TENSION AND HEAT PRODUCTION DURING ISOMETRIC CONTRACTIONS AND SHORTENING IN THE ANTERIOR BYSSUS RETRACTOR MUSCLE OF *MYTILUS EDULIS*

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SUMMARY

1. Tension and heat production were measured during phasic isometric contractions and isovelocity shortening in the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* at 20 °C.

2. Isometric tension at l_0 was 550 ± 40 mN/mm² (s.D. for 173 observations in nine muscles), while the isometric maintenance heat rate was $1\cdot0 \pm 0\cdot2$ mW/g wet wt. (s.D. for seventy-eight observations in eight muscles).

3. Isometric tension and heat production were measured as functions of muscle length over a range of $0\cdot79$–$1\cdot14\ l_0$ and were found to bear a linear relation to each other.

4. The force–velocity relation was determined in isovelocity releases imposed during tetanic stimulation and was found to fit the Hill equation with parameters $a/P_0 = 0\cdot07 \pm 0\cdot01$ and $b/l_0 = 0\cdot016 \pm 0\cdot0007$ sec⁻¹ (s.E. from non-linear least-squares regression of the pooled data from seven experiments).

5. Heat production measured in the same experiments showed that shortening heat is produced with a shortening heat coefficient α/P_0 of $0\cdot15$. Shortening heat does not appear to be force-dependent, and separate experiments confirmed that it is a linear function of the amount of shortening.

INTRODUCTION

The anterior byssus retractor muscle (ABRM) of the mussel *Mytilus edulis* and other 'catch' muscles have been the object of considerable curiosity from muscle investigators, and much is now known about their physiology (Jewell, 1959; Lowy & Millman, 1963; Tameyasu & Sugi, 1976), their ultrastructure (e.g. Millman & Elliott, 1972; Sobieszek, 1973; Nonomura, 1974) and the nature of neuromuscular excitation and 'inhibition' (e.g. Hidaka, Osa & Twarog, 1967; Marchand-Dumont & Baguet, 1975). The ABRM can be made to contract in two different ways. Phasic contractions similar to those seen in vertebrate striated muscle can be elicited by a.c. stimulation, while catch or tonic contraction occurs in response to d.c. stimulation. Catch is characterized by the presence of tension or resistance to stretch in the absence of active state (i.e. the ability to redevelop tension after a release) and can be maintained for many hours (Jewell, 1959; Lowy & Millman, 1963). Twarog

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(1967 *a*, *b*) has shown that acetylcholine causes contraction and the development of catch, while serotonin causes relaxation from the catch state. Phasic contractions presumably result from the simultaneous stimulation of the two types of nerve endings that release acetylcholine and serotonin.

The ABRM is known for its ability to maintain a large amount of tension (500 mN/mm² or more), both in the catch state and in phasic contractions, with a very low rate of energy expenditure, as shown by the measurements of arginine phosphate hydrolysis by Nauss & Davies (1966) and oxygen consumption by Baguet & Gillis (1967, 1968). Heretofore no detailed study of heat production has been reported, although preliminary investigations (Abbott & Lowy, 1958; Baguet, Maréchal & Aubert, 1962) indicate that the ABRM produces much less heat than do striated muscles, even in the less economical phasic contractions. The ABRM seems to contract by a sliding filament mechanism just as striated muscles do (e.g. Millman & Elliott, 1972). However, its mode of activation by calcium is different (Lehman & Szent-Györgyi, 1975), its thick filaments are longer (Sobieszek, 1973) and it develops tension and shortens under a given load more slowly than do most striated muscles. The object of the present study was to measure isometric maintenance heat and shortening heat in phasic contractions as the first step towards a characterization of the amount and time course of heat production by the ABRM.

<div align="center">METHODS</div>

Unpaired muscles were used. The animals were collected from near the mouth of the River Tamar and kept in the Laboratory's system of circulating natural sea water for no more than 10 days before use. All experiments were performed at room temperature (about 20 °C). Muscle weights ranged from 52 to 113 mg.

<div align="center">*Dissection and determination of* l_o</div>

Mussels were opened by slipping the blade of a scalpel between the two halves of the shell and cutting through the large posterior adductor muscle. The length of the ABRM from its attachment on the shell to its byssal thread was measured quickly with the shell still only partially open, and this length was taken to be l_o. It corresponded within 1–2 mm to the length at which the muscle developed the maximum isometric force. The range of l_o was 37–43 mm. The two halves of the shell were separated, and extraneous tissue, including the basal ganglion, was removed from the preparation. A rectangular piece of shell which included the muscle's origin was cut out with a hacksaw, and the muscle was fixed to the thermopile by means of a clamp attached to the piece of shell. A fine silver chain or epoxy-coated thread leading to the force transducer was tied to the byssal threads at the posterior end of the muscle. The thermopile was then fitted into a chamber-reservoir assembly which was submersed in a large bucket of water to provide temperature stability. Natural sea water bubbled with oxygen was passed into the chamber during 'washings' and returned to the reservoir during stimulation.

<div align="center">*Stimulation*</div>

To reduce stimulus heat, the stimulus was applied only at the two ends of the muscle, by means of two pairs of platinum electrodes. Since the electrodes of a pair were 1 mm apart, only the small part of the muscle between the electrodes of a pair experienced joule heating from the stimulus. Separate experiments showed this method of stimulation to be as effective in producing high tension and rapid relaxation as stimulation with a multi-electrode assembly with electrodes placed every 3 mm along the entire length of the muscle or massive stimulation through the bathing medium from large electrodes placed on each side of the muscle along its entire length. Stimulus heat was measured at the end of each experiment after the muscle

had been made inexcitable with isotonic KCl containing serotonin (5 μg/ml.) to prevent the persistence of catch after the potassium contracture.

The muscles were very sensitive to the kind of treatment they received from the very beginning of the experiments, and preliminary experiments showed that the balance between the duration of tetani and the length of rest periods, in air and in oxygenated sea water, was critical for obtaining reproducible mechanical and thermal responses. A possible reason is that the level of serotonin in the interstitium is critical. (Exogenous serotonin was not added; it is presumably produced by nerve endings remaining on the muscle.)

At the beginning of an experiment the muscle was stimulated for 5 sec at 8–10 V, 2–5 msec, 8–10 pulses/sec every 1–2 min without washing until the amount of tension developed in successive contractions had increased to a maximum and levelled off. These stimulus parameters were established after early experiments showed the futility of trying to fix optimal stimulus conditions, since increases in pulse width, strength and frequency did produce stronger contraction and faster relaxation but also caused the preparation to deteriorate rapidly. After the initial 5 sec contractions, the muscle was given several preliminary series of three 10–12 sec tetanic stimulations, with 2–3 min rest periods in air between the tetani of a series and 5–8 min 'washes' between series. The first contraction (I) of a series always produced only 60–80% as much tension as was produced in the latter two (II and III), which were very nearly identical. Therefore II and III were used as control and experimental contractions. During these preliminary series minor adjustments in muscle length were made as necessary to optimize tension development, and small quick releases were applied in experimental contractions to measure series compliance and thermoelastic heat as described previously (Gilbert & Matsumoto, 1976). During experiments with shortening at different velocities, durations of tetani during the experimental series were 12–20 sec, being longer for slower velocities of release. The amount of tension developed in the last contraction of an experiment was never less than 75% of that in the first contraction of the experimental run. In some experiments the tension produced in the preliminary series in which stiffness and thermoelastic heat were measured was somewhat larger than in the rest of the experiment, but in experiments in which 12 sec tetani were used throughout, tension in the last contraction was at least 90% of the tension at the beginning of the experiment. The experiments generally lasted 5–6 hr.

Recording of tension and heat production

Tension was recorded with a RCA 5734 triode mounted on the arm of a Levin–Wyman ergometer, which was used for the isovelocity releases.

Heat production was recorded with a Hill–Downing type thermopile with constantan-chromel thermal junctions. The junctions were arranged in three banks, the 5 mm centre bank having twenty junctions and a total resistance of 36 Ω and the top and bottom 2·5 mm banks having ten junctions and a resistance of 18 Ω each. The sensitivity of the thermopile, as determined by the method of Kretzschmar & Wilkie (1975), was 58 μV/°C per junction. The temperature changes measured by the thermopile were converted to heat by measuring the heat capacity of each muscle at the end of the experiment using the Peltier effect and the calibration curve of the thermopile.

Heat records made from the top bank of the thermopile showed evidence of movement artifacts during release, but records from the centre and bottom banks were virtually indistinguishable with respect to time course. One experiment in which the centre and bottom banks were both used showed that the quantities of heat measured from the two were not substantially different (expt. 27 July 1976, Table 2). A piece of silver (167 mg, heat capacity 39 mJ/°C) was applied to the reverse side of the thermopile, since paired muscles were not used. The use of silver frequently resulted in a rather complicated heating lag correction, but since the events recorded were very slow in these experiments, the effect was not a problem.

The temperature signal was amplified by an Ancom 15C-3 amplifier. Heat loss was exponential with a rate constant of 0·03–0·05 sec^{-1} and was corrected for analytically.

Tension and heat production during each contraction were recorded with a Devices recorder. In addition, the signals during the period of release and tension redevelopment were recorded on a Tektronix 5103N storage oscilloscope for visual monitoring, and permanent records were made by a 35 mm camera from the face of a second oscilloscope. Shortening heat, isometric maintenance heat rate and force for the force–velocity relation were read from enlarger images of the film records.

Tension and heat production in isometric contractions

Fig. 1 shows tension (P) and heat production (h) during a 20 sec tetanus, which was the worst case for contamination of the heat records with stimulus heat (h_{stim}, bottom trace). Stimulus heat did not appear in the part of the muscle over the active region of the thermopile until 5–6 sec after stimulation had begun and reached maximum a few seconds after the end of stimulation, during relaxation. The total amount of stimulus heat was never more than 40% of the total heat produced and was less than that for shorter tetani. h_{cor} is the heat record measured at 1·2 sec intervals after subtraction of stimulus heat and correction for heat loss.
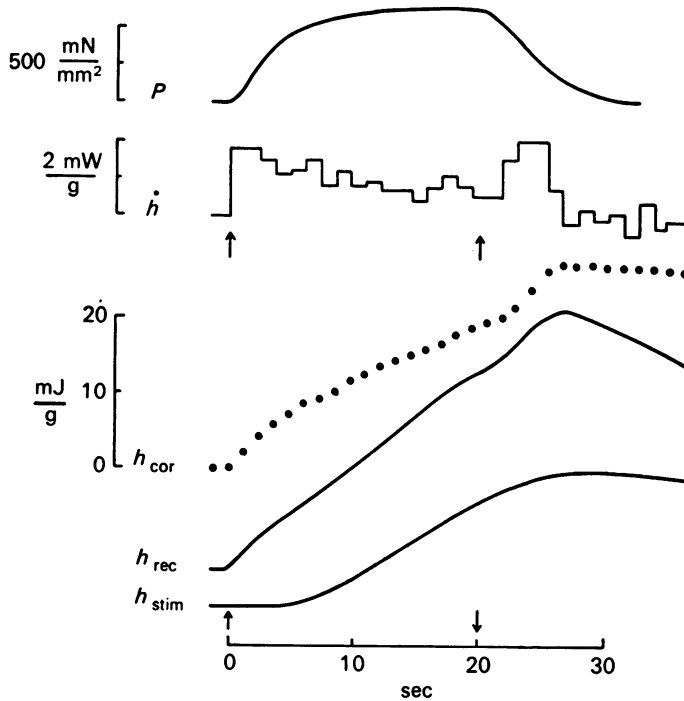

Fig. 1. Tension (P) and heat production (h) during a 20 sec phasic contraction. Stimulus, 8 V, 5 msec, 8/sec, shown by arrows. h_{stim}, stimulus heat recorded after treating the muscle with isotonic KCl at the end of the experiment; h_{rec}, heat production as recorded; h_{cor}, heat time course after subtraction of h_{stim} and correction for heat loss (rate constant = −0·04/sec); $\dot{h}$, heat rate. Scales for h_{stim} and h_{rec} are the same as for h_{cor}. Experiment 17 July 1976, l_o = 40 mm, m = 68 mg.

The heat rate ($\dot{h}$) was high during tension development, which required 5–6 sec. In this experiment heat production began as soon as tension development did, but in some experiments the production of heat came only several hundred milliseconds after the onset of mechanical activity. Heat production was never observed to precede the onset of tension development. The heat rate during the tension plateau was about 0·7 mW/g in this experiment. $\dot{h}$ was measured in nine experiments in tetani of 12, 15, 17 and 20 sec, from 7 sec after the beginning until the end of

stimulation, and was not found to vary within that time period with the duration of the tetanus. The average $\dot{h}$ for seventy-eight measurements in nine experiments was 1.0 ± 0.2 mW/g (S.D.).

The amount of heat produced during relaxation (7.7 mJ/g in Fig. 1) is approximately equal to the thermoelastic heat from the fall in tension (1.5 mJ/g) plus the degraded elastic energy (6.5 mJ/g), which were calculated from the thermoelastic ratio R and the stiffness curve of the compliant elements in series with the contractile apparatus of the muscle.
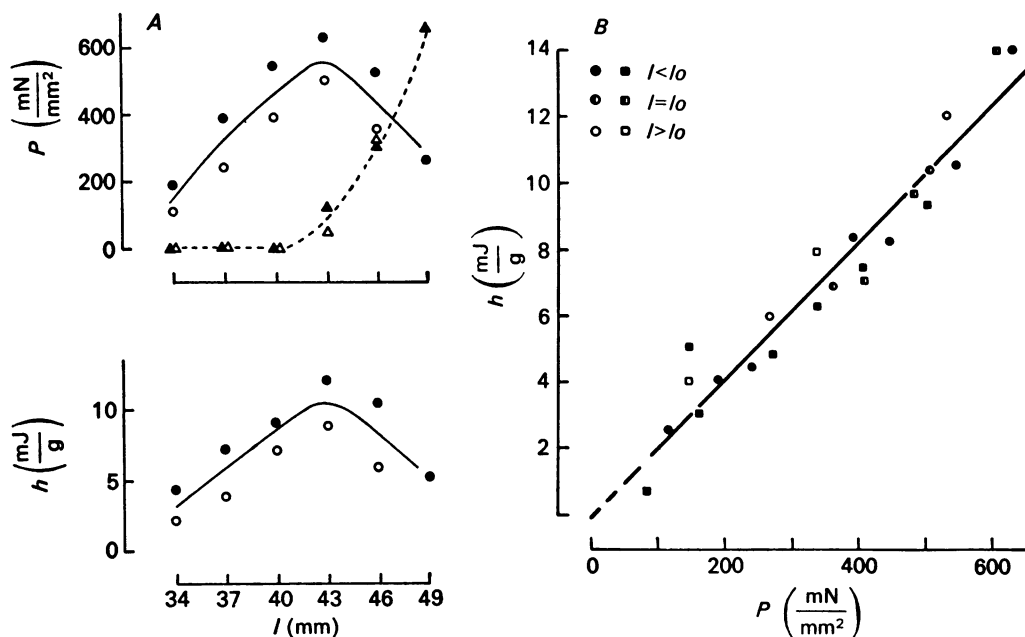

Fig. 2. Tension (P) and heat production (h) at various muscle lengths. A, length–tension and length–heat production curves for one experiment. Filled and open circles represent first and second excursions through muscle length, respectively, beginning at 34 mm. Triangles, passive tension. P is maximum tension recorded, h is total heat after 10 sec of stimulation, corrected for stimulus heat and heat loss. Experiment 2 August 1976, $l_0 = 43$ mm, $m = 81$ mg. B, tension dependence of heat production in two experiments. Circles, experiment 2 August 1976 as in A; squares, experiment 16 August 1976, $l_0 = 40$ mm, $m = 83$ mg. Line from linear regression, intercept = 0.01 mJ/g, slope = 0.02 mJ/g per mN.mm^{-2}, $r = 0.965$. 10 sec tetani.

The relation between heat production and tension is shown in Fig. 2. The tension was made to vary by changing the length of the muscle (Fig. 2A). The tension values plotted as circles in the Figure are 'active' tension, whose magnitude was determined by subtracting from the total tension the 'passive' tension remaining at the end of tetanus after relaxation. This tension level was used because spontaneous activity resulting in a degree of catch was observed at the longer lengths. The total tension was lowest just after relaxation at these lengths, as would be expected if catch were abolished by a.c. stimulation during the tetanus.

The lower curve in Fig. 2A is the total heat produced in 10 sec of stimulation plotted against the muscle length. After a preliminary series of contractions to

optimize tension with respect to length, the muscle length was reduced to 34 mm and heat recorded in two series of 10 sec contractions. The length was increased in 3 mm increments up to 49 mm. The filled circles are the average of tension or heat production in contractions II and III in the second series at each length. The process was repeated (open circles), starting again at the short length. Spontaneous activity was observed at lengths of 46 and 49 mm, and neither tension nor heat production
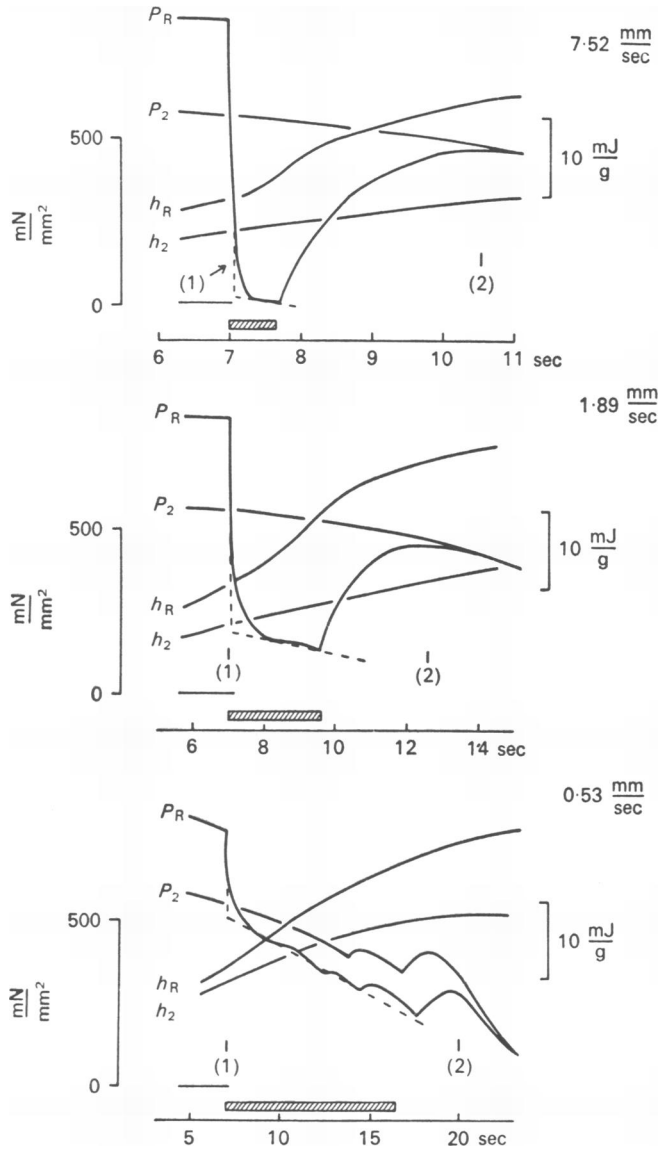

Fig. 3. Tension and heat production during 5 mm releases at three velocities, traces from 35 mm film records. Time base, seconds after the beginning of stimulation. P_R, h_R, tension and heat production during release; P_2, h_2, tension and heat production at the short length l_2. Shaded bars indicate period of isovelocity release. Dashed lines, extrapolation for force–velocity relation (see text). Stimulation was for 12, 15 and 20 sec. Experiment 19 July 1976, $l_o = 39$ mm, $m = 72$ mg.

recovered to the levels observed in the first excursion through length. The total heat produced in 10 sec of stimulation is plotted against tension for two experiments in Fig. 2*B*. It shows that the dependence of heat production on tension is the same in the length range $l > l_0$ as in the range $l < l_0$. There is also the suggestion that very little if any tension-independent heat is produced by the muscle.

The force–velocity relation in isovelocity releases

A series of tension (P) and heat (h) records at different release velocities is shown in Fig. 3. The amount of release was 5 mm in every case, and release began 7 sec after the onset of stimulation. P_R is tension in the contraction with release, and P_2 is the tension at l_2, the short length. Tension drops rapidly during the first few tens of milliseconds of release and then declines more slowly. The early rapid tension drop was found to fit exactly the predicted tension time course calculated from the release velocity and the length–tension curve of the compliant elements in series with the contractile apparatus of the muscle and is thus the result of shortening in that series compliance during the time required for the muscle to begin active shortening. The dashed lines show the extrapolation used to determine the force for the force–velocity relation. The total length of the muscle at the point of intersection of the dashed lines is only 0·4–0·5 mm less than the initial length l_1 (equal to l_0), so that the force–velocity relation determined in this way represents the relation near l_0 (Fig. 4).

The lowermost tension records in Fig. 3 show evidence of uncoupling between the tetanic stimulation and mechanical activity. This behaviour was seen in about half the muscles used but only during the long tetani (20 sec) required for very slow releases. Once shorter tetani were again used, the muscle's 'normal' response to the stimulus returned. This sort of behaviour was also observed at muscle lengths greater than l_0, as described above, and in the presence of exogenous serotonin.

The force–velocity curve for seven experiments is shown in Fig. 4. The curve represents the Hill equation,

$$\frac{P}{P_0} = \frac{b - (a/P_0)v}{v + b}$$

with parameters of $a/P_0 = 0·07 \pm 0·01$ and $b/l_0 = 0·016 \pm 0·0007$ sec^{-1} (s.e. from regression) obtained from a non-linear least-squares regression of the pooled normalized data. The paucity of points for low velocity and high values of P/P_0 is the result of using as large releases as possible to make the shortening heat large and as short tetani as possible to minimize spontaneous activity. This precluded the use of velocities of less than 0·5 mm/sec.

Shortening heat during isovelocity release

Heat production during release is shown as h_R in Fig. 3. The base line against which shortening heat (h_s) was determined was the isometric heat produced in a control contraction at l_2 during the time corresponding to shortening and tension redevelopment in the contraction with release, labelled h_2, such that

$$h_s = \{h_R(2) - h_R(1)\} - \{h_2(2) - h_2(1)\},$$

where (1) and (2) correspond to the times indicated by the short vertical bars in the Figure. These records show that there seems to be no dependence of h_8 on the force maintained during release. Table 1 summarizes the results of six experiments in which h_8 was measured at different release velocities. Only one experiment, 27 July, shows a consistent dependence of h_8 on P/P_0. In that experiment h_8 was
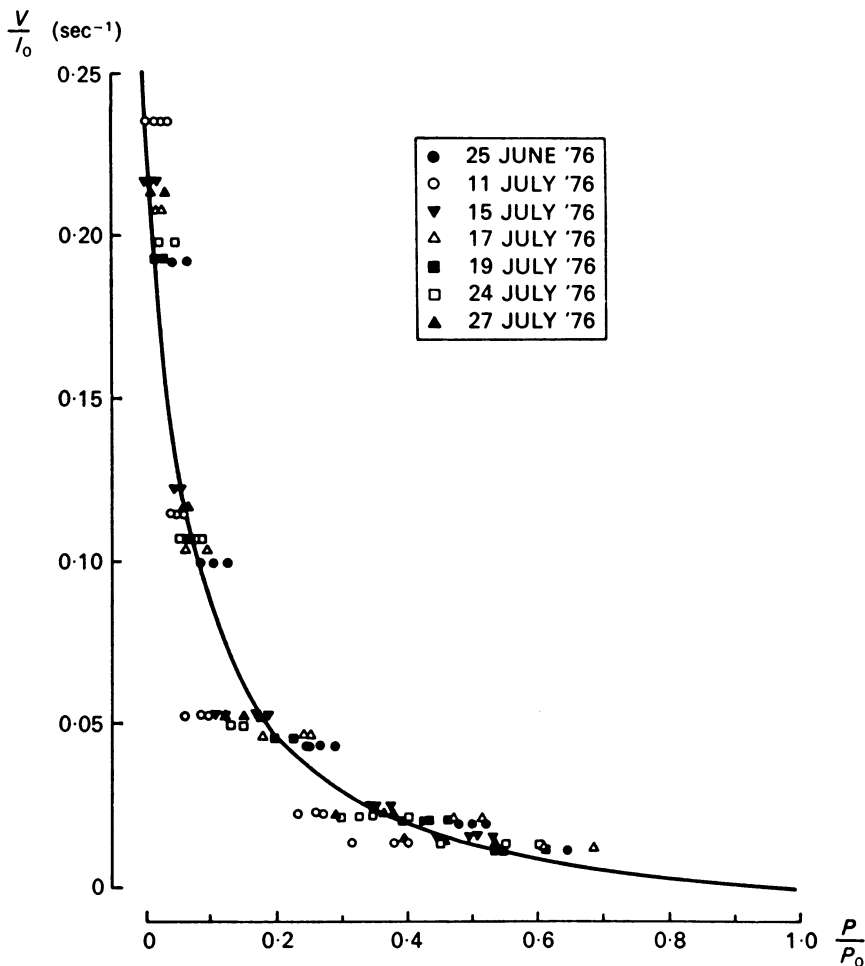

Fig. 4. Force *vs.* velocity in seven experiments. The curve is the result of a non-linear least-squares regression of all the data to fit the Hill equation, with P/P_0 as the dependent variable, $a/P_0 = 0\cdot07 \pm 0\cdot01$, $b/l_0 = 0\cdot016 \pm 0\cdot0007$ (standard errors from regression). Separate regressions were made on each experiment to produce the parameters a and b shown in Table 2.

measured from the bottom bank of the thermopile (data shown in Table 1) as well as from the centre bank which showed the same trend, with values of h_8 of 11·6, 11·8, 15·9 and 18·9 mJ/g for the first four values of P/P_0, respectively. A similar trend over the same range of P/P_0 is seen in the experiment of 17 July, but there h_8 is less at a still larger force. It is possible that the experiment of 27 July would have shown the same behaviour had the force range been as large. The average

value of the shortening heat coefficient α from 116 determinations in six experiments is 83 ± 13 mN/mm² (s.d.).

Shortening heat is a linear function of the distance shortened in the ABRM, as shown in Fig. 5. Sample records of tension and heat production from one experiment during releases of 1, 3 and 5 mm are shown in Fig. 5A. Data from two experiments are shown in Fig. 5B. The values of α, 87 and 77 mN/mm², obtained from the slopes of the lines, are in good agreement with 83 mN/mm² obtained from the six experiments discussed above.

TABLE 1. Shortening heat, h_s ($\pm$ s.d.), and tension, P/P_0, for 5 mm releases at different velocities. Each value of h_s (in mJ/g) represents the mean of three to five determinations on that muscle at a single release velocity

11 July 1976		15 July 1976		17 July 1976	
P/P_0	h_s	P/P_0	h_s	P/P_0	h_s
0·02	4·8 ± 0·3	0·01	8·5 ± 0·6	0·02	15·8 ± 0·9
0·05	4·8 ± 0·5	0·05	7·7 ± 1·5	0·07	18·8 ± 3·2
0·08	5·7 ± 0·7	0·15	8·7 ± 0·9	0·23	21·2 ± 2·5
0·25	5·3 ± 0·1	0·35	9·0 ± 1·9	0·50	24·6 ± 4·1
0·35	5·9 ± 0·7	0·49	6·1 ± 1·5	0·65	19·4 ± 3·4
19 July 1976		24 July 1976		27 July 1976	
P/P_0	h_s	P/P_0	h_s	P/P_0	h_s
0·02	8·6 ± 0·6	0·03	8·3 ± 0·8	0·02	8·6 ± 0·6
0·07	9·4 ± 0·9	0·07	8·0 ± 0·9	0·06	8·4 ± 1·0
0·22	10·5 ± 0·9	0·15	8·6 ± 0·9	0·15	11·4 ± 0·5
0·43	9·2 ± 0·6	0·34	7·2 ± 0·9	0·35	13·7 ± 0·4
0·58	8·1 ± 0·6	0·53	7·4 ± 0·4	0·47	16·6 ± 2·2

Table 2 summarizes the mechanical and thermal properties of the ABRM determined from these experiments. From these data it is possible to calculate the ratio of power to rate of enthalpy liberation. The range of maximum values of this ratio is 0·57–0·78. The length of the compliant elements in series with the contractile apparatus, designated l_{sc} in the Table, and the thermoelastic heat:tension ratio R were measured in small quick releases (0·5–3·0 mm, 30 mm/sec), as described previously (Gilbert & Matsumoto, 1976). l_{sc} is the amount of release (mm) required to reduce the tension to zero and was 4–6 % of l_0 in these experiments. The dependence of the decrease in force upon the decrease in length was exponential in each muscle with 'length constants' in the range 0·3–0·8 mm.

The thermoelastic ratio R is $(1·2–5·0) \times 10^{-3}$ in this muscle, somewhat lower than in frog striated muscle (about 8×10^{-3}; Gilbert & Matsumoto, 1976). In a few experiments R was determined in phasic contractions and in catch in the same muscles and was found to be the same.

DISCUSSION

Tension and heat production in isometric contractions

If sufficient attention is given to the stimulation regimen, it is possible to obtain consistent phasic contractions from the ABRM for several hours. The muscle can
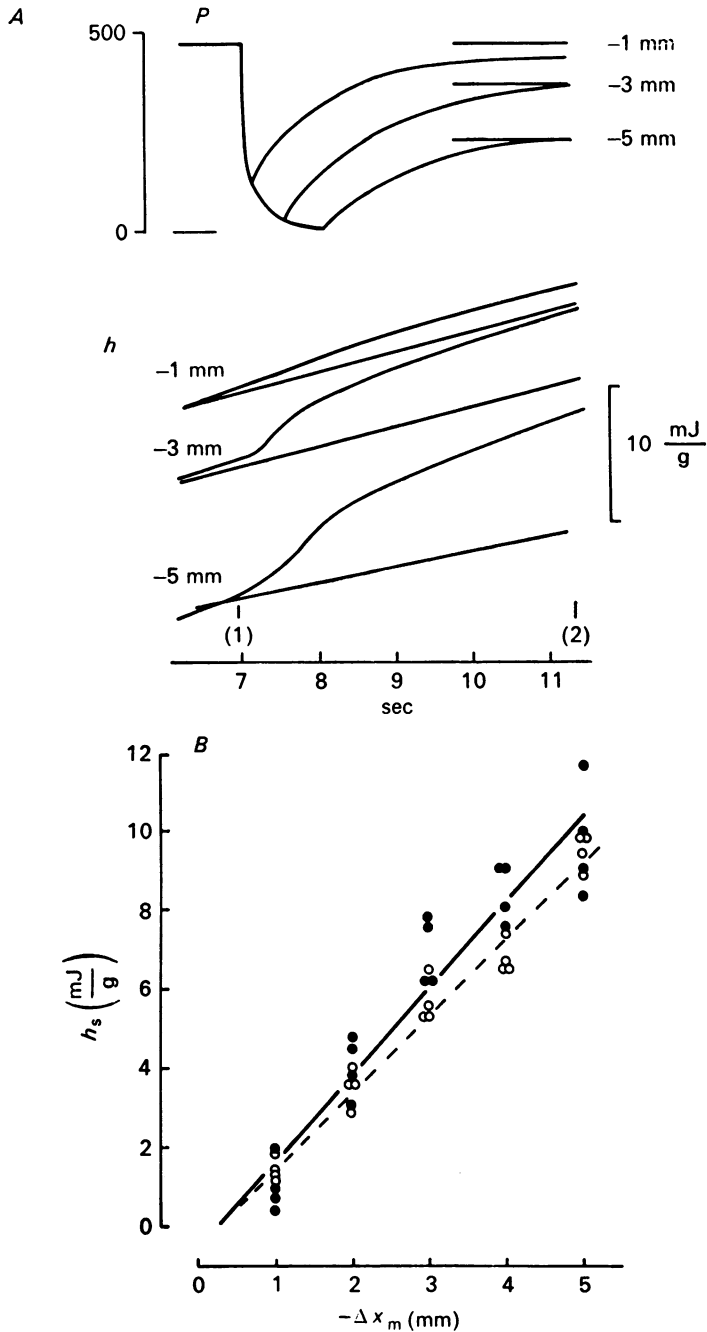
Fig. 5. Shortening heat for different amounts of release. A, traces from film records of tension (P, in mN/mm²) and heat production (h) during isovelocity releases of 1, 3 and 5 mm. Horizontal bars at right of tension traces show P at l_2. Lower heat trace of each pair shows heat production at l_2. Experiment 21 August 1976, $l_o = 40$ mm, $m = 64$ mg, $P_o = 473$ mN/mm², release velocity = 5 mm/sec. B, shortening heat (h_s) vs. amount of release ($-\Delta x_m$) from two experiments. Filled circles, experiment 29 July 1976, $l_o = 40$ mm, $m = 129$ mg, $P_o = 517$ mN/mm²; continuous line from linear regression of this experiment with intercept = -0.5 mJ/g, slope = 2.2 mJ/g/mm, to give $\alpha = 87$ mN/mm², correlation coefficient $r = 0.951$. Open circles, experiment 21 August 1976 as in A; dashed line from linear regression with intercept = -0.42 mJ/g, slope = 1.9 mJ/g/mm, to give $\alpha = 77$ mN/mm², correlation coefficient $r = 0.984$.

TABLE 2. Summary of mechanical and thermal properties of the ABRM in phasic contractions. Data from nine experiments. l_o, length at which maximum isometric force was developed and from which isovelocity releases were made; m, muscle mass; P_o, average isometric force at l_o during each experiment; a and b, parameters of Hill equation obtained by non-linear least-squares regression of P upon V; V_{max}, $b.P_o/a$; l_{sc}, amount of quick release (at 30 mm/sec) required to reduce active isometric force to 0; R, thermoelastic heat:tension ratio; h, average isometric heat rate in each experiment from 7 sec after the beginning to the end of stimulation at l_o; α, the average shortening heat coefficient in each experiment. Values represented as averages are given ± 1 s.E. for the number of observations shown in parentheses below. a and b are given ± 1 s.D. for regression.

Expt.	l_o (mm)	m (mg)	P_o (mN/mm²)	a (mN/mm²)	b (mm/sec)	V_{max} (mm/sec)	l_{sc} (mm)	$10^3 R$	h (mW/g)	α (mN/mm²)
25 June 1976	37	56	480±30 (16)	39±8	0·88±0·04	12	—	—	—	—
11 July 1976	40	69	610±20 (20)	14±5	0·32±0·02	14	2·0	1·1±0·2 (6)	0·7±0·1 (10)	53±4 (20)
15 July 1976	37	112	360±30 (20)	31±5	0·63±0·04	7	1·5	5·0±1·3 (4)	1·1±0·2 (7)	60±10 (18)
17 July 1976	40	68	500±60 (18)	69±14	1·05±0·09	8	2·5	3·2±0·5 (6)	0·7±0·1 (9)	160±20 (16)
19 July 1976	39	72	770±50 (21)	63±9	0·71±0·03	9	2·0	1·4±0·4 (7)	1·1±0·3 (11)	71±5 (21)
24 July 1976	42	69	510±40 (20)	31±12	0·61±0·06	10	2·0	1·2±0·4 (7)	0·7±0·1 (10)	67±7 (18)
27 July 1976	39	73	720±50 (18)	41±12	0·57±0·04	10	2·0	1·6±0·4 (5)	0·9±0·1 (10)	90±9 (19)
									1·3	130±30
29 July 1976	40	129	520±20 (20)	—	—	—	2·7	2·7±0·2 (5)	1·8±0·1 (10)	87
21 August 1976	40	64	470±10 (20)	—	—	—	2·0	1·6±0·6 (4)	1·0±0·2 (10)	77

be satisfactorily stimulated by pairs of electrodes at each end of the muscle. This property is expected from the work of Twarog, Dewey & Hidaka (1973), who showed that although the fibres of the ABRM are only about 1–2 mm long, they are electrically continuous and are arranged in bundles that run the entire length of the muscle. The augmentation of the mechanical response produced by repeated tetanic stimulations is expected from the work of York & Twarog (1973), who observed this effect and postulated its cause to be the accumulation of serotonin in the extracellular space.

The ABRM produces so little heat that it is essential that the stimulus produce as little heating of the muscle as possible. The arrangement of the thermopile and stimulating electrodes used in these experiments ensured that this was the case.

The spontaneous activity observed at $l > l_0$ and with prolonged stimulation is probably due to membrane instability associated with mechanical distortion and to the prolonged presence of serotonin, respectively. Lowy & Millman (1963) also observed this effect at $l > l_0$, and Hidaka *et al.* (1967) have reported that serotonin causes spontaneous electrical activity in the ABRM.

The ability of the ABRM to produce a large isometric tension has been well known for many years. The values reported here (Table 2) are within the range reported by previous workers (Abbott & Lowy, 1958; Jewell, 1959; Lowy & Millman, 1963; Baguet & Gillis, 1967). Tensions as high as 1500 mN/mm² were observed in some early experiments with very strong stimulation, but the muscles did not produce such tensions in repeated contractions. One expects the ABRM to produce more tension than frog striated muscle, since its thick filaments are 25 μm long (Sobieszek, 1973) and therefore allow more cross-bridges to act in parallel.

Although the pattern of heat production by the ABRM during isometric contractions is similar to the pattern observed in striated muscle, two interesting differences should be noted. First, the ABRM seems not to produce the very early burst of heat preceding mechanical activity called activation heat in frog muscle nor the tension-independent isometric maintenance heat which may also be associated with activation processes (Homsher, Mommaerts, Richhuiti & Wallner, 1972; Smith, 1972). These observations are interesting in view of the work of Lehman & Szent-Györgyi (1975), who have reported that calcium sensitivity in the byssus retractor of *Mytilus* is associated with myosin rather than actin and that this muscle does not appear to contain any troponin. The absence of activation heat in a muscle which also contains no troponin provides further circumstantial evidence for the association of activation heat with the interaction of calcium and troponin (Yamada, Mashima & Ebashi, 1976).

The second difference between heat production in frog striated muscle and the ABRM is the very low maintenance heat rate of the latter muscle, which at about 1 mW/g is one-tenth the rate of tension-dependent heat in frog muscle at 0 °C. This result is in agreement with the observations of Baguet *et al.* (1962) and Abbott & Lowy (1958) in the ABRM. It is also close to the value predicted by Baguet & Gillis (1967) from oxygen consumption measurements, although perhaps this is fortuitous, given the assumptions necessary for that prediction. To the extent that tension-dependent heat production in any muscle can be considered to be the result of reactions in which cross-bridges participate, one can conclude that these reactions

occur much more slowly in the ABRM at 20 °C than in frog striated muscle at 0 °C. Thus the rate of cross-bridge cycling during the maintenance of isometric tension may be much lower in the ABRM than in frog muscle.

The force–velocity relation in isovelocity releases

The force–velocity relation shown in Fig. 4 has parameters of $a/P_0 = 0.07$ and $b = 0.016\ l_0$/sec. The value of a/P_0 is less than the values reported previously (Abbott & Lowy, 1958; Tameyasu & Sugi, 1976), although b is about the same. Thus in the present investigation V_{max} as determined by extrapolation from the Hill equation is $0.23\ l_0$/sec, which is higher than reported by the other two groups. It seems not unlikely that their lower values of V_{max} were the result of a degree of catch in their muscles. Abbott & Lowy stimulated electrically at 2–4/sec, and it is known that low frequencies of stimulation can produce catch (Lowy & Millman, 1963). Tameyasu & Sugi activated their muscle fibre bundles with acetylcholine in the absence of serotonin and measured length changes following force steps during the rise in tension and also later during the tension plateau after some catch had developed. A comparison of the force–velocity relations they obtained in the two circumstances shows that a/P_0 becomes greater and V_{max} less after catch develops, which is what one would expect if the rate of detachment of cross-bridges were to become slower. Since serotonin, which releases catch, seems not to have been present at all in their preparations, it is reasonable that some cross-bridges could have had low detachment rates even as tension was developing.

Shortening heat

Shortening heat is produced during shortening by the ABRM (Figs. 3 and 5). The shortening heat coefficient α is about twice the size of the force–velocity parameter a. As defined here, the shortening heat appears ,in most cases not to be force-dependent, but in other respects it is qualitatively like the shortening heat observed in vertebrate striated muscle. The lack of force-dependence is not unique, since shortening heat is also not force-dependent in the tortoise muscle (Woledge, 1968).

The shortening heat is also quantitatively similar in the ABRM and in frog striated muscle, if both are normalized to P_0, since $\alpha/P_0 = 0.15$ in the ABRM and is in the range of 0.16–0.34 in the frog sartorius, depending on the load (Hill, 1964). This quantitative similarity could be coincidental, or it could suggest that shortening heat results from a reaction (or reactions) whose extent depends on the number of cross-bridges acting in parallel in both muscles. This quantitative similarity is also interesting in view of the striking difference in the isometric maintenance heat rates in the ABRM and in frog striated muscle, for it suggests that while maintenance heat may result from cyclic reactions that occur at different rates in the two muscles, the shortening heat may result from a non-cyclic reaction whose extent depends on the extent of shortening and whose rate is therefore a function of the velocity of shortening.

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