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FACTORS AFFECTING AEROBIC RECOVERY HEAT PRODUCTION AND RECOVERY RATIO OF FROG SARTORIUS

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SUMMARY

1. Sartorius muscles of *Rana temporaria*, equilibrated at 20 °C in Ringer solution buffered with phosphates, were stimulated isometrically for 0·2 up to 0·75 s at lengths varying from 1·03 to 1·48 times rest length, L_0. The aerobic recovery heat was measured for 10·5 min after contraction.

2. The recovery heat production had a complex time course, showing a variable delay to maximum, declining thereafter. In most cases, the rate of heat production did not decrease monotonically; attention was focused on the slow exponential decay which only persisted from 1·5–5 min after contraction. This latter part of the time course was considered as strictly aerobic and characterized by the time constant τ_s.

3. Increasing the tetanus duration from 0·2 to 0·75 s increased initial heat Q_i and recovery heat Q_r in proportion, so that the recovery ratio R (Q_r/Q_i) did not change; it was equal to $1{\cdot}29 \pm 0{\cdot}03$ (S.E.M.; $n = 44$) for muscles at about L_0. The kinetics of heat production were modified with longer tetani; in particular, τ_s was increased from 2·2 to 5·2 min.

4. When muscles were stretched beyond L_0, as long as there was no increase of the resting heat rate (stretch response or 'Feng effect'), recovery heat production had a similar evolution to that in muscles at about L_0; R was constant and equal to $1{\cdot}21 \pm 0{\cdot}03$ ($n = 46$).

5. When muscles were sufficiently stretched to develop a stretch response, R increased proportionally to the stretch response. The effect seemed independent of the contractile machinery, as it vanished concomitantly with the stretch response, while force and Q_i remained unchanged for the length considered. The kinetics were also modified – the delay to maximum was no longer detected and τ_s most likely increased.

6. Substitution of 60% of the NaCl of the Ringer solution by NaI (mol/mol) produced a significant increase of R, mainly due to the increase of Q_r.

7. The results show that neither the time course nor the amount of aerobic recovery heat Q_r are strictly determined by the amount of initial heat Q_i. The hypothesis is discussed that Q_r might include a variable fraction due to processes which are not directly implicated in the actin–myosin interactions, possibly those involving the cytosolic Ca^{2+} concentration and the rate of resting metabolism.

INTRODUCTION

Since its first description by A. V. Hill (1912), recovery heat has been extensively analysed and the literature was thoroughly reviewed by Hill himself in 1965. Two aspects dominate the picture: the amount and the kinetics of heat evolved. Regarding the first, it has been generally accepted that the aerobic recovery heat is about equal to the initial heat (Hill, 1965). The constancy of the ratio of recovery to initial heat – the recovery ratio, R – and its value – between 1 and 1·2 – led to a tight connection between energy expenditure and energy restoration. However, later experiments indicated that, in amphibian muscles, the recovery ratio is dependent on the species, the season, the innervation, the buffer of the physiological solution (Godfraind-De Becker, 1971, 1973), on metabolic modulators (Dawson, 1975), and in the mouse, on the type of muscle (Elzinga & Leijendekker, 1988). These facts suggested that the metabolic cost of contraction, as estimated by the recovery ratio R, could no longer be considered constant.

With respect to the kinetics, they appear not only complex, due to the simultaneous occurrence of heat absorbing and heat producing processes, but also variable. One important determinant is the amount of initial heat produced – the larger the initial heat, the greater the early rate of aerobic recovery heat production (Hill, 1965).

Recovery heat has always been considered as indicative of the replenishment of the energy stores used up by contractile activity. The parallel time course of O_2 consumption and of strictly aerobic recovery heat at 0 °C was first described by D. K. Hill (1940*a*). More recently, the time course of O_2 uptake has been reinvestigated in whole muscles at 0 °C (Kushmerick & Paul, 1976 *a*, *b*) and at 20 °C (Mahler, 1978, 1985), as well as in single- and multifibre preparations at 18 °C (Elzinga, Langewouters, Westerhof & Wiechmann, 1984). The relation between the initial energy output and the kinetics of O_2 uptake has been especially scrutinized, with discordant results: Kushmerick & Paul (1976*a*) observed that the time course of O_2 uptake was slowed down with longer duration of stimulation, while Mahler (1978, 1985) and Elzinga *et al.* (1984) did not notice any change, except when the O_2 consumption became limited by diffusion. However, D. K. Hill (1940*b*) who analysed the aerobic recovery heat after various durations of stimulation, could not ascertain a change at 0 °C, while A. V. Hill (1965) favoured an acceleration of its evolution with increasing duration, whatever the temperature.

In this paper the problem is re-examined, first by analysing how the amount of initial heat, which varies with stimulus duration and muscle length, influenced the quantity of recovery heat and its time course. The question of whether the initial heat is the unique determinant of recovery was investigated by comparing recovery heats produced after similar amounts of initial heat obtained either by simultaneously modifying the muscle length and the duration of stimulation, or by substituting part of the sodium chloride of the physiological solution by sodium iodide, a type A potentiator that lowers the mechanical threshold (Ebashi & Endo, 1968; Sandow, 1973).

It has been observed that, at constant muscle length, the duration of tetanus – between 0·2 and 0·75 s – only changes the kinetics of aerobic recovery heat without

affecting the recovery ratio. However, increasing the resting length beyond a definite threshold corresponding to the development of a stretch response (Feng, 1932) modifies the kinetics and the recovery ratio. Iodide too modifies both. It is further shown that the amount of initial energy is not the only factor governing the evolution of recovery – for a same initial heat, the amount and kinetics of recovery heat depend on the rate of resting heat production i.e. on the length of the muscle, and on the major anion of the physiological solution. The cost of contraction, estimated by the measurement of recovery heat, is thus influenced by conditions prevailing both before and during activity. The possibilities that higher than usual cytosolic Ca^{2+} might affect it and that stimulation could evoke a transient increase of resting metabolism are considered.

METHODS

Physiological material and solutions

The experiments were performed from September to April on paired sartorii of *Rana temporaria*. The muscles were dissected from pithed frogs, mounted at about the resting length, L_0, on both sides of a thermopile and equilibrated at 20 °C for 1 h in Ringer solution (mM: NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; NaH_2PO_4 and Na_2HPO_4, 1·9) bubbled with 100% O_2 (pH 7·3). In some experiments 60% of the NaCl of the Ringer solution was substituted by NaI (mol/mol). Higher concentrations of NaI could not be tested as they induced a contracture after a brief tetanus. During the actual measurements, the solution was withdrawn and a constant flow of water-saturated O_2 (50 ml min^{-1}) was passed through the muscle chamber.

The muscles were stimulated isometrically (stimulation rate, 100 s^{-1}) by alternate condenser discharges. To analyse the effect of stimulus duration, they were tetanized at 17 min intervals for two series of five tetani (two durations) or three series of four tetani (three durations). The results of a first 0·5 s tetanus in each series were ignored. Thereafter, the durations were distributed to cancel out the effect of fatigue (series and return or Latin square designs). Between each series, the muscles were allowed to rest for at least 30 min in the oxygenated Ringer solution.

The experiments in which the effect of length was tested followed a series and return design. The muscles were mounted at about L_0 and then tetanized for 0·2 or 0·5 s to get a reference for initial heat and force at that length. Thereafter, they were stretched by steps of 1 mm to a length at which maximum resting tension approached the twitch tension at L_0 and again stimulated for 0·2 or 0·5 s, giving another reference for initial heat and force at the longest length. If the muscles did not tear, it was usually possible to go through the experimental design without apparent muscular damage, as indicated by the reproducibility of force and initial heat at each length. The latter was estimated as follows: sarcomere spacing was measured by laser diffraction at the shortest length tested in the coxal, mid and tibial parts of the muscles; the mean of all measurements (at maximum 4% variation) was expressed relatively to L_0, assumed to correspond to 2·23 μm. Longer lengths were estimated from the macroscopic length changes of the muscles.

Measurements

Force. This was measured by means of strain gauges on a Sanborn Twin-Viso Recorder.

Heat. The thermopile (Hill–Downing type) consisted of eighty Nichrome–constantan couples connected in series, with a sensitivity of 3522 $\mu V\ °C^{-1}$ at 20 °C. Its output was amplified (Amplispot, Sefram, France) and fed into a chart recording galvanometer (Graphirac, Sefram, France).

The initial heat, Q_i, was measured as the maximum deflection of the temperature curve from the baseline immediately prior to stimulation. As the galvanometer had a response time of 1·2 s and the tetani were brief (< 1 s) relative to the time scale of recovery, Q_i might be considered as virtually instantaneous and independent of heat losses. In view of the slowness of heat loss (time constant: 25–40 s) and of recovery metabolism, no correction was made for the delay in thermal conduction from the muscles to the thermopile, nor for the lag introduced by the galvanometer.

To estimate the possible deficit of Q_i in such a type of measurement, some temperature curves were read with very brief and constant time intervals from the maximum deflection. Assuming that the initial decay of temperature was exponential, the readings were extrapolated to 0 time. The extrapolation (overestimate) was equal to 1·02–1·03 times the maximum reading for a 0·5 s tetanus. Stimulus heat increased the apparent Q_i by only 2% for a 0·5 s tetanus. Thus, under- or overestimates of Q_i are limited to a few per cent and can be disregarded.

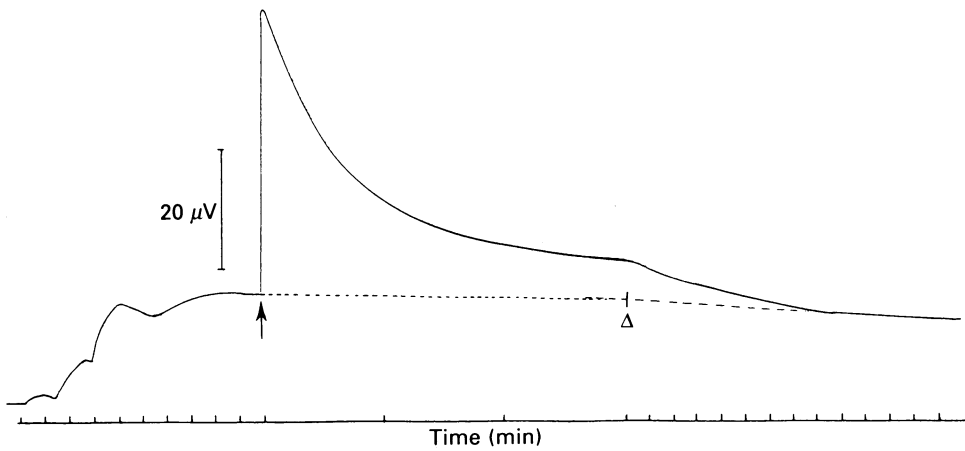

Fig. 1. Example of a temperature record during stretch, stimulation and recovery. The tracing has not been corrected for heat losses. At the beginning, the muscle pair was stretched by steps from 1·25 to 1·41 times rest length, L_0 (irregular tracing). The change of baseline after stretching indicates the presence of a stretch response. The arrow corresponds to a 0·2 s tetanus at 1·41 L_0. During the first 3 min after contraction, the recording speed was increased 5 times (time markers, 1 min). The baseline of temperature was reconstructed in two steps: (i) the final baseline recorded at low speed was extrapolated back (hatched line) up to the time Δ (vertical bar) where the recording speed was changed. If the baseline had shifted, as here, an angular coefficient was determined; (ii) from the point reached by the baseline at time Δ, the same angular coefficient was used for extrapolating a baseline for the high speed back to the time of stimulation (dotted line). The baseline at high speed nicely intersects the temperature trace at the time where the muscles were stimulated, indicating a uniform temperature shift.

For the determination of the recovery thermogenesis the temperature curves were read from the final baseline, either with intervals of 0·5 min after the maximum deflection up to 10·5 min, or 0·2 min for the first 3 min and 0·5 min from 3 to 10·5 min (Fig. 1). The return to the baseline before stimulation was generally completed within 2% of the maximum deflection; if more than 3% displacement existed, the experiments were discarded. This was particularly important when length changes were considered.

Stretching the muscles above a certain length does produce an increase of resting heat production (Feng, 1932) known as the 'stretch response' or 'Feng effect'. The maximum change of the temperature curve occurred in approximately 1 min and overshot the new level at which the baseline eventually stabilized. When the muscles were shortened, the temperature curve rapidly reached a minimum and then more slowly returned to a level intermediate between this minimum and the level at longer length. These transient changes precluded comparison of experimental situations such as stimulating around L_0 and following recovery in stretched muscles or stimulating at long length and analysing recovery at L_0. After a length change, sufficient time (3–25 min) was allowed to reach a stable temperature. It was thus assumed *a priori* that the estimate of the recovery heat, Q_r, was made in conditions strictly equivalent to those prevailing at about L_0, i.e. as the heat produced after mechanical activity above the new resting level (Fig. 1).

Heat losses were estimated from the exponential temperature decay after heating by high-frequency AC pulses at the end of each series of stimulations when muscle length was kept constant,

or after stimulation at each new length when the latter was changed during a series. Several controls were recorded in the course of each experiment, individual estimates of the constant were 0·98–1·02 times the mean.

The temperature curves were corrected for heat loss by using the logarithmic mean of two successive readings (Godfraind-De Becker, 1972) and Q_r was taken to correspond to the sum of the heat productions per time interval, ΔQ_t, without any assumption about the kinetics of Q_r production. As approximately half the total recovery heat was already produced during the first 3 min at about L_0, it was important to check the accuracy of the measurement during that period. Intervals of 0·2 or 0·5 min were compared. The recovery heat production calculated by the more accurate intervals of 0·2 min represented 0·95–0·99 of the heat estimated by the more usual intervals of 0·5 min in all cases tested. Using the intervals of 0·5 min thus led to overestimation of Q_r by 2 or 3%. This roughly compensated for the heat evolved after 10·5 min that was not taken into account here (Godfraind-De Becker, 1972). Further, calculating the amount of Q_r with a time constant of heat loss of 0·98–1·02 times that used, changed Q_r by at most 5%. On the whole, the estimated Q_r should only be in error by a few per cent (about 5%) of the heat actually produced. The precision of the recovery ratio R (Q_r/Q_i) would be of the same order.

The time constant of the slow component of aerobic recovery heat. After a delay ranging from 1 to 5 min, the recovery heat production followed a single-exponential time course of the type $\Delta Q_t = \Delta Q_0 e^{-t/\tau_s}$ (Fig. 2; Hill, 1965; Godfraind-De Becker, 1972, 1973) where ΔQ_t represents the heat production per time interval and ΔQ_0 the extrapolated heat production in the first time interval related to this exponential component. Its time constant, τ_s, and ΔQ_0 were approximated by exponential adjustment solving a set of linearized equations (normal 'Gauss' equations). This procedure introduced a subjective criterion as it had to be settled when the ΔQs, plotted on semilogarithmic co-ordinates, aligned on a straight line. Some comparisons were made with an automatic method of multi-exponential fitting (de Prony, 1795) and in most cases the two approximations did not differ appreciably.

RESULTS

Effects of tetanus duration

Seven pairs of muscles were repeatedly tetanized at fixed length (1·03–1·08 times L_0) for 0·2, 0·5 and 0·75 s. The longest duration was only used in two of them. The results (±S.E.M.) are summarized in Table 1: part A corresponds to the mean values

TABLE 1. Initial heat and aerobic recovery thermogenesis of frog sartorius muscles (*R. temporaria*, 20 °C, phosphate Ringer solution)

	Tetanus duration (s)	Q_i (mJ g^{-1})	Q_r (mJ g^{-1})	R	τ_s (min)
A	0·2	80·5±2·3	98·8±3·6	1·23±0·04	2·4±0·1
(n = 19)	0·5	143·9±4·3	193·8±8·1	1·35±0·05	3·4±0·2
B	0·2	81·8±1·4	98·0±4·9	1·20±0·06	2·2±0·2
(n = 6)	0·5	145·0±2·6	188·7±11·2	1·31±0·09	3·6±0·4
	0·75	190·1±3·2	240·7±11·0	1·27±0·07	5·2±0·2

Estimates (±S.E.M.) given in A were established on seven pairs of muscles, in B on the two pairs out of the seven in which 0·75 s duration was used; n is the number of tetani for each condition; Q_i is the initial heat, Q_r the recovery heat, R the recovery ratio (Q_r/Q_i) and τ_s the time constant of the slow exponential component of aerobic recovery heat production.

obtained on all the muscle pairs; part B only concerns those of the two pairs in which three durations of stimulation were tested.

Quantities

The initial heat Q_i and the aerobic recovery heat Q_r increased with stimulus duration (Table 1; Fig. 2). The increase of Q_r was proportional to that of Q_i, so that the ratio Q_r/Q_i, or recovery ratio R, remained approximately constant; the mean of all estimates was $1{\cdot}29 \pm 0{\cdot}03$ ($n = 44$).

Kinetics

Figure 2 (*A* and *B*) represents the mean recovery heat rate (heat production in each 0·5 min interval; $n = 3$ for each curve) at three durations in two pairs of muscles of the same batch of frogs, tested at a one week interval.

It is clear that the kinetics of the recovery heat production were affected by the tetanus duration. One can roughly distinguish two phases. The early one, which lasted for 1–5 min, depending on the tetanus duration, had a complex time course. For short tetani (0·2 s), the heat production rose to its maximum at between 0·5 and

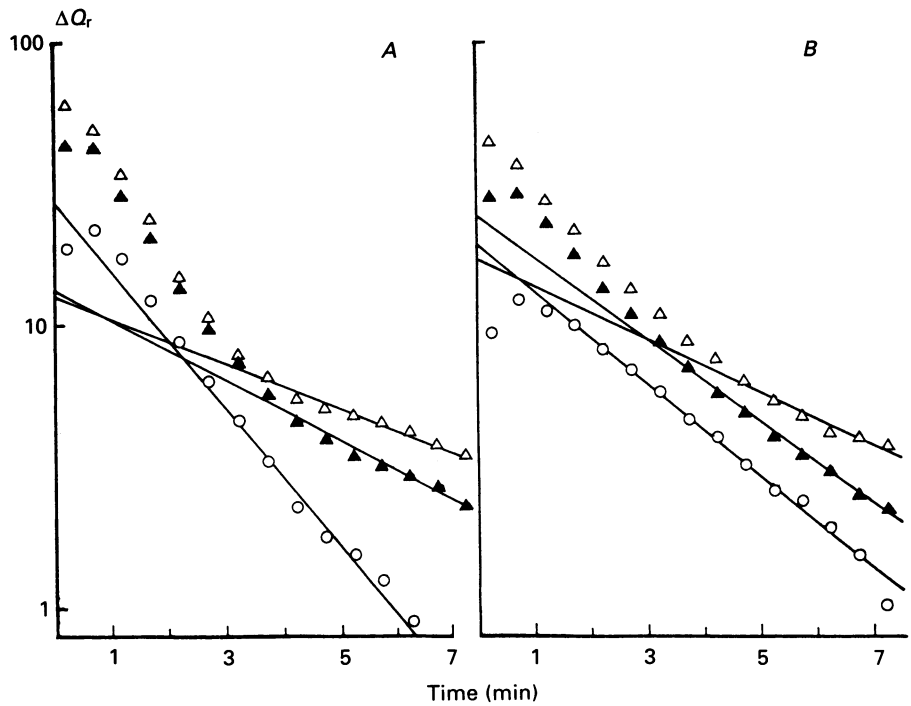

Fig. 2. Time course of aerobic recovery heat production after different stimulus durations. *A* and *B* correspond to the pairs of sartorii, the mean thermal characteristics of which are given in Table 1*B*. Abscissa: time in minutes; ordinate (log scale): recovery heat production, ΔQ_r in mJ g^{-1} (0·5 min interval)$^{-1}$. Each curve is the mean of three estimates. Muscles were tetanized (stimulation rate, 100 s^{-1}) for 0·2 s (○), 0·5 s (▲) and 0·75 s (△). The curves fitting the slow exponential decay of thermogenesis were computed as described in Methods.

1 min and declined thereafter. For longer tetani (0·75 s), the heat production was already maximum in the first 0·5 min and declined afterwards. Maximal values of heat production were roughly proportional to tetanus durations and thus to the initial heat Q_i (Fig. 2): they increased 2·8–3·7 times when the tetanus duration increased from 0·2 to 0·75 s. The first phase of the kinetics did not show saturation for the tetanus durations tested here.

The second phase was manifest early (at 1·5–2·0 min) after short tetani, but started later (at 4–5 min) after long tetani. It could be nicely approximated by a decreasing exponential function. Similar time courses have already been reported (Hill, 1965; Godfraind-De Becker, 1972, 1973). The time constant, τ_s, of this slow process increased with stimulus duration. In Fig. 2*A*, the mean estimates of τ_s were 1·8, 4·1 and 5·5 min for 0·2, 0·5 and 0·75 s tetani; in Fig. 2*B*, they were respectively, 2·5, 3·0 and 4·7 min. Even if individual variations of τ_s existed, as exemplified by Fig. 2, the effect of duration was always in the same direction, independently of the number of tetani and the sequence of durations followed. The mean time constants of the slow exponential evolution were $2·4 \pm 0·1$ min ($n = 19$) for the 0·2 s tetani and $3·4 \pm 0·2$ min ($n = 19$) for 0·5 s tetani. The medians were 2·4 and 3·3 min respectively, indicating that the distributions were hardly skew. The difference between the means is highly significant (paired t test; $P < 0·01$).

The observations reported above confirm that the amount of Q_i determines the amount of Q_r and its time course; for tetanic stimulations of 0·2 up to 0·75 s at 20 °C, Q_i and Q_r increase in proportion when excitation lasts longer, therefore R is almost constant although the kinetics of the delayed thermogenesis are modified.

Effects of length

Initial heat also depends on the length of the muscle. As the relation between Q_i and length is linear beyond L_0 (Aubert, 1956; Homsher, Mommaerts, Ricchiuti & Wallner, 1972), recovery heat production has been investigated in the range 1·0–1·48 L_0.

Stretching results in the development of resting tension and in an increase of resting heat production; both decrease with time. They were evaluated when they had stabilized, i.e. at various times after the stretch was completed (3–25 min, according to the magnitude of the stretch). Stimulations were only delivered in such steady-state conditions in order to avoid any interference between heat production due to activity – especially recovery heat – and resting heat transients (see Methods and Fig. 1). Seven pairs of muscles, which showed no impaired mechanical and thermal responses (Q_i) in the course of the experiments, could be used for the analysis of the recovery. Most tetani lasted for 0·5 s, but some other durations (0·2, 0·3 and 0·75 s) were also tested.

The 'stretch response'

As can be seen in the inset of Fig. 4, the stretch response developed at different lengths in the various pairs of muscles and had a broad scatter. A stable increase in resting heat could be detected from 1·18 L_0 onwards and the steady heat excess ranged from 4·4 up to 65·3 mJ g^{-1} min^{-1} according to length and stretch repetition. The stretch response might indeed fade away when stretch is repeated. This absence of

reproducibility was already noticed by Feng (1932) who observed that the stretch response depended on previous activity. It was further emphasized by Clinch (1968), who considered it as due to irreversible damage produced by overstretching. But, after the fading of the stretch response at a given length, stimulation evoked the

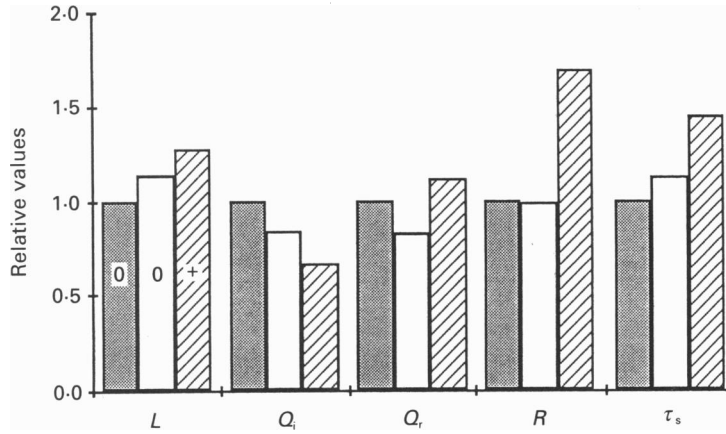

Fig 3. Relative values of thermal parameters in stretched muscles. Muscles were stimulated for 0·5 s at 1·15 ▨, 1·31 □ and 1·47 ▨ L_0. The symbols in the L columns (length) indicate the absence (0) or the presence (+) of a stretch response; the latter amounted to 26·5 mJ g^{-1} min^{-1} in steady-state conditions. The mean value ($n = 2$) of each parameter is reported relative to the corresponding estimate at 1·15 L_0. Absolute values of the references are: initial heat, $Q_i = 148$ mJ g^{-1}; recovery heat, $Q_r = 180$ mJ g^{-1}; recovery ratio, $R = 1{\cdot}22$; $\tau_s = 3{\cdot}05$ min.

same force and Q_i as when the stretch response was present. The possible damage would thus significantly affect neither the contractile machinery nor the initial energy sources. Further, the reproducibility of force and Q_i at a given length argued against gross uncontrolled length changes at the sarcomere level in the course of the experiments.

There was no evident relation between the stretch response and the appearance of resting tension. The latter had a lower threshold (1·08 L_0) and in no case could a stable excess of resting heat production be detected with less than 10 mN mm^{-2} resting tension. Moreover, fading or even disappearance of the stretch response at a given length was only associated with a comparatively small decrease (at most 20 %) of the resting tension. Feng (1932) already noticed the dissociation between resting tension and stretch response. It is especially clear in the species *Rana esculenta* where the latter is almost non-existent.

Quantities

Stretching beyond L_0 evoked a linear decrease of Q_i and active force, as described by Aubert (1956) and Homsher *et al.* (1972), but Q_r was differently affected by length. Figure 3 shows, as an example, the relative changes of the analysed parameters in one experiment for 1·15, 1·31 and 1·47 L_0, the values obtained at 1·15 L_0 being taken as the reference. As illustrated, Q_r decreased in proportion to Q_i, as long as no stretch response was detected. Once the latter was manifest Q_r ceased to decrease, on the contrary, it began to rise so that, for the longest lengths tested, the absolute amount

of Q_r might equal or even exceed that produced at 1–1·15 L_0 for the same tetanus duration. Thus R was no longer constant but increased.

As described above and confirmed in this series of experiments, R did not significantly change with tetanus duration at a given length; the results were thus pooled in this analysis. Table 2 gives the R values for groups of lengths tested. No significant difference existed between the first three groups ($L < 1{\cdot}3\ L_0$) nor between

TABLE 2. The recovery ratio R as a function of length

L/L_0	n	R
1·0–1·09	22	1·26 ± 0·04
1·1–1·19	16	1·40 ± 0·09
1·2–1·29	10	1·25 ± 0·09
1·3–1·39	16	1·78 ± 0·21*
1·4–1·48	12	2·11 ± 0·19**

Values of R are the mean ± S.E.M., n is the number of tetani in each group of lengths. The results of each length group were compared to those of the 1·0–1·09 L/L_0 group by the Student's t test: * corresponds to $P = 0{\cdot}01$, ** to $P < 0{\cdot}001$.

the last two groups ($L \geqslant 1{\cdot}3\ L_0$). However the mean R value at $L \geqslant 1{\cdot}3\ L_0$ was 1·92 ± 0·14 ($n = 28$), significantly larger (Student's t test; $P < 0{\cdot}001$) than that of 1·31 ± 0·04 ($n = 48$) obtained at $L < 1{\cdot}3\ L_0$. When each experiment was considered independently, it appeared that the increase of R was mainly related to the presence of a stretch response.

Such a point of view was further strengthened by the following observation. In four pairs of muscles, the stretch response faded or even disappeared when stretch was repeated (return series; see inset of Fig. 4). There was a coincident decrease of R and, in three out of the four pairs, R was lessened to the value estimated at about L_0. By stretching to greater lengths nevertheless, a stretch response was again manifest and so was the increase of R. This suggested that increase of R and stretch response were directly associated. As shown in Fig. 4, there existed a good correlation ($r = 0{\cdot}86$; $t = 16{\cdot}8$) between these parameters, the orthogonal regression corresponding to $R = 1{\cdot}17 + 0{\cdot}0294$(stretch response, mJ g^{-1} min^{-1}). (It should incidentally be noted that the slope corresponds to (34 mJ g^{-1} $min^{-1})^{-1}$.)

The increase of R appeared thus concomitantly with the stretch response, which was only detected beyond a threshold length (Feng, 1932; Clinch, 1968; these experiments). Indeed, the mean R value for all tetani in the absence of a stretch response, whatever the length, was 1·21 ± 0·03 ($n = 46$). This estimate was not significantly different from the mean R value in the 1·0–1·09 L_0 group, from that obtained in the variable duration experiments, nor from the extrapolation to the origin (stretch response = 0) of the orthogonal regression of Fig. 4 (99% confidence limits of R, 1·06–1·28).

The relationship of R to resting tension ($R = 1{\cdot}06 + 0{\cdot}033$(resting tension in g); $r = 0{\cdot}82$; $t = 12{\cdot}3$) had a broader scatter than to the stretch response, as could be expected from the different thresholds and the fact that resting tension was less labile than the stretch response. Nevertheless, due to the large dispersion, there was no significant difference between both correlations.

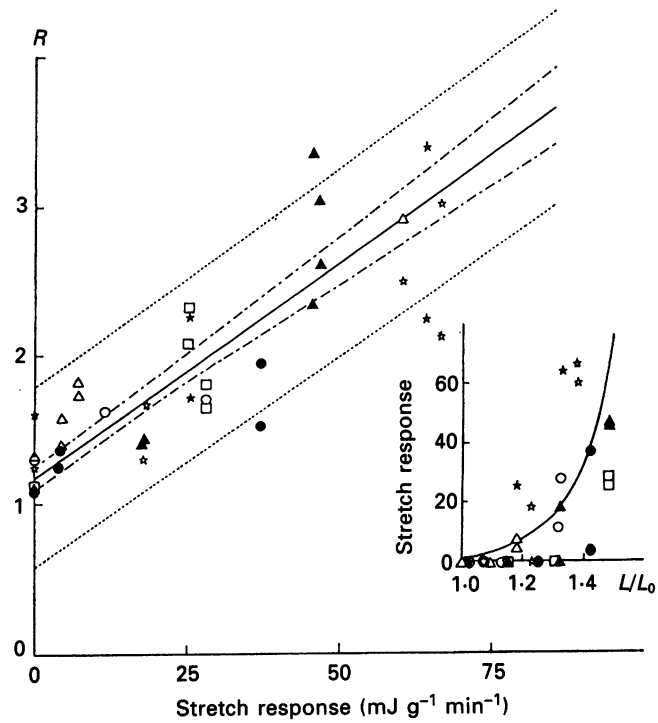

Fig. 4. Relation between the recovery ratio R and the stretch response. Abscissa: stretch response in mJ g^{-1} min^{-1}; ordinate: R (Q_r/Q_i). Each pair of muscles is represented by a different symbol. The stretch response was measured in steady-state conditions after stabilization of the temperature curve, i.e. at various times after step-by-step stretching or releasing (from 3 to 25 min). The continuous line corresponds to the calculated orthogonal regression: $R = 1{\cdot}17 + 0{\cdot}0294$ (stretch response in mJ g^{-1} min^{-1}) ($n = 76$; correlation coefficient = 0·859). For all short lengths where no stretch response could be detected, only the mean R value for each muscle pair is shown; the seven points at the intercept thus represent forty-six estimates on seven pairs. The 95% confidence limits of the regression are indicated by the dashed lines, those of the individual samplings by the dotted lines. Inset: relation between the stretch response (ordinate: mJ g^{-1} min^{-1}) and the length (abscissa: L/L_0). The line is fitted from the semilogarithmic orthogonal regression. When two identical symbols are given for the same length, the smaller estimate of the stretch response always corresponds to the second measurement.

Kinetics

In the presence of a stretch response, the recovery heat production was always maximum in the first half-minute, sometimes even in the first 12 s (Fig. 5, upper trace), a time interval when little or no heat was detected in the absence of a stretch response. Further, after tetani of 0·5 s, τ_s was increased from $3{\cdot}38 \pm 0{\cdot}18$ min ($n = 21$; no stretch response) to $4{\cdot}81 \pm 0{\cdot}65$ min ($n = 11$; presence of a stretch response; Student's t test, $P \approx 0{\cdot}01$).

Is the amount of initial heat the only factor governing recovery?

Different combinations of length and stimulus durations allow the comparison of Q_r after tetani producing about the same initial heat. Figure 5 illustrates one such pair of observations. For the muscles used here, a tetanus of 0·2 s at 1·15 L_0 gave out

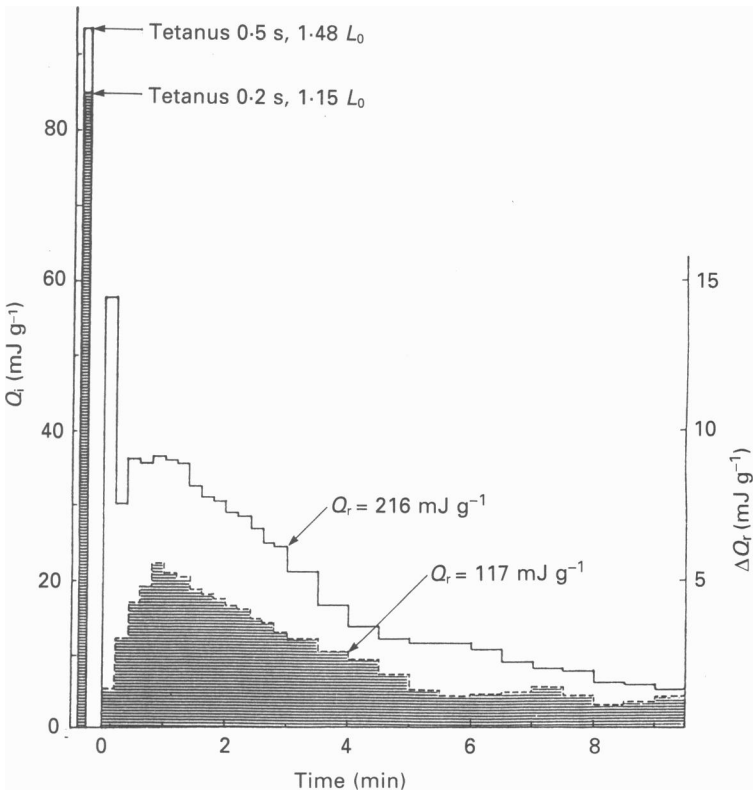

Fig. 5. Aerobic recovery heat production at different lengths and similar initial heats (change of stimulus duration). Recovery heat production, ΔQ_r (right ordinate, mJ g^{-1}), is given for intervals of 0·2 min for the first 3 min after stimulation and for 0·5 min intervals thereafter, as a function of time (abscissa, min). Initial heat, Q_i (left ordinate, mJ g^{-1}), is given as a block (without time scale). □, tetanus of 0·5 s (stimulation rate, 100 s^{-1}) at 1·48 L_0; Q_i = 93·4 mJ g^{-1}; Q_r = 216·2 mJ g^{-1}; tension = 180 kN m^{-2}; stretch response: 25·2 mJ g^{-1} min^{-1}. ▤, tetanus of 0·2 s (100 s^{-1}) at 1·15 L_0; Q_i = 84·9 mJ g^{-1}; Q_r = 116·7 mJ g^{-1}; tension = 320 kN m^{-2}.

91 % as much heat (Q_i) as was produced during a stimulation of 0·5 s at 1·48 L_0, the stretch response being 25·2 mJ g^{-1} min^{-1}. In contrast, the recovery heat was increased from 116·7 to 216·2 mJ g^{-1} and the recovery ratios (R) were 1·37 and 2·31 respectively.

It is obvious that the small disparity between the Q_is could not be responsible for the large Q_r difference: with an R of 1·37, as measured at 1·15 L_0, and no change due to the stimulus duration (see above), only a difference of about 12 mJ g^{-1} instead of 100 could be expected. Some 10–15 % of the excess heat appeared in the first 12 s but the remaining was produced thereafter at a rate of about 45 mJ g^{-1} min^{-1} and

decayed with a time constant of nearly 5 min, instead of 3 min at the shorter length. If the heat produced during the first 12 s was possibly not due to recovery but only reflected slower mechanical relaxation (Jewell & Wilkie, 1960; Mittenthal & Carlson, 1971; Fraser & Carlson, 1973) – an extreme and rather unplausible hypothesis – after correction of Q_i and Q_r for that amount there should still remain an excess of about 60 mJ g^{-1} recovery heat, i.e. a quantity roughly equal to 0·6 Q_i.

Thus, the production of Q_r is influenced by the presence of a stretch response and the amount of Q_i is not the sole factor influencing the recovery thermogenesis.

Effect of sodium iodide, a twitch potentiator

As the stretch response is affected by many drugs which modify the cycling of Ca^{2+} (Clinch, 1965, 1968), it was interesting to investigate whether NaI, which increases the release and slows the reuptake of Ca^{2+} by the sarcoplasmic reticulum (Ebashi & Endo, 1968; Sandow, 1973), affects the recovery processes in a similar way to stretch.

Three pairs of muscles were stimulated for 0·2 and 0·5 s at 1·03–1·06 L_0 after a rest period either in the usual Ringer solution (Cl^-) or in a solution where 60% (mol/mol) of the NaCl was substituted by NaI (I^-). To test for reversibility, three series of excitations were delivered following the sequence $Cl^-(1)$, $I^-(2)$, $Cl^-(3)$.

Quantities

As reported in Table 3, Q_i increased after I^-; the force development and its maintenance were essentially unaffected but the relaxation was clearly delayed (not shown) and might be responsible for the increase of Q_i. The recovery heat was increased still more; both effects were more manifest for the shorter tetani. As a consequence, the recovery ratio R was significantly larger after I^-, especially with the 0·2 s stimulation (Table 3).

TABLE 3. Effect of iodide on recovery heat production

	Anion	Q_i (mJ g^{-1})	Q_r (mJ g^{-1})	R	τ_s (min)
Tetanus duration 0·2 s	Cl^- (1)	74·8 ± 3·9	94·1 ± 6·0	1·26 ± 0·05	2·47 ± 0·23
	Cl^- (3)	72·6 ± 4·3	98·7 ± 6·8	1·36 ± 0·07	2·65 ± 0·26
	Cl^- (1+3)	73·7 ± 2·8	96·4 ± 4·6	1·31 ± 0·05	2·56 ± 0·17
	I^- (2)	90·3 ± 5·2**	186·2 ± 16·9**	2·07 ± 0·16**	3·08 ± 0·41
Tetanus duration 0·5 s	Cl^- (1)	132·7 ± 7·7	185·8 ± 18·2	1·39 ± 0·09	3·87 ± 0·33
	Cl^- (3)	129·2 ± 8·5	187·9 ± 12·3	1·46 ± 0·08	2·93 ± 0·20
	Cl^- (1+3)	130·9 ± 5·5	186·9 ± 10·5	1·43 ± 0·06	3·40 ± 0·23
	I^- (2)	141·0 ± 9·1	244·4 ± 19·5*	1·74 ± 0·11*	5·01 ± 0·42**

The sequence of the series of excitations is given in parentheses; Cl^-, chloride Ringer solution; I^-, solution where 60% of the NaCl has been substituted by NaI (mol/mol). Each estimate is the mean (± S.E.M.) of six measurements obtained on three pairs of muscles. The results after I^- were compared to those after Cl^- for the same durations by Student's t test: * corresponds to $P = 0{\cdot}01$, ** to $P < 0{\cdot}001$.

Kinetics

The time course of recovery heat production after I^- was modified, as if the tetani were longer. By comparison with Cl^-, the delay to maximum heat production was shortened, the heat production during the first half-minute was greater and τ_s might have been increased (0·5 s tetanus; Table 3).

These experiments show striking similarities to the evolution of recovery in the presence of a stretch response. They suggest that, despite the similar kinetics after a 0·5 s tetanus in Cl^- and one of 0·2 s in I^- (Table 3), there are important differences in the recovery processes as indicated by the magnitude of the recovery ratios.

DISCUSSION

Kinetics of aerobic recovery heat production

The experiments described here show that the kinetics of recovery heat production are dependent on the conditions prevailing during activity (tetanus duration, sarcomere length) and before contraction (stretch response, I^- substitution). These factors can simultaneously affect the time course of Q_r (Figs 2 and 5; Tables 1 and 3) so that no fixed relation emerged between Q_r kinetics and the initial energy output, Q_i. Instead, it was observed that the kinetics were similar when the amounts of Q_r produced were the same. This was the case, with widely differing Q_is, for a tetanus of 0·5 s (Cl^-) and one of 0·2 s after I^- substitution (Table 3) as well as for a 0·75 s tetanus (Cl^-, Fig. 1; Table 1 B) and a 0·5 s tetanus after I^- (Table 3).

Rather than being determined by the amounts of Q_i, the kinetics of Q_r thus seem largely dependent on the factors that determine the amounts of Q_r. Apparently, their changes do not reflect major modifications of the recovery processes; they may occur without changes in the recovery ratio (Table 1) and, conversely, different recovery ratios (see below) can be obtained for the same time courses of Q_r (Table 3).

The time constant τ_s

The time constant τ_s characterizes the slower phase of Q_r production, which is present in all types of kinetics and is most probably related to pure oxidative processes. This component of Q_r disappears in anaerobiosis and is almost unaffected by iodoacetate (IAA, 0·4 mM) poisoning (Godfraind-De Becker, 1973). Here, τ_s was increased by procedures that increase Q_r: longer stimulus duration (Table 1), presence of a stretch response (Fig. 5) and presence of I^-, at least for the 0·5 s tetani (Table 3).

If τ_s was related to the speed of pure oxidative reactions, the results obtained here for increased durations of activity confirm the observations of Kushmerick & Paul (1976*a*) who described an increase of the time constant of O_2 consumption with tetanus duration at 0 °C. However, they are at variance with those of D. K. Hill (1940*a*, *b*), Mahler (1978, 1985) and Elzinga *et al.* (1984) who measured the time constant of O_2 consumption at 0, 20 and 18 °C respectively and did not find any change with longer tetanus durations.

A possible explanation for the difference might be that O_2 consumption was

limited by diffusion when Q_r increased, but this is unlikely for the following reasons. Kushmerick & Paul (1976*a*), who also measured lactate production, considered that no part of the muscles was anoxic for tetani lasting less than 20–40 s at 0 °C, a duration corresponding to 2–4 s at 20 °C. Durations longer than 1 s were also assayed by Mahler (1978) at 20 °C without apparent anoxia. Further, with the muscles used here, the amount of O_2 dissolved at rest in the deepest tissue layers (Hill, 1965, chap. 6) should have been sufficient to account for at least 95% of the heat output. According to Mahler, Louy, Homsher & Peskoff (1985), who showed that O_2 solubility in muscle is greater than previously accepted, the O_2 concentration would have been about 50% larger than expected from Hill's data (1965) and not at all limiting (even with a stretch response). Moreover, as a long contraction (0·75 s) had little, if any, influence on the time course of the aerobic recovery heat production of the next shorter tetanus, the O_2 concentration in the deepest layers of the tissue would have been recovered between tetani (Hill, 1965, p. 230).

Finally, it has been shown (Godfraind-De Becker, 1973) that for Q_r kinetics similar to those observed after a 0·5 or 0·75 s tetanus in chloride Ringer solution, IAA poisoning changes the time course of Q_r production but does not reduce it to one of a single exponential, as is that of O_2 consumption (D. K. Hill, 1940*a*, *b*; Mahler, 1978, 1985; Elzinga *et al.* 1984). In the range tested here, the higher the Q_r, the larger the τ_s. The slow recovery processes do not obey simple first-order kinetics as does O_2 consumption (Mahler, 1985). This may reflect either the differences between non-specific measurements, such as heat, and specific ones, such as O_2 consumption, or the better time resolution and sensitivity of the former.

Amounts of recovery heat; relation to initial heat

The accuracy of the heat measurements (Q_i and Q_r) has already been validated (see Methods). The Q_i values fall within the range of many other estimates (Hill & Woledge, 1962; Godfraind-De Becker, 1973) and, when muscles were contracted at about L_0, the recovery ratio R had the usual magnitude (1·2–1·3; Hill, 1965, Table 4.1; Woledge, Curtin & Homsher, 1985, Table 4.V). Further, in all cases, Q_r was measured from a stable baseline (however, with the procedure used here, it could not be determined whether the baseline after iodide was the same as in chloride).

Regarding Q_r and R, the results reported here can be divided into two groups. In the first, exemplified by the effect of tetanus duration (Table 1), the amount of Q_r remained proportional to Q_i so that R was fairly constant. In the second group, in the presence of a stretch response or of I^-, Q_r was much larger than expected from Q_i and R was significantly increased (Tables 2 and 3). Dawson (1975) described a third group, with a decrease of Q_r for a given Q_i, resulting in a smaller R. Altogether, these observations show the complexity of recovery processes and indicate that it is only in well-defined conditions – at about L_0 and with the usual physiological solutions – that there is a constant proportionality between Q_r and Q_i. Outside these restricted conditions, significantly different amounts of Q_r can be produced after similar amounts of Q_i (Fig. 5; Table 3). The effects of stretch and iodide further suggest that recovery processes are also influenced by the conditions prevailing before activity.

The reasons why stretch and I^- increase R (Tables 2 and 3) are unclear; however, both treatments have a common effect, namely, interference with Ca^{2+} movements.

Iodide increases the release of Ca^{2+} by the sarcoplasmic reticulum (SR) and slows its reuptake (Ebashi & Endo, 1968; Sandow, 1973). The stretch response might be due to a larger Ca^{2+} release by the SR (Clinch 1965, 1968; Lopez, Alamo & Caputo, 1985). It is indeed potentiated by caffeine, twitch potentiators and procaine in depolarizing solution, all treatments that provoke Ca^{2+} release, although by different mechanisms (Martonosi, 1984). But stretch has more side-effects: alkalinization (Margaria, 1934; Dubuisson, 1940; Distèche, 1960) that also affects Ca^{2+} transits (Duggan & Martonosi, 1970), stimulation of the Na^+–K^+ pump (Harris, 1954; Vandenburgh & Kaufman, 1981) and modifications of protein turnover (Etlinger, Kameyama, van der Westhuyzen, Erlij & Matsumoto, 1981). It is likely that the increase of R might be due to modifications of Ca^{2+} cycling. The values obtained after I^- and stretch are rather similar, but I^- mainly increases heat production during the early phase of recovery (not shown) and stretch acts throughout the whole recovery period (Fig. 5); this could well indicate the difference between a transient or a persisting Ca^{2+} overload, as well as the additional influences of the other effects of stretch just cited, which might themselves be related to Ca^{2+} movements.

Thus, when contractions are elicited in muscles where the control of the cytosolic Ca^{2+} is most likely perturbed, a greatly exaggerated amount of recovery heat is liberated and the overall metabolic cost of those contractions is higher than expected.

Significance of the recovery ratio

Some fraction of the initial energy output has no metabolic equivalent. It is known as the 'unexplained enthalpy' (for references, see Woledge *et al.* 1985). On the other hand, post-contractile O_2 consumption exceeds that needed to resynthesize the amount of phosphorylcreatine (PC) split during contraction (Kushmerick & Paul, 1976*a*, b; Paul, 1983) and accounts for more Q_r than produced (Paul, 1983). However, in a complete contractile cycle (contraction–recovery), an energy balance is achieved (Paul, 1983). Thus recovery returns the muscle to its pre-contraction state, i.e. reverses all initial changes, including those responsible for the unexplained enthalpy. On this basis, Woledge *et al.* (1985) developed a formalism in which the recovery ratio r would correspond to

$$r = r_0 - z(1 + r_0)(1 - Y).$$

In this expression, r_0 is the recovery ratio when the initial energy output can be totally accounted for by PC splitting and the recovery heat by the oxidative resynthesis of the PC split. The fractional contribution of unexplained enthalpy to the initial energy expenditure is represented by z; the reaction(s) responsible for it is reversed at the expense of later PC splitting (Paul, 1983; Homsher, Lacktis, Yamada & Zohman, 1987) and Y represents the ratio of the heat produced by the PC splitting required to drive the reversal of the unexplained enthalpy to that absorbed by the reversal.

The equation of Woledge *et al.* (1985) can be rewritten as

$$r = (1 - z)\, r_0 + z[Y(1 + r_0) - 1],$$

where the first term represents the contribution to r of the explained enthalpy (PC

splitting) and the second that of the unexplained one. The term within square brackets is the recovery ratio for the unexplained enthalpy. As the latter might arise from more than one reaction, r could be expanded in a series of terms

$$r = (1-z)r_0 + n_1 r_1 + n_2 r_2 + \ldots + n_n r_n$$

where $\Sigma n_i = z$ and r_i is the proper recovery ratio of each individual reaction i ($i = 1, 2, \ldots n$) which is reversed; r is thus a weighted mean of all its possible components.

In the above expressions, r_0 corresponds to $[(\Delta H_{O_2}/p) - \Delta H_{PC}]/\Delta H_{PC}$ where ΔH_{O_2} and ΔH_{PC} are the molar enthalpy changes for substrate oxidation (-460, -470 kJ mol^{-1}; Carpenter, 1939; Woledge, 1971) and for PC splitting (-34 kJ mol^{-1}; Curtin & Woledge, 1978); p is the coupling ratio between PC resynthesis and O_2 usage ($P/O_2 = 6{\cdot}5$; Woledge, 1971), so that r_0 equals 1·08–1·13. In most cases, the experimentally estimated R is definitely larger than r_0 (Hill, 1965, Table 4.1; Woledge *et al.* 1985, Table 4.V; this paper, Tables 1, 2 and 3), suggesting that there is some unexplained enthalpy ($z \neq 0$) and that its coupled reversal is exothermic ($Y > 1$).

It is now widely accepted that the exchange of Ca^{2+} for Mg^{2+} on parvalbumins (PA) is responsible for at least part of the unexplained enthalpy (Gillis, Thomason, Lefèvre & Kretsinger, 1982; Homsher *et al.* 1987). During recovery, this Ca^{2+}–Mg^{2+} exchange is reversed (with concomitant PC splitting; Homsher *et al.* 1987), as Ca^{2+} is eventually reaccumulated in the SR (Gillis & Gerday, 1977). Taking the molar enthalpy change for the Ca^{2+}–Mg^{2+} exchange as -25 kJ mol^{-1} (Potter, Hsu & Pownall, 1976; Curtin & Woledge, 1978; Smith & Woledge, 1982; Tanokura & Yamada, 1985) and the stoichiometry of the Ca^{2+} pump as 2 Ca^{2+}:1 ATP (Hasselbach, 1964), Y equals $(-34 \text{ kJ mol}^{-1}/2)/(-25 \text{ kJ mol}^{-1}) = 0{\cdot}68$ and r_{PA} is $0{\cdot}68(2{\cdot}13) - 1 = 0{\cdot}45$. The coupled reversal of these ionic exchanges is thus endothermic ($Y < 1$) and its contribution to recovery would make $r < r_0$ ($1 - Y > 1$; $r_{PA} < r_0$). Further, to cope with the kinetics of the unexplained enthalpy (Curtin & Woledge, 1979; Homsher, Kean, Wallner & Garibian-Sarian, 1979), one should expect that r would tend to r_0 for increased stimulus durations. Obviously, the reversal of the Ca^{2+}–Mg^{2+} exchanges on PA cannot account for the experimental values of R ($> r_0$). Some other reaction(s) might play a role. Assuming that the unexplained enthalpy represents 0·3 of the initial energy output in a contraction of 0·5 s at 20 °C (Woledge *et al.* 1985) and that the Ca^{2+}–Mg^{2+} exchanges on PA are responsible for half of it (Gillis *et al.* 1982; Woledge *et al.* 1985), a single additional reaction with a molar enthalpy change of about -20 kJ mol^{-1} (the coupling factor of PC splitting and its reversal being 1) would fulfil the experimental requirements ($R = r = 1{\cdot}2$–$1{\cdot}3$). Nevertheless, the coupled reversal of such a reaction would be strongly exothermic ($Y_x = 2{\cdot}3$–$2{\cdot}9$). A possible candidate might be the exchange of the Mg^{2+} accumulated in the sarcoplasmic reticulum during Ca^{2+} release (Somlyo, Kitazawa, Gonzalez-Serratos, McClellan & Somlyo, 1985). Unfortunately, nothing is known about the thermodynamics of this exchange.

But the relation between R and the stretch response suggests another possibility – that R could include some fraction determined by the metabolic conditions at rest. It is tempting to speculate that the relation illustrated in Fig. 4 might be generalized

to usual conditions ($L \approx L_0$; chloride as the major anion of the physiological solution) stating that $R = r + 0{\cdot}0294$ (resting rate of heat production, in mJ g^{-1} min^{-1}). As the latter amounts to 10–11 mJ g^{-1} min^{-1} (Hill, 1965; Clinch, 1968) at 20 °C and L_0, its contribution to R would be 0·3 and r would lie in the range 0·9–1·0, a value quite compatible with an endothermic coupled reversal of the Ca^{2+}–Mg^{2+} exchanges on PA, without further assumption. For a tetanus of 0·5 s at 20 °C ($z = 0{\cdot}3$; Woledge *et al.* 1985), r is indeed expected to be about 0·7 (1·13) + 0·3 (0·45) = 0·93.

This view implies that activity interferes with resting conditions and modifies the 'basal' metabolism of the muscle, most probably by transiently increasing the work of ion pumps (see Appendix). A reversible change of 'baseline' due to activity would be unperceivable on the time scale of a contraction–relaxation cycle, but could considerably affect long-term heat production as Q_r. Rough estimates on the results reported here indicate that each mJ g^{-1} min^{-1} resting heat production at 20 °C would contribute to recovery about 4·5 mJ g^{-1} in 10 min (3 mJ g^{-1} in 10 min after a 0·2 s tetanus and 6 mJ g^{-1} in 10 min after a 0·5 s tetanus at about L_0), i.e. approximately one-third of Q_r in the usual experimental conditions. The effect would be of minor importance at low temperature (with a Q_{10} of 2·5 and a resting heat rate of 1 mJ g^{-1} min^{-1} at 0 °C, 3–6 mJ g^{-1} would be delivered in 60 min). It could thus account for $R \approx 1$ at 0 °C (Hill, 1965, Table 4.1; Paul, 1983), but it would considerably increase the overall cost of contraction at high temperature, for example in man during physical performance. Whatever the mechanism at play, it is clear that the cost of contraction, as estimated by the recovery ratio R, exceeds that involved in actin–myosin interactions and in ionic exchanges on parvalbumins.

APPENDIX

Na^+ and Ca^{2+} pumps: their possible contribution to 'recovery heat'

Na^+–K^+-ATPase of the plasma membrane

Let us assume that, in frog muscle, there are 2500 Na^+–K^+-ATPase sites per square micrometre of membrane and that the maximal reaction rate v_{max} is almost the same in all tissues (De Weer, 1985) i.e. 100 s^{-1} at 37 °C, about 900 min^{-1} at 20 °C with a Q_{10} of 3. In 1 g of muscle (density = 1·05) there should be about 7200 cells (supposed to be cylindrical: diameter, 75 μm; length, 3 cm) with a total plasma membrane surface of 508 cm^2 (for a thickness of 8 nm, this represents 0·0004 of the volume) or 127×10^{12} Na^+–K^+-ATPase sites per gram. As one pump cycle uses one ATP molecule, the Na^+ pump should hydrolyse $117{\cdot}7 \times 10^{15}$ ATP molecules g^{-1} min^{-1} or $196{\cdot}2 \times 10^{-9}$ mol ATP g^{-1} min^{-1} at v_{max}. In terms of PC equivalents ($\Delta H = -34$ kJ mol^{-1}), taking the overall efficiency of the pump as 0·5 (it seems rather less in muscle; Biron, Burger, Chinet, Clausen & Dubois-Ferrière, 1979), the energy dissipation would correspond to 3·3 mJ g^{-1} min^{-1}. The resynthesis of the PC split would produce 1·13 ($196{\cdot}2 \times 10^{-9}$ mol g^{-1} $min^{-1} \times 34$ kJ mol^{-1}) = 7·5 mJ g^{-1} min^{-1}. The total energy dissipation due to the Na^+ pump working at v_{max} would be about 11 mJ g^{-1} min^{-1}. As 5–8 % of the resting heat rate of striated muscle is ouabain-sensitive (Biron *et al.* 1979), the Na^+ pump could only be responsible for

0·5–0·9 mJ g^{-1} min^{-1} resting heat at 20 °C, indicating that it works at 0·05–0·08 times v_{max}. If contraction provoked a transient, impulse-like stimulation up to v_{max}, decaying exponentially with a time constant of 3 min, it could produce ± 30 mJ g^{-1} in 10 min.

Ca^{2+}-ATPase of the sarcoplasmic reticulum

As the Na^+ pump is only responsible for a small fraction of the resting heat rate, let us assume that the larger fraction of it – ± 10 mJ g^{-1} min^{-1} at 20 °C – is due to the cycling of the Ca^{2+} pump. A maximum uptake of 70 nmol Ca^{2+} (mg SR protein)$^{-1}$ s^{-1} has been obtained at 25 °C for mammalian SR (Inesi & Scarpa, 1972). With a Q_{10} of 3 (Weber, Herz & Reiss, 1966), 2 Ca^{2+}:1 ATP (Hasselbach, 1964), 10 mg SR (g muscle)$^{-1}$ (Peachey, 1965) and 0·7 of the SR weight being Ca^{2+} pumps, the maximum ATP consumption at 20 °C might reach the enormous amount of $8{\cdot}5 \times 10^{-6}$ mol g^{-1} min^{-1}. If the coupled reactions of reuptake were thermoneutral, the hydrolysed ATP still would have to be resynthesized, a reaction that should produce (PC equivalents) 1·13 ($8{\cdot}5 \times 10^{-6}$ mol g^{-1} $min^{-1} \times 34$ kJ mol^{-1}) = 327 mJ g^{-1} min^{-1}. To give out 10 mJ g^{-1} min^{-1}, the Ca^{2+} pump would work at 10/327 = 0·03 v_{max}. Its transient stimulation up to v_{max} and later decay with a time constant of 3 min could produce 946 mJ g^{-1} in 10 min.

Is the heat excess appearing during recovery compatible with stimulation of ionic pumps?

If both Na^+ and Ca^{2+} pumps were fully stimulated by a brief contraction, the maximum rate of heat production due to translocation of ions might reach ($11 + 327 =) \pm 340$ mJ g^{-1} min^{-1}. If such an increase of heat rate decayed with a time constant of 3 min, it should be associated with a total heat production of ± 1000 mJ g^{-1} in 10 min. In the experiments described here, the heat excess that is hypothetically associated with stimulation of resting metabolism, amounts to 30 mJ g^{-1} in 10 min for a 0·2 s tetanus, up to 60 for a 0·5 s and up to 65 for a 0·75 s tetanus. With a time constant of 3 min (τ_s, Table 1), this would imply an increase of heat rate production between 10·4 and 22·5 mJ g^{-1} min^{-1}, so that the total 'resting' heat rate would reach 20–33 mJ g^{-1} min^{-1}. This does not exceed 10% of the maximum rate that the pumps could be responsible for, so the hypothesis is plausible. In stretched muscles, the difference in Q_r may attain some 100 mJ g^{-1} in 10 min (Fig. 5), with stretch responses of 25–45 mJ g^{-1} min^{-1}. If the time constant of decay of stimulation of the pumps increased from 3 to 5 min (see Results: Effects of length, Kinetics), the heat rate should have to be increased by some 25 mJ g^{-1} min^{-1}, so that the total 'resting' heat rate (resting heat rate + stretch response + stimulation of pumps) would be in the range 60–80 mJ g^{-1} min^{-1} or, at maximum, one-quarter of what could be expected for pumps at v_{max}. Similarly, the difference between the effects of stretch and iodide could well be due to a smaller time constant for the decay of pump activity in iodide, concomitant with a larger stimulation. The heat excess measured (60–90 mJ g^{-1} in 10 min) still leaves a very large safety margin for the pump activities.

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