarcoplasmic calcium concentration does not change in this length range (Blinks, Rüdél & Taylor, 1978, Fig. 11) nor is there reason to suspect an increase in the rate of the calcium pump. The possibilities could be narrowed somewhat by measuring the length-dependence of phosphoryl creatine hydrolysis in this region, to see whether substrate utilization shows the same behaviour as energy liberation.

These results have implications concerning the interpretation of several types of energetics experiments. Energy balance experiments, whose object is to compare the energy liberated during contraction with that expected from the quantity of substrate utilized, are usually made by measuring substrate utilization and energy liberation in different muscles (e.g. Gilbert *et al.*, 1971; Homsher *et al.*, 1975). The present study shows that even if two muscles develop the same relative tension near l_0 , there could be a substantial difference in the energy liberated, depending on whether the muscle is on the long or the short side of l_0 . In properly randomized experiments, the effect would manifest itself as an increase in the error of measurement of energy liberation and perhaps substrate utilization as well, if the latter shows the same sort of length-dependence as the former. A length-dependence of substrate utilization could also affect results of studies in which this quantity is compared with substrate re-synthesis during recovery (e.g. Kushmerick & Paul, 1976*a, b*; DeFuria & Kushmerick, 1977). Potential difficulties could be avoided in both types of experiments if the muscles were held at lengths such that the sarcomere length is slightly greater than 2.2 μm . At that length there is an unambiguous relation between tension and energy liberation.

The results are also of interest concerning measurements of shortening heat (h_s). h_s is defined as the difference between the heat produced during shortening and that produced in a comparable isometric contraction *at the same length* (Hill, 1938). Since a shortening muscle is not at a given length, the experiments have usually been made in the region near l_0 such that the isometric tensions at the initial and final lengths were the same, with the implicit assumption that the isometric heats were also similar. The results of this study show that this assumption is not valid. Further, recent experiments in which h_s measured over length ranges just to the right and to the left of l_0 were compared have revealed rather dramatic differences in the amount and time course of total heat production during isovelocity shortening, particularly at low velocity (Gilbert, 1978, unpublished observations). These factors could have profound effects on our ideas about the time course of energy liberation in work-producing contractions.

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