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ENERGETICS OF ACTIVATION IN FROG AND TOAD MUSCLE

By I. C. H. SMITH

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

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SUMMARY

1. If activation heat reflects the operation of the calcium pump it should be independent of actomyosin activity. The semitendinosus preparation affords a technique for removing actomyosin activity since the muscle can be stretched till there is almost no overlap between the filaments.

2. Heat production, H, in twitches and tetani of stretched muscle fits the relation $H = A + M.P/P_{ot}$ where P/P_{ot} is the fraction of the optimal tension remaining at the stretched length and A and M are assumed to be the activation dependent and actomyosin dependent heat components.

3. For twitches the A component is early and fast and constitutes 0·26 (S.D. 0·09) of the heat production at normal muscle lengths. Its time course is similar in both frog and toad muscle although both M and P are twofold slower in toad muscle. High concentrations of CO_2 slow only M and P_{ot}. The A component is associated with a normal recovery heat.

4. The twitch-tetanus tension ratio, after correction for the extra shortening that occurs during a tetanus, does not vary with the degree of muscle stretch: it is thus probable that twitch activation does not vary with muscle stretch.

5. Moderately hypertonic Ringer solution reduces M and P_{ot} but not A, but strongly hypertonic solution also reduces A. Zn^{2+}, No_3^- and second shock potentiation of a twitch increase A, M and P_{ot} in proportion to each other.

INTRODUCTION

Activation heat has often been thought of as the thermal sign of the movements of calcium out from and back into the sarcoplasmic reticulum (Davies, 1963; Sandberg & Carlson, 1966; Gibbs, Richiutti & Mommaerts, 1966; Bendall, 1969; Kushmerick, Larson & Davies, 1969). However Mommaerts (1969) and Woledge (1971) have pointed out that the various measurements of activation heat probably include a significant proportion of heat from actomyosin activity as well as the heat associated with the calcium activation process. On the basis of current ideas concerning the

activation process the estimated energetic cost of calcium pumping is surprisingly high. Assuming the stoichiometric efficiency of this pump to be 2 moles of calcium pumped per mole ATP (Weber, 1966), about 20% of the observed chemical break-down in an isometric twitch would be required to pump the amount of calcium which just activates the contractile machinery (Ebashi, Endo & Ohtsuki, 1969).

In frog semitendinosus muscle actomyosin activity can be all but eliminated by stretching the muscle till the overlap between the actin and myosin filaments is very small. This paper, and the adjoining one – an independent but similar study by Homsher, Mommaerts, Richiutti & Wallner (1972), exploit this fact which allows activation heat to be separated from the effects of actomyosin ATPase. Preliminary results have already been presented (Smith, 1970).

METHODS

Semitendinosus and sartorius muscles of the frog (*Rana temporaria*) and the semitendinosus of the toad (*Bufo bufo*) were used. The frogs were kept, unfed, in a cold store (about 2° C) and the toads were kept at room temperature. The dorsal part only of the semitendinosus was used; the length of the muscle fibres was about 13 mm, the proximal tendon (which was left attached to the pelvis) was about 3 mm and the distal tendon about 10 mm. The muscles were bathed at intervals in oxygenated unbuffered Ringer solution (NaCl: 115·5 mM, KCl: 2·5 mM, $CaCl_2$: 1·8 mM). All experiments were performed at 0° C.

Length and tension were recorded with a strain gauge lever (Jewell, Kretzschmar & Woledge, 1967) to which the muscle was connected by a glass rod. Length, tension and heat were recorded simultaneously on a Devices pen recorder and the data were also stored on magnetic tape for subsequent digital analysis. The compliance of the tension recording system was smaller than that of the muscle: for an average semitendinosus muscle (force developed, 250 mN or 100 mN.mm^{-2}) the system was stretched by 0·1 mm, the muscle's internal compliance was stretched by 0·18 mm (as estimated from the results of Jewell & Wilkie (1958) for sartorii) and the extension of the semitendinosus tendons was found to be less than 0·02 mm. Thus the total mechanical work done in an average 'isometric' contraction was about 0·8 mJ per gram of muscle: this is about 8% of the twitch heat and it was recorded as a heat produced during relaxation when the stored energy was degraded in the muscle.

Heat production was measured as a temperature change with a chromel-constantan thermopile, 47 mm long, having connexions to every sixth couple (about every 5 mm) allowing records to be made from selected regions of the pile. The equivalent half-thickness (Hill, 1965) of the pile, including the evaporated gold electrodes over its surface, was about 17 μm. The current from the thermopile was recorded with a galvanometer amplifier; its response to a 50 Hz current was about half its DC response.

The total initial heat production was measured about 2 sec after the last stimulus; there was no further change during the following 30 sec. The semitendinosus muscle is cigar-shaped and was found to have a smaller heat production at its ends compared with its middle. The thermopile was arranged to record from most of the muscle, so that errors arising from this non-uniformity were not significant.

Capacitor discharge stimulation (time constant about 0·05 msec) of nerve twigs,

i.e. of normal excised muscle, was used so as to minimize the stimulus energy. Preliminary studies had indicated that nerve-twig stimulation was reliable; neither the twitch and tetanic tension, nor their length dependence, were affected by changing to direct stimulation after addition of 20 μg. ml.$^{-1}$ curare to the wash solution. The stimulus used in each experiment was chosen to give a just maximal response at short muscle lengths. In a stretched muscle the just maximal stimulus was 10 % smaller, presumably because the muscle was then thinner.

With the gold stimulation electrodes practically all the stimulus energy was converted into heat. This was usually less than 5 % of the activation heat (i.e. less than 1 % of the normal twitch heat) and was subtracted from the observed heat production. Since most of the stimulus current flowed close to the surface of the muscle the stimulus heat observed just after the stimulus was much greater than its final value. The heat artifact thus made it difficult to obtain measurements of the latent period before muscle heat production started. However, if a measure of the latency alone was required, the heat was recorded from part of the muscle and it was stimulated in a separate region. The electrodes above the recording region could be used to simultaneously record the action potential.

The muscle was stimulated regularly (every 60–90 sec) and was washed for about 20 min before each series of 10–15 twitches. When the Ringer solution was modified by the addition of sucrose, nitrate or zinc the soaking period was increased to 45 min to ensure complete diffusion into the centre of the muscle. At least four or five series could be made with each muscle before any lack of repeatability became apparent. An interlaced sequence of length changes in each series was used, this confirmed that there was no fatigue. The first twitch in each series was disregarded as it was often anomalous, heat production being larger than in subsequent twitches.

Linc-8 computer programmes were developed to correct the heat records for thermal lag, using Hill's (1965, p. 314) method of analysis by factors, and for heat loss. The programmes measured peak tension development and enabled the exponential decay constant of any part of the record to be estimated. Records could be plotted in parametric series. The results were also corrected for the thermal mass of the heated portion of the thermopile (about 10 % of the muscle's thermal mass) and for the specific heat of the muscle (Hill, 1965).

RESULTS

The length relationship

Fig. 1 shows how muscle heat and tension varied in an isometric twitch according to the muscle's length. The heat production was at a maximum (H_0) at the length (l_0) for optimal twitch tension development (P_{0t}). At muscle lengths less than l_0 the tension decreased steeply to zero although shortening of the muscle was still visible. The heat production decreased only slightly. It was very rapid and terminated abruptly; both these features are characteristic of twitch contractions in which shortening heat is a major part of the total heat produced. At muscle lengths greater than l_0 the heat production decreased less rapidly than did tension. The early fast phase of heat production decreased only slightly; most of the decrease was in the later phase. A regression diagram of total initial heat against tension (e.g. Fig. 5) showed a linear relationship for each series; this was

characterized by the equation: $H = A + M.P/P_{ot}$, where H is the heat production for a given tension P. The tension-independent and tension-dependent components (A and M) were estimated by least-squares regression analysis, assuming all the errors to be associated with the heat production. The linearity was so high (the correlation coefficient was usually better than 0·99) that the best estimate of A (the zero tension intercept of the heat axis) would be very little affected if all the errors were in the tension measurement. The decrease of tension was linear with stretch and

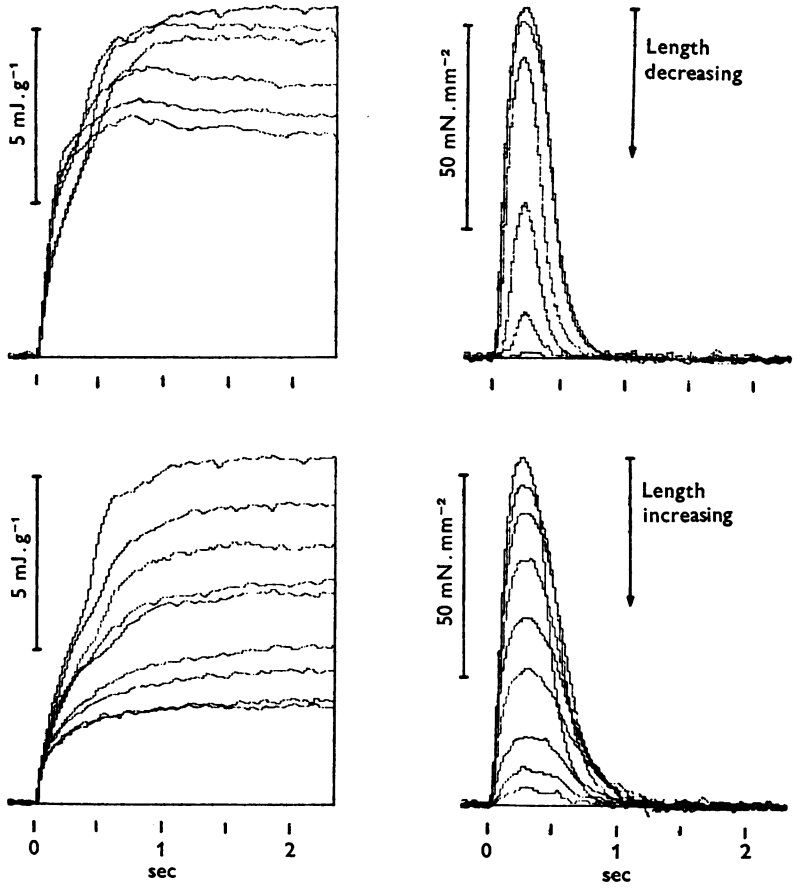

Fig. 1. Records of heat production (left-hand diagrams) and tension development (right-hand diagrams) at various muscle lengths. Measurements were for 1 mm length steps for lengths shorter than the optimal in the upper set of traces and for lengths longer than optimal in the lower set. Heat records have been corrected for heat loss and heat flow lag and plotted in 20 msec steps. *R. temporaria* (5 m J = 1·2 mcal; 50 mN.mm^2 = 0·51 kg.cm^{-2}).

the length at which the tension would become zero was about $1{\cdot}7\ l_0$. This is close to the figure found by Gordon, Huxley & Julian (1966*b*) for single fibres ($1{\cdot}74\ l_0$).

Mean values, for all experiments, of the tension-independent heat (expressed as a fraction of the total heat) and of the optimal twitch tension are

$$A/H_0 = 0{\cdot}26 \text{ (S.D. } 0{\cdot}09),$$
$$P_{ot} = 99 \text{ (S.D. } 38) \text{ mN.mm}^{-2} \quad (1{\cdot}01 \text{ kg.cm}^{-2}),$$
$$H_0 = 9{\cdot}9 \text{ (S.D. } 3{\cdot}7) \text{ mJ.g}^{-1} \quad (2{\cdot}4 \text{ mcal.g}^{-1}),$$
$$n = 43.$$

The extreme values of the ratio A/H_0 were 0·13 and 0·62. The latter figure was abnormal since the next largest was 0·43. Included in the average are seven experiments with the toad semitendinosus muscle and four with the frog sartorius. There was no significant difference between these groups. The accuracy obtainable with the sartorius was poor due to the relative inextensibility of this muscle which involved a long extrapolation to estimate A.

The limit of stretch was set by the criterion that all measurements should be repeatable: preliminary experiments showed that this criterion could be met if the resting tension in the stretched muscle was not allowed to exceed the maximum twitch tension. At this length (l_s) the active tension was reduced to $0{\cdot}25\ P_{ot}$ in the case of sartorius muscle and to about $0{\cdot}05\ P_{ot}$ for the semitendinosus muscle.

Stretch also changes the relaxation rate (Jewell & Wilkie, 1960). This rate was measured with the computer by fitting an exponential to the central region of relaxation. The change of relaxation rate with length, measured as the ratio of the rate at l_s to that at l_0, was variable (0·4–0·8 for semitendinosus and 0·15–0·25 for sartorius preparations). This ratio showed no significant correlation with the fraction of tension-independent heat (A/H_0); it is therefore unlikely that the change in relaxation rate is associated with a change in the activation process.

Analysis of variations

Using results from different muscles the tension-dependent heat component, M, was shown to be proportional to the optimal twitch tension, P_{ot}, over the wide range of tensions observed (correlation coefficient 0·80, $n = 43$, intercept not significantly different from zero). This relationship reinforces the interpretation that M measures the tension-producing reactions. In contrast, the activation component of the heat production, A, showed only a poor correlation (coefficient 0·24) with the optimal twitch tension. This lack of correlation can in part be explained by variations in

the tension developed from one batch of frogs to another. In Table 1 below the results have been divided into three groups according to the time of year in which the experiments were performed. The dates for the second group (mainly winter frogs) were chosen on the basis of the poor twitch tensions developed by this group. It can be seen that the change in the twitch tension was reflected by a proportional change in the M heat component but there was no significant difference in the A heat component between the groups. Thus the difference in tension is due not to a change of the degree of activation, but to a change in response to the activation. The effect is like that described for a small increase in tonicity of the Ringer solution (p. 591). Changes in optimal twitch tension within any one group showed the same degree of correlation with the A as with the M heat component, for example, in the first group the correlation coefficient of A to P_{ot} was 0·71 and that of M to P_{ot}, 0·78.

Time course of the activation heat

The time course of the activation heat component, A, can be estimated only from the heat production in fully stretched muscle, H_s. The M component of H_s was small, typically 13 % of the total, and has been ignored in studying the time course. The heat production (Fig. 2) has a latency of 30–40 msec from the stimulus, a fast rise and an exponential tail. There was a large variation of the time course, perhaps caused by a few damaged fibres on the surface of the muscles. One of the faster, and probably more reliable, records has been analysed for lag in the heat flow and in the galvanometer and for heat loss (lower diagram, Fig. 2). The corrected curve can be described as the sum of two exponentials; a fast phase (30 % of the heat) with a half-time of 30 msec and a slow phase (the final 70 % of the heat) with a half-time of 150 msec. Similar results are described by Homsher *et al.* (1972).

The inset of Fig. 2 shows a compound action potential recorded from the surface of a toad muscle. The velocity of the action potential was about 0·36 mm.msec^{-1} and its latency showed that about 25 msec of the

TABLE 1. Seasonal variation in M heat component

Date of experiment	n	P_{ot} mN.mm^{-2} S.E.	M mJ.g^{-1} S.E.	A mJ.g^{-1} S.E.	M/P_{ot} S.E.
June 1969–Nov. 1969	16	123 ± 10	9·28 ± 0·99	2·30 ± 0·21	0·074 ± ·005
Dec. 1969–Apr. 1970	14	85 ± 8	5·98 ± 0·54	2·51 ± 0·25	0·072 ± ·005
May 1970–Nov. 1970	13	92 ± 7	6·77 ± 0·29	2·76 ± 0·38	0·077 ± ·007

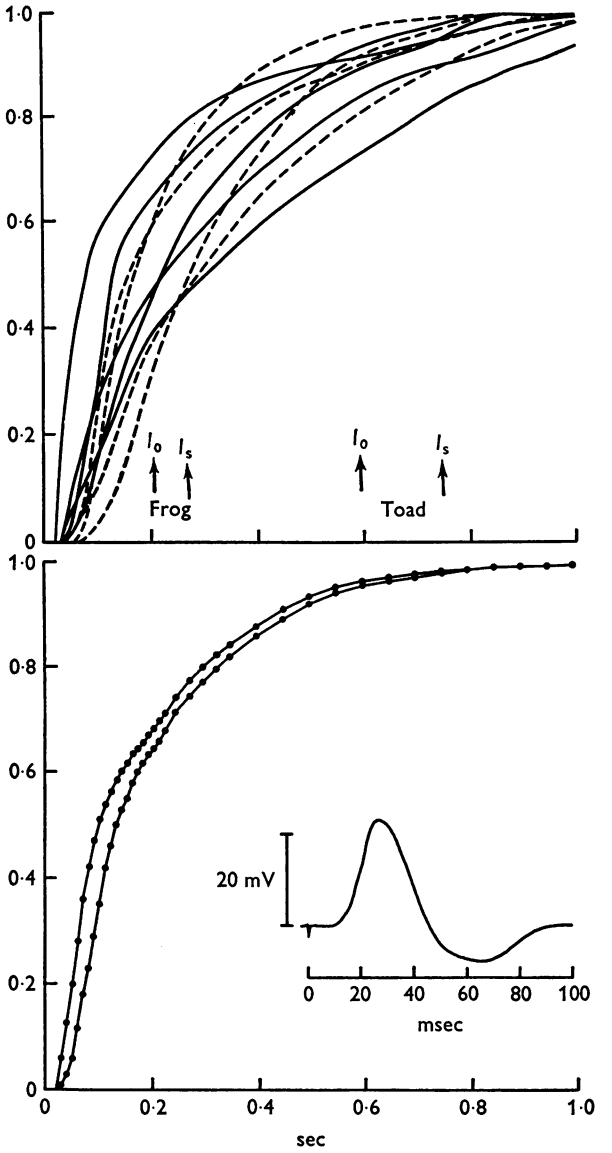

Fig. 2. Upper diagram; time course of heat production in stretched muscle of both frog (continuous lines) and toad (dashed lines). A single section of the thermopile, remote from the stimulating electrodes, was used in order to avoid any stimulus heat artifact. The records have not been corrected for heat loss or for lag due to the galvanometer or heat flow. Lower diagram; the effect of making these corrections can be seen from the typical record and its corrected version. Inset: a diphasic action potential recorded from the surface of a toad muscle at the same region as for the heat record. Average times for the development of peak tension at normal and stretched lengths are indicated on the upper diagram.

35–40 msec latency in heat production can be ascribed to the propagation delay. There was no detectable difference (resolution 5 msec) in the latencies of heat production at different muscle lengths.

Activation heat had the same time course in both frog and toad muscle (Fig. 2; compare also Fig. 1 with Fig. 3). Since the other form of heat production at normal length and the time course of tension change are twice as slow in toad as in frog, this result provides a clear indication that activation heat is due to a reaction not directly associated with tension development.

The time course of the tension-dependent heat was slowed by CO_2. On replacing pure oxygen by 50 % CO_2/ 50 % O_2 the heat and twitch tension decreased and became slower with successive shocks till the time to peak tension in a frog muscle had increased threefold to 750 msec. The activation heat, although reduced in size, showed no change of time course, half the heat being produced in 160 msec. Thus this result also suggests that activation heat and the tension-dependent heat are caused by separate reactions.

Twitch-tetanus tension ratio

The analysis of twitch heat as $H = A + M.P/P_{ot}$ implicitly assumes that the activation reaction is independent of length. Some support for this assumption can be obtained from studying the variation of the twitch-tetanus tension ratio with length. If the fused tetanus is assumed to represent the fully activated state a change of this ratio with length should indicate a change in degree of activation in the twitch. In six experiments the ratio was measured at all lengths. It had a maximum value of 0·63 (S.D. 0·06) at l_0 and showed a shallow but progressive decline with stretch. At maximum length, l_s, the ratio was 0·40. This low value was probably due solely to a shortening of the ends of the stretched muscle at the expense of its central region (Gordon, Huxley & Julian, 1966*a*). This presumably caused the slow increase in tension which was observed during a tetanus in stretched muscle. After correction for this effect by extrapolating the record backwards, the twitch-tetanus ratio no longer showed any significant change with stretch.

In contrast, at lengths less than l_0 the ratio declined steeply to zero; a substantial tetanic tension remaining even when the twitch had vanished. This was due to the muscle not being at the same internal length for twitch and tetanus. A muscle cannot be constrained at short lengths (Brown, Gonzalez-Serratos & Huxley, 1970), it must first shorten down on to the isometric stop and the time taken for this reduces the size of the twitch.

Recovery heat of the activation process

At 0° C recovery heat is produced too slowly to be measured easily so an experiment was made at room temperature (21·2° C) with toad muscle. The muscle was stimulated at 20 Hz for 3 sec at normal length and for 8 sec at stretched length so that the initial heats were of similar size. The ratio of recovery heat to initial heat at normal length was 0·94 (two observations) and was 0·83 (S.D. 0·11, $n = 5$) at the stretched length. Since 60 % of the initial heat at the stretched length was activation heat it is probable that this form of heat is followed by a normal amount of aerobic recovery heat.

Activation heat in a tetanus

Results obtained in the activation heat in a tetanus were in general agreement with the more comprehensive study reported in the adjoining paper (Homsher *et al.* 1972). The tension-independent and tension-dependent heat components for the steady heat rate produced in a tetanus occurred in the same ratio as that for twitches. In contrast the extra heat produced during the first 2 sec of a tetanus (labile heat, Aubert, 1956) consisted mainly of tension-independent heat.

Other measurements of 'activation' heat

The results described in this section are intended to show that all previous methods of measuring activation heat include a proportion of actomyosin-dependent heat production. The method of subtraction of shortening heat in an isotonic contraction (Hill, 1949) gives inconsistent results and is conceptually unsound (Woledge, 1971). With the method of preshortening (Gibbs *et al.* 1966) shortening heat was found to be produced. There may also be some deactivation (Taylor & Rüdel, 1970) at short muscle lengths. These two factors may, by chance, cancel but this does not always happen; for example, in Fig. 1 the heat in twitches with tension at a short muscle length is considerably greater than at long lengths.

Hypertonic Ringer solution

Hill (1958) showed that when muscle was exposed to a Ringer solution with a tonicity three times that of normal Ringer solution there was no twitch tension developed although the early fast phase of heat production remained. In Fig. 3 the upper traces (normal length) show similar results except for a small tension development even at high tonicity. However, as the muscle was stretched (lower curves) the heat production was even further decreased. A regression diagram of this effect is seen in Fig. 5 and Fig. 4*a* provides a summary of nine experiments using five muscles. The

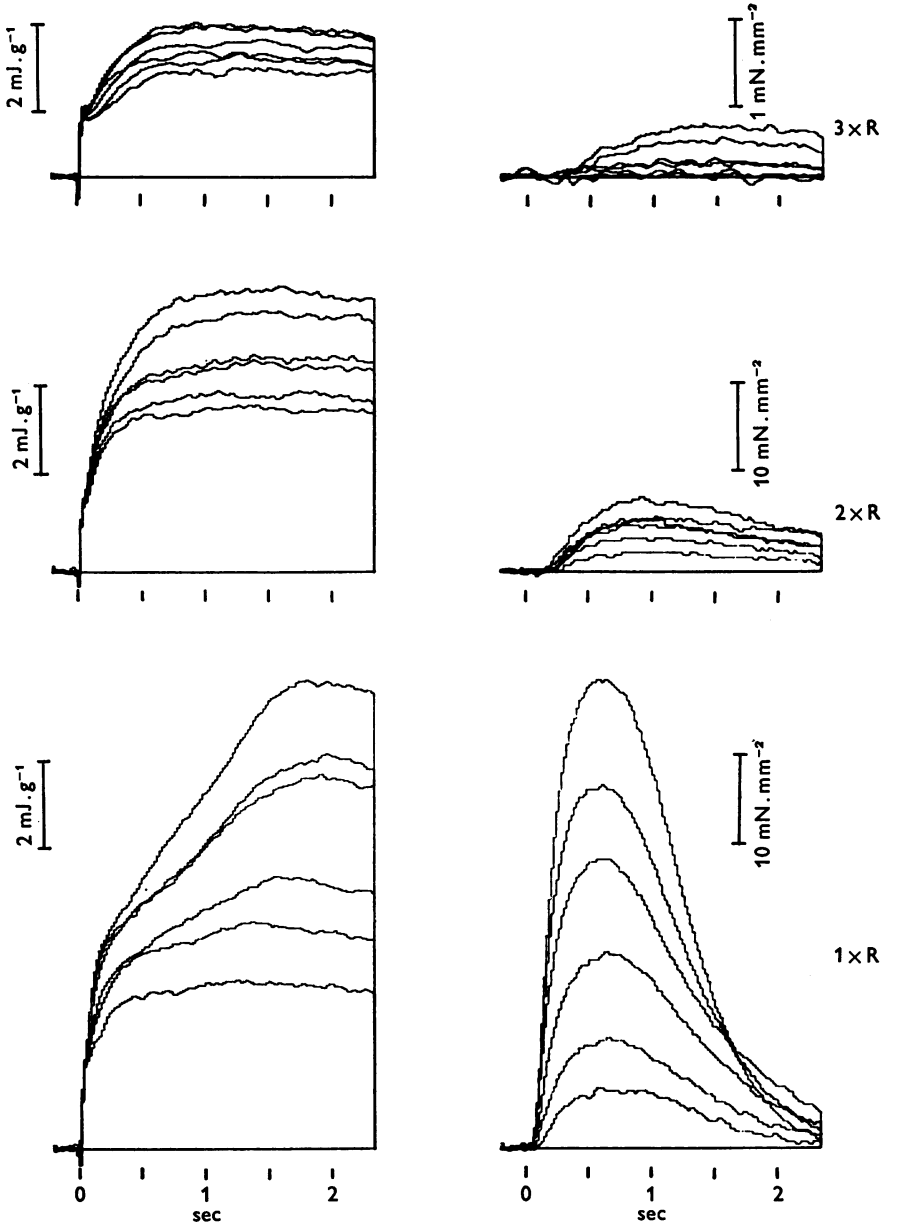

Fig. 3. Effect of hypertonic Ringer solution on heat production (left-hand diagrams) and tension development (right-hand diagrams) at muscle lengths greater than that for optimal tension development. Measurements were made in 1 mm length increments starting from top trace at l_0. Corrections for heat loss have been made in 20 msec steps. Bottom set of records were made with the muscle exposed to normal Ringer solution, the middle set with two times and the upper set with three times normal tonicity after addition of sucrose (2 mJ = 0·48 mcal; 10 mN.mm^{-2} = 0·102 kg.cm^{-2}).

heat productions have been corrected for the change in muscle weight (20% decrease) under hypertonic conditions (Dydyńska & Wilkie, 1963).

Tension-dependent heat (M) and tension development (P_{ot}) decreased steeply with increasing tonicity, activation heat (A) also decreased but to a lesser extent. Thus in an experiment at the same tonicity as that used by Hill (1958) (three times normal) the heat at normal length had decreased to a quarter of its value in normal Ringer solution. Two thirds of this heat

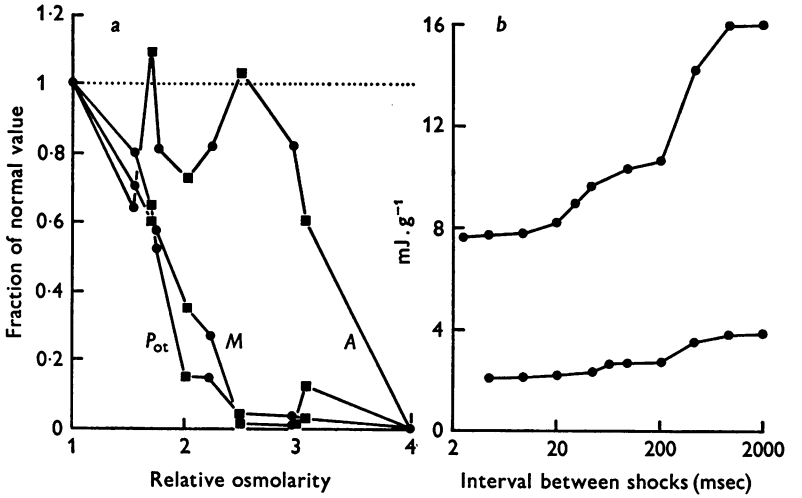

Fig. 4*a*. Effect of hypertonicity on optimal twitch tension development (P_{ot}), the activation heat component (A) and the actomyosin heat component (M). The values, collected from several muscles, are expressed as a fraction of their value in normal Ringer solution. ● *R. temporaria*, ■ *B. bufo*. *b*. Effect of interval between two shocks (log. scale) on heat produced at normal (upper line) and stretched (lower line) muscle lengths. *R. temporaria* (4 mJ = 0·96 mcal).

could be attributed to the activation reaction and one third to actomyosin activity; both A and M components had been reduced. Thus hypertonic solutions do not offer a reliable technique for measuring A.

Second shock technique

Gibbs *et al.* (1966) have reported that a plateau is reached in the amount of extra heat produced by a second shock when the interval is in the range 200–300 msec. They assumed that this plateau measured a second activation which was the same size as the first and, after correction for the extra tension developed, free from the effects of actomyosin activity.

The stretch technique allows both these assumptions to be tested. In two out of four experiments of this type a plateau effect was observed for

an interval of about 200 msec. However, in these two the activation process, measured as the heat produced in stretched muscle, also showed a plateau at a similar time (Fig. 4*b*). In all experiments the heats at normal and stretched length increased in proportion as the shock interval is increased. With an interval of 100 msec, a time corresponding to both the

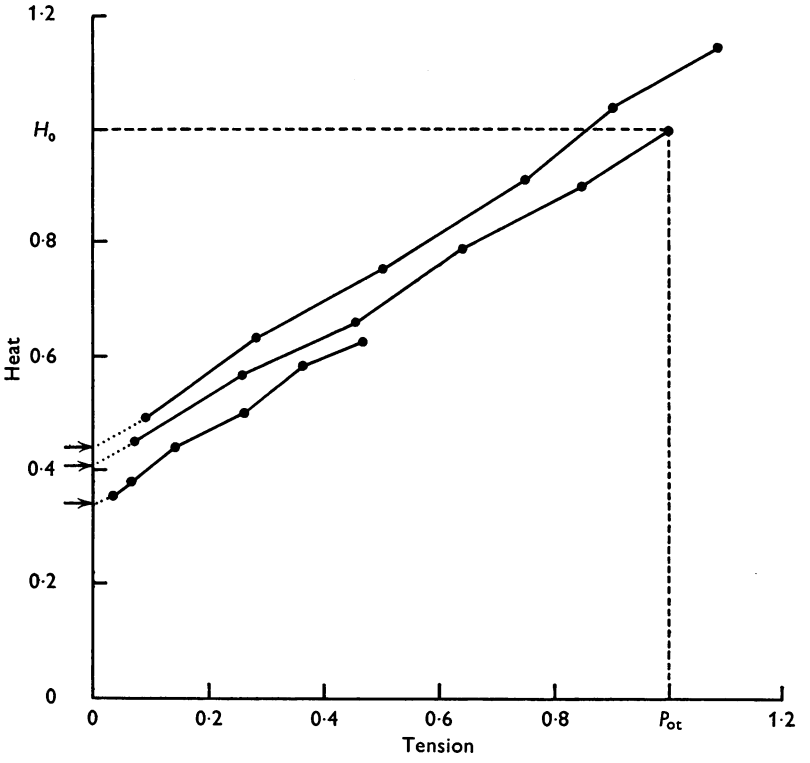

Fig. 5. Regression diagram of total heat against twitch tension for muscle lengths greater than l_0. Middle line, muscle exposed to normal Ringer solution. Upper line, effect of potentiation by nitrate Ringer solution (70 % NO_3', 30 % Cl'). Lower line, effect of sucrose hypertonic (1·75 times normal) Ringer solution. The arrows indicate the best estimate of the zero tension intercepts, i.e. the activation heats, of these lines. *R. temporaria*. H_0 = 5·9 mJ.g^{-1}, P_{ot} = 70 mN.mm^{-2}.

plateau time and to the tetanus fusion frequency, the activation process had recovered by 0·30 (S.D. 0·06, n = 4) and the actomyosin activity had been augmented by a similar amount. Thus the two stimuli method of Gibbs *et al.* (1966) does not in fact measure the heat produced by the activation process.

Potentiation of the activation heat

Both types A and B potentiators (Taylor, Preiser & Sandow, 1969) are assumed to be effective by increasing the calcium release from the reticulum and thus increasing the amount of actomyosin activity. Measurements of the activation heat as well as twitch tension allows each of these processes to be estimated separately. The regression diagram of heat and tension in stretched muscle (Fig. 5) shows that nitrate potentiation did not alter the slope of the regression (i.e. the actomyosin per unit tension) but the zero tension intercept (A, the activation heat) changed. Similar results were obtained using zinc potentiation (0·05 mM-Zn^{2+}) (14 % increase in twitch tension).

These small changes in activation could not be measured with much accuracy. A more pronounced 'potentiation' was obtained using a two shock stimulus (interval 100 msec) where both the 'twitch' tension and the activation heat were potentiated by about 45 %, again the slope of the regression line was almost unaltered (6 % decrease). Using the results from all three methods of potentiation the fractional increase in the activation heat was about the same as the fractional increase in tension. In contrast deactivation by hypertonicity decreased actomyosin activity much more than the degree of activation.

DISCUSSION

In stretched muscle the heat production (H) has been divided into tension-independent (A) and tension-dependent (M) components, $H = A + M.P/P_0$. The hypothesis that M represents actomyosin activity and that A, the activation heat, represents a separate reaction is supported by: (*a*) the linear decrease of heat production with stretch, which agreed with the variation predicted by the sliding filament theory, i.e. with the decrease of actomyosin overlap; (*b*) the time course of A was not dependent upon the time scale of M and of tension changes, the latter two being slower after exposure of the muscle to CO_2 and in toad muscle compared with frog muscle; (*c*) in mildly hypertonic Ringer solutions P_0 and M were much reduced in size whereas A was only slightly reduced; (*d*) in contrast twitch potentiators increased both A and M in proportion to each other.

The energy for the activation heat, which was shown to be followed by a normal aerobic recovery heat, probably comes from the activity of the reticular ATPase in its role as the calcium pump. The size of A, 25 % of the normal twitch heat, was close to the energy requirement of the calcium pump estimated earlier as at least 20 % of the twitch energy. Some energy may also be used in stretched muscles by the myosin ATPase, even though the myosin is no longer actin-activated. Indeed, Huxley (1971) has reported

a change in the X-ray diffraction of myosin filaments under these conditions, but the increase in activity is unlikely to be significant because of the much higher binding power for calcium of troponin and the reticulum compared with myosin (Ebashi *et al.* 1969). Likewise any increase in mitochondrial activity with increased calcium concentration (Chance, 1965) is probably small.

Length dependence of the activation heat

It has already been argued that the constancy of the twitch-tetanus tension ratio for muscle lengths greater than normal suggests that activation is not allowed by stretch. Furthermore, with Ringer solutions of high tonicity, in which the M component of heat production is small even at normal lengths, there was no evidence that stretch altered the size of the activation heat. Although the change in the rate of tension relaxation with stretch might be argued to indicate a change of activation, there was no correlation in different experiments between the change in relaxation rate and the size of A. In the experiments of Homsher *et al.* (1972) using *R. pipiens* there was no change in the relaxation rate with stretch. There may be a length dependence of activation for sartorius muscle: although normal activation heats were recorded in the four experiments reported in this paper, Fales (1969) using gradient layer calorimetry reported that tension-independent heat in a tetanus was probably very small. However, the repeatability of their measurements was insufficient to establish definite conclusions.

Activation heat in a tetanus

The burst of heat after the first shock in a tetanus corresponded roughly to the twitch activation heat and, as foreseen from several other experiments (see Woledge, 1971), the labile heat was found to consist mainly of the activation heat component. The labile phase occurs during the progressive decline of activation from 1/3 (after the 2nd shock) to 1/9 (after each shock in the steady state) of the activation in a twitch. However, when this steady state was reached the maintenance heat was neither entirely activation heat (cf. Hill's (1949) concept of maintenance heat being 'nothing more than the summated effect of the activation heat produced in response to each of a series of shocks') nor was it entirely tension-dependent heat (Aubert, 1956, 'the steady heat production tends towards zero at very stretched lengths'). About 25 % of it was activation heat, the same fraction as for twitches. In some experiments the regression line of labile heat against tension was not straight; it seems likely that this was due to mild stretch-deactivation.

Direct source of the activation heat

Activation heat, like the calcium transient (Jöbsis & O'Connor, 1966), is a short lasting event occurring early in the twitch, yet it has been assumed here that A measures the work done by the calcium pump during the relaxation process. Either one must assume, as Ashley & Ridgeway (1970) suggest, that calcium is pumped back early in the twitch after having briefly set the contractile system into an active state, or that the activation heat is produced by other processes which are in turn dependent on the ATPase activity of the calcium pump. The present results do not allow discrimination between these hypotheses but it seems likely that intermediate sources of heat production do exist. The heat of the unbinding of calcium from the reticulum may be important since probably more than 95% of reticular calcium is in bound form (Ogawa, 1970). Coleman (1952) has measured the heat evolved when $2K^+$ replace Ca^{2+} on an ion-exchange resin as 11 kJ.M^{-1} (2·6 kcal.M^{-1}; a binding heat of this size in a muscle twitch would correspond to about 50% of the activation heat. The cations displaced when calcium ions bind to the troponin system would, if they were hydrogen ions, produce a buffer heat of about 70% of the activation heat (Walsh & Woledge, 1970). In both cases the reactions would be reversed during the phase of active pumping. The buffer reactions might be associated with the early acidification observed in muscle (Jöbsis, 1968). The acidification cannot be caused by the splitting of ATP (Distèche, 1960) since there is no net splitting of ATP early in a contraction (Gilbert, Kretzschmar, Wilkie & Woledge, 1970).

Stoichiometry of the activation heat

The phosphocreatine break-down (Homsher *et al.* 1972) and recovery heat production in stretched muscle indicates that the A:M ratio (range 1:2 to 1:4 in both twitch and tetanus) measures the ratio of reticular to actomyosin ATPase activities. The former is believed to act as a calcium pump with an efficiency of 2 moles calcium moved per mole of ATP used (Weber, 1966), whilst the latter has been described as a cross-bridge movement for each ATP used. In these terms my experiments indicate a range of one to two cross-bridge movements occurring for each calcium ion pumped. This close agreement had been expected; indeed it would be somewhat foolish for a muscle to specialize to contract quickly and then to be unable to relax at a comparable rate. Since the work done in pumping calcium is, in terms of external work done, wasted energy, it is also reasonable for the ratio of calcium turnover to cross-bridge turnover to be somewhat less than unity: this is the experimental finding.

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