Energy Balance Studies in Frog Skeletal Muscles Shortening at One-Half Maximal Velocity

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ABSTRACT High-energy phosphate metabolism and energy liberated as heat and work were measured in 3-s tetani of frog sartorius muscle at 0°C. Two contraction periods were studied: (a) a 0.35-s period of shortening near half-maximum velocity beginning after 2 s of isometric stimulation, and (b) a 0.65-s isometric period immediately following the shortening. There were no significant changes in levels of ATP, ADP, or AMP in the two contraction periods. The observed changes in inorganic phosphate and creatine levels indicated that the only significant reaction occurring was phosphocreatine splitting. The mean rate of high-energy phosphate splitting during the shortening, 1.60 ± 0.23 \( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{s}^{-1} \) (n = 24), was about fivefold higher than that in the 1-s period in the isometric tetanus, 0.32 ± 0.11 \( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{s}^{-1} \) (n = 17), observed in our previous study. The mean rate in the post-shortening period, 0.46 ± 0.13 \( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{s}^{-1} \) (n = 17), was not significantly different from that in the 1-s period in the isometric tetanus. A large amount of heat plus work was produced during the shortening period, and this could be accounted for by simultaneous chemical changes. In the post-shortening period, the observed enthalpy was also accounted for by simultaneous chemical reactions. Thus, the present result is in sharp contrast to that obtained from a similar study performed at a shortening at \( V_{max} \), where an enthalpy excess was produced during shortening and an enthalpy deficit was produced during the period following the shortening.

INTRODUCTION

It has been known for a number of years that when a muscle shortens, the rate of energy liberation increases over that seen in an isometric tetanus, both as heat liberated (\( h \)) and mechanical work performed (\( w \)) (Hill, 1985). Since muscular contraction is a consequence of the interaction of the myofibrillar proteins actin and myosin with ATP and its hydrolysis to ADP and inorganic phosphate (\( P_i \)), it was natural to assume that the rate of enthalpy liberation by a muscle was a direct reflection of the enthalpy liberation associated with ATP hydrolysis. With

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the development of the cross-bridge hypothesis (Huxley, 1957), muscle contraction was viewed as a result of cyclical attachment and detachment of cross-bridges, with each working cycle involving the hydrolysis of one molecule of ATP. This view was reinforced by the development of the Lynn-Taylor (1971) model of the mechanism of ATP hydrolysis by in vitro actomyosin systems. Thus, some theories of muscle contraction (Huxley, 1957, 1973; Podolsky and Nolan, 1972; Eisenberg et al., 1980) have used the rate of enthalpy production as an index of the steady state rate of both ATP hydrolysis and cross-bridge turnover.

The assumption that high-energy phosphate (\(\sim P\)) hydrolysis is the sole source of enthalpy production during contraction can be tested using the energy balance technique. If the assumption is correct, the amount of energy liberated (as heat and work) must equal the amount of enthalpy liberated by the measured extent of high-energy phosphate hydrolysis. In the first study of the energy balance of shortening muscles, Curtin et al. (1974) compared the energy balance in muscles shortening at 0.3 \(V_{\text{max}}\) with that of an isometric contraction. In their experiments, the metabolism of a tetanized muscle was halted immediately after the muscle had completed a 1-s period of shortening. In a second set of experiments, the energy balance was measured in a muscle that had contracted isometrically for 1.7 s. The results showed that while both contractions produced a large and significant amount of unexplained enthalpy, the work performed during shortening could be explained by the observed high-energy phosphate hydrolysis; thus, one need not postulate the existence of some unknown reaction that contributes to the performance of work. The fact that both the isometric and shortening muscle produced similar amounts of unexplained enthalpy suggests, but does not prove, that the unexplained enthalpy is produced by a reaction(s) unrelated to shortening. It has since been shown (Homsher et al., 1979; Curtin and Woledge, 1979) that 10-40 mJ·g\(^{-1}\) of unexplained enthalpy is produced at the start of a tetanus, probably by processes independent of the cross-bridges (Curtin and Woledge, 1981; Homsher and Kean, 1982). Thus, it is not clear whether or not there is an energy balance in muscles shortening at an intermediate velocity.

Recently (Homsher et al., 1979; Curtin and Woledge, 1979), it was shown that the isometric unexplained enthalpy is produced only at the beginning of a tetanus and that the amount of unexplained enthalpy produced by \emph{Rana pipiens} sartorius muscles is small (as calculated by Curtin and Woledge, 1978, from the results of Homsher et al., 1975). On the basis of these observations, Homsher et al. (1981) demonstrated that in \emph{R. pipiens} the amount of unexplained enthalpy produced after 2 s of isometric tetanization was not different from zero and thus devised a method for unambiguously examining the energy balance during shortening.

Using an experimental design in which isometric unexplained enthalpy is depleted by 2 s of isometric contraction prior to shortening, Homsher et al. (1981) found that during a 0.3-s period of muscle shortening at \(V_{\text{max}}\), a large amount of heat plus work \((h + w)\) was produced, but more than half of it, 6.5 ± 2.6 mJ·g\(^{-1}\), could not be explained by \(\sim P\) hydrolysis. In the 0.7-s time interval following the shortening, when the muscle remained at constant length, the \(h + w\) production was 6.2 ± 2.6 mJ·g\(^{-1}\) less than that expected from the simultaneous
chemical reactions. Thus, over the combined shortening and post-shortening periods, there is an energy balance, but not at the time shortening occurs. These results were interpreted as indicating that rapid shortening produces an exothermic shift in the population distribution of two cross-bridge states during rapid shortening and that this shift is reversed in the subsequent isometric period by ATP hydrolysis. This model, first proposed by Irving and Woledge (1981), predicts that the rate of unexplained enthalpy production parallels the rate of ATP hydrolysis. Since the rate of ATP hydrolysis increases during shortening at intermediate velocities, the rate of unexplained enthalpy production should increase as well. Therefore, the energy balance during and after shortening at a velocity equal to $\frac{1}{2} V_{\text{max}}$ was measured using an experimental design and protocol similar to that used by Homsher et al. (1981). The results of these experiments showed that upon shortening at $\frac{1}{2} V_{\text{max}}$, in spite of the fact that the rate of energy liberation and ATP hydrolysis is four- to fivefold greater than the isometric rate, there was an energy balance both during and immediately after shortening. Preliminary reports of some of these results have been presented (Homsher et al., 1982).

METHODS

Frogs (R. pipiens) weighing 25-30 g were obtained in a single shipment from Nasco Biological Co. (Fort Atkinson, WI) and kept in moist tanks at 6°C for at least 14 d before use. On the evening before an experiment, pairs of sartorius muscles were dissected. If fiber damage or parasite infestation was detected on visual inspection, the pair was discarded. The remaining muscles were aerated overnight with 95% O$_2$, 5% CO$_2$ in Ringer's solution containing 95.0 mM NaCl, 25.0 mM NaHCO$_3$, 2.5 mM KCl, 1.0 mM MgCl$_2$, and 1.0 mM CaCl$_2$ (pH 7.2) at 4°C.

The methods used to measure their sarcomere length, record and analyze force and displacement, measure the amount of high-energy phosphate hydrolysis, and estimate the amount of explained and unexplained enthalpy were as previously described (Homsher et al., 1981).

Measurement of the Uniformity of Muscle Length Changes

Recent studies (Julian and Morgan, 1979; Kobayashi and Sugi, 1982) have shown that during contraction, under certain conditions, nonuniformity of shortening can arise along the length of the muscle or muscle fiber. Because the heat production in the present experiments was sampled from a muscle segment amounting to 62-78% of the total muscle length (extending from the pelvic origin toward the tibial tendon), while shortening was controlled only at the tibial end of the muscle, the possibility of a systematic error, caused by nonuniform shortening, existed. To test this possibility, single sartorius muscles were dissected and treated as described above and fine carbon particles (0.03-0.3 mm diam) were sprinkled over the dorsal surface of the muscle. The carbon particles adhere to the surface of the muscle fibers and act as markers of specific points on the muscle as the muscle is lengthened and shortened. The muscle was then mounted on a dummy thermopile, immersed in a Ringer's solution held at 0-1°C, and the sarcomere length was adjusted to 2.4 μm. After a 20-min equilibration, the Ringer's solution was rapidly drained, and the muscle was stimulated and allowed to shorten under conditions identical to those used in the energy balance experiments. Using an electronically triggered Nikon (Garden City, NY) F-3 camera, equipped with an MD-4 motor drive, 1/125-s exposures were made
of the muscle before the tetanus, 100 ms before shortening began, 175 and 350 ms after the beginning of shortening (the former corresponds to the midway of shortening and the latter to the cessation of shortening), 650 ms after the cessation of shortening (when tension had fully redeveloped), and after relaxation. Photographic enlargements of the negatives were used to measure the position of the markers. If shortening is uniform along the length of the muscle, then the relative length of different sections of the muscle (measured from a stationary reference point, the pelvic origin) will not differ from one another throughout the contraction. In the myothermal studies, the muscle temperature is initially sampled (before shortening) from 62 (starting from the pelvic tendon) to 78% (by the end of shortening) of the muscle length ($l_0$). Therefore, markers located near 0.62 and 0.78 from the pelvic origin were identified and their movement was compared with that of the knot joining the tibial tendon to the transducer connection. The length of each muscle section immediately before shortening was scaled to a value of 1.000. The results of measurements from seven different muscles are tabulated in Table I. These results show that the relative length of the various segments and the overall muscle length are not statistically different at all times.

RESULTS

Chemical changes and $h + w$ production were measured under identical conditions at 0°C in different muscle pairs of frogs from the same batch. Fig. 1 illustrates the experimental design used in these experiments and shows a typical set of myothermal records. All muscles were held at a 2.4-μm sarcomere length (as measured in the resting muscle) for the first 2 s of the tetanus. They were then released at 1.42 μm·s⁻¹/sarcomere for 0.35 s to a sarcomere length of 1.9 μm. The muscles remained at this length for the remainder of the tetanus. During shortening, the tension fell to 26.1 ± 1.7% ($n = 17$) of the isometric value (Fig. 1), and the rapid upturn in the heat record at the start of the

| Relative shortening distance | Knot to pelvic edge | 78% muscle segment* | 62% muscle segment* |
|------------------------------|---------------------|---------------------|---------------------|
| Before stimulation           | 1.002±0.001         | 1.001±0.003         | 1.003±0.003         |
| Just before shortening       | 1.000               | 1.000               | 1.000               |
| At the midway of shortening  | 0.892±0.006         | 0.889±0.006         | 0.888±0.004         |
| At the end of shortening      | 0.793±0.010         | 0.778±0.011         | 0.776±0.009         |
| After tension redevelopment  | 0.793±0.010         | 0.776±0.011         | 0.763±0.011         |
| After relaxation              | 0.796±0.011         | 0.784±0.011         | 0.778±0.012         |

* The 62 and 78% muscle segments correspond to the fractions of the muscle length from which heat production was measured. Photographs of the muscle surface were taken at various stages during a 3-s tetanus. The lengths of muscle segments, determined by measuring the distances between carbon particles, were expressed relative to those just before the shortening. See text for details.
shortening reflects the increased rate of heat production associated with the shortening.

Chemical changes during the 0.35 s of shortening were separately estimated from the difference in metabolic contents between a muscle frozen at the start of shortening (dotted line A in Fig. 1) and the contralateral muscle frozen 0.35 s later, i.e., at the end of shortening (dotted line B in Fig. 1). The mean maintained tension during shortening averaged 23.6 ± 1.0% (n = 41) of the isometric value and was not different from the value observed in the heat experiments. Mea-
measurements over the 0.65-s period after shortening were made by freezing one muscle of a pair at the end of shortening (dotted line B in Fig. 1) and the other at 0.65 s (dotted line C in Fig. 1) after the end of shortening.

**High-Energy Phosphate Metabolism**

The changes in metabolite levels associated with the two contraction periods are given in Table II. As noted earlier, oxidative and glycolytic recovery occurs to a negligible extent in brief tetani of frog muscles at 0°C (Curtin and Woledge, 1978), so these reactions were not monitored. Changes in the content of ATP, ADP, AMP, P<sub>i</sub>, creatine (C<sub>F</sub>), and total creatine (C<sub>T</sub>) were measured so that the extent of ATP and phosphocreatine (PCr) splitting and the myokinase reaction could be determined. The values in Table II have been normalized by the total creatine content of each muscle.

There was no significant change in the level of ATP, ADP, or AMP in either contraction period. Consequently, the extent of the myokinase reaction is small and insignificant, as is the observed ATP hydrolysis (ξ<sub>ATP</sub>), calculated from the change in ATP and ADP. The extent of PCr splitting (ξ<sub>PCr</sub>) was significantly different from zero in each case. The total ATP utilization was calculated for each muscle pair as ξ<sub>ATP</sub> + ξ<sub>PCr</sub>, and the last line in Table II shows the mean rate of ATP utilization in each contraction period. During shortening, the mean rate of ATP utilization, 43.9 ± 5.77 nmol·μmol<sup>-1</sup> C<sub>T</sub>·s<sup>-1</sup> (mean ± SEM, n = 24), was substantially greater than that in a comparable isometric contraction period (length = 1.8 μm/sarcomere), 7.97 ± 2.59 nmol·μmol<sup>-1</sup> C<sub>T</sub>·s<sup>-1</sup> (n = 17), observed in a previous study from this laboratory (Homsher et al., 1981). However, the mean rate of ATP utilization in the post-shortening period, 12.55 ± 3.39 nmol·μmol<sup>-1</sup> C<sub>T</sub>·s<sup>-1</sup> (n = 17) (or 0.46 ± 0.12 μmol·g<sup>-1</sup>·s<sup>-1</sup>), was not significantly different from that in an isometric contraction. We conclude that there is a significant fivefold increase in the rate of high-energy phosphate splitting during

| TABLE II | Chemical Changes Normalized by Total Creatine Content (C<sub>T</sub>) |
|-----------------------------|---------------------------|
| Shortening at ½ V<sub>max</sub> (2.0-2.35 s) | Post-shortening period (2.35-3.0 s) |
| C<sub>F</sub>/CT nmol·μmol<sup>-1</sup> | 15.41±2.78 | 7.06±2.02 |
| P<sub>i</sub>/CT nmol·μmol<sup>-1</sup> | 16.44±1.84 | 8.94±2.77 |
| ATP/CT nmol·μmol<sup>-1</sup> | 0.44±0.72 | -0.13±0.85 |
| ADP/CT nmol·μmol<sup>-1</sup> | -0.04±0.72 | 0.06±0.50 |
| AMP/CT nmol·μmol<sup>-1</sup> | -0.20±0.34 | -0.02±0.12 |
| ξ<sub>PCr</sub>/CT nmol·μmol<sup>-1</sup> | 15.76±2.02 | 8.06±2.14 |
| ξ<sub>ATP</sub>/CT nmol·μmol<sup>-1</sup> | -0.40±0.50 | 0.10±0.53 |
| R<sub>ATP</sub>/CT nmol·μmol<sup>-1</sup>·s<sup>-1</sup> | 43.9±5.77 | 12.55±3.39 |

All values for chemical change given as experimental – control. C<sub>F</sub>, free creatine; P<sub>i</sub>, inorganic phosphate; PCr, phosphocreatine, denotes extent of reaction (ATP or PCr splitting, according to the subscript) calculated as described under Methods. R<sub>ATP</sub> is the total mean rate of ATP utilization, (ξ<sub>ATP</sub> + ξ<sub>PCr</sub>)·s<sup>-1</sup>. The total creatine per gram of muscle (wet weight) was 36.43 ± 0.63 μmol·g<sup>-1</sup>. Data in the table are means ± SEM for the following numbers of observations: column 1 (shortening), n = 24; column 2 (post-shortening), n = 17.
shortening at $V_{max}/2$, but with the cessation of shortening the rate of ATP hydrolysis returns to levels similar to those seen in isometric contractions.

**Enthalpy Production**

The sum of heat and work production (the observed enthalpy) is shown in Table III (top row). In the shortening period, the external work production was $207.5 \pm 12.4 \mu J \cdot \mu mol^{-1} C_T (n = 17)$, which is $\sim 40\%$ of the total enthalpy liberation. In the post-shortening period, the work done by the muscles on the series elastic structures was calculated (see Methods) to be $24.6 \pm 1.4 \mu J \cdot \mu mol^{-1} C_T (n = 17)$, or $<9\%$ of the energy liberated in this period.

The mean rate of enthalpy liberation during shortening at $V_{max}/2$, $1.48 \pm 0.07 \text{mW} \cdot \mu \text{mol}^{-1} C_T (n = 17)$, was $\sim 4.5$ times that in an isometric contraction period, $0.33 \pm 0.02 \text{mW} \cdot \mu \text{mol}^{-1} C_T (n = 17)$, which was reported earlier (Homsher et al., 1981). To estimate the amount of shortening heat produced during shortening, the rate of isometric heat production for the 200 ms preceding shortening was measured, and the amount expected in the period with shortening was estimated by linear extrapolation. This value was then subtracted from the observed heat production to yield the estimated shortening heat, which was $172.1 \pm 12.6 \mu J \cdot \mu mol^{-1} C_T$, or $\sim 33\%$ of the observed $h + w$. While this technique does not take into account the sarcomere length dependence of isometric heat rate (Homsher et al., 1983), the error introduced by this approximation is small, resulting in an overestimate of the shortening heat production of $<5\%$. The rate of enthalpy production in the post-shortening period, $0.45 \pm 0.02 \text{mW} \cdot \mu \text{mol}^{-1} C_T (n = 17)$, is slightly, but significantly ($P < 0.05$), greater than a purely isometric contraction and is probably a reflection of the performance of internal work and shortening during force redevelopment in the post-shortening period. Nevertheless, as expected, a large increase in enthalpy production occurs during shortening.

**Energy Balance Calculations**

Combining the observed extents of reaction (Table II) with the molar enthalpy change of each reaction under physiological conditions (Curtin and Woledge,

| TABLE III |
| Enthalpy ($\mu J$) Normalized by the Total Creatine Content |
| Shortening at $Vmax (2.0\text{--}2.35 \text{s})$ | Post-shortening period (2.35--3.0 s) |
|--------|--------|
| Observed enthalpy | $517.1 \pm 23.8$ | $295.1 \pm 14.3$ |
| ($\mu J \cdot \mu mol^{-1} C_T$) | ($n = 17$) | ($n = 17$) |
| Explained enthalpy | $516.6 \pm 72.7$ | $278.5 \pm 76.6$ |
| ($\mu J \cdot \mu mol^{-1} C_T$) | ($n = 24$) | ($n = 17$) |
| Unexplained enthalpy | $0.5 \pm 76.5$ | $16.6 \pm 77.9$ |
| ($\mu J \cdot \mu mol^{-1} C_T$) | |

All values are given as the mean $\pm$ SEM. Observed enthalpy is the sum of the heat and work production in each measurement period. Explained enthalpy was calculated from the extents of the reactions measured in the corresponding period (see Table I) by the procedure described in the Methods. Unexplained enthalpy is the difference between the observed and explained enthalpies.
FIGURE 2. Enthalpy liberation (mJ·g⁻¹), mean ± SEM; the number of observations was as given in Table III. Open bars, observed enthalpy (heat + work); diagonally shaded bars, explained enthalpy calculated as described in the text; stippled bars, unexplained enthalpy (observed − explained). Columns from left to right show results for the period of shortening at \( \frac{1}{2} V_{\text{max}} \) (S), the post-shortening period (PS), and the sum of these two periods (S + PS).

1978), one can calculate the explained enthalpy, i.e., the enthalpy produced by the measured chemical reactions. If the difference between the observed enthalpy and the explained enthalpy is nonzero, some other reaction of energetic significance must be occurring. The results of such an energy balance calculation are shown in Table III and Fig. 2. The unexplained enthalpy produced during shortening was \( 0.5 \pm 76.5 \mu\text{J}\cdot\mu\text{mol}^{-1} \cdot \text{CT} \), and during the post-shortening period it was \( 16.6 \pm 77.9 \mu\text{J}\cdot\mu\text{mol}^{-1} \cdot \text{CT} \). Neither of these values is significantly different from zero (\( P > 0.5 \)). Thus, an energy balance is maintained both during and after shortening at \( \frac{1}{2} V_{\text{max}} \). Fig. 2 shows that in the time interval between seconds 2 and 3, a total of \( 29.58 \pm 0.98 \text{ mJ} \cdot \text{g}^{-1} \) of energy was liberated by the muscles, and \( 28.96 \pm 3.84 \text{ mJ} \cdot \text{g}^{-1} \), or ~98% of it, can be explained by the high-energy phosphate hydrolysis.

DISCUSSION

The major result of the experiments described above is that both during shortening at \( \frac{1}{2} V_{\text{max}} \) and during the period immediately following shortening, no significant amounts of unexplained enthalpy are produced. This fact, plus the energy balance observed in the isometric contractions between 2 and 3 s (Homsher et al., 1981), suggest that at velocities less than \( \frac{1}{2} V_{\text{max}} \) the rate of energy liberation during and immediately after shortening is a direct consequence of the hydrolysis of high-energy phosphate. This result is in stark contrast to the results of energy balance studies in muscles shortening near \( V_{\text{max}} \) in which there is a significant excess of enthalpy produced during shortening and a
significant deficit produced after shortening (Homsher et al., 1981). In Fig. 3, the rate of total enthalpy production ($h + \dot{w}$), the rate of energy liberation derived from the hydrolysis of high-energy phosphate ($\dot{H}_p$), and the rate of unexplained enthalpy production ($\dot{U}E$) for isometric and for shortening at $\frac{1}{2} V_{\text{max}}$ and $V_{\text{max}}$ contractions are compared both during shortening (Fig. 3A) and immediately after shortening. This figure facilitates the following discussions.

Rate of Total Energy Liberation

The rate of total energy liberation in each case is significantly different from zero ($P \ll 0.01$). The energy liberation rates for the isometric contraction, during the shortening at $\frac{1}{2} V_{\text{max}}$ and during shortening at $V_{\text{max}}$, are significantly different from each other ($P \ll 0.001$). During shortening at $\frac{1}{2} V_{\text{max}}$ and $V_{\text{max}}$, the rate of energy liberation increases to four and three times, respectively, that of the isometric rate. The rate of energy liberation during tension redevelopment after shortening at either velocity is significantly ($P < 0.01$) greater than that of an isometric contraction. However, if the rate of internal work performance during tension redevelopment (1.4 mW·g$^{-1}$) is subtracted from the total, the rate of energy liberation by the muscles that had shortened is no longer significantly ($P > 0.2$) different from the wholly isometric contraction. The rates of work production during shortening at $\frac{1}{2} V_{\text{max}}$ and $V_{\text{max}}$ were, respectively, 40 and 13% of the total energy liberation rate. These results are in good accord with earlier work of Hill (1964).

![Figure 3](image)

**Figure 3.** Comparison of the rate of observed enthalpy production ($h + \dot{w}$), explained enthalpy production ($\dot{H}_p$), and unexplained enthalpy production ($\dot{U}E$), during isometric contraction during and after shortenings at $V_{\text{max}}$ and $\frac{1}{2} V_{\text{max}}$. Diagonally shaded bars, isometric; open bars, $\frac{1}{2} V_{\text{max}}$; stippled bars, $V_{\text{max}}$. 


Rate of Energy Liberation Produced by High-Energy Phosphate Hydrolysis

Using the data in Tables II and III, the \((\mathcal{H}_{\text{P}})\) can be calculated. The results show that in each case, \((\mathcal{H}_{\text{P}})\) is significantly different from zero \((P < 0.01)\), except for muscles shortening at \(V_{\text{max}}\) \((P < 0.07)\). Isometric \((\mathcal{H}_{\text{P}})\) is significantly \((P < 0.02)\) less than that observed both during shortening at \(\frac{1}{2} V_{\text{max}}\) and after shortening at \(V_{\text{max}}\). However, neither \((\mathcal{H}_{\text{P}})\) during shortening at \(V_{\text{max}}\) nor \((\mathcal{H}_{\text{P}})\) after shortening at \(\frac{1}{2} V_{\text{max}}\) is significantly different from the isometric value \((P > 0.5)\). The rate of high-energy phosphate hydrolysis during shortening at \(\frac{1}{2} V_{\text{max}}\) \((1.60 \pm 0.23 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{s}^{-1})\) is in good agreement with the value observed by Kushmerick et al. (1969) under comparable conditions.

Rate of Unexplained Enthalpy Production

The rates of unexplained enthalpy production in the isometric contraction or muscles shortening at \(\frac{1}{2} V_{\text{max}}\) are all small and are not significantly different from zero \((P > 0.5)\). However, both during and after shortening at \(V_{\text{max}}\), \(\mathcal{U}\) is large and significantly different from zero \((P < 0.025)\). Although there is a tendency for the rate of \(\mathcal{U}\) production by muscles shortening at \(V_{\text{max}}\) to be different from that of a muscle shortening at \(\frac{1}{2} V_{\text{max}}\) (both during and after shortening), the difference is not significant \((P < 0.08\) in the former case and \(P < 0.1\) in the latter).

Aside from the comparisons made above, the results of the present experiments have direct bearing on two points of interest in the energetics of muscles shortening at velocities near \(V_{\text{max}}\). First, one could have argued that the low rate of hydrolysis of muscles shortening near \(V_{\text{max}}\) was a consequence of an inability of amphibian actomyosin to cleave ATP at a rate sufficient to keep pace with cross-bridge cycling. The present results show that this hypothesis is untenable because the muscle can hydrolyze high-energy phosphate at a rate more than three times that observed at \(V_{\text{max}}\). This point is emphasized by expressing the rate of ATP hydrolysis as a turnover rate (per myosin S1 head) while shortening at \(V_{\text{max}}\) or \(\frac{1}{2} V_{\text{max}}\) and while under isometric contraction: these rates are \(1.7 \pm 0.9, 5.8 \pm 0.8,\) and \(1.1 \pm 0.4 \text{ s}^{-1}\), respectively (assuming the muscle contains 0.28 \(\mu\text{mol myosin S1 heads per gram of muscle}\) [Ebashi et al., 1969]). Thus, the rates of ATP hydrolysis and energy liberation are controlled by mechanical factors external to the muscle. Second, we (Homsher et al., 1981) had earlier noted the similarity in magnitude between the shortening heat and unexplained heat production. However, in the present work, the amount of shortening heat produced was \(6.27 \pm 0.46 \text{ mJ} \cdot \text{g}^{-1}\) as compared with an unexplained enthalpy production of \(0.02 \pm 2.78 \text{ mJ} \cdot \text{g}^{-1}\). The two values are significantly different \((P < 0.05)\), which argues against a common origin.

In considering the factors that could be responsible for the reduction of unexplained enthalpy production during shortening at a lower velocity, several possible factors can be identified: (a) the duration of shortening; (b) the sarcomere length range over which shortening takes place; (c) the distance shortened; and (d) the velocity of shortening. The first two possibilities are unlikely because in the present experiments the duration of shortening (350 ms) and sarcomere length range (2.4–1.9 \(\mu\text{m}\)) were selected so as to closely approximate the...
conditions (300 ms and 2.6–1.8 \mu m) used in the studies at $V_{\text{max}}$. With regard to the third possibility, if one were to hypothesize that the amount of unexplained enthalpy production were proportional to the distance shortened, the present experiments should have resulted in the production of 4.1 mJ·g$^{-1}$ (at $V_{\text{max}}$, 6.5 mJ·g$^{-1}$ of unexplained enthalpy was produced by a shortening of 0.8 \mu m/sarcomere), which is not significantly different from the value actually observed ($P < 0.2$). Given the errors inherent in studies using paired muscles, a shortening of $\sim 1.0$ \mu m/sarcomere at $\frac{1}{2} V_{\text{max}}$ would be required to test this hypothesis conclusively. If this hypothesis were correct, however, a very substantial reduction in the rate of ATP hydrolysis with further shortening at $\frac{1}{2} V_{\text{max}}$ would be required to generate significant unexplained enthalpy, since the observed enthalpy would continue to be produced at the same or reduced rate (Abbott, 1951). Kushmerick and Davies (1969) have found that the rate of ATP hydrolysis over displacements in excess of 1.5 \mu m/sarcomere at velocities near $\frac{1}{2} V_{\text{max}}$ is $\sim 1.6$ \mu mol·g$^{-1}$·s$^{-1}$. Therefore, it is unlikely that the presence of unexplained enthalpy production in muscles shortening near $V_{\text{max}}$ in our earlier work is specifically related to the distance shortened; it is more likely to be related to the velocity of shortening.

To account for the energy imbalance in muscles shortening near $V_{\text{max}}$, a hypothesis using the idea of incomplete thermodynamic cycles has been advanced (Homsher et al., 1981). It was assumed, as earlier suggested by Irving and Woledge (1981), that cross-bridges could exist in either an X or Y state; during isometric contraction, most cross-bridges would be in the X state, and during shortening an increasing fraction of the cross-bridge population would exist in the Y state. The net transition of X to Y was assumed to be spontaneous ($\Delta F$ is negative) and exothermic, and to occur at a rate, $k_8$, which increases during shortening. The transition from Y to X must then be powered by ATP hydrolysis and the Y-to-X transition would itself be endothermic. To reconcile the low rate of ATP hydrolysis with the reduced muscle stiffness during shortening at $V_{\text{max}}$ (Julian and Sollins, 1975), it was assumed that the Y state was a detached cross-bridge and the rate constant for the Y-to-X transition, $k_8$, was independent of velocity of shortening. While the model does account for the energetic behavior near $V_{\text{max}}$, it predicts that the rate of unexplained enthalpy production will parallel the rate of ATP hydrolysis. Fig. 3 shows that this expectation is contradicted at $\frac{1}{2} V_{\text{max}}$ shortening, and the model therefore fails. It is possible to fit the data by increasing the number of cross-bridge states to three. However, at least two different reaction schemes could be imagined using three states, and as no data are available to further constrain the models, it does not seem prudent to speculate any further.

The data in this work can be used to estimate the number of cross-bridges attached to the thin filaments and hydrolyzing ATP at any given time. If the filaments are sliding by each other at a velocity of 710 nm·s$^{-1}$/half-sarcomere at $\frac{1}{2} V_{\text{max}}$ and if the maximum distance a cross-bridge can remain attached to a given actin molecule is 15 nm (Ford et al., 1977), the cross-bridge cycling rate must be at least 48 s$^{-1}$. Since the ATP hydrolysis rate (per myosin head) is 5.5 s$^{-1}$, no more than 11% of the myosin head can be attached and splitting ATP at
any instant (there could be a large number of cross-bridges attached and not hydrolyzing ATP). A similar calculation for the results at $V_{\text{max}}$ indicated that as few as 2% of the myosin heads were attached and hydrolyzing ATP at a shortening velocity near $V_{\text{max}}$. The steady state force exerted during shortening at $\frac{1}{2} V_{\text{max}}$ and $V_{\text{max}}$ was 0.24 and 0.02 $P_c$, respectively. If there is a linear relationship between force and the number of attached and cycling cross-bridges, one would estimate that fewer than 46% of the myosin heads would be attached and splitting ATP at a given instant in an isometric tetanus.

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