Published May 1, 1979

The Time-Course of Energy Balance in an Isometric Tetanus

E. HOMSHER, C. J. KEAN, A. WALLNER, and V. GARIBIAN-SARIAN

From the Department of Physiology and the Jerry Lewis Neuromuscular Research Center, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

ABSTRACT Unpoisoned sartorius muscles of *Rana temporaria* were stimulated tetanically in isometric contractions lasting up to 20 s at 0°C. The observed enthalpy (heat + work) production and the chemical changes in these contractions were measured, and a comparison was made between the observed enthalpy and the enthalpy that could be explained by the chemical changes. Like earlier workers, we found that the only net known reaction of energetic significance that occurred was dephosphorylation of *n*-phosphoryl creatine (PC), and we found a significant evolution of unexplained enthalpy (UE), a portion of the observed enthalpy which could not be explained by the extent of PC dephosphorylation. We measured the total quantity and the rate of production of the UE, and we found that its rate of evolution, which was most rapid during the first 750 ms of contraction, fell progressively to zero by the 8th s of contraction; i.e., after 8 s of contraction, all the observed enthalpy is adequately explained by PC dephosphorylation. The time-course of evolution of the UE was slower than that of the labile enthalpy (a component of the enthalpy evolved in isometric contraction whose rate of production declines exponentially at $\sim 1\ s^{-1}$). We conclude that, although the magnitudes of these enthalpy quantities may be similar, they are not derived from the same chemical reaction in muscle.

INTRODUCTION

A number of investigations using the energy balance technique have shown that the enthalpy (heat + work, $h + w$) production during an isometric contraction in frog striated muscle cannot be completely accounted for by phosphoryl creatine (PC) dephosphorylation or other reactions known to occur in muscle (Gilbert et al., 1971; Curtin et al., 1974; Curtin and Woledge, 1974, 1975, 1977; Homsher et al., 1975). Similar results have been obtained in chelonian (Walsh and Woledge, 1970), avian (Bridge, 1976), and mammalian (Gower and Kretzschmar, 1976) muscles which indicate the generality of this finding. In addition, verification of the validity of the procedures used for measurement of the muscle enthalpy production (Wilkie, 1968; Kretzschmar and Wilkie, 1972; Homsher et al., 1975) and chemical change (Curtin and Woledge, 1974, 1975; Kushmerick and Paul, 1976; Dawson et al., 1977) and the reexamination of the molar enthalpy change for a number of reactions occuring in the muscle (Woledge, 1972) provide assurance that the result mentioned is real. This result

Downloaded from on April 11, 2017

Published May 1, 1979

indicates that at least one additional exothermic reaction occurs in muscle during contraction to yield the unexplained enthalpy (UE), that part of the enthalpy not accounted for by known chemical reactions. At present, neither the time-course nor the total amount of the unexplained enthalpy ultimately produced in a long duration tetanus at 0°C is known.

Recently Curtin and Woledge (1977) found that the amount of labile enthalpy (LE, an amount of heat + work produced during a tetanus, the rate of which declines exponentially at ~ 1 s^{-1} at 0°C [Aubert, 1956]) and the amount of UE were similar in 5-s tetani; they also found that the amount of both LE and UE were reduced to a similar extent in the second of a pair of consecutive 5-s tetani. The similarity in amount and behavior of the LE and UE led Curtin and Woledge (1977) to speculate that both phenomena were caused by the same reaction. If this idea were correct, one would predict that (*a*) the total amount of LE and UE would be the same; (*b*) the rate of evolution of both UE and LE would decline with the same time-course; and (*c*) all the enthalpy produced after ~ 5 s of stimulation by an isometric muscle should be accounted for by known chemical reactions, specifically the dephosphorylation of PC and (or) ATP. These experiments were designed to test these predictions.

METHODS

Sartorius muscles of *Rana temporaria* were used throughout this study. For the first series of experiments described below, a single shipment of male frogs purchased from Charles Sullivan of Nashville, Tenn. were used. A single shipment of animals of both sexes from Nasco (Fort Atkinson, Wis.) was used for the second series. On the evening before an experiment, muscles were dissected, the pelvic bone split, any damaged pairs discarded after examination under a dissecting microscope, and the others aerated overnight at 4°C as described previously (Homsher et al., 1975).

Myothermal Measurements

Inasmuch as the biochemical experiments were conducted over the course of several weeks, myothermal measurements were made at the start, middle, and end of the biochemical experiments. No systematic changes in the mechanical, myothermal, or chemical behavior of the muscles was found. For myothermal measurements, a pair of sartorius muscles was mounted on a thermopile at their standard body length (l_0) attached to an isometric force transducer, immersed in oxygenated Ringer solution at 0°C until thermal equilibration had occurred, and stimulated as described by Rall et al. (1976). Before stimulation of the muscles, the Ringer solution was drained from the system and the rate of heat loss by the muscle-thermopile system was determined by measuring the rate of the decline in temperature difference between the "hot" and "cold" junctions after passing a Peltier current into the thermopile (Kretzschmar and Wilkie, 1972). The muscles were then tetanically stimulated with square wave pulses of 3 ms duration and 16 v amplitude at 12 Hz for 20 s in the first series and 10 s in the second series of experiments. At the end of each experiment, the muscles were removed from the thermopile, weighed, blotted, reweighed, and then frozen in liquid nitrogen. The muscles were subsequently extracted and their total creatine contents were determined. The observed enthalpy could thus be expressed per unit of total creatine for comparison with the biochemical experiments. A number of these muscles were also used to measure the stimulus heat as previously described (Rall et al., 1976).

Two different fast thermopiles fabricated by the method of Ricchiuti and Mommaerts

Downloaded from on April 11, 2017

Published May 1, 1979

(1965) were used in this work. For the first series of experiments thermopile E10 was used whose overall length is 30 mm. E10 has multiple taps from the thermopile so that the active region may be selected at 20, 22, 24, 26, 28 or 30 mm. The sensitivity of the shortest region (20 mm) is 8.99 $mV \cdot °C^{-1}$. In the second series of experiments thermopile E6, which is 20 mm long with an active region 13.5 mm long and a sensitivity of 6.44 $mV \cdot °C^{-1}$, was used. The accuracy of the sensitivity of these thermopiles, which was determined as described by Hill and Woledge (1962), was verified by the independent calibration technique of Kretzschmar and Wilkie (1972). These tests as well as earlier calibrations of the absolute energy sensitivity (Homsher et al., 1975; Curtin and Woledge, 1978) using this type of thermopile lead us to believe that our estimates of the absolute heat production are accurate within ± 3%. Each face of E10 is covered with a grounded 4-μm-thick layer of aluminium foil which acts as an electrostatic shield to reduce the amplitude of the stimulus artifacts. The equivalent half thickness (Hill, 1965; p. 311) of E10 is 19 μm.

Analysis of Thermal Recordings

Amplification and display of the thermopile output, correction of temperature recordings for heat loss and stimulus heat, calculation of the absolute energy liberation, and correction for conduction of heat from the muscle to the thermopile were as described previously (Homsher et al., 1975). A thermoelastic heat coefficient of 0.01 was used as in earlier work (Gilbert et al., 1971; Homsher et al., 1975) and the quantity of energy stored as elastic work was estimated from the data of Hill (1970). A combined thermoelastic and stored elastic work correction of ~6 $mJ \cdot g^{-1}$ of blotted wet weight was made to the heat records which amounts to 18.5% of the total enthalpy at 0.75 s, and 2% at 20 s of tetanic stimulation.

After correction for stimulus heat, thermopile lag, and heat loss, the heat recordings were fitted to Eq. 1 after Aubert (1956):

$$h_t = \dot{h}_{A/\alpha} (1 - e^{-\alpha t}) + \dot{h}_B t, \quad (1)$$

where h_t is the heat production at time t after the onset of stimulation, $\dot{h}_{A/\alpha}$ is the maximum labile maintenance heat, α is a rate constant which determines the rate at which labile maintenance heat production declines with time, and $\dot{h}_B$ is the stable maintenance heat rate. Heat records were read every 0.5 s, and a linear regression line was fitted to the data between 5 and 10 s. The regression coefficient yielded $\dot{h}_B$ and the intercept at $t = 0$ gave $\dot{h}_{A/\alpha}$ (the average coefficient of correlation of this regression was 0.998). The logarithm of the difference between the regression line and the observed heat record was plotted as a function of time, and the rate constant α was estimated from the slope of this line, the average correlation coefficient for this line was −0.994. This analysis for the labile and stable maintenance heat differs from that of Aubert (1956) in that he omitted the first 0.5 s of a tetanus from his analysis. The thermoelastic energy and elastic work produced by the muscle was added to the labile maintenance heat thus yielding the labile (heat + work) (Curtin and Woledge, 1977, 1978) or the labile enthalpy (LE).

Chemical Experiments

Muscle pairs were mounted, thermally equilibrated to 0°C, aerated, stimulated, and frozen using the hammer apparatus described earlier (Homsher et al., 1975). The frozen muscles were then extracted and analyzed for their contents of free creatine (C_F), total creatine (C_T), ATP, ADP, AMP, and inorganic phosphate (P_i) as previously described (Mommaerts and Wallner, 1967; Homsher et al., 1972). Lactate contents were determined

Downloaded from on April 11, 2017

Published May 1, 1979

by a modification of the method of Gutman and Wahlefeld (1974). In the assay mixture, the NAD concentration is lowered to 1.6×10^{-4} M and the extent of its reduction by the lactic acid dehydrogenase reaction is measured fluorimetrically. The sensitivity of Gutman and Wahlefeld's method is increased by about 100-fold. Hexose monophosphate was measured in some experiments by the method of Scopes (1972). All chemical values were referenced to the muscle total creatine content (expressed as nanomoles per micromole C_T). The extent of the studied reactions that occurred in an experimental period was calculated as the difference in content between the muscles of each pair, using the assumption that the metabolite contents (C_F/C_T, P_i/C_T, ATP/C_T, etc) of both muscles of a pair were identical before stimulation. The explained enthalpy was calculated by multiplying the extent of PC dephosphorylation ($\Delta PC/C_T$ and $\Delta P_i/C_T$) by the molar enthalpy change for PC dephosphorylation (taken as -34 kJ· mol^{-1} (Woledge, 1972). There were a total of 114 muscle pairs examined in the chemical experiments of which 7 pairs were rejected on the criterion that the change in C_F, or ATP per micromole C_T was more than 2 SD away from the mean of their group with the suspected deviant value included.

TABLE I

HEAT EXPERIMENTS

	First series (n = 13)*	Second series (n = 13)*
$\frac{P_0 l_0}{M}$, $kN \cdot m^{-2}$	232 ± 2	225 ± 3
$\dot{h}_B$, $mJ \cdot g^{-1} \cdot s^{-1}$	13.9 ± 0.44	15.5 ± 0.36
$\frac{\dot{h}_A}{\alpha}$, $mJ \cdot g^{-1}$	44.2 ± 1.14	35.2 ± 1.46
α, s^{-1}	0.84 ± 0.03	0.83 ± 0.04
Total creatine content, $\mu mol \cdot g^{-1}$	35.3 ± 0.39	37.5 ± 0.51

* Values given as the mean ± 1 SEM; for normalization to muscle weight, the blotted wet weight was used. P_0 was the maximum isometric tension recorded at the standard length in the body (l_0).

RESULTS

The experiments described below were performed to measure, under identical conditions, the amount and time-course of the evolution of both the unexplained enthalpy and the labile enthalpy. Thus, LE and UE could be compared and a direct test of the predictions stated in the Introduction could be made.

Myothermal Results

Heat and enthalpy production in tetanic contractions at 0°C were measured and the records were analyzed as described earlier. The muscles were stimulated for 20 s in the first series, and for 10 s in the second series of experiments.

Table I contains a summary of the results of the myothermal measurements, and Fig. 1 shows a plot of the (h + w) production during the first 13 s of a 20-s tetanus. Eq. 1 fitted the records of heat production adequately as shown by the correlation coefficients given in the methods section. Examination of Table I shows that the force per cross-sectional area was similar in the two series of animals. The stable maintenance heat rate ($\dot{h}_B$) was slightly but significantly

Downloaded from on April 11, 2017

Published May 1, 1979

lower ($P < 0.05$) in the first series of animals than in the second series; conversely, there was significantly more labile maintenance heat ($\dot{h}_{A\alpha}$) produced by the first batch of animals, but the time constant for evolution of the labile heat (α) was similar in both series. The total creatine content of the muscles used for the myothermal experiments is also given in Table 1.

The solid line in Fig. 1 illustrates the technique used to estimate the labile enthalpy and shows that after 5 s of a long tetanus, the rate of enthalpy production is essentially constant. Extrapolation to $t = 0$ (as shown by the dotted line) gives an intercept on the ordinate whose magnitude corresponds to the LE, which amounted to 1.43 ± 0.036 mJ·μmol^{-1}C_T in the first series ($n = 13$) of experiments.

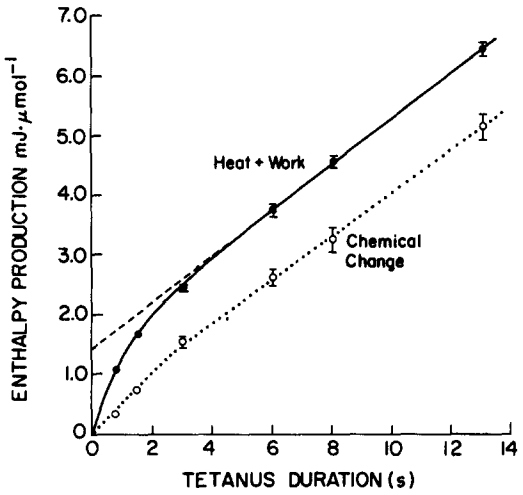

FIGURE 1. The solid curve shows the observed enthalpy (heat + work) produced plotted as mJ·μmol^{-1} of total creatine (ordinate) against tetanus duration in seconds (abscissa). The extrapolation of the linear part of the curve towards the ordinate gives the labile maintenance enthalpy as an intercept. The broken curve shows the net chemical change (phosphorylcreatine [PC] split) at various intervals. This has been multiplied by the molar enthalpy change (−34 kJ·mol^{-1} for PC dephosphorylation). Error bars represent 1 SEM.

Energy Balance Late in a Tetanus

We first tested the prediction that an energy balance would be found late in a tetanus by measuring the enthalpy production and chemical change that occurred in muscles in the intervals between 15 and 20 s and between 8 and 13 s after the onset of tetanic stimulation. These times were selected to allow at least six time constants of LE evolution to pass, thus ensuring that the enthalpy evolved was uncontaminated by LE. Enthalpy production was measured as the difference between the net enthalpy at 20 and 15 s, and 13 and 8 s, respectively in 13 muscle pairs stimulated for 20 s.

For the chemical experiments, the control muscles were frozen after 15 s or 8 s

Downloaded from on April 11, 2017

Published May 1, 1979

of stimulation; the experimental muscles in each group were stimulated for 5 s longer before freezing. The difference in chemical contents represents the chemical change in the 5-s intervals studied. The results of these experiments are shown in Tables II and III.

Table II shows that there was no significant difference between the change in C_F content and the change in P_i content in the muscles. As there was no change in the adenine nucleotide or lactate contents between 8 and 13 s, PC splitting was probably the only significant known reaction in this series. There was, however, a small increase in ADP and AMP between 15 and 20 s, which may

TABLE II

CHEMICAL CHANGES DURING TETANIC CONTRACTIONS

Tetanus duration Control	Experimental	Metabolite							
		ΔC_F	ΔP_i	ΔATP	ΔADP	ΔAMP	ΔLactate	ΔHMP	n
s		*Δ = (E − C) ± SEM in nmol/μmol total creatine*							
First series									
0	0.75	9.3	9.5	2.1	0.2	0.1	1.8	–	11
		±2.1	±2.4	±2.0	±0.6	±0.2	±2.5		
0	1.5	22.1	21.4	2.0	−0.6	0.0	−0.1	–	8
		±2.6	±1.8	±2.7	±0.6	±0.3	±1.5		
0	3.0	46.3	45.6	2.2	1.2	0.1	−0.9*	–	17
		±3.6	±2.2	±3.1	±1.1	±0.1	±1.6		
0	6.0	73.4	81.7	−0.2	−1.1	−0.4	0.7	–	6
		±5.4	±14.1	±5.2	±1.7	±0.4	±0.5		
3	8	54.3	47.7	3.0	−1.7	−0.3	0.2	–	10
		±5.2	±6.2	±3.8	±2.0	±0.8	±0.8		
8	13	60.0	50.6	−3.7	2.2	0.0	0.2	–	9
		±2.9	±7.1	±3.1	±2.6	±0.3	±1.1		
15	20	52.4	52.3	−2.0	2.3	0.8	1.3‡	1.5‡	12
		±6.4	±7.8	±1.8	±0.8	±0.3	±1.2	±0.4	
Second series									
0	5	60.0	57.8	−0.4	–	–	–	–	11
		±5.7	±5.7	±1.6	–	–	–	–	
5	10	41.4	47.6	4.7	−1.3§	1.8§	0.6§	–	16
		±4.4	±4.7	±2.1	±1.8	±1.0	±1.1		

* n = 14.
‡ n = 9.
§ n = 13.

reflect a decline in ATP and onset of some myokinase activity. There was also a slight increase in HMP content (1.5 ± 0.4 nmol·μmol$^{-1}C_T$) between 15 and 20 s, however, as there was no significant lactate change in these muscles (Table II), the contribution of glycolysis towards changes in ATP levels was negligible in these experiments.

Table III gives the estimated PC splitting, the enthalpy explained by the PC splitting, the enthalpy observed, and the difference between the two latter values or the unexplained enthalpy. It is quite clear from the Table that there was an energy balance between 8 and 13 s; i.e., the enthalpy evolved in this period was completely accounted for by PC splitting. Table III also shows a

Downloaded from on April 11, 2017

Published May 1, 1979

balance between the observed enthalpy and the enthalpy explained by PC splitting in the interval between 15 and 20 s of stimulation. This conclusion is not altered by addition of the enthalpy changes due to the small adenine nucleotide and HMP changes that we found in this period (see Table II) to the observed enthalpy from PC dephosphorylation (Table III) which increases the latter value by 6% and decreases the unexplained enthalpy to -0.13 ± 0.24. $mJ \cdot \mu mol^{-1} C_T$, which like the value given in Table III, is not significantly different from zero ($P > 0.6$).

TABLE III

ENERGY BALANCE CALCULATIONS

Stimulus duration		$\Delta PC/C_T$*‡	Explained‡ enthalpy	Observed§ enthalpy	Unexplained enthalpy
Control	Experimental	$nmol \cdot \mu mol^{-1} C_T$		$mJ \cdot \mu mol^{-1} C_T$	
s					
First series					
0	0.75	9.4	0.320	1.08	0.76
		±1.6	±0.055	±0.015	±0.057
0	1.5	21.8	0.740	1.67	0.93
		±1.9	±0.064	±0.021	±0.067
0	3	46.0	1.56	2.48	0.92
		±2.2	±0.076	±0.035	±0.084
0	6	77.5	2.64	3.77	1.13
		±4.7	±0.160	±0.058	±0.170
3	8	51.0	1.73	2.11	0.38
		±5.3	±0.180	±0.066	±0.192
8	13	55.3	1.88	1.90	0.02
		±3.8	±0.130	±0.050	±0.139
15	20	52.4	1.78	1.76	−0.02
		±7.0	±0.237	±0.047	±0.241
Second series					
0	5	58.9	2.00	3.16	1.16
		±4.9	±0.17	±0.06	±0.18
5	10	44.5	1.51	2.07	0.56
		±5.3	±0.15	±0.05	±0.16

* $\Delta PC/C_T$ is estimated as $\frac{(\Delta C_F + \Delta P_i)}{2}$.

‡ Number of estimates for $\Delta PC/C_T$ and explained enthalpy are those in final column of Table II.

§ Enthalpy observed is mean of 13 muscle pairs.

Magnitude of the Unexplained Enthalpy

The experiments already described showed that all the unexplained enthalpy must be evolved in the first 8 s of a tetanus at 0°C. We measured the amount of unexplained enthalpy in two ways. First, an estimate was made by measuring the UE produced in the first 3 s of stimulation, as well as the UE evolved between the 3rd and 8th s of stimulation, the sum of these quantities being the UE evolved in the first 8 s of contraction. Muscles were frozen after 3 s of stimulation, their controls being unstimulated; other muscles were stimulated

Downloaded from on April 11, 2017

Published May 1, 1979

for 8 s, and their controls were stimulated for 3 s. Second, the total UE evolved in the first 6 s of contraction was measured by freezing muscles after 6 s of tetanic stimulation, whereas the controls for this series were not stimulated.

The chemical changes that occurred in these intervals were calculated and are shown in Table II. Examination of Table II for the periods between 0 and 3 s, 3 and 8 s, and 0 and 6 s of stimulation shows that, as before, the changes in content of both C_F and P_i in each respective interval were similar. There were no significant changes in the content of the other measured metabolites in these intervals, and we concluded that the only net known reaction that occurred was PC dephosphorylation. The observed enthalpy, which was estimated in myothermal experiments at 3 and 6 s, and then at 8 s, was compared to the enthalpy explained by PC splitting. The results of the comparisons are shown in Fig. 1 and Table III. It may be seen in Table III that there was a highly significant ($P \ll 0.01$) evolution of UE in the period between 0 and 3 s and between 0 and 6 s, thus these frog muscles resembled muscles of previous studies (e.g., Gilbert et al., 1971; Homsher et al., 1975), because a significant quantity of the enthalpy observed during isometric contraction could not be explained by PC and (or) ATP splitting. The magnitude of the UE that was evolved in the first 8 s of contraction, obtained by addition as mentioned above, was 1.30 ± 0.21 $mJ \cdot \mu mol^{-1} C_T$. This estimate is not significantly different from the estimate obtained by measuring the UE evolved during the first 6 s, which was 1.13 ± 0.17 $mJ \cdot \mu mol^{-1} C_T$ (see Table III).

The Time-Course of UE Production

The experiments described in the last section showed that most of the UE was evolved in the first 3 s of contraction (see Table III). Between the 3rd and 8th s of stimulation, 0.38 ± 0.192 $mJ \cdot \mu mol^{-1} C_T$ of unexplained enthalpy was evolved which is not significantly different from zero ($0.1 > P > 0.05$). It seemed likely therefore that UE was evolved at a rapid rate early in the tetanus, and this rate declined thereafter to zero, inasmuch as the experiments described above showed an energy balance later in a tetanus. The time-course of the earlier phase of a tetanus was measured by performing energy balance experiments at times 3 s of stimulation. The chemical contents of muscles frozen after 0.75 and 1.5 s of stimulation were compared with those of unstimulated controls. The results of these measurements are shown in Table II, and in neither series was there any significant change in the contents of the adenine nucleotides or lactate. However, for each stimulus duration, the increase in C_F in the experimental muscles matched the increase in P_i, indicating that, as before, the only net known reaction was PC splitting. The observed enthalpy was determined at 0.75 and 1.5 s of stimulation in the myothermal experiments, and the results given together with the explained enthalpy in Table III (see also Fig. 1). The unexplained enthalpy at both times was highly significant ($P \ll 0.01$) and has been plotted in Fig. 2 (data connected by broken lines), together with the data calculated from the experiments described earlier. This figure shows that most of the UE is evolved in the early part of the tetanus, half or more of the total amount being evolved during the first 750 ms of stimulation at 0°C. This result

Downloaded from on April 11, 2017

Published May 1, 1979

is also illustrated in the inset to Fig. 2, in which the rate of evolution of UE has been plotted against the duration of stimulation. The values for this inset were obtained by dividing the unexplained enthalpy produced over each time interval studied by the duration of that interval and plotting the resultant rate at the middle of the time interval. This plot clearly shows that the rate of unexplained enthalpy production is greatest at the beginning of the tetanus and progressively falls as the tetanus progresses, finally reaching zero between 5 and 8 s.

The total amount of labile enthalpy in the first series of experiments as shown in Fig. 1 was 1.43 ± 0.036 mJ·μmol$^{-1}C_T$ for 13 observations. The time-course of

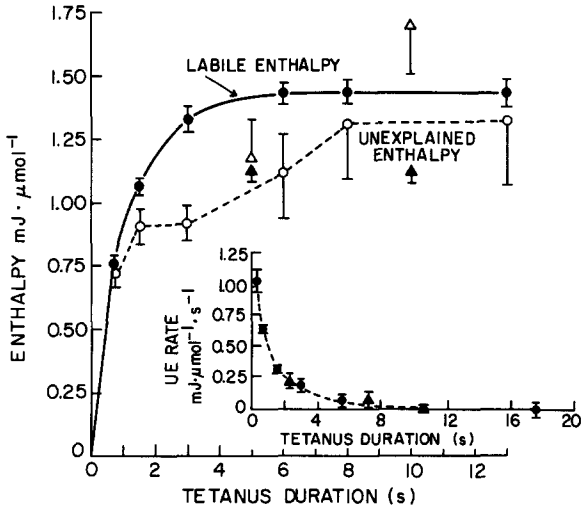

FIGURE 2. The ordinate is scaled in mJ·μmol$^{-1}C_T$ (enthalpy) and the tetanus duration (s) is shown as abscissa. The filled circles and unbroken curve show the labile enthalpy produced in the first series of experiments, which is compared with the unexplained enthalpy (hollow circles). The labile enthalpy and unexplained enthalpy measured in the second series of experiments are shown as the filled and open triangles, respectively plotted at 5 and 10 s. Inset shows the rate of evolution of unexplained enthalpy plotted as a function of tetanus duration. Circles represent the data from the first series of experiments; the triangles represent the second series. The curve was fitted by eye to the circles. All error bars represent 1 SEM.

evolution of the LE is shown as the unbroken line in Fig. 2, whereas comparison of the UE and LE in Fig. 2 shows that, at each point, the UE is less than the LE, and that this difference is only significant at the 3-s data point.

A Reexamination of the Identity of the Time-Course of the LE and UE

The previous series of experiments showed that, when all the UE and LE had been evolved, their magnitudes were not different. Although the time-course of evolution of the UE might be slower than that of the LE, the data do not allow one to make a definitive statement about the similarity of the time-courses of the UE and LE.

Downloaded from on April 11, 2017

Published May 1, 1979

The following experiment, carried out on a separate batch of frogs, was designed specifically to examine this question. Myothermal records were obtained from pairs of muscles stimulated for 10 s in order to obtain an estimate of the LE and its rate constant, α, in this group of animals. These results, together with those of other myothermal experiments obtained during and after the chemical experiments described below, are summarized in Table I in the column headed "Second series". The rate constant α for labile maintenance heat production in this group of frogs was 0.83 s^{-1}; thus, 98.5% of the LE would be evolved in a 5-s tetanus. Demonstration that significantly more UE than the residual LE was evolved in the second half of a 10-s tetanus would provide strong evidence that the UE is evolved more slowly than LE.

The chemical changes that occurred in muscles during the first and second 5 s of a 10-sec tetanus were measured by freezing experimental muscles after 5 and 10 s of stimulation, while their control muscles were unstimulated, or stimulated for 5 s, respectively, before they were frozen. The explained enthalpy was calculated from the extent of reaction, the observed enthalpy was obtained from the myothermal experiments, and the amounts of unexplained enthalpy was calculated as before.

The results from this experiment are summarized in Tables II and III (second series). In neither period of the tetanus was there a significant difference between the change in content of C_F and P_i. There was a small but significant ($P < 0.05$) apparent synthesis of ATP in the 5–10-s interval. We have omitted this ATP change in calculating the energy balance of this experiment because (*a*) we did not find a significant ATP change in any of the other 8 experiments summarized in Table II, (*b*) there was no significant change in ADP or AMP in this group of muscles; (*c*) the change in P_i content was larger than that of C_F, an opposite trend to that expected in the presence of ATP synthesis. It is clear from Table III that there was significant ($P << 0.01$) unexplained enthalpy produced in both intervals. The UE produced in the 0–5-s (1.16 ± 0.18 $mJ \cdot \mu mol^{-1} C_T$) interval is in excellent agreement with the LE evolved in this interval (1.08 ± 0.04 $mJ \cdot \mu mol^{-1} C_T$) as shown by the triangles in Fig. 2; this result is consistent with the data from the first series of experiments, in Fig. 2, and with the results of Curtin and Woledge (1977, 1978). However, between 5 and 10 s, there is significantly ($P < 0.01$) more unexplained enthalpy than the 0.017 $mJ \cdot \mu mol^{-1} C_T$ of LE evolved in this period. This result shows that the time-course of the unexplained enthalpy is slower than that of the labile heat. The amount of UE produced in the first 5 s of contraction, as well as the total quantity evolved in 10 s (1.73 ± 0.23 $mJ \cdot \mu mol^{-1} C_T$) have been plotted as open triangles in Fig. 2 to allow comparison with the data from the first series of experiments; the mean rate of evolution of UE in each 5-s period has also been plotted as triangles on the inset to Fig. 2. Examination of this figure shows that the amount and rate of evolution of UE of the second series is similar to the observations made with the first series of animals. The total LE (solid triangles in Fig. 2) observed in the second series of animals is significantly less ($P < 0.05$) than the total UE produced by this series of animals. This result is at variance with the similarity in the magnitude of the UE and the LE found in the first

Downloaded from on April 11, 2017

Published May 1, 1979

series, and also with the results of Curtin and Woledge (1978). It is possible that the similarity between the LE and UE observed earlier is coincidental.

Resting Levels of Metabolites

The mean concentrations of C_F, P_i, ATP, ADP, AMP, lactate, and HMP from the unstimulated muscles in the series of animals used for the experiments summarized in Figs. 1 and 2 have been calculated and are summarized in Table IV. These results are comparable to the values found by Curtin and Woledge (1977, 1978) in resting muscles. These authors found mean values of 175 and 170 nmol $C_F \cdot \mu mol^{-1} C_T$, 131 and 94 nmol $P_i \cdot \mu mol^{-1} C_T$, 86 and 122 nmol ATP $\cdot \mu mol^{-1} C_T$, respectively. The ADP level in this study is similar to the resting value of 15.4 nmol ADP$\cdot \mu mol^{-1} C_T$ found by Curtin andWoledge (1977), and the HMP value is similar to the value of 6.6 nmol HMP$\cdot \mu mol^{-1} C_T$ quoted by Curtin and Woledge (1978).

DISCUSSION

These experiments have shown that it is only during the first 8 s of an isometric tetanus that unexplained enthalpy is produced. At subsequent times (at least to

TABLE IV
CONCENTRATIONS OF METABOLITES IN UNSTIMULATED MUSCLES

	Metabolite						
	C_F	P_i	ATP	ADP	AMP	Lactate	HMP
					$nmol \cdot \mu mol^{-1} C_T$		
Mean	179.2	91.2	111.9	15.2	3.8	26.5	4.6
SEM	6.2	2.5	3.1	1.3	0.2	3.4	1.0
Number of observations	53	53	53	47	47	50	6

20 s), the only energetically significant reaction that occurs is PC dephosphorylation.

Although there are notable similarities in the magnitude and time-course of the LE and UE, we have shown instances in which their magnitudes were significantly different. Therefore, like Curtin and Woledge (1978), we conclude that it is doubtful that the UE and LE are identical in origin, but they may still be produced by related reactions.

Inasmuch as unexplained enthalpy is produced most rapidly early in a tetanus (see Fig. 2), the energy imbalance is most striking early in the contraction; i.e., the ratio of the total observed enthalpy to the explained enthalpy is greatest after a brief period of contraction and declines thereafter. This ratio in the current study declines rapidly during the first few seconds of stimulation, but after 3 s of stimulation, the decline towards the eventual asymptote, presumably of unity, is slow. For example, calculation of this ratio from the data used to construct Fig. 1 shows that the ratio [(h + w)/(ΔPC × 34 kJ/mol)] was 1.59 ± 0.08 at 3 s and 1.25 ± 0.06 after 13 s of stimulation. If such a decline in this ratio with

Downloaded from on April 11, 2017

Published May 1, 1979

increase in stimulus duration is real, one would expect to find that a fraction such as (observed enthalpy ÷ PC dephosphorylated), which is the same as the product of the molar enthalpy change for PC hydrolysis (-34 kJ mol^{-1} [(Woledge, 1972]) and the ratio above, would decline with an increase in tetanus duration. Such as trend is discernible in the results of Gilbert et al. (1971) and Homsher et al. (1975) with unpoisoned muscles, and with muscles poisoned by FDNB in the study of Curtin and Woledge (1975). The errors involved may have prevented statistical significance from being placed on such a decline in ratio with increase in tetanus duration, e.g., Table II in Homsher et al. (1975). However, this type of behavior was not evident in Wilkie's (1968) work inasmuch as such a decline would be lost in the scatter of his isometric data.

If one assumes that 37 mol of ATP are produced during the biological oxidation of 1 mol of glycosyl units by 6 mol of oxygen (McGilvery, 1973), or a theoretical $\Delta P:O_2$ ratio of 6.2, and that the unexplained enthalpy is eventually reversed by ATP splitting, and that no substrate cycling (Kushmerick, 1977) occurs during recovery from contraction, one can calculate (Homsher and Kean, 1978) that a $\Delta P:O_2$ ratio of 3.9 and 4.9 would occur after a 3-s and 13-s tetanus, respectively, in the muscles used for Fig. 1 (if one measured the PC dephosphorylated at the end of contraction and suprabasal O_2 utilized during recovery). Kushmerick and Paul (1976) found that the $P:O_2$ ratio was constant after tetani lasting from 5–20 sec in muscles of *Rana pipiens*. However, they state that they could not preclude changes within the range allowed by their mean 4.4 ± 0.5 (SD of a ratio). The agreement between our predicted $P:O_2$ ratios and those measured by Kushmerick and Paul (1976) is so good that we suggest that our experiments and theirs may be compatible, if the behavior of both species of frog are similar, and further suggest that the extent of any substrate cycling (Kushmerick, 1977) that may occur during recovery from a contraction is probably small.

Examination of the data in Table I shows that the isometric tetanic tension observed in this study is somewhat greater than the range of values from 160 to 190 $kN \cdot m^{-2}$ found by Aubert (1956), Hill and Woledge (1962), and Curtin and Woledge (1978) but is similar to the value found in this laboratory earlier (Homsher et al., 1975). The values for $\dot{h}_B$ and for α fall within the range of values between 11.5 and 21.9 $mJ \cdot g^{-1} \cdot s^{-1}$ and 0.71 and 0.89 s^{-1}, respectively, found in the literature (Aubert, 1956; Hill and Woledge, 1962; and Curtin and Woledge, 1978). These authors found values for $\dot{h}_{A/\alpha}$ in the range of 25.5–37.3 $mJ \cdot g^{-1}$; our first series of animals yielded a value that was somewhat higher than these values, but our second series gave a result that falls within this range.

The batch of frogs used for the main study in this series yielded a total amount of unexplained enthalpy (see Fig. 2, Table III) of 1.30 ± 0.21 $mJ \cdot \mu mol^{-1} C_T$. This quantity is not different from the total quantity (1.73 ± 0.24 $mJ \cdot \mu mol^{-1} C_T$) evolved by the batch of frogs used for the experiments summarized in Tables II and III as series 2; it is also similar to the quantity of unexplained enthalpy (1.18 ± 0.28 $mJ \cdot \mu mol^{-1} C_T$) found by Curtin and Woledge (1978) after 15 s of stimulation and is also similar to the amount (1.88 ± 0.22 $mJ \cdot \mu mol^{-1} C_T$) evolved in 5 s in an earlier study in this laboratory (Homsher et al., 1975).

Downloaded from on April 11, 2017

Published May 1, 1979

The quantity of unexplained enthalpy found in this study is also similar to the total amount evolved during two successive 5-s tetani separated by a 3-s interval (Curtin and Woledge, 1977). It is uncertain whether the unexplained enthalpy evolved during the second tetanus in that study is a production of enthalpy by the unknown reaction which had been reversed during the 3 s between tetani or whether it represents UE that had not been evolved before cessation of stimulation in the first contraction. The experiments of Curtin and Woledge (1974) would seem to suggest that the latter explanation is valid, but further studies of the time-course of the recovery of the unexplained enthalpy production are clearly needed.

Sources of the Unexplained Enthalpy

Inasmuch as our results show that all the unexplained enthalpy in a maintained contraction is evolved during the first 8 s of contraction and thereafter there is an energy balance, we may conclude that the unknown reaction is not stoichiometrically linked to PC dephosphorylation, and therefore we recant the suggestion about a possible stoichiometric linkage made earlier (Homsher et al., 1975). We conclude that the UE does not derive from a reaction such as acceptance or donation of protons and buffer changes associated with PC or ATP splitting.

Present data do not allow us to assign the unexplained enthalpy to other hypothesized sources. Two classes of hypotheses have previously been discussed by Homsher and Kean (1978). The first of these involves a variety of reactions associated with the release of activator calcium, its subsequent resequestration to the sarcoplasmic reticulum, and its return to the release site (Winegrad, 1968, 1970). A second hypothesis, proposed by Woledge (1977), suggest that the unexplained enthalpy production results from a difference in the steady-state distribution of myosin-nucleotide and actomyosin-nucleotide complexes between the resting and active muscles. These hypotheses could be discriminated by studies of the muscle length dependence of the unexplained enthalpy production. With currently available data, however, further speculation seems premature.

We thank Drs. Nancy Curtin, Michael Ferenczi, Martin Kushmerick, Merlin Kretschmar, and Roger Woledge for very helpful discussions about this manuscript.

This work was supported by grant HL 11351 from the U.S. Public Health Service and grant LA 525 from the American Heart Association. Dr. Homsher is supported by Research Career Development Grant AM 70792 from the National Institutes of Health.

Received for publication 10 October 1978.

REFERENCES

AUBERT, X. 1956. Le Couplage Énergétique de la Contraction Musculaire. Éditions Arscia, Brussels. 320 pp.

BRIDGE, J. H. B. 1976. A thermochemical investigation of chicken latissimus dorsi muscle. Ph.D. Thesis. University of California, Los Angeles, California, 139 pp.

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

Downloaded from on April 11, 2017

Published May 1, 1979

CURTIN, N. A., and R. C. WOLEDGE. 1974. Energetics of relaxation in frog muscle. *J. Physiol. (Lond.).* **238:**437–446.

CURTIN, N. A., and R. C. WOLEDGE. 1975. Energy balance in DNFB-treated and untreated frog muscle. *J. Physiol. (Lond.).* **246:**737–752.

CURTIN, N. A., and R. C. WOLEDGE. 1977. A comparison of the energy balance in two successive isometric tetani of frog muscle. *J. Physiol. (Lond.).* **270:**455–471.

CURTIN, N. A., and R. C. WOLEDGE. 1978. Chemical change and energy production during contraction of frog muscle. How are their time-courses related? *J. Physiol. (Lond.).* In press.

DAWSON, J. M., D. G. GADIAN, and D. R. WILKIE. 1977. Contraction and recovery of living muscles studied by ^{31}P nuclear resonance. *J. Physiol.(Lond.).* **267:**703–735.

GILBERT, C., K. M. KRETZSCHMAR, D. R. WILKIE, and R. C. WOLEDGE. 1971. Chemical change and energy output during muscular contraction. *J. Physiol. (Lond.).* **218:**163–193.

GOWER, D. C., and K. M. KRETZSCHMAR. 1976. Heat production and chemical change during isometric contraction of rat soleus muscle. *J. Physiol. (Lond.).* **258:**659–671.

GUTMAN, I., and A. W. WAHLEFELD. 1974. L-(±)-Lactate determination with lactate dehydrogenase and NAD. *In* Methods of Enzymatic Analysis. H. U. Bergemeyer, editor. Academic Press, Inc., New York. 1464–1468.

HILL, A. V. 1965. Trails and Trials in Physiology. The Williams & Wilkins Company, Baltimore, Md. 374 pp.

HILL, A. V. 1970. First and Last Experiments in Muscle Mechanics. Cambridge University Press, Cambridge. 141 pp.

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

HOMSHER, E., and C. J. KEAN. 1978. Skeletal muscle energetics and metabolism. *Annu. Rev. Physiol.* **40:**93–131.

HOMSHER, E., W. F. H. M. MOMMAERTS, N. V. RICCHIUTI, and A. WALLNER. 1972. Activation heat, activation metabolism and tension-related heat in frog semitendinosus muscles. *J. Physiol. (Lond.).* **220:**601–625.

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.

KRETZSCHMAR, K. M., and D. R. WILKIE. 1972. A new method for absolute heat measurement utilizing the Peltier effect. *J. Physiol. (Lond.).* **244:**18–19P.

KUSHMERICK, M. J. 1977. Energy balance in muscle contraction: a biochemical approach. *Curr. Top. Bioenerg.* **6:**1–37.

KUSHMERICK, M. J., and R. J. PAUL. 1976. Relationship between initial chemical reactions and oxidative recovery metabolism for single isometric contractions of frog sartorius at 0°C. *J. Physiol. (Lond.).* **254:**711–727.

MCGILVERY, R. W. 1973. The use of fuels for muscular work. *In* Metabolic Adaptation to Prolonged Physical Exercise. H. Howald and J. R. Poortsmans, editors. Birkhäuser Verlag, Basel.

MOMMAERTS,W. F. H. M., and A. WALLNER. 1967. The breakdown of adenosine triphosphate in the contraction cycle of the frog sartorius muscle. *J. Physiol. (Lond.).* **193:**343–357.

RALL, J. A., E. HOMSHER, A. WALLNER, and W. F. H. M. MOMMAERTS. 1976. A temporal

Downloaded from on April 11, 2017

Published May 1, 1979

dissociation of energy liberation and high energy phosphate splitting during shortening in frog skeletal muscles. *J. Gen. Physiol.* **68**:13–22.

RICCHIUTI, N. V., and W. F. H. M. MOMMAERTS. 1965. Technique for myothermic measurements. *Physiologist.* **8**:259. (Abstr.)

SCOPES, R. K. 1972. Automated fluorometric analysis of biological compounds. *Anal. Biochem.* **49**:73–87.

WALSH, T. H., and R. C. WOLEDGE. 1970. Heat production and chemical change in tortoise muscle. *J. Physiol. (Lond.).* **206**:457–469.

WILKIE, D. R. 1968. Heat, work and phosphorylcreatine breakdown in muscle. *J. Physiol. (Lond.).* **195**:157–183.

WINEGRAD, S. 1968. Intracellular calcium movement of frog skeletal muscle. *J. Gen. Physiol.* **51**:65–69.

WINEGRAD, S. 1970. The intracellular site of calcium activation of contraction in frog skeletal muscle. *J. Gen. Physiol.* **55**:77–88.

WOLEDGE, R.C. 1972. In vitro calorimetric studies relating to the interpretation of muscle heat experiments. *Cold Spring Harbor Symp. Quant. Biol.* **37**:629–634.

WOLEDGE, R. C. 1977. Calorimetric studies of muscle and muscle proteins. *In* Applications of Calorimetry in Life Sciences. I. Lamprecht and B. Schaurschmidt, editors. Walter De Gruyter & Co., Berlin. 183–197.

Downloaded from on April 11, 2017