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HEAT PRODUCTION AND CHEMICAL CHANGE DURING ISOMETRIC CONTRACTION OF RAT SOLEUS MUSCLE

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SUMMARY

1. Methods are described whereby the soleus muscle of the rat may be used for the investigation of initial processes in the absence of oxidative recovery.

2. The anaerobic conditions employed had no effect on the concentration of phosphocreatine in resting muscle or the mechanical response during contraction.

3. Muscles were stimulated tetanically for 10 s at 17–18° C. Measurements were made of the heat production and metabolic changes that occurred during a 13 s period following the first stimulus.

4. There was no detectable change in the concentration of ATP. Neither was there detectable activity of adenylate kinase or adenylate deaminase. The changes in the concentration of glycolytic intermediaries were undetectable or very small.

5. The change in the concentration of phosphocreatine was large and amounted to $-127 \pm 11{\cdot}4$ μmol/mmol Ct (mean and S.E. of the mean, negative sign indicates break-down, Ct = free creatine + phosphocreatine) which is equivalent to about $-2{\cdot}13$ μmol/g wet weight of muscle. The heat production was 6549 ± 408 mJ/mmol Ct (mean and S.E. of mean) which is equivalent to about 110 mJ/g.

6. About 30% of the observed energy output is unaccounted for by measured metabolic changes.

7. The ratio of heat production (corrected for small amounts of glycolytic activity) to phosphocreatine hydrolysis was $-49{\cdot}7 \pm 5{\cdot}6$ kJ/mol (mean and S.E. of mean), in agreement with previous results using comparable contractions of frog muscle, but different from the enthalpy change associated with phosphocreatine hydrolysis under *in vivo* conditions (-34 kJ/mol).

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8. The results support the notion that the discrepancy between energy output and metabolism is an indication of an unidentified process of substantial energetic significance that is common to a number of species.

INTRODUCTION

During the past few years it has become increasingly clear that there is a discrepancy between *in vivo* measurements of the chemical and thermal changes accompanying muscular contraction and *in vitro* measurement of the relevant enthalpy changes. *In vivo* measurements, under conditions where hydrolysis of phosphocreatine (PCr) is thought to be the only net reaction, yield a ratio of heat + work ($h+w$) to phosphocreatine change (ΔPCr) of about -46 kJ/mol or of greater magnitude, see for example Wilkie (1968) and Gilbert, Kretzschmar, Wilkie & Woledge (1971). In contrast calorimetric determinations of the enthalpy change associated with PCr hydrolysis under *in vivo* conditions suggest a value of -34 kJ/mol (Woledge, 1971, 1972, and personal communication). It seems unlikely that this discrepancy is a result of experimental error (Kretzschmar, 1975).

Up to the present nearly all energy balance studies *in vivo* have employed frog muscle, although Walsh & Woledge (1970) used tortoise muscle. An energy balance sheet has been drawn up by Edwards, Hill & Jones (1975) using human muscle, but it was not possible to test whether PCr splitting yielded -34 or -46 kJ/mol. Separate chemical or thermal measurements, using various mammalian preparations, have been made by: Herrmann, Nicholas & Vosgian (1949); Goldspink, Larson & Davies (1970); Spande & Schottelius (1970); Stainsby & Barclay (1971); Gibbs & Gibson (1972); Wendt & Gibbs (1973). However comparison between different sets of data is problematic, and therefore it is difficult to draw up a balance sheet for mammalian muscle using published data.

This paper describes both the energy output and the concurrent chemical changes that result from a contraction of a mammalian muscle, namely the soleus muscle of the rat. The aim was to design the experimental conditions such that oxidative and anaerobic recovery processes were absent and the net initial processes consisted solely of PCr hydrolysis. Results of such an experiment are the simplest to interpret in terms of energy balance and they allow an interesting comparison with previous work on frog muscle.

METHODS

The experimental procedure. Soleus muscles were dissected from male Sprague–Dawley rats, aged between 4 and 6 weeks which were bred in the Department of Physiology, U.C.L. Care was taken to prevent muscles becoming anoxic during

dissection: the rats were anaesthetized with urethane and therefore continued to respire. As soon as the blood supply to a soleus muscle was cut off, the muscle was cooled to about 20° C using oxygenated Krebs solution in order to slow resting metabolism. The length of the muscle *in situ* (l_0) was measured with the foot set at right angles to the leg. To allow full recovery after dissection muscles were immersed for at least 1 hr in bicarbonate-buffered Krebs solution (composition in mmol/l: NaCl, 115; KCl, 5; $CaCl_2$, 5; $NaHCO_3$, 25; $MgSO_4$, 2; KH_2PO_4, 1; glucose, 6) at room temperature, bubbled with 5% CO_2/95% O_2. During the main series of experiments the muscles were then mounted on a thermopile (for thermal measurements) or on an apparatus designed to arrest metabolism rapidly (for chemical measurements) and were immersed in Krebs solution bubbled with 5% CO_2/95% N_2 for exactly 15 min at 17–18° C, after which the muscles were removed from the solution but remained in the oxygen free gas at 17–18° C for a further 2–3 min before a 10 s stimulation, using alternating capacitor shocks (0·04 μF, 18 or 24 V, 20/s). Only the first contraction after dissection and recovery of each muscle was used for the measurement of energy balance. Two indices of muscle size were used, as appropriate: the total creatine content (free creatine + phosphocreatine) of the muscle (Ct) and the wet weight of the muscle after dissecting off the tendons (m).

Mechanical measurements. All contractions were isometric at l_0. The tension developed by all muscles (including unstimulated controls) was measured (Jewell, Kretzschmar & Woledge, 1967). Control muscles were monitored in order to detect accidental stimulation. All experiments in which the peak isometric tension was less than 10 Nm/mmol Ct (equivalent to about 17 N cm^{-2}) were discarded. The tension was normalized by the factor l_0/Ct.

Heat measurement. Heat was measured using a thermopile (K2) described previously by Curtin, Gilbert, Kretzschmar & Wilkie (1974). The output was connected to a low-noise amplifier (Ancom, type 15C-3a) and displayed on a U.V. recorder (Bryans Southern, type 10-430). A single muscle was mounted on one face of the thermopile, on the other there was a thin sheet of silver (24 mm × 2 mm × 25 μm) which covered the region of the thermopile that was electrically active. The presence of the silver strip provides for rapid temperature equilibration along the length of the muscle and allows accurate absolute calibration. For each muscle, the thermopile/muscle assembly was calibrated using a method employing the Peltier effect (Kretzschmar & Wilkie, 1972, 1975). The heat records were corrected for stimulus heat, which was measured using a 'dummy muscle' of approximately the same size and thermal capacity as a genuine muscle.

Chemical experiments. In each experiment a pair of soleus muscles from a single rat were mounted on the hammer apparatus (Kretzschmar & Wilkie, 1969; Kretzschmar 1970; Gilbert *et al.* 1971). One muscle was stimulated for 10 s, the other remained an unstimulated control. The muscles were frozen 13 s after the first stimulus.

Chemical analyses. Protein free extracts were prepared (Dydynska & Wilkie, 1966) and were analysed on a Technicon Autoanalyzer for phosphocreatine (PCr) and total creatine (Ct = free creatine + phosphocreatine) (Curtin *et al.* 1974) for ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), glucose-1-phosphate (G1P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1, 6-diphosphate (F16DP), lactate, pyruvate and phosphoenolpyruvate (PEP) (Scopes 1972). Total adenine and total inosine were measured spectrophotometrically (Curtin & Woledge, 1975). An individual measurement (normalized for muscle size) was excluded from the data presented in this paper if it was more than ± 2 S.D. away from the mean of its group. The mean and S.D. of an observation used for this test were those calculated using all the data in the group including the value thought to be aberrant (Curtin *et al.* 1974, p. 460).

RESULTS

The effects of the anoxia and low temperature

Before a muscle was stimulated either on the thermopile or the hammer apparatus, it was subjected to a period of anoxia as described in the Methods. Results are presented in this section to show: firstly, that this treatment prevented oxidative recovery but did not affect the contractile response of the muscle; and secondly, that the levels of metabolites in the muscle immediately before stimulation were characteristic of a normal resting muscle.

Concentrations of metabolites

Results given in Table 1 indicate that there is no significant difference between the levels of PCr in oxygenated and anoxic muscle. Likewise the concentrations of glucose-6-phosphate and fructose-1,6-diphosphate were unaltered by anoxia. There were small but significant increases in the lactate and pyruvate contents of muscle during the period of treatment with nitrogen, indicating glycolytic activity.

The chemical results in Table 1 were derived from oxygenated muscle frozen by immersion, and anoxic muscles (the control muscles of the main series of experiments) frozen in the hammer apparatus. The method of freezing is not expected to affect the concentrations of compounds in the glycolytic pathway, but may alter the concentration of PCr. Slow freezing (by immersion) has been shown to cause a small amount of PCr breakdown (Kretzschmar, 1970; Gilbert *et al.* 1971), and indeed the present results indicate a slightly lower concentration of PCr in muscles frozen by immersion, although this difference is not significant.

Oxidative recovery

Recovery heat was measured in the presence of oxygen and after the standard period of anoxia. The heats produced during the period from 13 to 33 s after the first stimulus of a 10 s isometric tetanus are shown in Table 1. Recovery heat is easy to detect in the presence of oxygen, and was observed many times during preliminary experiments using a variety of contractions. After the standard period of anoxia recovery heat was absent, the mean given in Table 1 indicates a small heat absorption which is not significantly different from zero ($P \simeq 0{\cdot}4$). If oxygen was readmitted to an anoxic muscle after a contraction, then recovery heat production commenced. The observation that lactate and pyruvate accumulate during the treatment with nitrogen showed that at least some parts of the muscle were anoxic. It remains possible that other parts (if there be any) contained just sufficient oxygen to allow some recovery

TABLE 1. The effects of anoxia

	Oxygenated			Anoxic			Difference		
	Mean	S.E. of mean	n	Mean	S.E. of mean	n	Mean	S.E. of mean	P
(Recovery heat)/m (mJ/g)	36·6	9·9	2	−1·1	1·2	6	—	—	—
$(PCr/Ct)_c$ (μmol/mmol Ct)	671	13	6	687	19	11	16·1	23·1	~ 0·5
$(G6P/Ct)_c$ (μmol/mmol Ct)	9·7	0·4	6	10·6	0·5	12	1·0	0·6	~ 0·2
$(F16DP/Ct)_c$ (μmol/mmol Ct)	0·8	0·5	6	0·7	0·6	12	−0·06	0·8	~ 0·9
$(lactate/Ct)_c$ (μmol/mmol Ct)	14·8	3·2	6	22·5	1·9	12	7·7	3·7	= 0·05
$(pyruvate/Ct)_c$ (μmol/mmol Ct)	−0·4	0·4	6	0·9	0·3	12	1·3	0·5	< 0·05
Pl_0/m (N cm^{-2})	22·0	1·8	5	23·2	1·1	6	1·2	2·1	~ 0·6

All muscles were allowed to recover in oxygen. Subsequently anaerobic muscles were treated with nitrogen as described in the Methods. Recovery heat was measured during the period from 13 to 33 s after a 10 s isometric tetanus and was normalized by the wet weight (m) of the muscle. Phosphocreatine (PCr), glucose-6-phosphate (G6P), fructose-1,6-diphosphate (F16DP), lactate and pyruvate were normalized by the total creatine content of muscle (Ct = free creatine + PCr). The subscript c denotes that the muscles were controls, and had never been stimulated. The peak tension (P) during an isometric tetanus was normalized using the muscle length (l_0) and wet weight. P gives the significance of the difference between the appropriate mean and zero.

during the 13 s contraction, but not thereafter. There was no evidence to support this hypothesis. Thus it seems that the duration of the treatment with 95% N_2/5% CO_2 is adequate to ensure that oxygen was excluded from the muscle.

The mechanical response

Isometric contractions were performed at a muscle length of l_0 where the tension developed was approximately maximal. There is no sign that nitrogen treatment changes the size of the response; the mean values for peak tension given in Table 1 are not significantly different from each other. The average fibre length in a soleus muscle of the rat is about 71% of the muscle length (Close, 1964), therefore the force developed per cross-sectional area of the muscle is about 16 N cm^{-2}.

Heat production and metabolism during a 10 s isometric tetanus

The mechanical response

A comparison between chemical and thermal experiments is required, even though the measurements were made on different sets of muscles. These two sets were treated identically, to avoid systematic differences; but as a check on their similarity the peak isometric tensions produced during the two types of experiment were measured. The tension produced during the thermal experiments was 1410 ± 100 N cm $(mmol)^{-1}$ (mean and S.E. of mean, $n = 6$; normalized by the factor l_0/Ct), that during chemical experiments was 1330 ± 70 N cm $(mmol)^{-1}$ ($n = 12$). The two means agree closely and are not significantly different ($P \simeq 0{\cdot}5$), which suggests that comparison of thermal and chemical measurements is valid. The Ct content of these muscles was $16{\cdot}76 \pm 1{\cdot}03$ μmol per gram wet weight of muscle (mean and S.E. of mean, $n = 6$), and so the tensions developed may be expressed less accurately, but in more conventional units (Pl_0/m), as 23·2 and 22·3 N cm^{-2} for thermal and chemical experiments respectively.

Heat production

Measurement of heat production in mammalian muscle is complicated by the fact that the stimulus heat can become large compared with the heat produced by the muscle itself. There is some uncertainty as to the exact quantity of stimulus heat that appears in the heat record, and therefore large stimulus heats are undesirable. To minimize the stimulus heat, the stimulus voltage was set just supramaximal (as measured during preliminary experiments), and the stimulus frequency was set to provide a fused tetanus, but not maximal tension. Peak isometric tension could have been increased somewhat by increasing the stimulus frequency from 20 s^{-1} to 50 s^{-1}. Under the conditions of this experiment the stimulus

heat was only about 16% of the total heat recorded, and 86% of the energy dissipated by the stimulus appeared as measured heat. Thus a small error in the correction for stimulus heat would produce a negligible error in the final result.

The heat produced during a period of 13 s following the first stimulus of a 10 s tetanus is given in Table 2.

Initial processes

The isometric contraction resulted in a large and highly significant break-down of PCr (Table 2) that amounted to about 18% of the PCr in a resting muscle (Table 1). In contrast the change in ATP (Table 2) was small and the mean was in the direction of synthesis. The mean change was less than 2% of the change in PCr and was not significantly different from zero ($P \simeq 0{\cdot}6$).

Activity of adenylate kinase and adenylate deaminase

In a muscle where the ATP concentration is maintained close to resting levels, activity of adenylate kinase and adenylate deaminase is not expected; certainly it does not occur in frog muscle under these conditions (Curtin & Woledge, 1975). Nevertheless total adenine, total inosine, ADP and AMP were estimated, and Table 2 shows that there were no significant changes in the concentrations of these compounds and that mean changes were small compared to changes in PCr.

Anaerobic glycolysis

The changes in concentration of a number of compounds in the glycolytic pathway are shown in Table 2. All changes were small, as were the standard errors of the means. Only the change in the concentration of fructose-1,6-diphosphate was significant at the 5% level, although lactate and glucose-6-phosphate productions were close to significance. Assuming all three changes to be genuine *pro tem.*, glycolytic activity would resynthesize 2·1 μmol/mmol Ct of ATP. Thus it is clear that the amount of glycolytic activity was small compared with PCr break-down. About 98% (or more) of the total ATP turn-over resulted in observed PCr break-down and about 2% or less was accounted for by glycolysis.

Energy balance

The only measured reaction that occurred to a large extent was PCr hydrolysis. Thus it is legitimate, at least as a first approximation, to calculate the ratio of heat to PCr break-down (h/ΔPCr). This ratio is $-51{\cdot}6 \pm 5{\cdot}6$ kJ/mol (mean and S.E. of mean) and is not significantly different from -46 kJ/mol ($P \simeq 0{\cdot}4$), but is significantly different from -34 kJ/mol ($P < 0{\cdot}02$). The heat productions expected to result from the observed

TABLE 2. Heat production and metabolism during a 10 s isometric tetanus

		μmol/mmol Ct												
		Initial processes		Glycolysis							Adenylate kinase and adenylate deaminase activity			
	mJ/mmol Ct *h*	Δ PCr	Δ ATP	Δ lactate	Δ F16DP	Δ G6P	Δ pyruvate	Δ PEP	Δ HMP	Δ(F6P +G1P)	Δ ADP	Δ AMP	ΔA	ΔI
Mean	6549	−126·9	2·0	2·4	1·5	0·9	−0·5	−0·3	1·0	1·0	4·2	−0·04	10·1	2·0
S.E. of mean	408	11·4	3·6	1·2	0·5	0·5	0·4	0·3	0·6	0·6	3·0	0·4	7·4	3·6
n	6	10	10	11	12	12	12	11	3	3	5	6	11	10
P	—	—	~ 0·6	< 0·1	< 0·02	< 0·1	~ 0·3	~ 0·4	> 0·2	> 0·2	> 0·2	> 0·9	0·2	~ 0·6

All measurements were normalized by the total creatine content of muscle (Ct = free creatine + phosphocreatine). The means are the changes that occurred during a 13 s period following the first stimulus; a negative sign indicates a reduction. Here: *h* is heat; PCr, phosphocreatine; F16DP, fructose-1,6-diphosphate; G6P, glucose-6-phosphate; PEP, phosphoenolpyruvate; HMP, hexosemonophosphate; F6P, fructose-6-phosphate; G1P, glucose-1-phosphate; ADP, adenosine diphosphate. AMP, adenosine monophosphate; A, total adenine; I, total inosine. *P* gives the significance of the difference between the appropriate mean and zero.

reactions are shown in Table 3. The first row includes all changes that are significant, also included are the lactate and glucose-6-phosphate changes that are not quite significant at the 5% level. The expected heats are the products of the relevant chemical changes and enthalpy changes (with a change of sign if required). The total heat expected (the sum of the expected heats from the chemical changes listed) is significantly different ($P < 0{\cdot}01$) from the heat observed, and accounts for about 70% of that heat (see Table 4). The difference between observed and expected heat represents the heat that is unaccounted for. These results are affected little if the changes

TABLE 3. The heat productions expected to result from the observed reactions

		Phospho-creatine	Lactate	Glucose-6-phosphate	Fructose-1,6-di-phosphate
	Mean	−126·9	2·4	0·9	1·5
Chemical change	S.E. of mean	11·4	1·2	0·5	0·5
(μmol/mmol Ct)	*n*	10	11	12	12
	P	—	< 0·1	< 0·1	< 0·02
ΔH (kJ mol^{-1})		−34·0	−96·0	−12·5	~ 0
Heat expected	Mean	4315	229	11	0
(mJ/mmol Ct)	S.E. of mean	388	111	6	0

The values for the enthalpy changes (ΔH) are those given by Woledge (1971, 1972 and personal communication): chemical changes were normalized by the total creatine content of muscle (Ct = free creatine + phosphocreatine). *P* gives the significance of the difference between the appropriate mean and zero.

TABLE 4. Comparison of the energy output expected and observed

	Total heat expected (mJ/mmol Ct)	Heat observed (mJ/mmol Ct)	Difference (mJ/mmol Ct)
Mean	4555	6549	1994
S.E. of mean	404	408	574
P	—	—	< 0·01

The total heat expected is the sum of the expected heats given in Table 3; all measurements were normalized by the total creatine content of muscle (Ct = free creatine + phosphocreatine). *P* gives the significance of the difference between the appropriate mean and zero.

in lactate and glucose-6-phosphate are omitted from the calculation, indeed the discrepancy between observed and expected heat is slightly larger and more significant.

Tables 3 and 4 may also be used to calculate a heat:ΔPCr ratio. Of the total observed heat, 240 ± 111 mJ/mmol Ct (mean and S.E. of mean) may

be accounted for by processes other than PCr splitting. The remaining heat amounts to 6309 ± 423 mJ/mmol Ct (mean and S.E. of mean), and the ratio of this heat to ΔPCr is −49·7 ± 5·58 kJ/mol (mean and S.E. of mean). This figure is significantly different from −34 kJ/mol ($P < 0{\cdot}02$) but is not significantly different from −46 kJ/mol ($P \simeq 0{\cdot}5$), a conclusion identical to that drawn from the simpler calculation above.

DISCUSSION

The tissue as an experimental preparation

In the present work the temperature was chosen so that the mechanical response was normal and reproducible, and so that the rate of recovery was slow compared with the rate of energy liberation during contraction. The former condition requires temperatures between 15 and 37° C (D. K. Hill, 1972). The latter condition requires the use of low temperatures, since the Q_{10} for recovery processes is larger than that for initial processes (Wendt, 1974). The temperature adopted (17–18° C) has the additional advantage that resting metabolism is slow and therefore oxygen, diffusing from the bathing solution, is able to maintain the muscle in a fully recovered state. Small muscles (from young rats) were employed, to ensure adequate oxygen supply in the core of the muscle. The peak tension produced during an isometric tetanus at 17–18° C was about 16 N/cm² cross-sectional area. This figure might seem low (a satisfactory figure for frog muscle is 20 N cm^{-2}) and might seem to indicate inexcitable or chronically anoxic muscle. This is not thought to be the case since peak tension is reduced by cooling to 17–18° C and by the use of a stimulus frequency below that giving a maximal response. At higher temperatures and higher stimulation rates it was possible to achieve tensions of about 20 N cm^{-2} using this preparation.

It is practically impossible to draw up a balance sheet for oxidative processes. Therefore it is essential that oxidative recovery is absent during the experimental period. Treatment of soleus muscle with nitrogen for about 25 min at 22° C decreases the tension during isometric tetanus by 23 % (Wendt, 1974) and the effect is not eliminated by cooling to 12° C and treating with nitrogen for about 30 min (Wendt, 1974). This result was confirmed in preliminary experiments for the present work, in which muscles were treated with nitrogen for about 30–40 min at 17° C. However, if the exposure to nitrogen is limited to the 17–18 min immediately before contraction then there was no detectable reduction in tension, despite the fact that oxidative recovery was avoided.

The initial heat production resulting from a 10 s isometric contraction at 17–18° C was 110 mJ/g. Other workers have not used this temperature; but Wendt (1974) found initial heat production (corrected for drop in

tension) at 12° C to be 7·4 mJ g^{-1} s^{-1} during isometric tetanus, and Gibbs & Gibson (1972) found 21·3 mJ g^{-1} s^{-1} at 27° C. Performing a linear interpolation between these figures, the heat production during a 10 s contraction at 17·5° C is calculated to be 125 mJ/g. This figure agrees remarkably well with our measurements in view of the approximations involved in the calculation. Concentrations of glycolytic intermediates remained very low during the period of anoxia, as is characteristic of resting, fully recovered muscle. Only lactate and pyruvate concentrations showed small increases. The concentration of PCr remained unchanged and is in good agreement with figures given for resting levels in human quadriceps muscle *in situ* (Edwards *et al.* 1975) and for mouse soleus muscle in oxygen (D. A. Jones, personal communication). The level of PCr is a particularly sensitive indicator of muscle damage or lack of recovery: by this or any other criterion investigated, the preparation seems to have been in a satisfactory condition. The results presented in this paper show that a suitable choice of experimental conditions allows the investigation of initial processes during contraction of mammalian muscle, almost uncontaminated by recovery.

Errors of measurement

One of the reasons for commencing this work was that frog muscle had proved to be variable: the magnitude of parameters associated with heat production and metabolism varied significantly from one batch of frogs to another (Kretzschmar, 1970; Gilbert *et al.* 1971), so that any comparison had to be made within a single batch. This complicates experimental design. Perhaps this variation is not so surprising: frogs in different batches may be of different age, may have been in captivity (and therefore fasting) for different times, and may have lived far apart in the wild. In addition there are probably seasonal effects (E. Homsher, personal communication). It was hoped that systematic variations of the sort observed in frog muscle could be avoided by the use of a single strain of rats, at a fixed age, of a single sex, isolated from seasonal changes, and fed a standard diet.

Of prime importance when evaluating the suitability of a preparation, are the magnitudes of the random errors involved in the measurements of interest. The S.D. of a particular chemical measurement varies somewhat from laboratory to laboratory (Kretzschmar, 1970), but broadly speaking standard deviations of observations are similar when using a single batch of frogs (Kushmerick & Davies, 1969; Gilbert *et al.* 1971; Curtin *et al.* 1974; Homsher, Mommaerts, Ricchiuti & Wallner, 1972) or when using rats (present work). A comparison of a single batch of frogs with rats, using identical experimental methods in our laboratory, shows that the S.D. of

an observation between paired muscles is almost always smaller for rat muscle than for frog muscle. An exception is Δ(PCr/Ct), the S.D. of which is of the same order or perhaps 50 % larger for rat muscle. However the mean value for Ct is considerably smaller in rat muscle so that a given amount of PCr hydrolysis would yield a larger value of Δ(PCr/Ct): this more than compensates for the larger S.D. The rats were not 'batched', but were simply obtained from breeding stock as required. Thus it would seem that the problem of batch variation is avoided.

Energy balance

It is quite clear that there is insufficient measured chemical change to account for the observed energy output during contraction of the rat soleus. The value for the ratio of heat to ΔPCr (−49·7 kJ/mol) is similar to that obtained using long complete contractions of frog muscle (Wilkie, 1968). Contraction of tortoise muscle (Walsh & Woledge, 1970) results in a ratio that is perhaps a little larger than −49·7 kJ/mol, although the difference is not statistically significant. However short contractions of frog muscle yield ratios that are often considerably larger than −49·7 kJ/mol (Gilbert *et al.* 1971), although this is not always the case (Homsher, Rall, Wallner & Ricchiuti, 1975). Thus long complete contractions provide similar values for (heat + work)/ΔPCr, irrespective of the species employed, whereas within a single species the ratio may vary according to the contraction pattern under investigation. This conclusion supports the notion that the energy discrepancy observed after a long contraction is an indication of an unidentified process that is common to a number of species, and is therefore a fundamental property of muscular contraction.

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