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

EARL HOMSHER, TAKENORI YAMADA, ALFRED WALLNER, and JAMES TSAI

From the Department of Physiology, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024

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 $\mu mol \cdot g^{-1} \cdot s^{-1}$ ($n = 24$), was about fivefold higher than that in the 1-s period in the isometric tetanus, 0.32 ± 0.11 $\mu mol \cdot g^{-1} \cdot s^{-1}$ ($n = 17$), observed in our previous study. The mean rate in the post-shortening period, 0.46 ± 0.13 $\mu mol \cdot g^{-1} \cdot s^{-1}$ ($n = 17$), 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, 1983). 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

Address reprint requests to Dr. Earl Homsher, Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, CA 90024.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/84/09/0347/13 $1.00 347
Volume 84 September 1984 347–359

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 Lymn-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 (~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_{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 $mJ \cdot g^{-1}$ 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 *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 *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_{max}, a large amount of heat plus work ($h + w$) was produced, but more than half of it, 6.5 ± 2.6 $mJ \cdot g^{-1}$, could not be explained by ~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 $mJ \cdot g^{-1}$ 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 $\frac{1}{2} V_{max}$ 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 $\frac{1}{2} V_{max}$, 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 l_0 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 $\mu m \cdot s^{-1}$/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

TABLE I

Changes in the Length of Muscle Segments with Shortening at ½ V_{max}

	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	0.770±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.

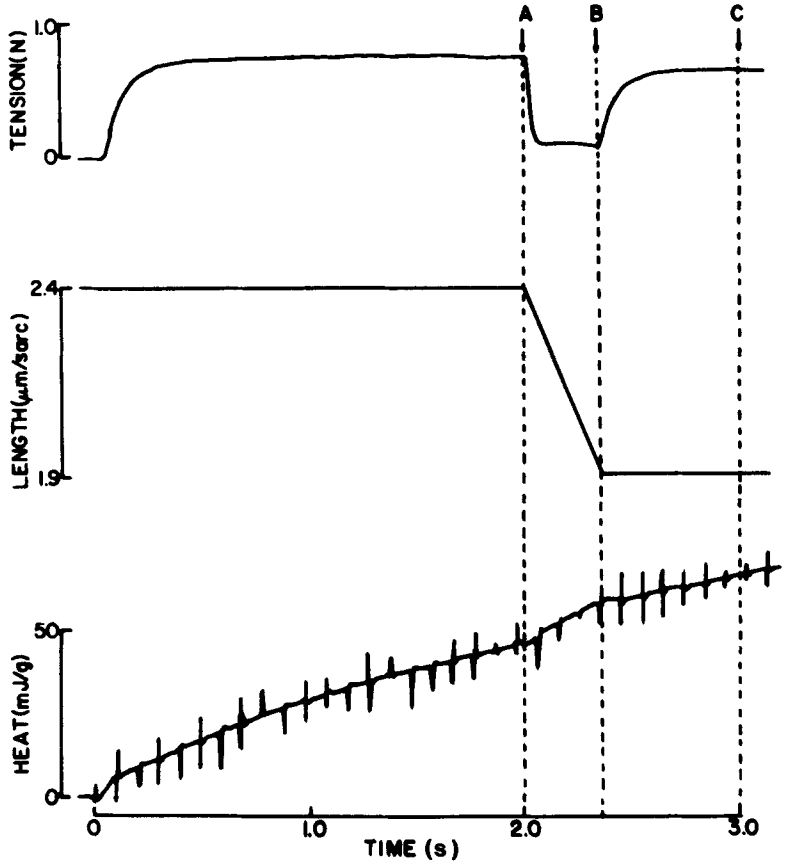

FIGURE 1. Traces of original records of muscle force, displacement, and heat production in a 3-s tetanus. After 2 s of isometric contraction at a sarcomere spacing of 2.4 μg/sarcomere (*A*), the muscle pair was released at a constant velocity of 1.42 $\mu m \cdot s^{-1}$/sarcomere and shortened to a sarcomere spacing of 1.9 μm/sarcomere in 0.35 s (*B*). For the remainder of the tetanus, the muscle contracted isometrically at this length. Chemical changes during shortening were estimated by freezing one muscle of a pair at *A* and its contralateral mate at *B*. Changes in the post-shortening period were determined from a comparison of paired muscles frozen at *B* and *C*. The heat recording has not been corrected for heat loss or conduction lag; the vertical spikes are stimulus artifacts. The blotted weight of the muscle pair was 141.2 mg; muscle length at 2.2 μm sarcomere length was 30.3 mm.

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-

surements 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_i, creatine (C_F), and total creatine (C_T) 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_{ATP}), calculated from the change in ATP and ADP. The extent of PCr splitting (ξ_{PCr}) was significantly different from zero in each case. The total ATP utilization was calculated for each muscle pair as $\xi_{ATP} + \xi_{PCr}$, 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^{-1} C_T·s^{-1} (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^{-1} C_T·s^{-1} ($n = 17$), 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^{-1} C_T·s^{-1} ($n = 17$) (or 0.46 ± 0.12 μmol·g^{-1}·s^{-1}), 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_T)

	Shortening at ½ V_{max} (2.0–2.35 s)	Post-shortening period (2.35–3.0 s)
C_F/C_T nmol·μmol^{-1}	15.41±2.78	7.06±2.02
P_i/C_T nmol·μmol^{-1}	16.44±1.84	8.94±2.77
ATP/C_T nmol·μmol^{-1}	0.44±0.72	−0.13±0.85
ADP/C_T nmol·μmol^{-1}	−0.04±0.72	0.06±0.50
AMP/C_T nmol·μmol^{-1}	−0.20±0.34	−0.02±0.12
ξ_{PCr}/C_T nmol·μmol^{-1}	15.76±2.02	8.06±2.14
ξ_{ATP}/C_T nmol·μmol^{-1}	−0.40±0.50	0.10±0.53
R_{ATP}/C_T nmol·μmol^{-1} s^{-1}	43.9±5.77	12.55±3.39

All values for chemical change given as experimental − control. C_F, free creatine; P_i, inorganic phosphate; PCr, phosphocreatine, denotes extent of reaction (ATP or PCr splitting, according to the subscript) calculated as described under Methods. R_{ATP} is the total mean rate of ATP utilization, $(\xi_{ATP} + \xi_{PCr})$/s. The total creatine per gram of muscle (wet weight) was 36.43 ± 0.63 μmol·g^{-1}. 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 ½ V_{max}, 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 ± 12.4 $\mu J \cdot \mu mol^{-1}$ C_T (n = 17), which is ~40% of the total enthalpy liberation. In the post-shortening period, the work done by the muscles on the series elastic structues was calculated (see Methods) to be 24.6 ± 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 ½ V_{max}, 1.48 ± 0.07 $mW \cdot \mu mol^{-1}$ C_T (n = 17), was ~4.5 times that in an isometric contraction period, 0.33 ± 0.02 $mW \cdot \mu mol^{-1}$ 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 ± 12.6 $\mu J \cdot \mu mol^{-1}$ C_T, or ~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 ± 0.02 $mW \cdot \mu mol^{-1}$ 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 (μJ) Normalized by the Total Creatine Content

	Shortening at ½ V_{max} (2.0–2.35 s)	Post-shortening period (2.35–3.0 s)
Observed enthalpy ($\mu J \cdot \mu mol^{-1}$ C_T)	517.1±23.8 (n = 17)	295.1±14.3 (n = 17)
Explained enthalpy ($\mu J \cdot \mu mol^{-1}$ C_T)	516.6±72.7 (n = 24)	278.5±76.6 (n = 17)
Unexplained enthalpy ($\mu J \cdot \mu mol^{-1}$ C_T)	0.5±76.5	16.6±77.9

All values are given as the mean ± 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.

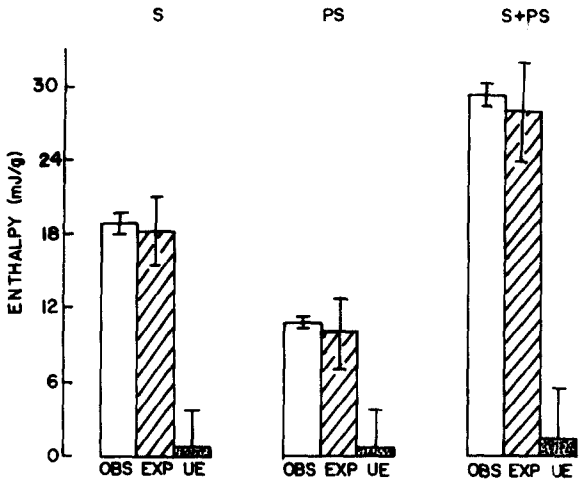

FIGURE 2. Enthalpy liberation ($mJ \cdot g^{-1}$), mean ± SEM; the number of observations was as given in Table III. Open bars, observed enthalpy (heat + work); diagonally shaded bars, explained enthalpy calculated as described in the text; stippled bars, unexplained enthalpy (observed − explained). Columns from left to right show results for the period of shortening at ½ V_{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 ± 76.5 $\mu J \cdot \mu mol^{-1}$ C_T, and during the post-shortening period it was 16.6 ± 77.9 $\mu J \cdot \mu mol^{-1}$ C_T. Neither of these values is significantly different from zero ($P > 0.5$). Thus, an energy balance is maintained both during and after shortening at ½ V_{max}. Fig. 2 shows that in the time interval between seconds 2 and 3, a total of 29.58 ± 0.98 $mJ \cdot g^{-1}$ of energy was liberated by the muscles, and 28.96 ± 3.84 $mJ \cdot g^{-1}$, 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 ½ V_{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 ½ V_{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_{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 ($\dot{h} + \dot{w}$), the rate of energy liberation derived from the hydrolysis of high-energy phosphate ($\dot{H}_{\sim P}$), and the rate of unexplained enthalpy production ($\dot{U}E$) for isometric and for shortening at ½ V_{max} and V_{max} contractions are compared both during shortening (Fig. 3*A*) 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 ½ V_{max} and during shortening at V_{max}, are significantly different from each other ($P \ll 0.001$). During shortening at ½ V_{max} and V_{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 $mW \cdot g^{-1}$) 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 ½ V_{max} and V_{max} were, respectively, 40 and 13% of the total energy liberation rate. These results are in good accord with earlier work of Hill (1964).

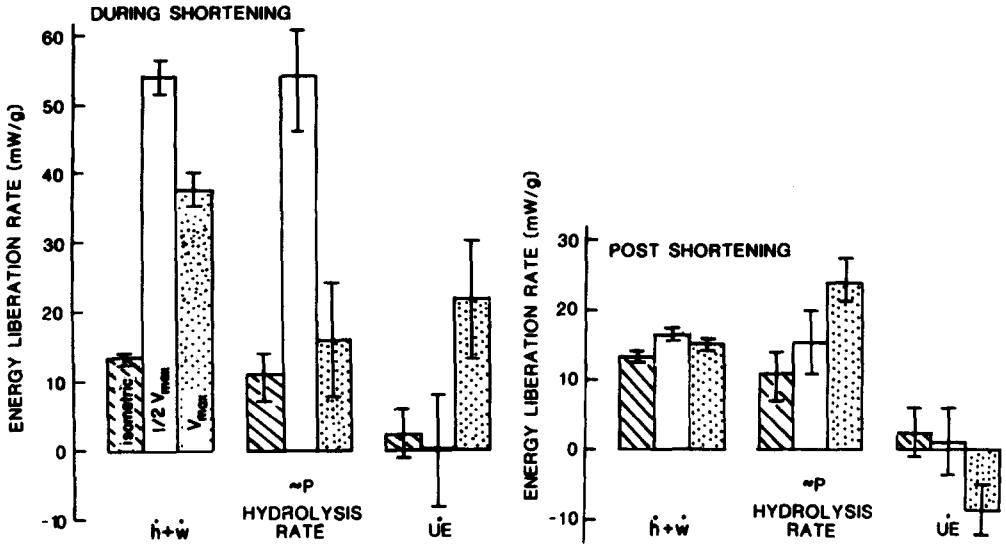

FIGURE 3. Comparison of the rate of observed enthalpy production ($\dot{h} + \dot{w}$), explained enthalpy production ($\dot{H}_{\sim P}$), and unexplained enthalpy production ($\dot{U}E$), during isometric contraction during and after shortenings at V_{max} and ½ V_{max}. Diagonally shaded bars, isometric; open bars, ½ V_{max}; stippled bars, V_{max}.

Rate of Energy Liberation Produced by High-Energy Phosphate Hydrolysis

Using the data in Tables II and III, the ($\dot{H}_{\sim P}$) can be calculated. The results show that in each case, ($\dot{H}_{\sim P}$) is significantly different from zero ($P < 0.01$), except for muscles shortening at V_{max} ($P < 0.07$). Isometric ($\dot{H}_{\sim P}$) is significantly ($P < 0.02$) less than that observed both during shortening at ½ V_{max} and after shortening at V_{max}. However, neither ($\dot{H}_{\sim P}$) during shortening at V_{max} nor ($\dot{H}_{\sim P}$) after shortening at ½ V_{max} is significantly different from the isometric value ($P > 0.5$). The rate of high-energy phosphate hydrolysis during shortening at ½ V_{max} (1.60 ± 0.23 $\mu mol \cdot g^{-1} \cdot s^{-1}$) 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 ½ V_{max} are all small and are not significantly different from zero ($P > 0.5$). However, both during and after shortening at V_{max}, $\dot{U}E$ is large and significantly different from zero ($P < 0.025$). Although there is a tendency for the rate of UE production by muscles shortening at V_{max} to be different from that of a muscle shortening at ½ V_{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_{max}. First, one could have argued that the low rate of hydrolysis of muscles shortening near V_{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_{max}. This point is emphasized by expressing the rate of ATP hydrolysis as a turnover rate (per myosin S1 head) while shortening at V_{max} or ½ V_{max} and while under isometric contraction: these rates are 1.7 ± 0.9, 5.8 ± 0.8, and 1.1 ± 0.4 s^{-1}, respectively (assuming the muscle contains 0.28 μmol 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 ± 0.46 $mJ \cdot g^{-1}$ as compared with an unexplained enthalpy production of 0.02 ± 2.78 $mJ \cdot g^{-1}$. 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 μm) were selected so as to closely approximate the

conditions (300 ms and 2.6–1.8 μm) used in the studies at V_{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 $mJ \cdot g^{-1}$ (at V_{max}, 6.5 $mJ \cdot g^{-1}$ of unexplained enthalpy was produced by a shortening of 0.8 μm/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 ~1.0 μm/sarcomere at ½ V_{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 ½ V_{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 μm/sarcomere at velocities near ½ V_{max} is ~1.6 $\mu mol \cdot g^{-1} \cdot s^{-1}$. Therefore, it is unlikely that the presence of unexplained enthalpy production in muscles shortening near V_{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_{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 (ΔF is negative) and exothermic, and to occur at a rate, k_s, 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_{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_r, was independent of velocity of shortening. While the model does account for the energetic behavior near V_{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 ½ V_{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 $nm \cdot s^{-1}$/half-sarcomere at ½ V_{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_{max} indicated that as few as 2% of the myosin heads were attached and hydrolyzing ATP at a shortening velocity near V_{max}. The steady state force exerted during shortening at ½ V_{max} and V_{max} was 0.24 and 0.02 P_o, 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.

This work was supported by National Institutes of Health grant AM30988 and by a grant from the Muscular Dystrophy Association of America.

Received for publication 19 April 1982 and in revised form 27 February 1984.

REFERENCES

Abbott, B. C. 1951. The heat production associated with the maintenance of a prolonged contraction and the extra heat produced during large shortening. *J. Physiol. (Lond.)*. 112:438–445.

Curtin, N. A., C. Gilbert, K. M. Kretzschmar, and D. R. Wilkie. 1974. The effect of the performance of work on total energy output and metabolism during muscular contraction. *J. Physiol. (Lond.)*. 238:455–472.

Curtin, N. A., and R. C. Woledge. 1978. Energy changes and muscular contraction. *Physiol. Rev.* 58:690–761.

Curtin, N. A., and R. C. Woledge. 1979. Chemical change and energy production during contraction of frog muscle: how are their time courses related? *J. Physiol. (Lond.)*. 288:353–366.

Curtin, N. A., and R. C. Woledge. 1981. Effect of muscle length on energy balance in frog skeletal muscle. *J. Physiol. (Lond.)*. 316:453–468.

Ebashi, S., M. Endo, and I. Ohtsuki. 1969. Control of muscular contraction. *Q. Rev. Biophys.* 2:351–384.

Eisenberg, E., T. L. Hill, and Y. Chen. 1980. Crossbridge model of muscular contraction. Quantitative analysis. *Biophys. J.* 29:195–227.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol. (Lond.)*. 269:441–515.

Hill, A. V. 1938. The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B Biol. Sci.* 126:136–195.

Hill, A. V. 1964. The effect of load on the heat of shortening of muscle. *Proc. R. Soc. Lond. B Biol. Sci.* 159:297–318.

Homsher, E., M. Irving, and J. Lebacq. 1983. The variation in shortening heat with sarcomere length in frog muscle. *J. Physiol. (Lond.)*. 345:107–121.

Homsher, E., M. Irving, and A. Wallner. 1981. High-energy phosphate metabolism and energy liberation associated with rapid shortening in frog skeletal muscle. *J. Physiol. (Lond.)*. 321:423–436.

Homsher, E., and C. J. Kean. 1982. Unexplained enthalpy production in contracting skeletal muscles. *Fed. Proc.* 41:149–154.

Homsher, E., C. J. Kean, A. Wallner, and V. Garibian-Sarian. 1979. The time-course of energy balance in an isometric tetanus. *J. Gen. Physiol.* 73:553–567.

Homsher, E., J. A. Rall, A. Wallner, and N. V. Ricchiuti. 1975. Energy liberation and chemical change in frog skeletal muscle during single isometric tetanic contractions. *J. Gen. Physiol.* 65:1–21.

Homsher, E., T. Yamada, A. Wallner, and J. Tsai. 1982. Energy balance studies in skeletal muscle shortening at ½ V_{max}. *Biophys. J.* 37:123*a*. (Abstr.)

Huxley, A. F. 1957. Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* 7:255–318.

Huxley, A. F. 1973. A note suggesting that the crossbridge attachment during muscle contraction may take place in two stages. *Proc. R. Soc. Lond. B Biol. Sci.* 183:83–86.

Irving, M., and R. C. Woledge. 1981. The dependence on extent of shortening of the extra energy liberated by rapidly shortening frog skeletal muscle. *J. Physiol. (Lond.).* 321:411–422.

Julian, F. J., and D. L. Morgan. 1979. The effect of tension of non-uniform distribution of length changes applied to frog muscle fiber. *J. Physiol. (Lond.).* 293:379–392.

Julian, F. J., and M. R. Sollins. 1975. Variation of muscle stiffness with force at increasing speeds of shortening. *J. Gen. Physiol.* 66:287–302.

Kobayashi, T., and H. Sugi. 1982. Segmental length changes in stimulated frog sartorius muscle during dynamic mechanical responses. *Jpn. J. Physiol.* 32:817–830.

Kushmerick, M. J., and R. E. Davies. 1969*a*. The chemical energetics of muscle contraction. II. The chemistry, efficiency, and power of maximally working sartorius muscles. *Proc. R. Soc. Lond. B Biol. Sci.* 174:315–353.

Kushmerick, M. J., R. E. Larson, and R. E. Davies. 1969*b*. The chemical energetics of muscle contraction. I. Activation heat, heat of shortening and ATP utilization for activation-relaxation processes. *Proc. R. Soc. Lond. B Biol. Sci.* 174:293–313.

Lymn, R. W., and E. W. Taylor. 1971. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry.* 10:4617–4624.

Podolsky, R. J., and A. C. Nolan. 1972. Muscle contraction transients, crossbridge kinetics, and the Fenn effect. *Cold Spring Harbor Symp. Quant. Biol.* 37:661–668.