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LABILE HEAT AND CHANGES IN RATE OF RELAXATION OF FROG MUSCLES

BY MICHELLE PECKHAM* AND R. C. WOLEDGE

From the Department of Physiology, University College London, Gower Street, London WC1E 6BT

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SUMMARY

1. Observations were made of the labile heat and the progressive slowing of relaxation as a function of tetanus duration (Abbott effect) during isometric tetani of frog extensor longus digiti IV (e.l.d. iv) and sartorius muscle.

2. Both the labile heat and slowing of relaxation are less marked in e.l.d. iv than in sartorius muscle.

3. Both effects are depressed to the same extent in the second of two closely spaced tetani in sartorius muscle.

4. The repriming of both effects follows the same time course in sartorius muscle.

5. The hypothesis is discussed that both effects result from binding of calcium to parvalbumin during a tetanus, and that their repriming is due to the removal of calcium from parvalbumin by the sarcoplasmic reticulum.

INTRODUCTION

Amphibian muscles relax more slowly after a long tetanic contraction than after a short one. Abbott (1951) described the time course of this effect (which we propose to refer to as the Abbott effect) in toad sartorius muscle and also showed that there was, over the same period, a progressive slowing in the rate of heat production. Aubert (1956) studied the time course of this heat production in more detail and described a component of the heat rate which decays exponentially over several seconds. This he called the 'labile heat'. He showed that the labile heat is relatively independent of sarcomere length and therefore unlikely to be connected with actomyosin interaction. The labile heat was later found not to be due to ATP or phosphocreatine (PCr) hydrolysis (Curtin & Woledge, 1978). It was also discovered that in the second of two closely spaced tetani, the labile heat was much less than in the first (Aubert, 1967; Curtin & Woledge, 1977) and that the 'repriming' of the labile heat was rather slow.

An hypothesis that would explain these facts is that both the Abbott effect and the labile heat are due to the interactions of calcium with the calcium-binding protein paravalbumin. This protein is present in high concentration in amphibian muscle

* Present address and to whom correspondence should to be sent: Dr Michelle Peckham, Department of Biophysics, 26–29 Drury Lane, London WC2R 2LS.

(Gosselin-Rey & Gerday, 1977). This paper describes two experiments based on this hypothesis. (1) We discovered during other experiments (unpublished observations) that the extensor longus digiti IV (e.l.d. iv) muscle produces less labile heat than sartorius muscle and therefore measured the Abbott effect in this muscle. (2) We have observed the 'repriming' of the Abbott effect and compared it with that of the labile heat in sartorius muscle.

METHODS

Pairs of sartorius muscles still attached to the pelvic bone, or single e.l.d. iv muscles from the frog *Rana temporaria* were used. They were dissected and mounted on the thermopile, and placed in a chamber so that they could be bathed in aerated Ringer solution (115·0 mM-NaCl, 2·5 mM-KCl, 1·8 mM-$CaCl_2$, 1·0 mM-$MgCl_2$, 1·0 mM-NaH_2PO_4, 2·0 mM-Na_2HPO_4, pH 7·0). The chamber was placed in a stirred ice bath and left for 30 min to equilibrate. After this time, Ringer solution was drained away from the muscle into a reservoir also at 0 °C. The muscle length was then adjusted so that force production was maximal. This was done by measuring force production for a series of twitches given to the muscle at 30 s intervals, while the muscle length was periodically changed by 0·5 mm. Two twitches were obtained at each length. In most experiments sarcomere length was measured using a He–Ne laser before mounting the muscle on the thermopile. The muscle was used at a resting sarcomere length of 2·2 μm. Taking into consideration a minimum amount of internal shortening of 5 % on stimulation, the overlap between thick and thin filaments should be optimum for force production at this sarcomere length. Muscles were stimulated, via two platinum electrodes, by supramaximal square-wave pulses of 1–5 ms duration at 10–13 Hz. All the experiments were carried out at 0 °C.

Tension and heat were recorded in isometric contractions using a Nicolet 4094 oscilloscope. Tension was measured by strain gauge transducers as described by Jewell, Kretzschmar & Woledge (1967). Heat production was recorded by thermopiles similar to those described by Hill (1965). For e.l.d. iv muscle experiments an evaporated metal film thermopile (as described by Mulieri, Luhr, Trefry & Alpert, 1977) was used with an active region of 5·8 mm. For sartorius muscle, an electroplated thermopile (Ricchiuti & Mommaerts, 1965) was used with a variable active region which was adjusted to record from approximately 80 % of the muscle. Heat loss was observed by Peltier or by Joule heating (100 kHz). The heat loss was always exponential and heat loss correction was carried out by the method described by Hill (1965). In the case of sartorius muscle, the heat capacity of the part of the muscle from which heat was recorded, was estimated from the wet weight of the muscle, its length and that of the active region of the thermopile. For e.l.d. iv muscle, heat capacity was estimated from the initial slope of Peltier heating (as described by Kretzschmar & Wilkie, 1972, 1975). The heat records were corrected appropriately for stimulus heat where this was not negligible using observations of stimulus heating of muscles made inexcitable by 20 mM-procaine. No correction was made for time lag as it was negligible with the long tetani used in these experiments. Corrections for internal work and thermoelastic heat were not appropriate.

To measure the rate of heat production in the muscles, they were given isometric tetani of 7–10 s duration. To estimate any change in rate of relaxation as tetanus duration increased, the muscles were given 'interrupted' tetani. During this interrupted tetanus, the muscle is given a short period of stimulation (A) followed by a short period without stimulation (B). Both periods (A and B) were kept at a constant duration and repeated, usually 10 times. The length of period A most commonly used was 0·8 s and this was usually just long enough for the muscle to have produced its maximum force during the first period of stimulation. The length of period B was made long enough for the muscle to have relaxed by at least a 5 % drop in force during each period B. This time was usually about 0·4 s.

Measurements of the rates of heat production and of relaxation were made on the same muscle or pair of muscles (usually two observations of each type were made) and the order in which ordinary and 'interrupted' isometric tetani were given to the muscle was varied at random. Muscles were allowed to rest for at least 20 min, bathed in aerated Ringer solution, between tetani.

The effects of previous activity on the amount of labile heat and change in relaxation rate during a tetanus were investigated for sartorius muscle only. Four or five pairs of ordinary or 'interrupted' tetani were given in each experiment, separated by time intervals of 10, 20, 40, 60, 300 and 600 s.

The muscles were bathed in aerated Ringer solution for 20 min between the pairs of tetani. The order in which pairs of tetani for each time interval were given was varied at random. Peltier heating was carried out for each pair of ordinary tetani, in order to estimate heat loss. Heat capacity was estimated as described above.

Muscle weight used for normalization is that of the blotted muscles. The dry weight to blotted weight ratio was found to be $0{\cdot}234 \pm 0{\cdot}007$ ($n = 11$) for sartorius muscle and $0{\cdot}220 \pm 0{\cdot}017$ ($n = 11$) for e.l.d. iv muscle.

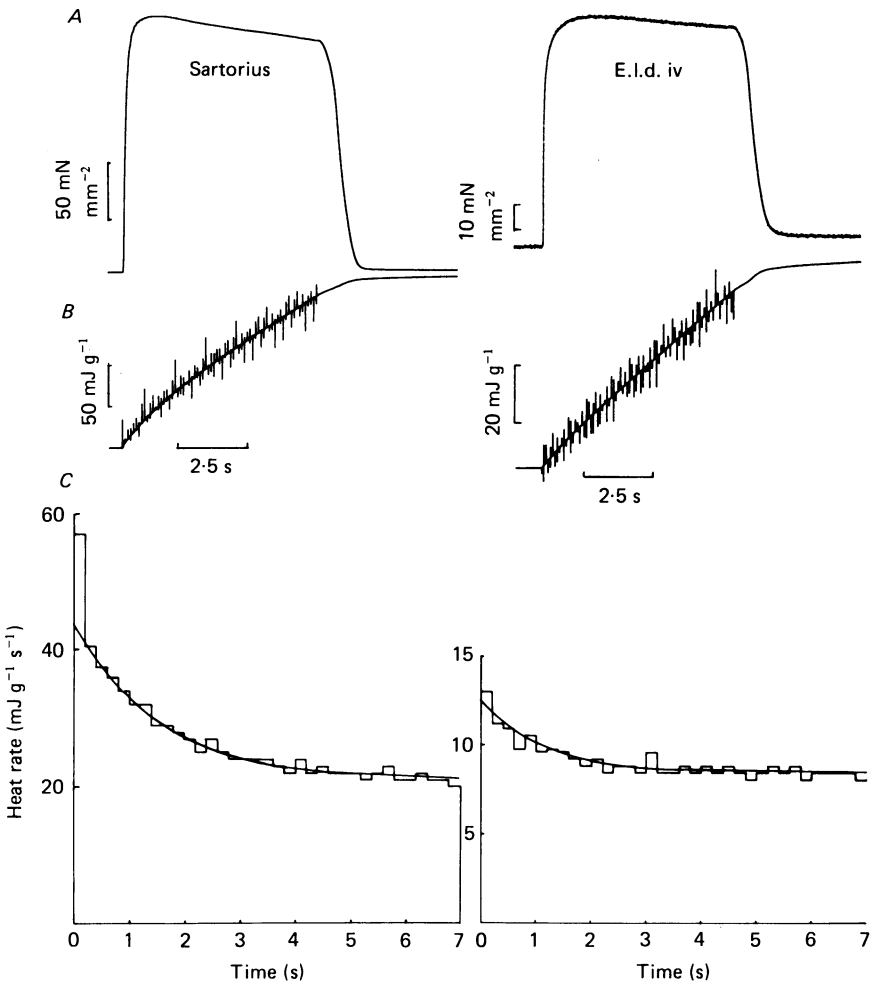

Fig. 1. Analysis of labile and stable heat production: Force (*A*) and heat production (*B*) during tetanic contractions of sartorius and e.l.d. iv muscles. *C* shows heat rate for sartorius and e.l.d. iv muscle during a 7 s isometric tetanus. The full line in *C* represents the heat rate as described by eqn. (1) in the text.

RESULTS

Comparison of sartorius and e.l.d. iv muscles

Fig. 1 shows records of the force and heat produced during isometric tetani of sartorius and e.l.d. iv muscles. The heat produced (H) up to time t has been fitted with

the following equation, derived from that suggested by Aubert, 1956:

$$H = A + h_a \tau_h (1 - e^{-t/\tau_h}) + h_b t, \quad (1)$$

by adjusting h_a (the labile heat rate), τ_h (its time constant), h_b (the stable heat rate) and A (an initial burst of heat production occurring in the first time interval). The total amount of labile heat is $h_a \tau_h$. This equation fits the observations adequately in both muscles, as can be seen by comparing the observed and predicted heat rates (Fig. 1*C*). In these examples, sartorius muscle is seen to produce more labile heat than

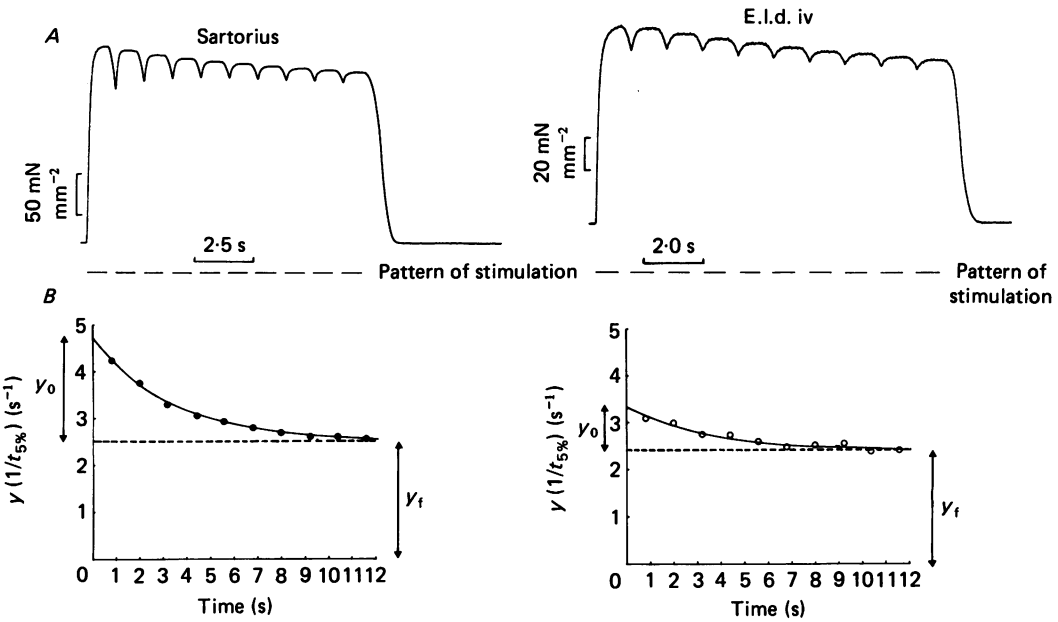

Fig. 2. Analysis of the Abbott effect. *A*, force production during an interrupted tetanus in sartorius and e.l.d. iv muscles. Pattern of stimulation is shown below each tension trace. *B*, the reciprocal of time taken for force to fall by 5 % at the end of each stimulation period ($1/t_{5\%}$) is plotted against the tetanus duration. The full lines represent y as described by eqn. (2) of the text.

e.l.d. iv. The mean, from thirty-two experiments (Table 1), shows that this is typical with little overlap between the values for the two muscles.

An 'interrupted' tetanus for each type of muscle is shown in Fig. 2. As observed by Abbott (1951), the fall of tension becomes progressively slower in each successive period of relaxation. The time required for tension to drop by 5 % during each period ($t_{5\%}$) was measured and the reciprocal of $t_{5\%}$ (y) is plotted against time (t) in Fig. 2*B*. y falls exponentially to a steady value (y_f). These observations have been fitted (as shown) with the equation:

$$y = y_0 e^{-t/\tau_r} + y_f, \quad (2)$$

where y_0 is the initial 'extra' rate of relaxation at $t = 0$ s and τ_r is the time constant for the effect. This equation is analogous to the differentiated version of eqn. (1). It can be seen that y_0 is greater for sartorius than for e.l.d. iv muscle. Mean results in

Table 1 show that this difference is consistently observed and that there is little overlap between the results for the two muscles. These results therefore show a similar trend to those for the labile heat.

The choice of a 5% tension decrement in analysing these results is, of course, arbitrary. The effect of using other sizes of decrement is shown in Fig. 3 in which records for the periods of relaxation are normalized and superimposed on an expanded

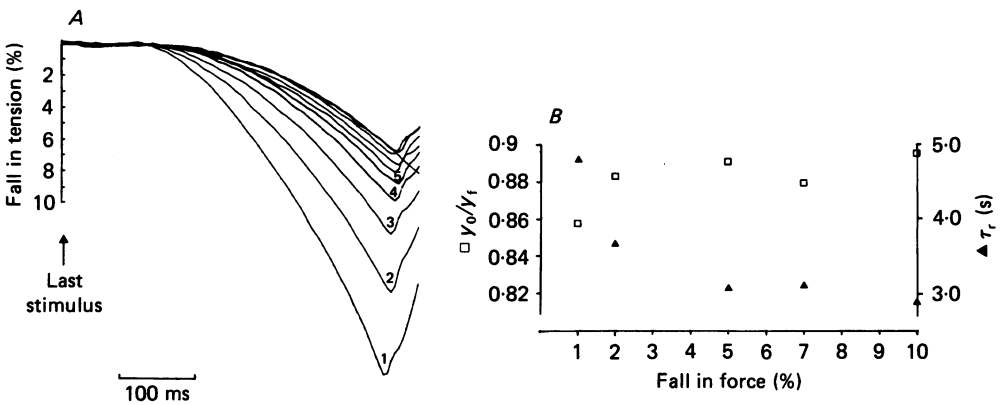

Fig. 3. *A*, records of each relaxation period for an interrupted tetanus in sartorius muscle, normalized and superimposed on an expanded scale. *B*, the relation of the magnitude of relaxation slowing (y_0/y_f) and its time constant (τ_r) for $1/t_{1\%}$ to $1/t_{10\%}$ as a function of the selected percentage fall in force.

scale. The change in relaxation that occurs is in both the delay before tension drops and in the rate at which it declines. Fig. 3*B* shows that the ratio y_0/y_f is almost independent of the selected percentage fall of force, but τ_r is somewhat less for larger values of the decrement. The data could however be described reasonably well using a single value of τ_r. In other experiments y was measured only for a 5% decrement. The relation of these measurements to the rates of the underlying processes will be discussed below.

The analysis of the results for interrupted tetani has used total time from the start of the tetanus as the independent variable. This is appropriate only if the process(es) causing changes in relaxation continue while stimulation is interrupted. To test this point, the two separate experiments illustrated in Fig. 4 were performed. In these experiments tetani were compared in which the length of the periods of stimulation was different but the relaxation periods were the same. The results are more consistent if the y values are plotted against total time (Fig. 4*A* and *C*) rather than the total period of stimulation (Fig. 4*B* and *D*). This suggests that the relaxation slowing processes do continue after stimulation has stopped.

As well as the differences in labile heat and in y_0 already noted, there are other significant differences between sartorius and e.l.d. iv muscles. These are summarized in Table 1. In particular both the normalized force (P) and the stable heat rate (h_b) are less in e.l.d. iv. The ratio of heat to force (h_b/P) is about 10% greater in e.l.d. iv, but this difference is not significant ($P \simeq 0{\cdot}1$). This contrasts with the differences between the muscles in the labile heat ($h_a\tau_h$) which is reduced in e.l.d. iv far more

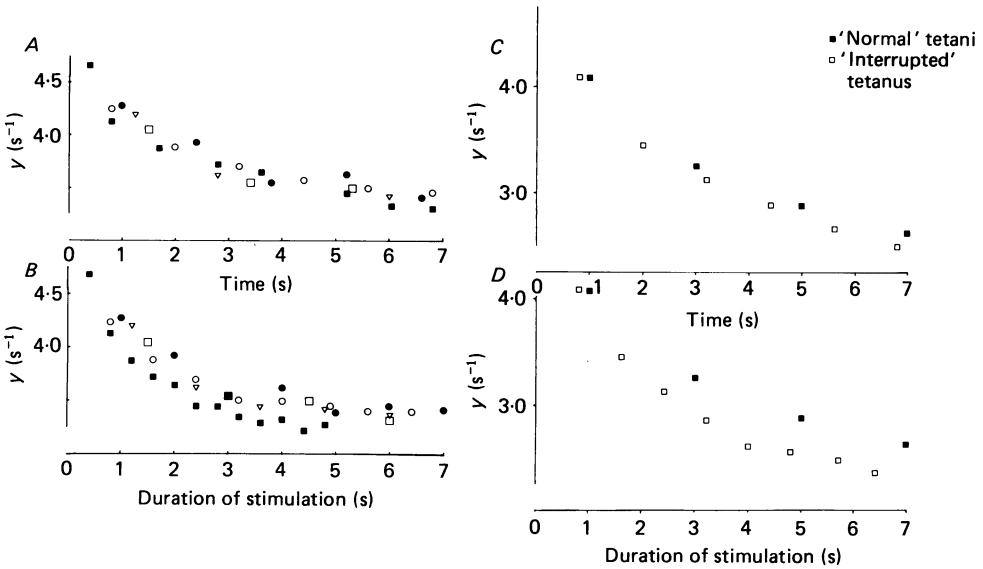

Fig. 4. A comparison of two alternative time bases in experiments with interrupted tetani. In *A* and *B*, five tetani were given to the muscle with period A different for each tetanus (■, 0·4 s; ○, 0·8 s; ●, 1·0 s; ▽, 1·2 s; □, 1·5 s) while keeping period B constant. *A*, shows the results as a function of total time, the correlation coefficient for the best exponential fit to all the data, *r*, is 0·965. *B*, shows the results as a function of total period of stimulation, $r = 0{\cdot}949$. In a second experiment (*C* and *D*) four normal tetani of different duration are compared to a single uninterrupted tetanus with period A at 0·8 s and period B at 0·4 s. *C*, shows the results as a function of total time, $r = 0{\cdot}999$. *D*, shows the results as a function of total period of stimulation $r = 0{\cdot}987$.

than are the tension or stable heat rate; the ratio h_a/h_b in e.l.d. iv is only 55% of the value in sartorius muscle ($P < 0{\cdot}001$). In addition e.l.d. iv muscle sometimes shows a component of the active tension that decays very slowly (Fig. 1). This is due to the presence of tonic fibres (Gray, 1958) and does not occur if the muscles are treated with curare (10^{-5} g m^{-1}). Curare was used in about half of our experiments with e.l.d. iv muscle. No differences, other than the absence of the slow component of the tension were noted.

Since both y_0 and $h_a\tau_h$ are less in e.l.d. iv muscle than in sartorius, it might be expected that the variations about the mean of these two quantities would be correlated within each type of muscle. To test this point, the mean value of y_0 and $h_a\tau_h$ (for each type of muscle) were subtracted from the observed values and the correlation coefficient (r) between the remainders was calculated. The result ($r = 0{\cdot}353$) is significant at the 5% level. Thus there is evidence that the factor that causes y_0 and $h_a\tau_h$ to be less in e.l.d. iv muscle is responsible for some (about 12%) of the variance in these quantities within each type of muscle.

Effect of previous activity on the mechanics and energetics of sartorius muscle

Fig. 5*A* shows force and heat production in two tetani separated by a 20 s interval. The labile heat in the second or 'test' tetanus is less than that for the first or

TABLE 1. Mean values for e.l.d. iv and sartorius muscles: y_0, y_b and τ_r are the mean values for the Abbott effect described by eqn. (2). A, h_a, h_b and τ_h are the mean values for the heat production as described by eqn. (1). P is the peak force normalized per cross-sectional area. S.E. is the standard error. Nineteen observations of each quantity on sartorius and thirteen on e.l.d. iv

	Sartorius		E.l.d. iv	
Quantity	Mean	S.E.	Mean	S.E.
y_0 (s^{-1})	1·89	0·1	0·84	0·11
τ_r (s)	2·51	0·13	3·53	0·61
y_f (s^{-1})	2·59	0·08	2·96	0·12
h_a (mJ g^{-1} s^{-1})	16·5	1·16	6·31	0·82
τ_h (s)	1·90	0·12	1·45	0·11
h_b (mJ g^{-1} s^{-1})	14·7	0·5	11·1	0·9
P (mN mm^{-2})	190·0	6·9	125·9	8·7
h_b/P (s^{-1})	0·078	0·003	0·088	0·005
h_a/h_b	1·09	0·07	0·58	0·06
A (mJ g^{-1})	1·73	0·59	0·72	0·32
$h_a \tau_h$ (mJ g^{-1})	25·8	2·1	8·9	1·4

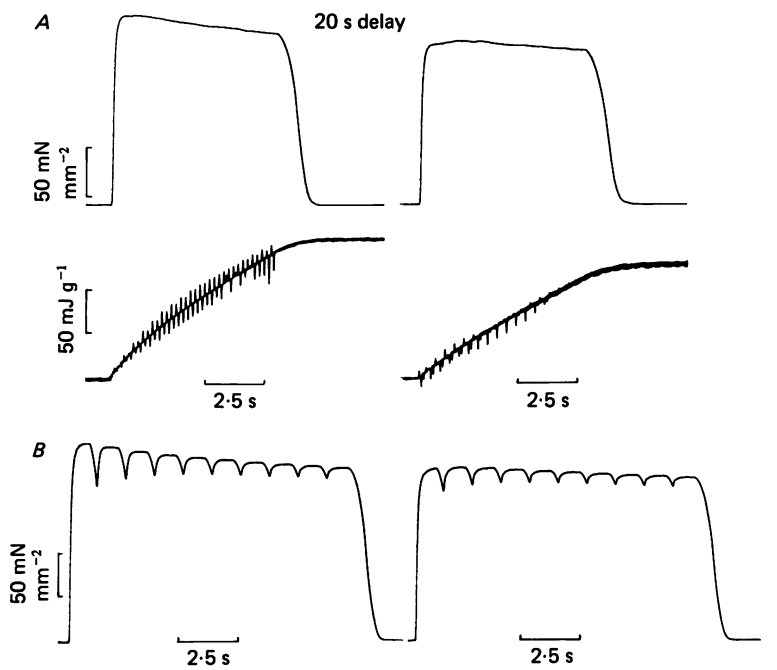

Fig. 5. Effects of conditioning tetani. *A*, force and heat production in two tetani separated by a 20 s interval (sartorius muscle). *B*, force in two interrupted tetani separated by a 20 s interval (sartorius muscle).

'conditioning' tetanus (as observed by Aubert (1967) and Curtin & Woledge (1977)). The heat records for both tetani were fitted by eqn. (1). For this observation, the initial rate of labile heat (h_a) for the test tetanus is only about half that in the conditioning tetanus. The corresponding experiment for a pair of 'interrupted' tetani separated by a 20 s interval is shown in Fig. 5 *B*. The change in relaxation rate is

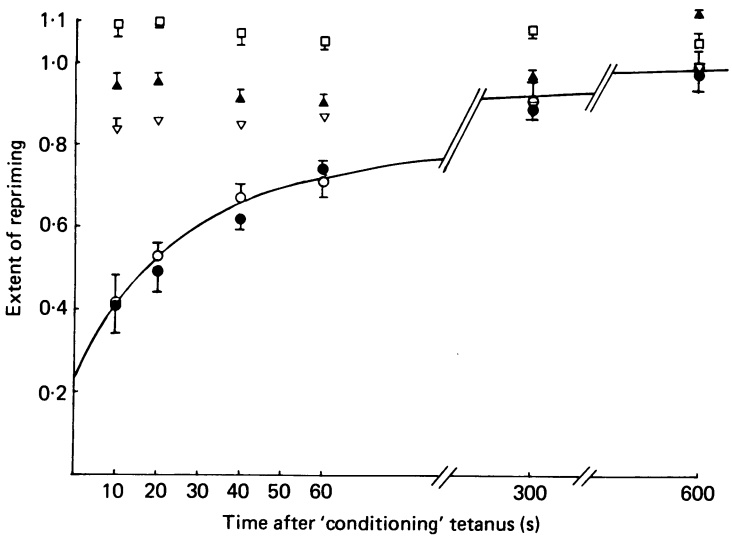

Fig. 6. Time course of repriming of y_0, h_a, P, h_b and h_b/P (see text). Results are shown as means ± S.E. of mean, n is between 4 and 14. ●, $h_{a,2}/h_{a,1}$; ○, $y_{0,2}/y_{0,1}$; ▽, P_2/P_1; ▲, $h_{b,2}/h_{b,1}$; □, $[h_b/P]_2/[h_b/P]_1$.

less in the test tetanus than in the conditioning tetanus. The value of y_0 (obtained as described above, eqn. (2)) in the test tetanus is only about half that of the conditioning tetanus.

An estimate of the extent of repriming of the labile heat is the ratio of the initial rate of labile heat in the test tetanus to that of the conditioning tetanus. That for y_0 can be made similarly. The time courses of repriming for the labile heat and y_0 have been compared in Fig. 6. They are almost identical with no significant difference in the extent of repriming at any of the six time points.

The time course for the extent of repriming (E) of these two effects cannot be adequately described by a single exponential but can be described by an equation with two exponential terms:

$$E = 1-(a\,e^{-t/\tau_1}+b\,e^{-t/\tau_2}), \tag{3}$$

where a and b are constants and τ_1 and τ_2 are the two time constants. For the line shown in Fig. 6, the values for a and b are 0·42 and 0·36 and for τ_1 and τ_2 are 19·5 and 203 s. However, there are other values of the constants in this equation that would give an almost equally good description of the data. In particular, the extent and time course of the slower component of the repriming was not accurately obtained by this experiment because only a few long time intervals were used. It should also be noted that, in these experiments, observations were repeated at intervals of 20 min. If, as seems possible, repriming is not complete in this time, such observations would systematically underestimate the amplitude and the time constant of the slower component of the repriming processes.

Repriming of force and stable heat rate

Fig. 6 also shows the repriming of force (P), of stable heat rate (h_b) and of their ratio (h_b/P). On average, force and stable heat rate are generally reduced in the test tetanus compared to the conditioning tetanus. The ratio of h_b/P, however, remains constant. Force production and stable heat rate gradually recover as the time interval between the test and conditioning tetanus increases. It was also noticed that after the peak, the fall of force was usually greater during the conditioning tetanus than in the test tetanus; that is force was better maintained in the test tetanus.

DISCUSSION

The experiments described in this paper have demonstrated two new connexions between the labile heat and the Abbott effect. Both are less in e.l.d. iv muscle than in sartorius; both 'reprime' after a conditioning tetanus with the same time course. This strengthens the case for a common origin for these two effects. Our working hypothesis is that they are both due to the uptake of calcium ions by parvalbumin. The binding sites on this protein are considered to be largely occupied by magnesium in resting muscle and to become progressively saturated with calcium as a tetanus proceeds (Gillis, 1985). As this occurs the heat rate falls because of the diminishing rate of the exothermic calcium-binding process and the relaxation rate falls because the rate of removal of calcium from the sarcoplasm by parvalbumin is diminishing. This hypothesis is also supported by the following observations.

(1) During a tetanus there is an increase in the total amount of calcium located in the myofibrillar space which is much greater than can be accounted for by increases in free calcium, or calcium bound to the myofibrils (Somlyo, Gonzalez-Serratos, Schuman, McClellan & Somlyo, 1981). This is evidence that, during a tetanus, calcium is binding to either parvalbumin or some unknown calcium binding site in the myofibrillar space.

(2) Observations using aequorin as a calcium indicator (Blinks *et al.* 1978; Cannell, 1983) have shown that the free calcium level does fall more slowly after a long tetanus than a short one. This suggests that the change in relaxation rate is due to changes in calcium uptake rather than (for instance) changes in actomyosin kinetics.

(3) *In vitro* experiments have shown that parvalbumin is capable of removing calcium from myofibrils, and that preparations of fragmented sarcoplasmic reticulum are capable of removing calcium from parvalbumin (Gillis & Gerday, 1977).

(4) Calcium binding to parvalbumin in exchange for magnesium produces heat equivalent to about 50 kJ mol^{-1} protein (Smith & Woledge, 1985). The amount of heat that would be produced by saturation of all the parvalbumin in muscle (0·35 μmol g^{-1}, Gosselin-Rey & Gerday, 1977) with calcium is 18 mJ g^{-1}, which is of the same order as the amount of labile heat in sartorius muscle (20–40 mJ g^{-1}) (see below).

The reason for the smaller extent of the Abbott effect and the labile heat in e.l.d. iv muscle is unknown, but there are three obvious possibilities (not mutually exclusive) which should be tested.

(1) E.l.d. iv muscle might contain less parvalbumin than sartorius muscle.

Although a pilot experiment we have performed (Heizmann, Peckham & Woledge, 1984) did not support this suggestion, it cannot yet be eliminated.

(2) E.l.d. iv muscle might contain a different isoform of parvalbumin having a higher apparent affinity for calcium. It is known that frog muscles contain two forms of parvalbumin and that these forms differ in calcium affinity.

(3) The free calcium level might be higher, or the free magnesium might be lower in e.l.d. iv muscle.

If either (2) or (3) were the case, the proportion of parvalbumin binding sites occupied by calcium in resting muscle would be greater in e.l.d. iv muscle, and therefore there would be fewer sites available to provide the labile heat during a tetanus. In the absence of any evidence to the contrary this possibility certainly cannot be eliminated. To explain the difference between e.l.d. iv and sartorius muscle a 6-fold difference (0·77 log unit) in either the resting $[Ca^{2+}]/[Mg^{2+}]$ ratio or of the apparent affinity of the parvalbumin would be required. (For example the free calcium concentration in e.l.d. iv muscle could be raised to 12×10^{-8} M compared to 2×10^{-8} M in sartorius muscle, a difference barely measurable by current methods.) It should be remembered that parvalbumin in muscle has a higher apparent affinity for calcium than troponin (Gillis & Gerday, 1977). There is thus no reason why a *relaxed* muscle should not contain parvalbumin largely saturated with calcium. Indeed this is presumed to be the situation in muscle immediately after a long tetanus.

Besides the need to determine the underlying nature of the differences between e.l.d. iv and sartorius, our hypothesis faces two further difficulties in explaining the results we have described.

(1) The time constant for the Abbott effect, (τ_r) is significantly greater than that for the labile heat (τ_h) (Table 1).

(2) The relative change, during a tetanus, in the part of the heat rate attributable to calcium uptake is much greater than the proportional change in the time required for partial relaxation, as can be seen from the following considerations.

The stable heat rate is due to splitting of ATP (Curtin & Woledge, 1979) partly by the myofibrils and partly by the sarcoplasmic reticulum (s.r.). The contribution of the latter can be estimated from the sarcomere length dependence of the stable heat rate (Homsher & Kean, 1978) and is about 40% of the total. The initial rate of labile heat in sartorius muscle is 1·1 times the stable heat rate (see Table 1) and thus 2·75 times the heat rate produced by the s.r. calcium pump. In our hypothesis, the heat produced per mole of calcium taken up is 17 kJ for the s.r. calcium pump (Curtin & Woledge, 1978) and 25 kJ (Smith & Woledge, 1985) for parvalbumin. Thus we have to suppose that calcium uptake by parvalbumin at the start of a long tetanus is proceeding at $2{\cdot}75 \times 17/25 = 1{\cdot}9$ times faster than the rate of s.r. calcium uptake. In contrast, the extra rate of relaxation seen at the start of contraction is only 0·73 times the final rate (y_0/y_f from Table 1) and this is not dependent on the extent of relaxation (between 1 and 10%) examined (Fig. 3). These difficulties may be due to the possibility that the Abbott effect reflects only indirectly the changes in the rates of calcium uptake at the time stimulation ceases.

On the very simple assumptions that after stimulation ceases, calcium is removed at a constant rate during the time required for tension to drop by the measured amount, and that only the rate of calcium removal influences the time required for

relaxation, then the quantity y would be proportional to the rate of calcium uptake. In reality, however, the rate-limiting processes during relaxation are likely to be more complex than this. For example, part of the time required for relaxation may be independent of tetanus duration perhaps because it is related to actomyosin kinetics rather than calcium removal. This would have the effect of lengthening the apparent time constant (τ_r) because the effect of the fixed delay would be greater when the variable delay is small. The fixed delay would also mean that a given change in calcium-uptake rate would cause a proportionally smaller change in the time required for relaxation.

To test the plausibility of this idea, we selected nine records of the Abbott effect, each from a different experiment and fitted the results using the equation below:

$$y' = y'_0 e^{-t/\tau_h} + y'_f. \quad (4)$$

This is equivalent to eqn. (2) but the time constant is now fixed at the value of τ_h for the same muscle. The new variable y' is related to the observations of $t_{5\%}$ by the equation:

$$y' = 1/(t_{5\%} - t_s),$$

where t_s is chosen to obtain the best fit. The proportional change, during a tetanus, in the remainder of the time ($t_{5\%} - t_s$) required for the tension to drop by 5% (and therefore in the *total* rate of calcium removal) became, on average, 3·2-fold. Thus, in this model, the *extra* calcium removal process runs initially at 2·2 times that of the steady process, more or less what is required to explain the extra heat rate. The mean value of t_s was 0·18 s, which was about half of $t_{5\%}$. The predicted time course of the Abbott effect still matched the observations very well (mean value of $r = 0{\cdot}99$). Thus, both the difficulties described above could be resolved if it is assumed that about half the total delay in relaxation is due to processes other than calcium uptake. Therefore, a critical examination of the question whether the labile heat is strictly paralleled by changes in the rate of calcium uptake would require the use of a more direct technique to observe the rate of removal of calcium from the myofibrils and the pool of free calcium. At the moment no such technique exists. We conclude that the explanation we offer is plausible but for the moment untestable.

Kinetics of calcium binding and release from parvalbumin

Can the kinetics of calcium binding and release from parvalbumin explain the time course of the labile heat and of its repriming? Simulation of the calcium release and uptake in frog muscle (Cannell & Allen, 1984) has shown that (for a temperature of 20 °C), the parvalbumin takes up calcium during a tetanus exponentially with a time constant of 0·65 s. Assuming a Q_{10} of 3 (Aubert (1956, p.159) gives a Q_{10} of 2·8 for the time constant of the labile heat) this would give a value for the time constant of 2·4 s at 0 °C, similar to that observed. Cannell & Allen did not describe, in their model, the time course with which calcium is removed from parvalbumin at the end of a tetanus. We have therefore run a version of their model in order to study this question using the rate constants given in their paper. As the process of calcium removal from parvalbumin at the end of a tetanus is slow, it is unnecessary in our simulation to consider the diffusion processes involved, nor changes in the concentration of free parvalbumin – that is parvalbumin bound to neither calcium nor

magnesium ions. Our simulation showed that the predicted time course of calcium removal from parvalbumin could well be described as a double exponential:

$$\text{proportion of parvalbumin bound to } Ca^{2+} = 1-a(1-e^{-t/\tau_1})-b(1-e^{-t/\tau_2}),$$

where $a = 0{\cdot}284$, $b = 0{\cdot}422$, $\tau_1 = 1{\cdot}3$ s, and $\tau_2 = 4{\cdot}4$ s. Thus, (1) the repriming process is predicted to be much slower than the development of the effect as is observed. (2) It cannot be described by a single exponential process but has a 'long tail', again as is observed. (3) If the Q_{10} of the recovery process is about 3 (there is little evidence on this point) then the predicted time course of calcium removal from parvalbumin would have a time course similar to the recovery processes observed. Therefore, the kinetics of both calcium uptake and release from parvalbumin might well provide an explanation for the labile heat and its repriming if more precise information about their kinetics at 0 °C were available.

Some of these studies arose from, and are consistent with, preliminary experiments concerning the relation between labile heat and relaxation rate by N. A. Curtin, some of which were reported to the Physiological Society in March 1976. We would like to acknowledge Dr G. Elzinga for the generous gift of the evaporated metal film thermopile used. Michelle Peckham was an M.R.C. research scholar.

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