Simultaneous Heat and Fluorescence Measurements on Mammalian Slow-twitch Skeletal Muscle.

Angus W. Stewart

Department of Physiology, Monash University, Clayton, Victoria, 3168 - Australia.

Abstract

A technique has been developed for simultaneously monitoring heat output and pyridine nucleotide fluorescence from contracting mouse hindlimb muscle. Measurements were carried out on mouse soleus muscle contracting isotonically and tetanically under a variety of loads in the presence of either glucose, pyruvate or lactate in 10 millimolar (mM) concentrations.

The fluorescence transients displayed complex kinetics which did not correlate in any simple way with the evolution of heat arising from muscular contraction. Thus, at high rates of energy expenditure, the fluorometric technique cannot be used to quantitate the flux of adenosine diphosphate (ADP) through the respiratory transport chain. It is believed that the activation of intermediary metabolism by the elevation of intracellular ADP is responsible for the complex fluorescence transients and that a significant portion of tissue fluorescence is of cytoplasmic origin.

The presence of pyruvate produced an increase in the total enthalpy, external work performed and energetic efficiency, however this was not reflected by a variations in the Hill force velocity parameters.

Key words: myothermic fluorometric, skeletal muscle, energetics

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The fluorometric technique is a valuable tool for monitoring the level of nicotinamide adenine dinucleotide (NADH) in whole muscle [9], brain tissue [6] and has generated qualitative and quantitative data relating the rate of energy turnover with contractile activity in amphibian muscle [38,39], cardiac muscle [12,13], and mammalian skeletal muscle [16,52,58]. Since tissue fluorescence reflects the ratio of NADH to NAD+, the production of recovery heat should be accompanied by an equivalent fluorescent response. While there have been separate studies of heat and fluorescence in mammalian muscle [58,60], there have been few investigations where the benefits of both these approaches have been combined [17]. An examination of the time courses of heat evolution and fluorescence transients requires the simultaneous recording of both in single muscles, and this is the subject of this paper.

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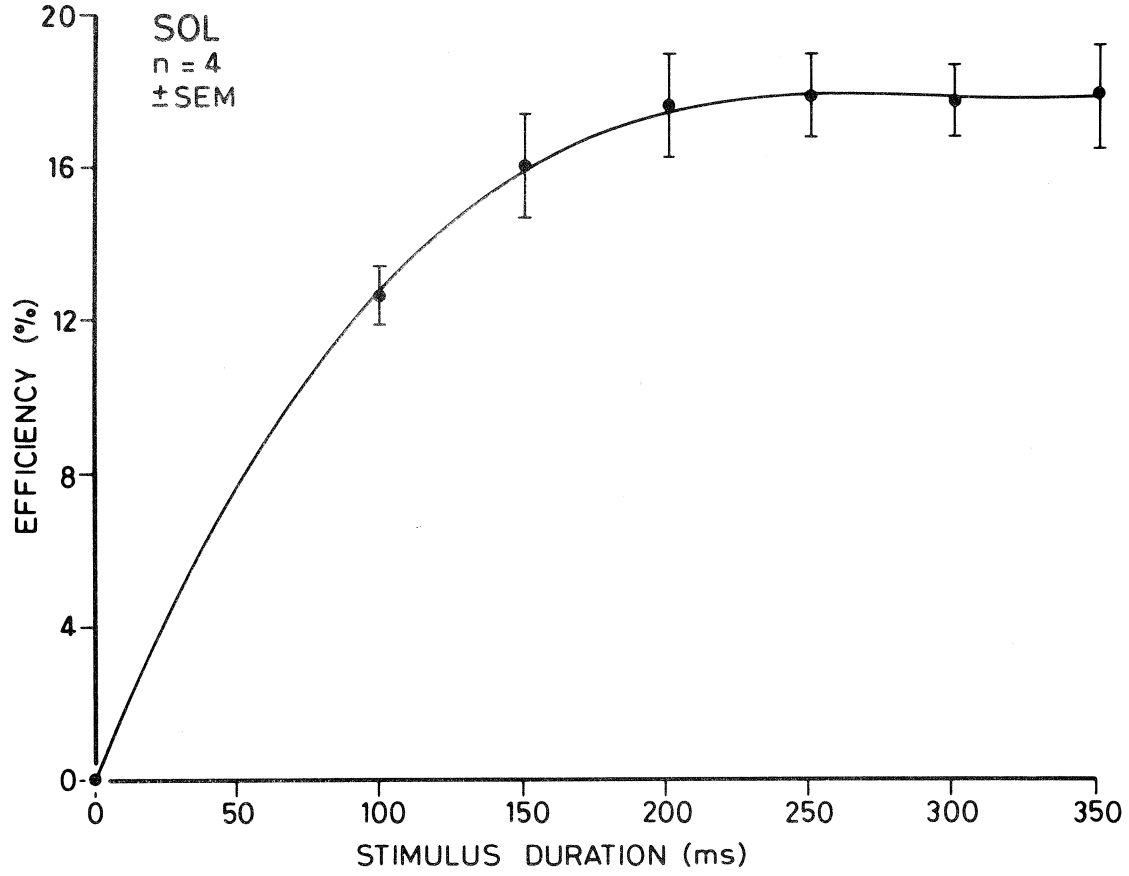

Figure 1. Mechanical efficiency (work/total enthalpy) plotted against stimulus duration for 4 soleus muscles ± standard error of the mean (SEM). From this plot 250 milliseconds was selected as a suitable stimulus duration for tetanic stimulation.

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The thermopile system was calibrated by discharging into the muscle a capacitor that had been charged to a known voltage [55]. The temperature change of the muscle (Δ T) was calculated by

$$\Delta T = (Df \,.\, Cs) / (Sn \,.\, G) \qquad (1)$$

where Df = chart recorder deflection, Cs = chart recorder sensitivity, Sn = thermopile sensitivity in millivolts per °C, and G = nanovolt amplifier gain. The muscle heat produced during contraction expressed in Joules/gram is

$$Q = (\Delta T - \Delta Ts).F.[(MmCm) + (MpCp) + (MsCs)]/Mm \qquad (2)$$

where ΔTs is the stimulus heat, F is an efficiency factor calculated for the thermopile, Mm, Mp and Ms are the masses of the muscle, thermopile and adhering solution respectively, and Cm, Cp and Cs are the specific heat capacities of the muscle, thermopile and adhering solution respectively [55].

Stimulus parameters

It has been observed that altering the stimulus parameters will influence the relationship between load and enthalpy [59]. This will affect any estimation of muscular efficiency (defined here as external work divided by the total enthalpy produced). To circumvent any possible problems, a number of experiments was performed to determine practical stimulus parameters that would result in maximum muscular efficiency. Pulse duration was set at 1.0 milliseconds, and the frequency that produced a fused tetanic contraction was determined to be 50 Hz. The muscles were then allowed to shorten isotonically during stimulation with an afterload equivalent to 0.5 Po, (where Po is the maximum isometric tension), while varying the stimulus duration from 100 to 350 milliseconds. On the basis of the data in Figure 1, 250 milliseconds was selected as a suitable stimulus duration. This parameter was found to produce maximal muscular efficiency while minimizing fatigue. Shorter duration times were rejected, as these did not permit maximum shortening of the muscle under

heavy loads. Contractions were either isometric or afterloaded isotonic, with loads approximating 0.1, 0.2, 0.4, 0.6 and 0.8 of Po. All loads were selected in a randomized manner. For each contraction, the total heat production, consisting of initial and recovery phases in addition to the resting heat, was measured. Load and shortening were also recorded; from these, external work and mechanical efficiency were calculated and the Hill force velocity relationship fitted.

Statistical design

A one way analysis of variance (ANOVA) was used to examine work, enthalpy, efficiency and the time for completion of recovery heat to determine whether any significant differences existed at a given load owing to substrate influences. The time for completion of recovery heat data was also examined using a randomized block design (RBD) ANOVA to find any differences between loads for one substrate group. Each substrate group was treated separately with one muscle representing one block. Statistical significance resulted in further testing using Duncan's new multiple range test (DMRT) to differentiate between either substrate groups or loads [51]. Student's t test for 2 means was used to examine some of the steady state fluorescence data. All tests were performed at the 5% significance level.

Dynamic fluorescence measurements

All fluorescence recordings were made using a microfluorometer based on the original design by Chance and Legallais [10], incorporating modifications by Jobsis and Stainsby [40]. Fluorescence was induced by illumination from a water cooled 1000 watt mercury arc lamp. After passing through a heat filter, light with a wavelength of 366 nm was isolated using a filter with a waveband width of 30 nm. This was reduced to a 2 mm diameter field and focussed onto the surface of the muscle by means of an epi illumination microscope attachment (Leitz, Wetzlar, Germany).

The light was collected by a x6.5 objective lens and split with a 95% to 5% beam splitter. The 95% fraction was passed through a Wratten 2C gelatin filter to eliminate any scattered or reflected 366 nm light, and to select the fluorescent light which peaks in the 465 nm region. This filtering arrangement has been used in previous studies of single muscle fibres [59].The 5% fraction was passed through a second 366 nm filter and provided a measure of the reflected excitation light. This was used to provide a control for movement artifacts that might occur in the optical records.

Light was detected using end window photomultiplier tubes (EMI type 9524B); the resulting signals were demodulated using a chopper amplifier system and the output DC voltages displayed on a Brush Mark 280 chart recorder. Fluctuations arising from instability of the UV lamp were reduced by use of a third photomultiplier tube which directly monitored the output of the arc lamp. The output of the third tube was kept constant by means of a negative feedback circuit acting on the high voltage supplying it. As all three photomultiplier tubes were supplied from the same voltage source, both fluorescence and reflectance outputs were compensated for any arc lamp fluctuations (refer to Figure 2). This technique is suitable for small muscle preparations of around 1 mm in thickness such as mouse soleus muscle [54] and has been used on larger preparations [49].

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The apparatus and mechanical arrangements used were similar to those devised by Chapman et al, [17]. The restriction imposed by a limited working distance from the front of the microscope (16 mm) was overcome by constructing a special glass envelope with a quartz window, thus allowing close positioning of the thermopile against the inner wall of the water bath. The clearance between the two quartz windows was never greater than 1 mm but this arrangement did not interfere with the thermal isolation of the thermopile.

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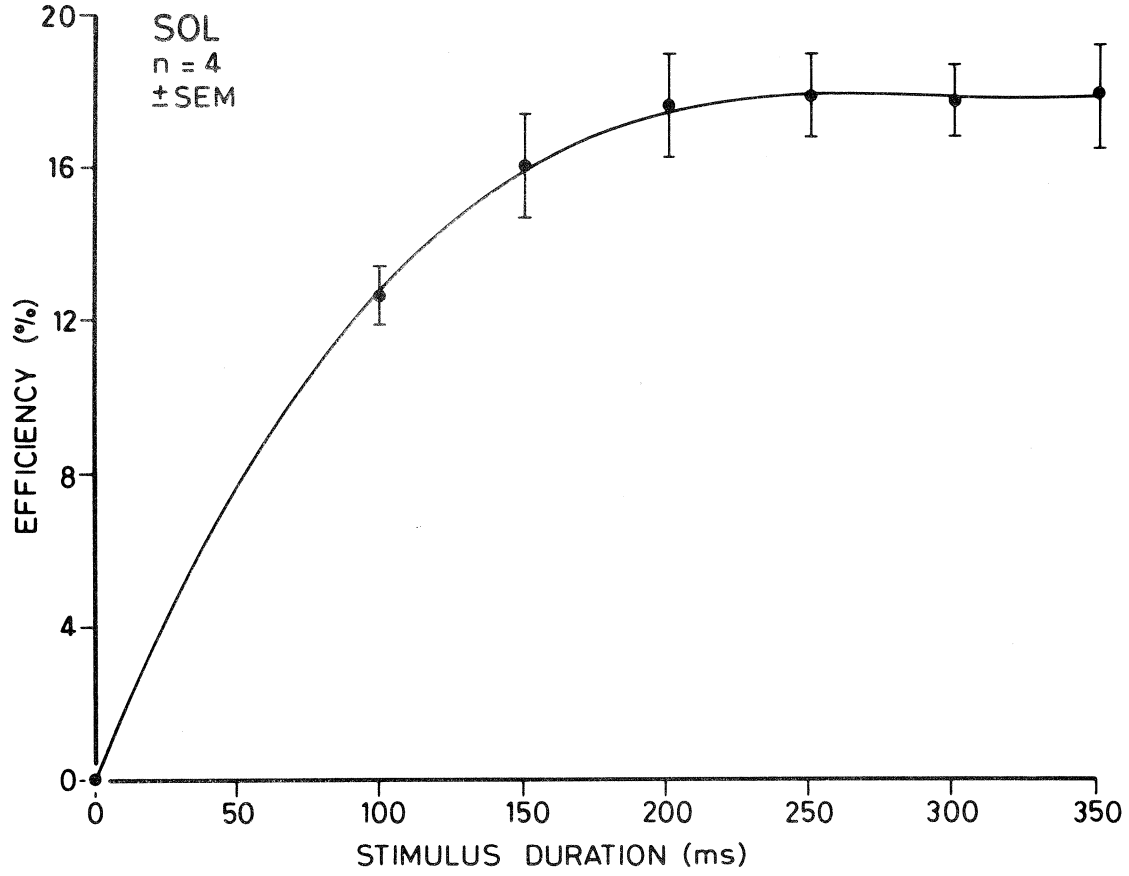

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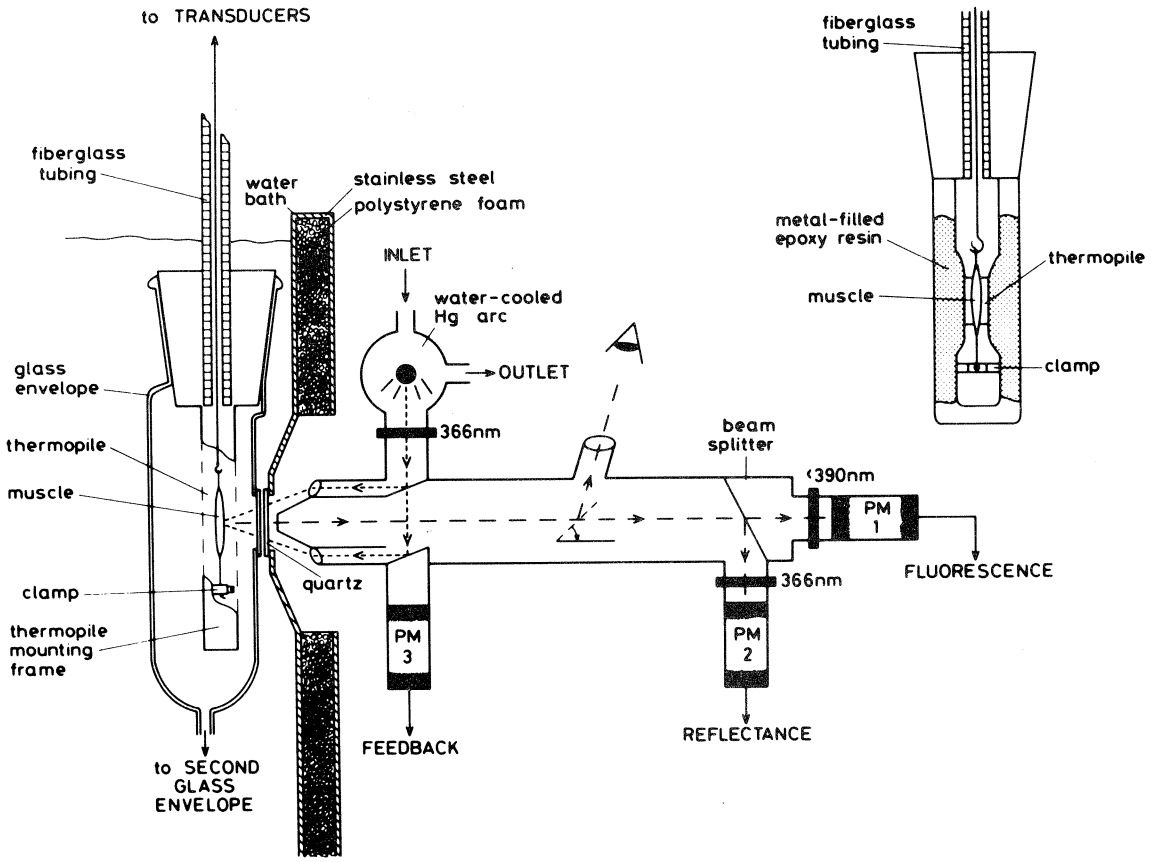

Figure 2. Schematic diagram showing the arrangement of the microfluorometer and the thermopile. PM 1 to 3 represent the three photomultiplier tubes. The insert on the upper right of the figure shows the position of the muscle on the thermopile.

Table 1. Results of steady-state fluorescence measurements. All results are expressed as a percentage of the resting fluorescence level (100%) in substrate to free conditions (aerobic), and are ± SEM. Measurements were taken 15 to 20 minutes after switching from a substrate free solution. The number of muscles is indicated in brackets on the right.

MEDIUM	FLUORESCENCE	
10 mM PYRUVATE	109.6 +/- 1.7	(11)
10 mM GLUCOSE	100.0 +/- 0.0	(5)
10 mM LACTATE	106.3 +/- 1.6	(11)
N_2/NO SUBSTRATE	132.8 +/- 4.8	(5)
N_2/10 mM PYRUVATE	** 115.4 +/- 3.1	(5)
N_2/10 mM LACTATE	130.0 +/- 5.4	(5)

** = statistically significant at 5% confidence level when compared to N_2/NO SUBSTRATE group, (Student's t test for two means).

with fine silk suture (Ethicon Mersilk 6/0). The suture attached to the proximal end of the muscle was held in a stainless steel clamp at the bottom of the thermopile frame (Figure 2), while the distal suture was attached to a length and tension monitoring system via a stainless steel tube. One muscle was mounted on the thermopile system while the other was held under light tension and kept submerged. The solution was held in a system of two sealed glass envelopes fixed at different levels. One of these was fitted with a quartz window so that muscle fluorescence could be monitored (refer to Figure 2). The thermopile frame had a tapered seal which fitted one of the glass envelopes and the whole arrangement was submerged in a circulating water bath, the temperature of which was regulated by a Haake NBS ultra thermostat. At the conclusion of the first series of measurements, the first muscle was removed and weighed, the second muscle placed on the thermopile and the experimental protocol repeated. The muscles had an average weight of 9.6 mg within a range of 13.8 to 8.4 mg and varied in length between 10.5 and 12.0 mm with an average length of 11.0mm. The average tetanic tension developed by these muscles was 145.5 ± 6.8 millinewtons per mm^2 ± S.E.M. (standard error of the mean). The experiments

were performed in 3 groups of 10. For each group, 10mM glucose and 0.01 IU/ml insulin, 10 mM pyruvate or 10 mM lactate was present in the solution as substrate. The muscles were always equilibrated for 45 minutes before any measurements were recorded. Muscle length was set to be optimal for twitch tension development and this was defined as Lo. All experiments were run at 27°C.

Results

Steady state fluorescence measurements

All results are summarized in Table 1 where the steady state fluorescence levels are expressed as a percentage of the (100%) resting aerobic level in substrate free solution. The greatest increase in fluorescence was obtained by switching

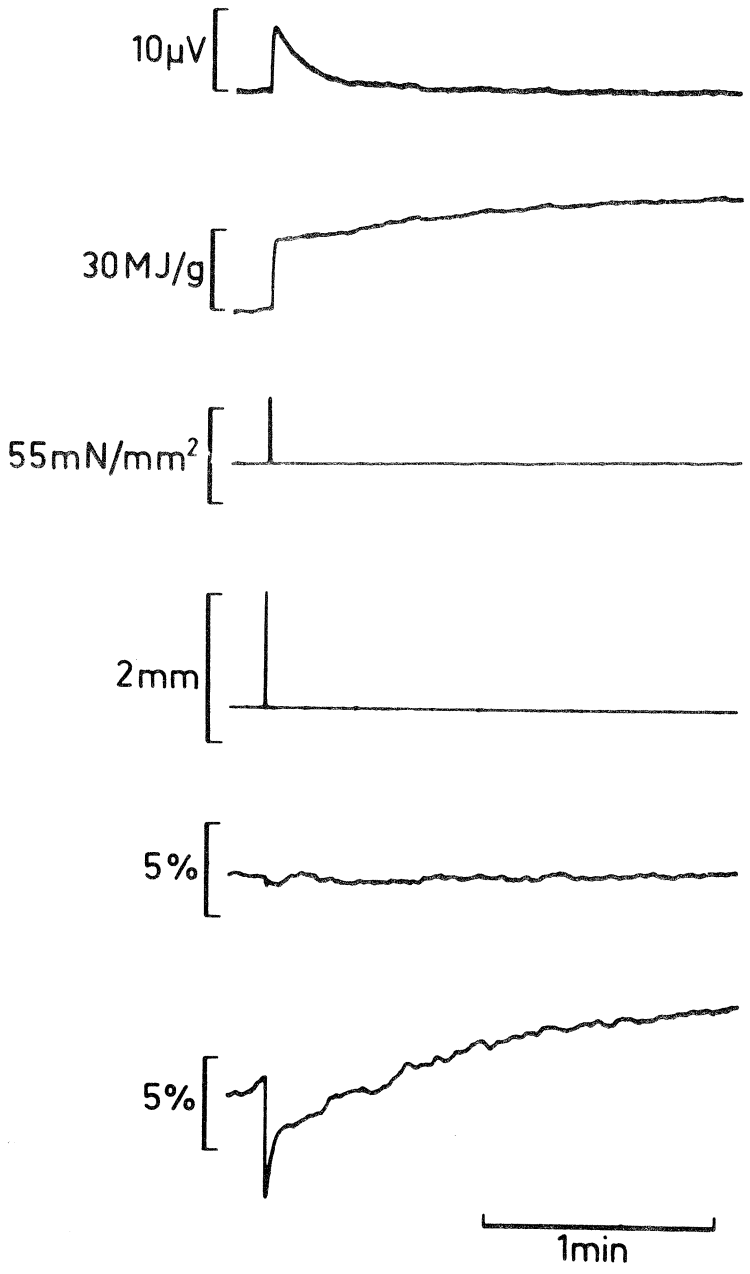

Figure 3. A section of a chart recording showing (from top to bottom) thermoelectric output, corrected heat, tension, displacement, fluorescence and reflectance for a soleus muscle contracting under a load of 0.2 Po in the presence of 10 millimolar glucose.

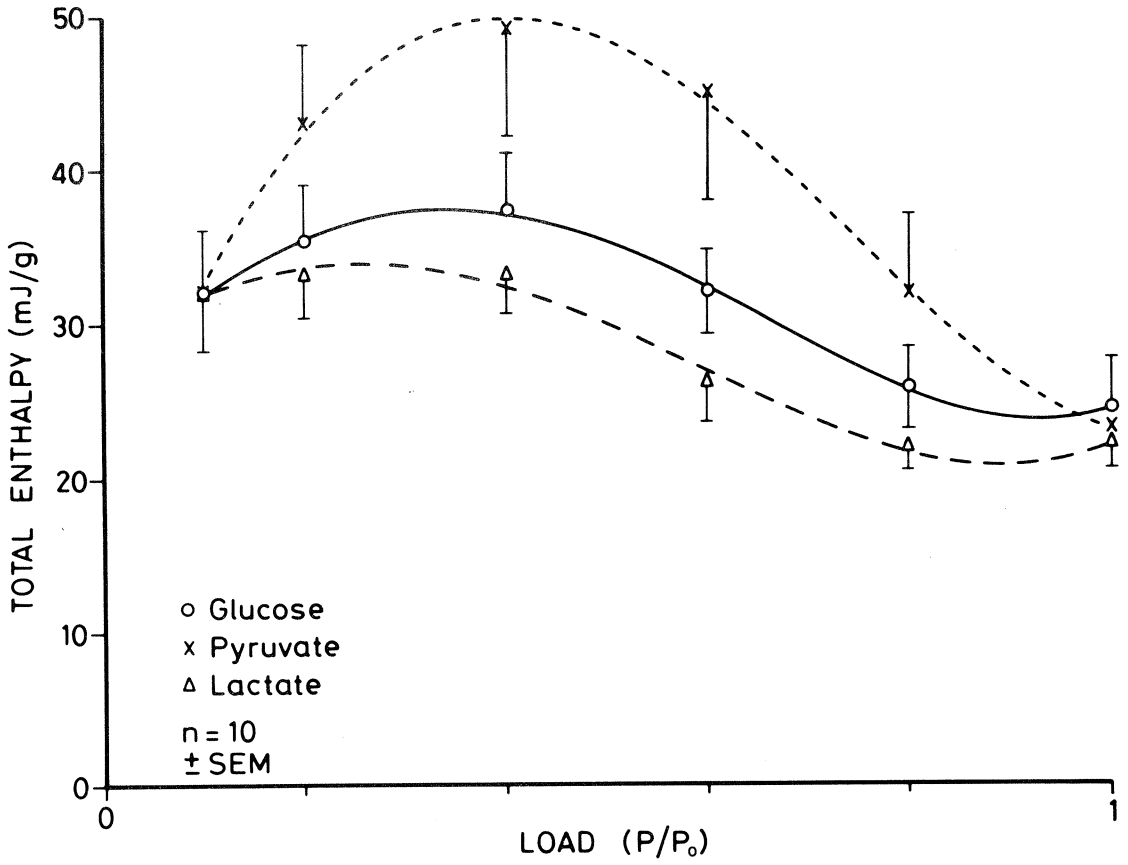

Figure 4. Total enthalpy plotted against load expressed as a fraction of Po. All points are the mean of 10 measurements (10 muscles) and are ± SEM.

Table 2. Time for completion of recovery heat in seconds ± SEM (in brackets) following isometric and isotonic contractions in different substrates. Loads (P) are expressed as a fraction of Po, (maximum isometric tetanic tension).

	LOAD (P/Po)					
	0.1	0.2	0.4	0.6	0.8	1.0
GLUCOSE	78	85	93	80	76	66
	(14)	(16)	(10)	(11)	(13)	(16)
PYRUVATE	79	70	57	78	71	76
	(12)	(13)	(10)	(12)	(11)	(14)
LACTATE	61	57	68	55	57	59
	(19)	(22)	(20)	(14)	(22)	(14)

from 95% oxygen, 5% carbon dioxide to 95% nitrogen, 5% carbon dioxide in the absence of substrate. This amounted to an increase of 32.8% and indicated that approximately one third of the total tissue fluorescence was metabolically labile. It is interesting to compare this result with those values obtained from anoxic muscles after switching to pyruvate or lactate, both of which are indicative of a more oxidized metabolic state. Student's t test was used to compare these data. Significance was found at the 5% confidence level between the means obtained in pyruvate and those obtained under anoxic substrate free conditions.

The muscles used throughout these experiments occupied a variable portion of the fluorometer observation field, therefore an absolute comparison of the fluorescence between different muscles was not possible [54]. For each preparation, the resting level of fluorescence (100%) was defined as that obtained while the muscle was quiescent, and immediately after having been removed from gassed Krebs Henseleit solution. Changes in fluorescence were expressed as percentage changes of this resting level. Care was taken when mounting muscles in the field of observation not to include any tendon, as this fluoresces strongly under UV light and would have given rise to large optical artifacts.

Dynamic fluorescence data

Figure 3 shows an original record obtained for an isotonic tetanic contraction against a load of 0.2 Po in the presence of glucose. This response was typical of those observed for both isometric and isotonic contractions. Both fluorescence and reflectance traces were filtered with a time constant of 400 milliseconds. Most optical records contained a movement artifact associated with each contraction. No attempt was made to reduce or remove this. In most cases the reflectance

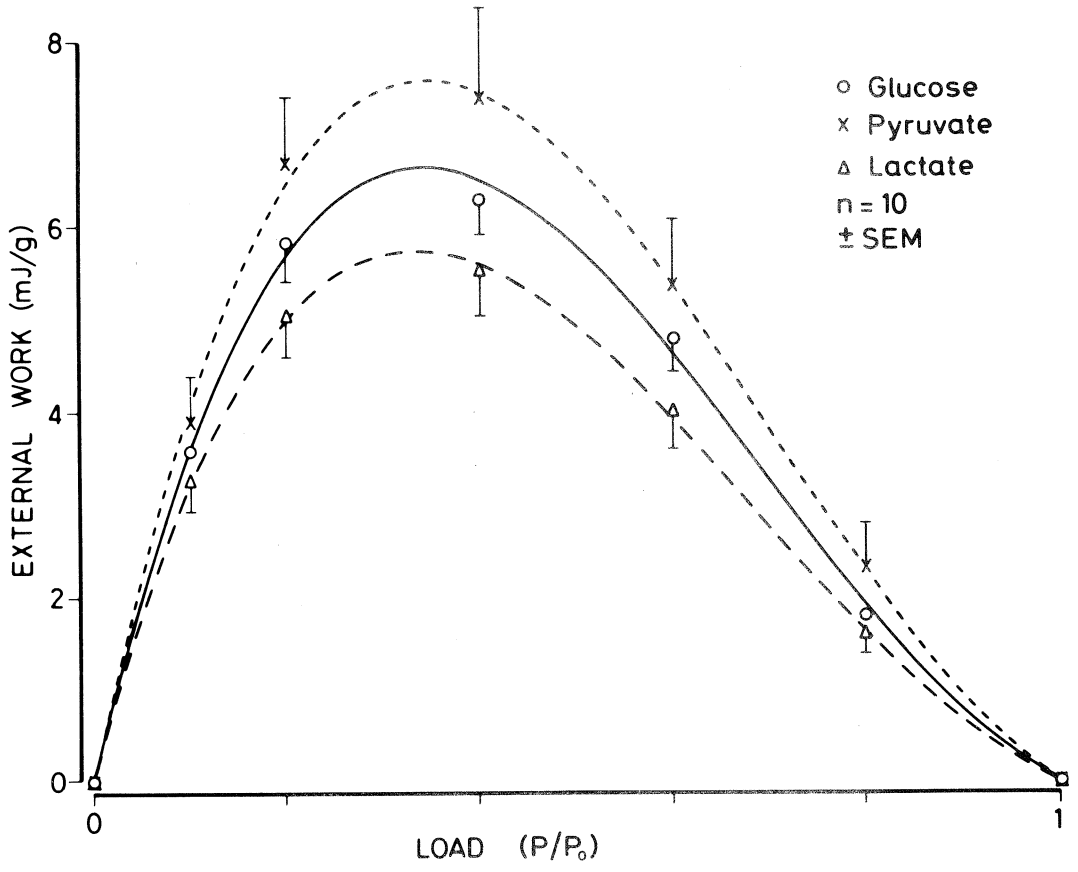

Figure 5. External work plotted against load expressed as a fraction of Po. All points are the mean of 10 measurements (10 muscles) and are ± SEM.

Table 3. Mechanical efficiency (defined as a percentage of external work/total enthalpy). All calculations are ± SEM (numbers in brackets). Loads (P), are expressed as a fraction of Po, (maximum isometric tetanic tension).

	LOAD (P/Po)				
	0.1	0.2	0.4	0.6	0.8
GLUCOSE	12.12	17.20	17.86	16.19	7.32
	(1.60)	(1.26)	(1.44)	(1.35)	(1.23)
PYRUVATE	16.97	16.29	16.04	14.25	7.00
	(1.88)	(1.43)	(1.66)	(2.08)	(1.37)
LACTATE	10.58	15.96	17.28	15.00	7.07
	(1.05)	(1.80)	(1.70)	(0.83)	(0.77)

Table 4. Hill force velocity parameters Vmax, a/Po and b calculated for each substrate group. Po is the maximum isometric tetanic tension. Calculations are ± SEM (figures in brackets).

	V_{max} (muscle lengths per second)	a/Po	b
GLUCOSE	1.72	0.48	0.67
	(0.27)	(0.09)	(0.08)
PYRUVATE	1.98	0.27	0.69
	(0.24)	(0.05)	(0.11)
LACTATE	1.48	0.47	0.65
	(0.11)	(0.07)	(0.06)

signal (which was dependent on the reflective properties of the muscle surface), returned to its control value after each contraction.

Following stimulation, the fluorescence signal increased reaching a peak of approximately 2% in 5 to 7 seconds, indicating a net increase in the reduction level of NAD^+. This was usually followed by a swing below baseline of 2 to 3%, peaking approximately 25 seconds after stimulus. The kinetics of the fluorescence transients did not show any reproducible correlation with the time course for the production of recovery heat although for about one quarter of the responses, the point of baseline return coincided with the cessation of heat production. Muscle contractions in the presence of lactate produced similar fluorescence transients, displaying complex kinetics with no discernible qualitative or quantitative differences from those seen in the presence of glucose. Occasionally, following isometric tetanic stimulation, the fluorescence signal displayed a monophasic drop of 1 to 2% before returning to resting level. Such transients have been obtained from rat extensor digitorum longus muscle stimulated at low rates of energy expenditure, and arise mainly from the oxidative reactions that underlie the production of recovery heat [16].

For muscles contracting in the presence of pyruvate, the amplitude of the fluorescence transients was reduced by at least half, although the time course did not differ with those obtained with glucose. This diminution of amplitude was more pronounced when the muscle was contracting either isometrically or against loads of 0.1, 0.2 and 0.8 Po.

Myothermic data

A typical myothermic recording is also represented by Figure 3. There were qualitative similarities between the net heat output and that observed for frog sartorius [31]; for rat soleus [23], and for mouse soleus and extensor digitorum longus [60]. There was an initial rapid phase of heat production which accompanied the mechanical response, followed by a prolonged recovery heat phase. After an initial upstroke the thermoelectric output dropped briefly before reaching maximum value. This occurred for half of the myothermic responses and was due to the transfer of heat from the muscle to the adhering Krebs Henseleit solution. The initial heat phase was succeeded by the slower phase of recovery heat. The time for completion of recovery heat was recorded for all contractions and is presented in Table 2. For muscles contracting in the presence of either glucose and lactate, the recovery heat times were maximal with a load of 0.4 Po. Minimal times were observed with isometric contractions and with a load of 0.8 Po for lactate and glucose respectively. However, with pyruvate as available substrate, the largest times were recorded with loads of 0.4 Po and the smallest with 0.1 Po. ANOVA failed to show significance between loads (F values for glucose, pyruvate and lactate were 0.80, 0.82 and 0.73 respectively for 5 degrees of freedom), although differences were significant between substrates for loads of 0.4 Po (F value = 4.22 for 2 degrees of freedom). DMRT confirmed significance between the times recorded in the presence of glucose and pyruvate.

In Figure 4 the total enthalpy (heat + work) is plotted against load (expressed as a fraction of Po), for each substrate group. For this investigation the total enthalpy is the sum of the initial and recovery heat components plus the energy performed as external work returned to the muscle during relaxation as heat. All data displayed a distinct "Fenn effect" [21], with maximal energy liberation occuring with loads in the range of 0.2 to 0.6 Po for pyruvate and glucose, and 0.2 to 0.4 for lactate. Minimal energy output was observed at loads of 0.1 Po for all substrates. The enthalpy/load curves are qualitatively similar to those obtained for rat soleus contracting in the presence of glucose, although for these muscles total enthalpy production was maximal in the range 0.1 to 0.2 Po. ANOVA produced significance between substrates contracting under a load of 0.6 Po (F value = 4.16 for 2 degrees of freedom), and DMRT confirmed significances between the lactate and pyruvate data.

The external work performed by each muscle is plotted against load in Figure 5. The work output increased in the substrate order lactate, glucose and pyruvate for each load, although statistical significance was not detected.

The mechanical efficiency of the isotonic contractions over the complete cycle of contraction, relaxation and recovery is shown in Table 3. The isotonic efficiencies are not as high as those reported for frog sartorii [32], but do compare with those observed for rat soleus [23]. ANOVA produced significance between substrates at a load of 0.1 Po (F value = 4.63 for 2 degrees of freedom), and DMRT detected differences between the results obtained with pyruvate and lactate, and pyruvate and glucose.

It was proposed by Woledge [63], that mechanical efficiency can be related to the Hill force velocity relation: (P+a)V = b(Po-P), where P = load, V = speed of shortening, Po = maximum isometric tension and a and b are constants. The hyperbolic form of the equation was fitted to the data using the method of Gibbs and Loiselle [24], and the calculated parameters listed in Table 4. Both force and velocity data were normalized in terms of force per cross sectional area and muscle lengths per second respectively. As soleus is a fusiform muscle with a staggered attachment of fibres to one tendon, it is difficult to estimate the exact fibre length, although the fibre length/muscle length ratio is approximately 80% [19]. None of the Hill parameters produced statistical significance when examined with ANOVA, although significance occurred at the 10% level for the a/Po data (F value = 3.17 for 1 degree of freedom). DMRT demonstrated significant differences between the mean values of a/Po for pyruvate and glucose, and pyruvate and lactate.

For the measurement of resting heat the muscle thermopile system was first brought to a thermal steady state while the muscle was submerged. After adjusting the thermoelectric output to zero the solution was drained. In consequence, the thermoelectric output increased until stabilizing at a new steady state level. After first setting the time constant for the exponential function of heat loss, the heat loss corrector was activated and the resting heat rate calculated from the increasing thermoelectric output. Calculated resting heat rates in milliwatts/gram ± SEM are 1.75 ± 0.48, 2.33 ± 0.33 and 1.45 ± 0.12 for glucose, pyruvate and lactate. ANOVA failed to detect any significance.

Discussion

Fluorescence data

There are several distinct fractions of pyridine nucleotides that exist within muscle tissue, each contributing to the fluorescence excited by 366 nm light. The fraction kinetically most active in the process of oxidative phosphorylation is designated $NADH_1$ and is directly involved in the transfer of reducing equivalents from substrates to the flavoproteins of the respiratory chain in the mitochondria [12]. The other mitochondrial fraction $NADH_2$, is reduced indirectly from substrates such as succinate and glycerophosphate via flavoproteins, although the contribution of this fraction to the total tissue fluorescence is only 1 or 2 % in amphibian muscle [38]. The involvement of NADPH in the production of tissue fluorescence is also believed to be insignificant, as the total fraction of NADPH is only about 3% of the total nicotinamide coenzyme concentration. Furthermore, it has been suggested that mitochondrial NADPH has a fluorescence efficiency 3 to 4 times smaller than that of the $NADH_1$ fraction [2], and that its kinetics of reduction are too slow to produce any interference with the kinetics of the fluorescence changes associated with mechanical activity [11].

There is another pyridine nucleotide fraction that functions in conjunction with the glycolytic chain and there is some uncertainty as to whether this fraction contributed to the total tissue fluorescence. Jobsis and Duffield originally argued that the binding of mitochondrial NADH to enzymes enhances its quantal efficiency of fluorescence so as to render any contribution from cytoplasmic NADH insignificant. This was restated by Chapman [12] who proposed that less than 0.03% of the labile tissue fluorescence is of cytoplasmic origin. Freeze clamp investigations of total NADH content of rat soleus are not inconsistent with this [48], although an opposing view was proposed by Godfraind De Becker [26] who suggested that glyceraldehyde 3 phosphate dehydrogenase activity is an important factor in rapidly increasing the cytoplasmic NADH concentration, and that this reaction can account for the initial fluorescence changes observed following contractile activity in toad sartorius muscle [45].

In the steady state fluorescence experiments, both pyruvate and lactate produced increases in the ratio of NADH to NAD^+ under aerobic conditions, indicating stimulation of the tricarboxylic acid (TCA) cycle. This is not consistent with the finding that skeletal muscle high oxidative fibres readily oxidize pyruvate and lactate [4,36]. Further net reduction of NAD^+ was possible by the induction of anoxia, thus blocking respiration and preventing the dehydrogenation of NADH. However, it has been documented that the lactate/pyruvate ratio in rat soleus muscles did not vary significantly during induced anoxia [48]. Of interest with these results is the comparison between data obtained under anoxic conditions in the presence of pyruvate and lactate. The reduced fluorescence signal indicated that pyruvate must be acting as an oxidant of NADH. This could occur via the isoenzyme lactate dehydrogenase which catalyses the formation of NAD^+ in the cytoplasm. If so, then a significant proportion of total tissue fluorescence may indeed be of cytoplasmic origin [45].

At any instant, the level of mitochondrial NADH will depend on the difference between the inflow of reducing equivalents arising from metabolism of carbohydrate or endogenous substrate (equation 3), and the flow of electrons along the respiratory chain from NADH to molecular oxygen (equation 4).

$$NAD^+ + SUBSTRATE{*}H_2 \leftrightharpoons NADH + H^+ + SUBSTRATE \quad (3)$$

$$NADH + 3ADP = 3P + H^+ + 1/2O_2 \leftrightharpoons NAD^+ + 3ATP + H_2O \quad (4)$$

where ATP and ADP are adenosine triphosphate and adenosine diphosphate respectively and Pi represents inorganic phosphate. In quiescent muscle the electron flow would be governed by the availability of ADP. Following muscular contraction the resulting increase in ADP should stimulate the second reaction shifting the equilibrium to the right. This will decrease the reduction level of NAD^+, producing a drop in tissue fluorescence.

In this and prior studies [58], it was observed that the first response to tetanic stimulation is an increase in fluorescence, followed by a drop to below baseline which eventually returns to steady state levels. One explanation for these complex kinetics is that the elevated levels of ADP and inorganic phosphate are enhancing the activity of the intermediary enzymes phosphofructokinase and glyceraldehyde 3 phosphate dehydrogenase [50]. This would increase the availability of reducing equivalents, thus stimulating the TCA cycle whish would result in net reduction of the respiratory coenzymes. It is also possible that a direct glycolytic effect is responsible for the initial increase in fluorescence. This is supported by evidence that iodoacetic acid (IAA) an inhibitor of glycolysis significantly modifies the recorded fluorescence

signal in contracting toad muscle [26], as well as observations that such muscles can still produce fluorescence transients in the presence of nitrogen [25,26]. Observations by Wendt and Chapman [58] have shown that IAA prevents the initial fluorescence increase normally observed in tetanically contracting rat soleus. Furthermore, it has been determined that cytoplasmic rather than mitochondrial pyridine nucleotides underlie most of the fluorescence response in the electric organ of Electrophorus [1], and that the activation of glycolysis will significantly augment the fluorescence signals recorded from single cells [41]. With regard to the occasional appearance of monophasic transients, it is possible that the resulting net change in ADP concentration was insufficient to significantly influence intermediary metabolism.

10mM pyruvate had the effect of reducing the amplitude of the fluorescence response. The exogenous pyruvate stimulated the TCA cycle enzymes, thus elevating the levels of NADH and lactate dehydrogenase which oxidizes NADH. The predominant effect is mitochondrial as indicated by the data in Table 1 which show net reduction of NAD^+. So it is not unreasonable to believe that as the $NADH/NAD^+$ ratio is already elevated, any influence on this ratio from ADP arising from contraction (via equation 4), will be lessened. Early computer simulations of cardiac muscle energetics have indicated an accumulation of larger amounts of creatine phosphate in the resting state with exogenous pyruvate, relative to that in the absence of substrate [12]. This would provide greater buffering of ADP levels, lessening any influence on the electron transport chain. 10mM lactate produced a resting fluorescence level intermediate between those produced by substrate free and pyruvate conditions. The equilibrium of the lactate dehydrogenase reaction is sufficiently biased towards lactate production [43] such that the amount of pyruvate produced will not dominate the established equilibrium with mitochondrial NADH.

Myothermic data

It is clear from examination of the chart recordings that the time courses for the evolution of heat do not correlate in any simple way with the time courses of the fluorometric responses. The only reproducible correlation observed was that return of the fluorescence signal to baseline (indicating movement of the respiratory enzymes to a more reduced state), always occurred during the production of recovery heat which is believed to be generated by aerobic metabolism [27,34,35]. In other studies, the time required for complete evolution of recovery heat from mammalian muscle was not greatly influenced by substrate, although under conditions of high work load pyruvate did produce a significant lessening [23]. An explanation for this effect arises from the hypothesis that the kinetics of ADP phosphorylation are faster with a more reduced respiratory chain [12]. Mouse soleus contains 75% type I and 25% type IIa fibres [20], and it was anticipated that 10 mM pyruvate would greatly accelerate the metabolic recovery processes. So it is assumed that (for most work loads) 10 mM lactate and glucose resulted in the production of satisfactory amounts of reducing equivalents so as not to limit oxidative metabolism.

The overall shape of the total enthalpy/load curves is similar to the data of Fenn [21], for amphibian skeletal muscle after recalculation to correct for calibration errors [46]. A distinct "Fenn effect" which was further enhanced by 10 mM pyruvate was evident at most workloads. In this study it was observed that the total enthalpy produced with the lightest loads exceeded the maximum isometric value, contrasting with the results of Gibbs and Gibson [23], for rat soleus. One factor that increases isotonic energy liberation at light loads is a higher twitch to tetanus ratio [22,46]. This may well account for the above observation, as the measured twitch/tension ratios (0.25, 0.31 and 0.29 for glucose, pyruvate and lactate respectively), are higher that those reported by Close [18] for rat soleus.

The increase in total enthalpy produced by pyruvate is paralleled by similar changes in external work performed by the muscles. The likelihood that this is a direct result of ATP limitation is small. Tissue analysis of rat myocardium by Williamson [62] demonstrated that pyruvate has no significant effect on the levels of ADP or ATP. An alternative explanation arises from the observation that calcium ions are transported by the mitochondria, and that there is a reversible link between such transport and the redox level of the respiratory coenzymes [8]. Evidence that calcium ions accumulate in the mitochondria of skeletal muscle [7] leads to the proposition that mitochondria may play a dynamic role in the intracellular calcium movements associated with muscle contraction. However there still remains the difficulty of reconciling the decrease in work and enthalpy output observed with 10 mM lactate with the net reduction of NAD^+ in the steady state (Table 1). It is possible that for the 25% of fibres that depend to some extent on the oxidation of glucose, this source of energy is being severely limited due to gluconeogenisis, thus restricting the amount of ATP available for hydrolysis by the myosin ATPase enzymes [5,30,44].

In the past there have been conflicting theories as to the definition of muscle efficiency [47,61,64]. For these experiments, mechanical efficiency was defined as the ratio of the work done to the total enthalpy liberated over the entire cycle of contraction, relaxation and recovery. The efficiency values reported here compare with those obtained for rat soleus [23], although those data were not maximum values. Wendt [56] indicated that the maximum mechanical efficiency of rat soleus is approximately 23%. The most noticeable effect of substrate on mechanical efficiency in this study was an increase in the presence of pyruvate in comparison to those data obtained with glucose and lactate (at a load of 0.1 Po). Since the value for total enthalpy at this load are practically identical, this would appear to be due to an enhancement of contractile activity, as is indicated by the increase in maximum speed of shortening (Table 4). On the basis of biochemical studies, Goldspink and colleagues suggested (i) that mammalian slow muscles evolved for efficiency in maintaining isometric tension and (ii) that, owing to a longer cross bridge engagement time, the isometric efficiency of these muscles is less than that of fast muscle [3,28]. The data reported here are compatible with the first proposition but not the second.

While developing a kinetic model of cross bridge activity, Woledge [63] proposed that the mechanical efficiency of a muscle is inversely related to the curvature of the force velocity relation (as indicated by the a/Po ratio). The values for this ratio show variation in the response to the substrate present but suggest a less curved relationship than that found in rat soleus [23,56]. The efficiency values for mouse soleus are less than those calculated for frog and tortoise over the whole cycle of contraction and relaxation [63]. This does not

support earlier views that mammalian slow-twich muscles appear to be a compromise between true slow muscles (which have a relatively slower rate of shortening and low isotonic efficiency), and fast muscles.

The rate of resting heat production was found to increase with substrate in the order glucose, lactate, pyruvate, and it is known that the resting heat rate of isolated papillary muscle can be doubled by changing substrate from 10 mM glucose to 10 mM pyruvate. The values reported here are comparable in range to those of Wendt and Barclay [57] for mouse soleus and have been confirmed by oxygen consumption measurements (C.L. Gibbs, personal communication), and are some 2 to 3 times greater than those obtained for rat soleus [56]. In comparison to frog sartorius, there is at least an eight fold difference in the resting heat rate. This appears to be related to the smaller size of the fibres in mammalian skeletal muscles since with a high surface to volume ratio, the energy required to maintain ionic gradients will be greater. However this cannot be the only factor, as ionic transport accounts for only a small fraction (less than 10% in cardiac muscle) of the total basal heat.

In conclusion, the simultaneous application of these techniques has shown that it is possible to correlate the heat evolution with the biochemical metabolic changes associated with muscular contraction, although activation of intermediary metabolism does confound the kinetics of the fluorescence signals. The relationship between heat and fluorescence remains to be investigated at lower rates of energy expenditure where this effect will be minimized.

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