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HIGH-ENERGY PHOSPHATE METABOLISM AND ENERGY LIBERATION ASSOCIATED WITH RAPID SHORTENING IN FROG SKELETAL MUSCLE

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SUMMARY

1. High-energy phosphate metabolism and energy liberated as heat and work were measured in 3 sec tetani of frog sartorius muscles at 0 °C.

2. Three contraction periods were studied: (*a*) shortening at near-maximum velocity for 0·3 sec from sarcomere length 2·6 to 1·8 μm, beginning after 2 sec of isometric stimulation, (*b*) the 0·7 sec isometric period immediately following such rapid shortening, (*c*) the period from 2 to 3 sec in an isometric tetanus at sarcomere length 1·8 μm.

3. There were no significant changes in levels of ATP, ADP or AMP in any contraction period. The observed changes in inorganic phosphate and creatine levels indicated that the only significant reaction occurring was phosphocreatine splitting.

4. The mean rate of high-energy phosphate splitting during rapid shortening, $0{\cdot}48 \pm 0{\cdot}24$ μmole/g . sec (mean $\pm$ S.E. of mean, $n = 29$; 'g' refers to blotted muscle weight), was not significantly different from that in the 1 sec period in the isometric tetanus, $0{\cdot}32 \pm 0{\cdot}11$ μmole/g . sec ($n = 17$). The mean rate in the post-shortening period, $0{\cdot}71 \pm 0{\cdot}10$ μmole/g . sec ($n = 22$), was greater than that in the 1 sec period in the isometric tetanus, and this difference is significant ($P < 0{\cdot}02$, t test).

5. A large quantity of heat plus work was produced during the rapid shortening period, but less than half of this could be accounted for by simultaneous chemical reactions. The unexplained enthalpy production was $6{\cdot}5 \pm 2{\cdot}6$ mJ/g (mean $\pm$ S.E. of mean). No significant unexplained enthalpy was produced in the 1 sec period in the isometric tetanus.

6. In the post-shortening period the observed enthalpy was less, by $6{\cdot}2 \pm 2{\cdot}6$ mJ/g, than that expected from the simultaneous chemical reactions.

7. The results are interpreted in terms of an exothermic shift in the population of cross-bridge states during rapid shortening. It is suggested that a relatively slow subsequent step prevents many of these cross-bridges from completing the cycle and splitting ATP until after the end of shortening.

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INTRODUCTION

Muscle contraction involves the interaction of the myofibrillar protein matrix with ATP and its hydrolysis products and co-factors. In living, unpoisoned muscle any ADP produced is rapidly rephosphorylated by creatine-phosphokinase, so that the net reaction is phosphocreatine (PCr) splitting (Carlson & Siger, 1959; Cain, Infante & Davies, 1962). Measurements of the extent of this reaction define the energy supply to the contractile system. The energy output of the muscle occurs as heat and mechanical work, and can also be readily measured under various mechanical conditions.

When a muscle is allowed to shorten the rate of energy liberation increases (Hill, 1938). In addition to any mechanical work output the rate of heat production is higher than that in isometric contraction. Hill's results were incorporated into a quantitative model of muscle contraction involving cyclical formation and breaking of cross-bridges between myofilaments (Huxley, 1957). It was assumed in this case that the rate of energy liberation was proportional to cross-bridge turnover rate. Subsequent theories, for instance those of Julian, Sollins & Sollins (1974) and Eisenberg, Hill & Chen (1980), have also used the energy liberation data as an index of the cross-bridge turnover rate.

Measurements of the chemical energy input do not support this simple interpretation of the heat and work measurements, however. Early experiments showed that there is extra PCr splitting associated with the performance of mechanical work by a shortening muscle, but there is no component associated with shortening *per se* (Cain *et al.* 1962; Mommaerts, Seraydarian & Maréchal, 1962; Carlson, Hardy and Wilkie, 1963). The latter result was surprising, because there is a component of heat production associated with shortening. The discrepancy is most apparent for the case of rapid shortening, when work output is very small.

In direct comparisons between rapidly shortening and isometric contractions made by Kushmerick, Larson & Davies (1969) and Rall, Homsher, Wallner & Mommaerts (1976) both groups found that the extent of PCr splitting in a brief lightly after-loaded contraction was *less* than that in an isometric contraction. In addition, Rall *et al.* confirmed directly the conclusion of Kushmerick *et al.*, that there is much more heat production during such shortening than can be accounted for by simultaneous PCr splitting.

We have measured heat and work production and PCr splitting during rapid shortening which began after 2 sec of isometric contraction, rather than from the start of stimulation as in the earlier experiments. In the present case shortening was allowed only after the muscle was fully activated; the consequences of the 'mechanical inactivation' due to shortening seen in twitches and short tetani (Edman, 1975) were thus avoided. The present design has another advantage. The heat production in excess of that expected from PCr splitting which has been observed during rapid shortening (Rall *et al.* 1976) occurred very early in the tetanus, when a similar 'unexplained' heat production is seen in isometric tetani (Gilbert, Kretzschmar, Wilkie & Woledge, 1971; Curtin & Woledge, 1979; Homsher, Kean, Wallner & Garibian-Sarian, 1979). This ambiguity may be removed by delaying the start of shortening until after 2 sec of isometric contraction, since heat plus work production

from sources other than PCr splitting is insignificant after this time in *R. pipiens* muscles (as calculated by Curtin & Woledge (1978) from the results of Homsher, Rall, Wallner & Ricchiuti (1975)).

We have also measured energy liberation and PCr splitting in a period of continuing stimulation following rapid shortening. An increased rate of PCr splitting at this time could explain some of the results of Irving & Woledge (1981*a*, *b*) who showed that the extra energy liberation due to shortening is reduced during extensive shortening, but recovers after several hundred msec of subsequent isometric stimulation. There does seem to be an increased rate of PCr splitting during relaxation after shortening (Rall *et al.* 1976).

Preliminary reports of some of these results have been presented (Homsher, Irving & Wallner, 1981; Irving, Homsher & Wallner, 1981).

METHODS

Rana pipiens weighing 25–30 g were obtained in a single shipment from Nasco Co. (Fort Atkinson, Wis.) and kept in moist tanks at 6 °C for at least 17 days before use. On the evening before an experiment pairs of sartorius muscles were dissected and the pelvic bones were split. If fibre damage or parasite infestation was detected under a dissecting microscope the pair was discarded (approximately 20% of the dissected muscles were rejected on this basis). The remaining muscles were aerated overnight with 95% O_2, 5% CO_2 in Ringer 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. For both myothermal and chemical experiments tetanic stimulation was applied via two Pt electrodes, one at each end of the muscle using 16 V, 3 msec square pulses at 10 Hz for 3 sec.

Muscle length measurements. Before the experiment one muscle from each pair was illuminated with the 1 mm diameter laser beam from a 0·5 mW He-Ne laser (Spectra Physics Inc., Mt. View, Calif.). The diffracted beam fell upon a screen which had been calibrated using a diffraction grating of known periodicity. Muscle length was adjusted so that the distance between the two first-order lines corresponded to a sarcomere length of 2·2 or 2·6 μm. The muscle length (the distance between the tie on the distal tendon and the pelvic bone) was measured at both sarcomere lengths. The muscle length at a sarcomere spacing of 1·8 μm, where the diffraction bands were very faint, was estimated by extrapolation from these values. All sarcomere length measurements were made in unstimulated muscles; mean sarcomere length is expected to decrease slightly on stimulation due to shortening of series elastic structures.

Mechanical measurements. Variable capacitance force transducers (Schilling, 1960) with resonant frequencies between 1×10^3 and 4×10^3 Hz were used. The compliance of the transducer, stainless steel wire connexion and 5–0 silk thread used to tie the muscle to the transducers was 0·12 mm/N. The muscle length was controlled with a speaker-coil ergometer described previously (Homsher & Rall, 1973), except in those biochemical experiments in which both control and experimental muscles shortened. In this case a Levin–Wyman ergometer with attached force transducer and position-sensitive potentiometer was used as a second ergometer, with compliance similar to that of the speaker-coil system.

Chemical experiments. These experiments were made over a time span of 9 weeks. The muscle pairs were mounted, thermally equilibrated at 0 °C, aerated, stimulated, and frozen using the hammer apparatus previously described (Homsher *et al.* 1975). The length of the muscle of each pair used for laser diffraction was adjusted to that corresponding to a sarcomere length of 2·6 μm. The length of the contralateral muscle was then adjusted so that both muscles had the same resting tension (mean value 0·04 N). After resting at 0 °C for 5 min the muscles were drained and after a further 90 sec they were stimulated. To reduce any random error arising from a difference in the chemical utilization in the 2 or 2·3 sec of tetanus preceding the experimental period, the eighteen muscle pairs (out of a total of eighty-six) in which the isometric tetanus tensions at 2·0 sec differed by more than 10% were discarded. The frozen muscle pairs were extracted and analysed for their contents of free creatine (C_F), total creatine (C_T), ATP, ADP, AMP, and inorganic phosphate (P_i) as previously described (Mommaerts & Wallner, 1967; Homsher, Mommaerts, Ricchiuti & Wallner,

1972). The change in these metabolites normalized by total creatine content ($\Delta C_F/C_T$, etc.) was determined for each experimental period as the paired difference between contralateral muscles (experimental-control) assuming that the metabolic contents (C_F/C_T, P_i/C_T, ATP/C_T, etc.) were identical at the beginning of the experimental period.

To evaluate the rate of high-energy phosphate splitting and calculate the energy balance in these experiments we have used the following three simplifications. First, there is no hexose monophosphate formation in a 1 sec contraction period; formation of glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate is small (less than 0·5 nmole/μmole C_T) and insignificant even in 5 sec tetani (Curtin & Woledge, 1975). Secondly, there is no significant formation of IMP (deaminase activity) since the change in total adenine nucleotide content between the experimental and control muscles was only $+0{\cdot}75 \pm 0{\cdot}69$ nmole/μmole C_T ($n = 48$) and insignificant. Thirdly, no significant myokinase activity is present, since the change in AMP content in these experiments is small and insignificant ($0{\cdot}02 \pm 0{\cdot}05$ nmole/μmole C_T, $n = 48$). Thus the only significant net measured reactions occurring in these experiments are the unreversed hydrolysis of ATP and PCr splitting:

$$ATP \rightarrow ADP + P_i \text{ (reaction 1)},$$

$$PCr \rightarrow C_F + P_i \text{ (reaction 2)}.$$

The net extent of reaction 1 (ξ_{ATP}) was calculated for each muscle pair as:

$$\xi_{ATP} = \left(\frac{\Delta ADP}{C_T} - \frac{\Delta ATP}{C_T}\right)\Big/2.$$

The extent of reaction 2 (ξ_{PCr}) can be estimated from either $\Delta C_F/C_T$ or ($\Delta P_i/C_T - \xi_{ATP}$) [since P_i is also formed by reaction 1]. Thus ξ_{PCr} was calculated as:

$$\xi_{PCr} = \left[\Delta C_F + \Delta P_i - \left(\frac{\Delta ADP - \Delta ATP}{2}\right)\right]/2C_T.$$

The explained enthalpy production was determined as ξ_{ATP} multiplied by -48 kJ . mol^{-1} plus ξ_{PCr} multiplied by -34 kJ . mol^{-1} (Curtin & Woledge, 1978). The total high-energy phosphate splitting is $\xi_{ATP} + \xi_{PCr}$.

Energy liberation measurements. Measurements of energy liberation were made in the 1st, 7th and 8th week of the 9 week experimental period. Heat production was measured with an electroplated thermopile (E-10, of Homsher *et al.* 1979) from a 20 mm region near the pelvic end of each muscle pair. A 10 mm protecting region of the thermocouples (Hill, 1938), adjacent to the recording region, ensured that the heat measurements would not be affected by the type of longitudinal temperature gradients in the muscles described by Irving, Woledge & Yamada (1979). The thermopile output was amplified by an Astrodata 120 nV amplifier. Calibration of heat values was as described by Homsher *et al.* (1975) following the method of Hill & Woledge (1962). Heat loss from the muscles was exponential with time constants between 10 and 24 sec and was determined by the Peltier method (Kretzschmar & Wilkie, 1972) at the muscle lengths used in the heat measurements; the time constants were consistently smaller at greater lengths. Experimental records were corrected for heat loss by the procedure of Hill (1965). For shortening muscles the time constant used was that appropriate for the current muscle length at each time point (0·1 sec intervals). A correction for conduction lag was also applied, using the method of Hill (1965). The equivalent half-thickness of the thermopile was 19 μm and a further 3·4 μm was allowed for adhering Ringer solution on each face. Heat dissipated from the stimulus current was measured at the end of six experiments by rendering muscles inexcitable with a Ringer solution containing 10 mM-procaine hydrochloride. The stimulus heat was $0{\cdot}68 \pm 0{\cdot}07$ mJ . g^{-1} sec^{-1} (mean $\pm$S.E. of mean) and was subtracted from the experimental records. The records were also corrected for thermoelastic heat production using a thermoelastic heat coefficient of 0·01 as suggested by Curtin & Woledge (1978). Thermoelastic heat production was calculated as $1{\cdot}64 \pm 0{\cdot}06$ mJ . g^{-1} (mean $\pm$S.E. of mean, $n = 14$) for the rapid shortening period (Fig. 1) and $-1{\cdot}41 \pm 0{\cdot}11$ mJ . g^{-1} ($n = 12$) in the post-shortening period. No correction was necessary in the case of the isometric tetanus period.

Muscles were mounted on the thermopile and attached to transducers at a sarcomere length of 2·2 μm. At least 40 min was allowed for thermal equilibration at 0 °C in aerated Ringer solution. The muscle length was adjusted to the desired value and after 5 min the Ringer solution was drained from the muscles. 90 sec later the muscles were stimulated and measurements of heat and work

production made. After recovery, heat loss time constants and stimulus heat were measured. Muscles were then removed from the thermopile, weighed, blotted, then reweighed. Enthalpy production values given below in mJ/g have been normalized by this blotted weight. Subsequently the muscles were frozen in an isopentane-liquid N_2 slush and stored in liquid N_2. The frozen muscles were reweighed, extracted, and the total creatine content determined as described above. This final weight determination was used in calculating the ratio of total creatine content to muscle mass.

External work production during isovelocity shortening was estimated as the product of the extent of shortening and the mean maintained force during shortening. The rapid fall in tension at the start of shortening (more than 90% complete in the first 30 msec) was not included in this mean as this component of work production is performed by series elastic structures. The mean external work production during shortening from 2·6 to 1·8 μm/sarcomere was 1·48 mJ . g^{-1}, which is small compared with the total enthalpy production in this period (11·3 mJ . g^{-1}). In the period after the shortening the muscles perform work on series elastic elements; the characteristics of these elements were determined from force-displacement recordings from nine muscle pairs released at 20 cm . sec^{-1} from the plateau of an isometric tetanus. Regression analysis showed that the relationship between series elastic work (W_s) and change in force (ΔF; N . cm^{-2}) was well-fitted by the expression:

$$W_s = 0{\cdot}023\ (\Delta F)^{1{\cdot}44}\ \text{mJ} \cdot \text{g}^{-1}.$$

The series elastic work performed in the 0·7 sec post-shortening period was calculated from this expression for each muscle pair.

Data analysis and rejection. Measurements of energy liberation and chemical change were normalized by total creatine content for each muscle or pair of muscles. The data appears in this original form in the Tables. The muscles used in the energy liberation measurements had a total creatine content of 40·71 ± 0·78 μmole/g blotted weight (mean ± S.E. of mean, $n = 21$), and this factor has been used to express the data per gram weight in the text and in Fig. 2. The small contribution of the error in the conversion factor was included in the S.E. calculated for these values. Significance tests for differences between means were performed as described by Snedecor & Cochran (1967, pp. 114–115) on the original data (normalized by C_T). The chemical data from one muscle pair were omitted from the sample because the change in one metabolite level was more than 3 S.D. from the mean (as calculated with this value included).

RESULTS

Chemical changes and enthalpy production were measured under identical mechanical conditions in different muscle pairs from the same batch. The muscles which were used for heat and work measurements were stimulated tetanically for 3 sec at 0 °C. In the experiments with shortening (Fig. 1) the muscle length was held at 2·6 μm/sarcomere (as measured in the resting muscle) for the first 2 sec of the tetanus. The muscles were then released at constant velocity for 0·3 sec to 1·8 μm/sarcomere, at which length they were held for the remainder of the tetanus. The velocity of shortening was close to the maximum, and the tension fell during shortening to a small fraction (0·026 ± 0·006, mean ± S.E. of mean, $n = 14$) of its isometric value (Fig. 1). Work production was consequently very small compared with total energy liberation.

The rate of heat production shows a clear increase during the rapid shortening period. Chemical changes in this period were determined by freezing one muscle of a pair at the start of shortening (Fig. 1 at *A*) and the other at the end (*B*); corresponding measurements on the 0·7 sec period after shortening were made by freezing one muscle of a pair at *B* and the other at *C*.

Chemical changes and enthalpy production were also measured in the period from 2 to 3 sec in isometric tetani at muscle length 1·8 μm/sarcomere.

High-energy phosphate metabolism

The changes in metabolite levels associated with the various contraction periods are shown in Table 1. Oxidative and glycolytic recovery and the formation of hexose phosphates occur to a negligible extent in brief tetani of frog muscle at 0 °C (Curtin & Woledge, 1978), so these reactions were not monitored. We measured ATP, ADP, AMP, inorganic phosphate (P_i), free creatine (C_F) and total creatine (C_T), so that the extents of ATP splitting, phosphocreatine (PCr) splitting and the myokinase reaction could be determined. The values in Table 1 have been normalized by the total creatine content of each muscle.

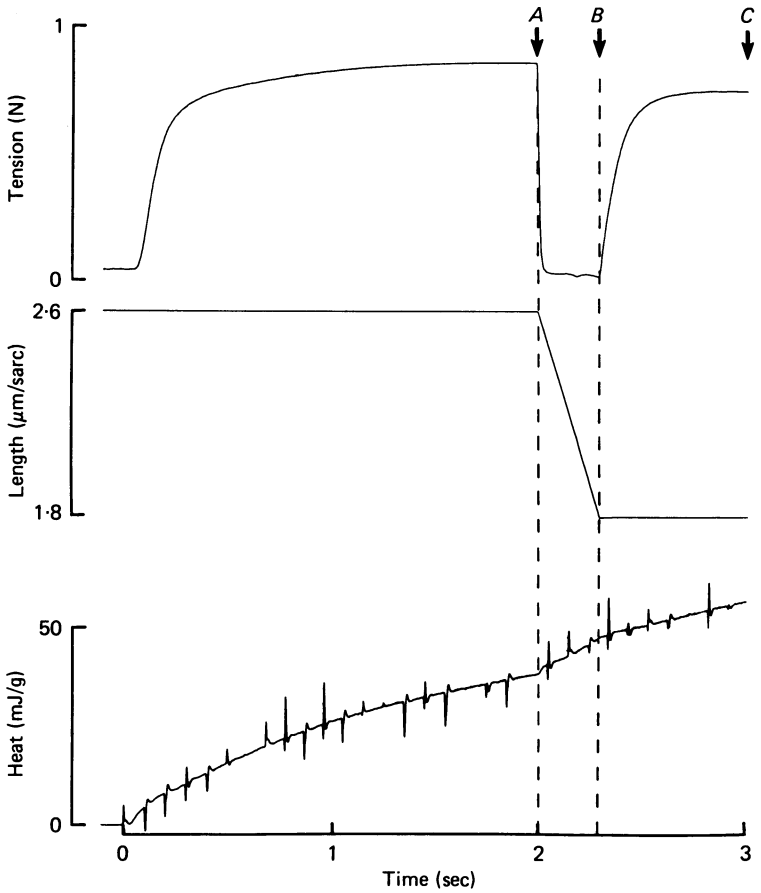

Fig. 1. Original records of muscle tension, length and heat production in a 3 sec tetanus. After 2 sec of isometric contraction at muscle length 2·6 μm/sarcomere, the muscle pair was released at constant velocity and shortened to 1·8 μm/sarcomere in the following 0·3 sec. For the remainder of the tetanus the muscles contracted isometrically at this length. The heat record has been normalized by blotted weight; it has not been corrected for heat loss or conduction lag. The spikes are electrical artifacts due to the stimulus. Blotted weight of muscle pair, 173·5 mg; muscle length at 2·2 μm sarcomere length, 36·7 mm. In the chemical experiments (on a separate group of muscles) the 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*.

There was no significant change in the level of ATP, ADP or AMP in any contraction period. The extent of the myokinase reaction was very small, as were ΔATP and ΔADP. The extent of PCr splitting (ξ_{PCr}) was however significantly different from zero in each case. The total ATP utilization was calculated for each muscle pair as ($\xi_{ATP}+\xi_{PCr}$), and the bottom line in Table 1 shows the mean rate of ATP utilization in each contraction period. During rapid shortening the mean rate

TABLE 1. Tension development (measured at 2 sec in the tetanus) and chemical changes normalized by total creatine content (C_T). There were no significant differences in tension between the three groups of muscles, though the measurement was made at muscle length 2·6 μm/sarcomere for the data in the first two columns and 1·8 μm/sarcomere for that in the third column. C_F, free creatine; P_i, inorganic phosphate; PCr, phosphocreatine. ξ denotes extent of reaction (ATP or PCr splitting, according to the subscript) calculated as described in the text (Methods). R_{ATP} is the total mean rate of ATP utilization: $(\xi_{ATP}+\xi_{PCr})/\Delta t$. The mean total creatine per gram of muscle (blotted weight) was 40·71 ± 0·78 μmole/g. Data in the table are means ± S.E. of mean for the following numbers of observations: column 1 (rapid shortening), $n = 29$ except for $\Delta AMP/C_T$ ($n = 20$); column 2 (post-shortening), $n = 22$; column 3 (isometric), $n = 17$ except for $\Delta AMP/C_T$ ($n = 6$).

Tension	N	Rapid shortening (2·6–1·8 μm/sarcomere, 2–2·3 sec)	Post-shortening (1·8 μm/sarcomere, 2·3–3 sec)	Isometric (1·8 μm/sarcomere, 2–3 sec)
		0·368 ± 0·015	0·330 ± 0·015	0·361 ± 0·022
$\Delta C_F/C_T$	nmol. μmol^{-1}	3·54 ± 2·58	13·13 ± 2·61	9·71 ± 3·80
$\Delta P_i/C_T$	nmol. μmol^{-1}	3·81 ± 1·96	11·56 ± 2·04	6·44 ± 2·67
$\Delta ATP/C_T$	nmol. μmol^{-1}	0·46 ± 0·70	0·65 ± 0·89	0·62 ± 0·89
$\Delta ADP/C_T$	nmol. μmol^{-1}	−0·02 ± 0·13	0·15 ± 0·20	0·19 ± 0·20
$\Delta AMP/C_T$	nmol. μmol^{-1}	0·10 ± 0·07	−0·07 ± 0·08	0·12 ± 0·18
ξ_{ATP}/C_T	nmol. μmol^{-1}	−0·24 ± 0·36	−0·25 ± 0·45	−0·22 ± 0·43
ξ_{PCr}/C_T	nmol. μmol^{-1}	3·80 ± 1·75	12·47 ± 1·59	8·19 ± 2·77
R_{ATP}/C_T	nmol. μmol^{-1}. sec^{-1}	11·85 ± 5·88	17·46 ± 2·49	7·97 ± 2·59

of ATP utilization expressed per blotted weight of muscle, 0·48 ± 0·24 μmole/g. sec (mean ± S.E. of mean, $n = 29$) was not significantly different ($P > 0·5$) from that, 0·32 ± 0·11 μmole/g. sec ($n = 17$), in the 1 sec measurement period in an isometric tetanus. However the mean rate of ATP utilization in the post-shortening period, 0·71 ± 0·10 μmole/g. sec ($n = 22$), was more than twice that during a similar period of an isometric tetanus at the same length, and this difference is significant ($P < 0·02$). Thus there is no evidence for an increased rate of PCr splitting during rapid shortening, but there is a clear increase during the post-shortening period.

Enthalpy production

The sum of the heat and work production (the observed enthalpy) is shown in Table 2 (top row). In the rapid shortening period the work produced by the muscles was 36·4 ± 7·0 μJ. (μmole C_T)$^{-1}$ (mean ± S.E. of mean, $n = 14$), which is less than 15% of the total energy liberated. In the post-shortening period the work done by the muscles on series elastic structures (see Methods) was calculated as 24·5 ± 2·2 μJ. (μmole C_T)$^{-1}$ ($n = 12$), or less than 10% of the energy liberation in this period. Thus in each contraction period almost all the liberated energy appeared as heat.

The mean rate of enthalpy liberation during rapid shortening expressed per gram (blotted weight) of muscle, 37·6 ± 2·5 mW/g (mean ± S.E. of mean, $n = 14$) was about

2·8 times that in the 1 sec period of an isometric tetanus, 13·3 ± 0·8 mW/g ($n = 7$). The rate in the post-shortening period, 15·2 ± 0·8 mW/g ($n = 12$) was not significantly different from that in the isometric tetanus. The large increase in enthalpy production occurs mainly during shortening, in contrast with the results on ATP utilization presented above, where the increase occurred mainly in the post-shortening period.

Energy balance

By combining the observed extents of reaction (Table 1) with the molar enthalpy of each reaction under physiological conditions (Curtin & Woledge, 1978) it is possible

TABLE 2. Enthalpy (μJ) (mean ± S.E. of mean) normalized by total creatine content (μmole). Mean total creatine per muscle blotted weight was 40·71 ± 0·78 μmole/g. Observed enthalpy is the sum of heat and work production in each measurement period. Explained enthalpy was calculated from the extents of reactions measured in the corresponding period (Table 1) by the procedure described in the text (see Methods). Unexplained enthalpy is difference between observed and explained enthalpy. *, $P < 0·02$; †, $P < 0·05$. Tension values (measured at 2 sec in the tetanus) for the three groups of muscles used for observed enthalpy measurements were as follows: rapid shortening, 0·317 ± 0·026 N; post-shortening, 0·329 ± 0·028 N; isometric, 0·322 ± 0·026 N (means ± S.E. of mean)

Enthalpy (μJ/μmole C_T)	Rapid shortening (2·6–1·8 μm/sarcomere, 2–2·3 sec)	Post-shortening (1·8 μm/sarcomere, 2·3–3 sec)	Isometric (1·8 μm/sarcomere, 2–3 sec)
Observed	277·1 ± 18·2 ($n = 14$)	261·0 ± 13·6 ($n = 12$)	327·0 ± 20·5 ($n = 7$)
Explained	117·6 ± 60·8 ($n = 29$)	412·1 ± 62·3 ($n = 22$)	267·8 ± 86·0 ($n = 17$)
Unexplained	159·5 ± 63·4*	−151·1 ± 63·7†	59·1 ± 88·4

to calculate the 'explained enthalpy', i.e. the enthalpy produced by measured chemical reactions. If the difference between the observed enthalpy and the explained enthalpy is not zero some other reaction of energetic significance must be occurring.

The results of such an energy balance calculation are shown in Table 2 and Fig. 2. From 2 to 3 sec in an isometric tetanus at length 1·8 μm/sarcomere the unexplained enthalpy production was 2·4 ± 3·6 mJ/g (mean ± S.E. of mean), which is not significantly different from zero. However during rapid shortening a significant ($P < 0·02$) amount of unexplained enthalpy, 6·5 ± 2·6 mJ/g, was produced. In the post-shortening period the calculated unexplained enthalpy was −6·2 ± 2·6 mJ/g ($P < 0·05$). This negative value means that the observed enthalpy production was *less* than that expected from the simultaneous chemical reactions. Taking the whole of the 2–3 sec period including shortening (Fig. 2, S + PS) there is an energy balance: the unexplained enthalpy is only 0·3 ± 3·7 mJ/g, compared with an observed enthalpy of 21·9 ± 0·9 mJ/g. The production of unexplained enthalpy during the 0·3 sec shortening period has thus been completely reversed within the 0·7 sec post-shortening period.

DISCUSSION

Comparison with previous work

Enthalpy production. The rate of enthalpy production was much greater during rapid shortening from muscle length 2·6 to 1·8 μm/sarcomere than in a period of

isometric contraction at the shorter length. Nearly all the excess enthalpy appeared as heat, and corresponds approximately to the shortening heat (Hill, 1938). The shortening heat production in the present experiments was estimated as the sum of the heat production in the 0·3 sec shortening period and the 0·7 sec post-shortening period, minus that produced in the 1·0 sec isometric period. (Work production was

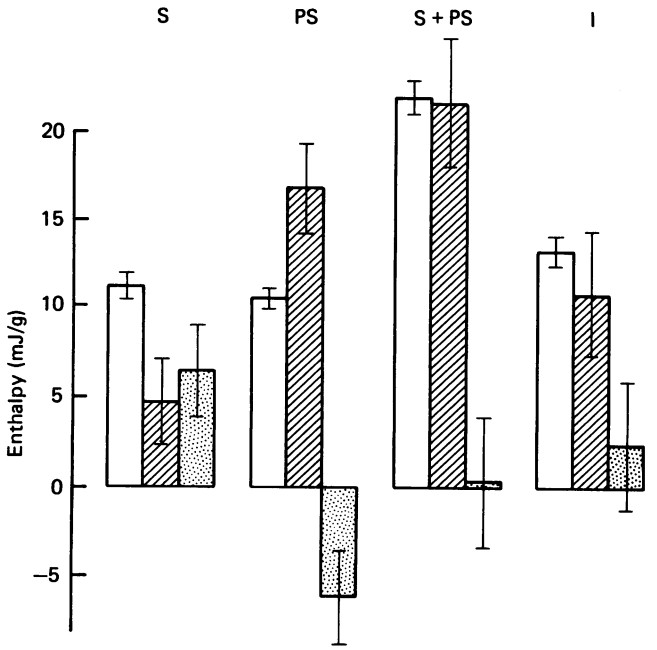

Fig. 2. Enthalpy liberation (mJ/g), means ±S.E. of mean, n values as in Table 2. Unshaded, observed enthalpy (heat plus work); diagonal shading, explained enthalpy calculated from the results of Table 1 as described in the text; dots, unexplained enthalpy (observed – explained). Columns from left to right show results for the rapid shortening period (S), post-shortening period (PS), the sum of these two values (S + PS) and the period (I) (of duration equal to that of S plus PS) of an isometric tetanus.

not included.) The resulting value, 5·70 ± 0·87 mJ/g, is similar in magnitude to the unexplained enthalpy produced during shortening, 6·5 ± 2·6 mJ/g (Fig. 2). The post-shortening period was included in the shortening heat estimate in order to take account of any delayed shortening-dependent heat production (Irving & Woledge, 1981*a*, *b*); a similar value was obtained for the rapid shortening period alone, however.

The dimensionless shortening heat constant (Hill, 1938), the shortening heat per unit shortening normalized by isometric tension (taking the value at 2·6 μm/sarcomere, before the release), was calculated as 0·11 ± 0·02. This is somewhat lower than previously-reported values for rapid shortening (Hill, 1964; Homsher & Rall, 1973), but this is expected for these long releases on the basis of the decrease in shortening heat constant with increasing extent of shortening reported by Irving & Woledge (1981*b*).

Chemical changes. The mean rate of ATP utilization during rapid shortening was not significantly different from that in the isometric tetanus. Our results are not

inconsistent with the small decreases in ATP utilization during rapid shortening reported by Kushmerick *et al.* (1969) and Rall *et al.* (1976) for the case of brief lightly after-loaded contractions.

Energy balance. During rapid shortening the enthalpy production was significantly greater than that expected from the simultaneous chemical reactions, by $6{\cdot}5 \pm 2{\cdot}6$ mJ/g (mean $\pm$S.E. of mean) (Fig. 2). Rall *et al.* (1976, Table 3, line *C*) found an unexplained enthalpy of $15{\cdot}4 \pm 4{\cdot}1$ mJ/g in brief lightly after-loaded contractions. The larger value in the experiments of Rall *et al.* is probably due to the fact that considerable unexplained enthalpy is produced at this time even in an isometric tetanus (Curtin & Woledge, 1979; Homsher *et al.* 1979). We have avoided this component by not allowing shortening until after 2 sec of isometric contraction. The unexplained enthalpy produced from 2 to 3 sec in an isometric tetanus was shown to be insignificant and the mean was sufficiently small that the amount expected from 2 to 2·3 sec was only 10 % of that observed when rapid shortening was allowed in the same period. Sources of unexplained enthalpy which are common to isometric and shortening contractions thus make a negligible contribution to the results of the present experiments.

Chaplain & Frommelt (1972) used an experimental design similar to that of the present study. These authors did not calculate unexplained enthalpy values, but it is possible to determine from their data that the unexplained enthalpy production in an 8 mm release at 30 mm.sec^{-1} was $4{\cdot}1 \pm 1{\cdot}1$ mJ/g. This is not significantly different from the value determined above (Fig. 2).

The idea that *less* enthalpy might be produced in the post-shortening period than could be explained by the simultaneous chemical reactions was predicted by Davies (1963) and received support from the results of Rall *et al.* (1976). This result has now been demonstrated directly. This is the first time a set of conditions has been found in which the enthalpy liberated by the muscle is less than that expected from the simultaneous chemical reactions.

The energetics of rapid shortening

The extra energy liberated during rapid shortening appears mainly as heat (the shortening heat) and cannot be explained by a simultaneous increase in PCr splitting. This shortening-dependent production of unexplained energy should not be confused with the unexplained energy produced during isometric tetani; the latter is confined to the first few seconds of the tetanus and shows no subsequent reversal with continued stimulation (Curtin & Woledge, 1979; Homsher *et al.* 1979).

Shortening heat production is proportional to the expected degree of overlap between contractile filaments as this is varied by changing the sarcomere length in the range 2·25–3·75 μm (Irving, Homsher & Lebacq, 1980). It therefore seems likely that shortening heat and the shortening-dependent unexplained energy are a property of the interaction between myosin-containing and actin-containing filaments. Many features of this interaction can be described in terms of a cycle of attachment and detachment of a myosin head (cross-bridge) to the actin-containing filament (Huxley, 1957). According to the biochemical model of Lymn & Taylor (1971), this corresponds to a reaction cycle containing transient binding of myosin to actin and the hydrolysis of ATP. In a complete cycle the net reaction would simply be ATP

hydrolysis, with the production of the molar enthalpy of this reaction. No complete cyclical reaction coupled to ATP hydrolysis could produce the shortening-dependent unexplained energy; it would have to be the result of *incomplete* cross-bridge cycles.

One might expect such incomplete cycles to occur in the transition from isometric contraction to rapid shortening. Suppose that during isometric contraction most cross-bridges were in state X, and during rapid shortening in state Y. If the net transition X to Y were exothermic, it could be responsible for the unexplained enthalpy observed in the rapid shortening period. In the *post*-shortening period the reverse transition Y to X would occur as the muscle returned to the isometric steady state. This would explain why the magnitude of the unexplained energy produced during shortening ($6{\cdot}5 \pm 2{\cdot}6$ mJ/g) is the same as that of the deficit in enthalpy production after shortening ($6{\cdot}2 \pm 2{\cdot}6$ mJ/g). Thus the incomplete cycle idea simply explains the results from both periods. It has also been shown to accurately describe several other properties of the heat production of shortening muscle (Irving & Woledge, 1981*b*).

The present results can be used to place some restrictions on the chemical identity of the intermediates X and Y in such a scheme. In the post-shortening transition ($Y \rightarrow X$) there is more PCr splitting than can be accounted for by the simultaneous energy liberation. In particular, there is a high rate of P_i and C_F production with no statistically significant change in adenine nucleotide levels in this period (Table 1). The transition $Y \rightarrow X$ must therefore include the step of the cross-bridge cycle in which ATP is hydrolysed. The P_i so produced need not have been released from actomyosin, since the acid extraction step employed in the analysis removes nucleotides or P_i bound to actomyosin (Sartorelli, Fromm, Benson & Boyer, 1966). The rate of C_F production in the post-shortening period is about twice that in the isometric tetanus period. Although the difference is not significant at the 5% level, it seems likely that there is a burst of C_F production following shortening. This would have to be a result of ADP release at this time, which was then available for rephosphorylation by creatine-phosphokinase. We conclude that the transition $Y \rightarrow X$ probably contains the ADP release as well as the hydrolysis step. It might be argued that the results could reflect changes in the level of phosphorylation of myosin light chains (Bárány, Bárány, Gillis & Kushmerick, 1979) rather than intermediate steps in ATP hydrolysis at the active site. The former, however, involve a covalently bound phosphate which would not be extracted from myosin with our technique, so changes in light chain phosphorylation could not produce the parallel changes in P_i and C_F seen in the post-shortening period.

It is implicit in our interpretation of the chemical results for the post-shortening period that creatine phosphokinase is able to rapidly phosphorylate any free ADP produced. This assumption is supported by the precise constancy of ADP levels in each contraction period (Table 1), with an additional implication. The ADP measurement includes that bound to actomyosin; this too would have to remain at a constant level. In the rapid shortening period, for example, the change in ADP corresponds to only $0{\cdot}0025 \pm 0{\cdot}019$ per myosin head (taking the concentration of the latter as 0·28 μmole/g (Ebashi, Endo & Ohtsuki, 1969)). A similar argument could be made for the constancy of bound ATP (the corresponding ATP change was $0{\cdot}067 \pm 0{\cdot}100$ per head).

During rapid shortening the mean rate of ATP utilization was $0{\cdot}48 \pm 0{\cdot}24$ μmole/g . sec, which corresponds to a turnover rate of $1{\cdot}7 \pm 0{\cdot}9$ sec^{-1} per myosin head. The rate for the isometric tetanus period was $1{\cdot}1 \pm 0{\cdot}4$ sec^{-1} per head. Although these values must include a small contribution from ATPases other than actomyosin, it is clear that the results are not consistent with the cross-bridge model of Eisenberg *et al.* (1980), which predicts that the ATPase rate during rapid shortening should be seven times greater than that in isometric contraction. At the velocity of shortening employed in the present experiments, the relative filament velocity is 1330 nm/sec. Assuming that the maximum filament displacement over which a cross-bridge may remain attached is 15 nm (Ford, Huxley & Simmons, 1977) the cross-bridge detachment rate must be *at least* 85 sec^{-1}, which is fifty times the ATP turnover rate. Either only 2% of cross-bridges are attached during rapid shortening, or cross-bridge detachment can occur without ATP hydrolysis. In the Lymn–Taylor model of the cross-bridge cycle (Lymn & Taylor, 1971) the hydrolysis step immediately follows cross-bridge detachment. In this model one could only achieve detachment without hydrolysis if the rate of the latter was slow compared with the duration of shortening (0·3 sec). This type of scheme, in which myosin . ATP is built up during shortening, has been used by Kodama & Yamada (1978) to describe the energetics of muscle shortening. The finding that the rate of the hydrolysis step for frog myosin at 0 °C is at least 5 sec^{-1} (Ferenczi, Homsher, Simmons & Trentham, 1978) argues against this explanation, but further biochemical experiments are required to resolve this point.

An alternative explanation for the low rate of ATP utilization during rapid shortening is that cross-bridge attachment takes place in two stages, the first of which is rapidly reversible, but both are required before ATP splitting can occur (Huxley, 1973). This would effectively reduce the ATPase rate at high velocity and, in terms of the Lymn–Taylor model, cross-bridges would accumulate in the myosin . hydrolysis products state (and an actin-bound state in rapid equilibrium with it). However there could not be a post-shortening burst of hydrolysis from this state in anything less than a complete cycle, in contrast to the present results. This type of explanation would thus require a more complicated hydrolysis mechanism than that proposed by Lymn & Taylor (1971).

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