Force-dependent and force-independent heat production in single slow- and fast-twitch muscle fibres from *Xenopus laevis*

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1. The origin of labile heat production, i.e. a heat component which rapidly decays after the onset of stimulation, and of stable (maintenance) heat production was investigated in intact single fast-twitch (type 1) and slow-twitch (type 3) iliofibularis muscle fibres from *Xenopus laevis*, at 20 °C, by varying stimulation frequency and by varying sarcomere length and the concentration of 2,3-butanedione 2-monoxime (BDM) added.

2. The labile heat produced consisted of a force-independent and a force-dependent part. The average parvalbumin (PA) content found in type 1 fibre bundles (0·84 ± 0·08 mM; mean ± S.E.M.; $n = 5$) and in type 3 fibre bundles (0·12 ± 0·02 mM; $n = 5$) indicates that the force-independent labile heat is explained by Ca^{2+}–Mg^{2+} exchange on PA, and amounts to a molar enthalpy change of −78 kJ (mol PA)$^{-1}$.

3. Force-dependent labile heat during fused contractions was similar to the calculated heat production resulting from the formation of force-generating cross-bridges, assuming an enthalpy change associated with cross-bridge formation of −30 kJ mol^{-1}.

4. Activation heat, i.e. the part of the total stable heat that is not related to the contractile apparatus, and of which the calcium sequestration by the sarcoplasmic reticulum is the most important contributor, determined by varying sarcomere length or BDM concentration, was identical. For fused contractions the fraction activation heat of the stable maintenance rate of heat production was 34 ± 4% (mean ± S.E.M.; $n = 13$) in type 1 fibres, and 52 ± 4% ($n = 15$) in type 3 fibres. In unfused contractions this was 48 ± 5% ($n = 13$) in type 1 fibres, and 35 ± 2% ($n = 11$) in type 3 fibres.

5. From the force-dependent stable rate of heat production the economy of cross-bridge cycling, expressed as the force–time integral for a single myosin head per ATP molecule hydrolysed, was calculated. It follows that cross-bridge interaction in type 3 fibres is more economical than in type 1 fibres, and that fused contractions are more economical than unfused contractions.

Heat production during a tetanic contraction in skeletal twitch muscle usually starts at a high rate and then declines in an exponential fashion to a constant value. This led Aubert (1956) to distinguish a rapidly decaying time-dependent component, which became known as labile heat, and a steady component, the stable (maintenance) heat. It has been suggested (e.g. Peckham & Woledge, 1986) that the origin of labile heat production is the enthalpy change of calcium binding to parvalbumin (PA) in exchange for magnesium. This idea is supported by the finding that the slowing down of labile heat production has a time constant similar to that of the slowing down of force relaxation with tetanus duration, and that both are influenced to the same extent by the time between contractions (Peckham & Woledge, 1986; Lännergren, Elzinga & Stienen, 1993). However, Lännergren *et al.* (1993) found that only part of the labile heat could be explained by calcium binding to PA. Furthermore, in other studies (Berquin & Lebacq, 1992), labile heat was shown to be present in muscles that were devoid of PA. This indicates that labile heat might originate from more than one process, as was suggested by the studies of Aubert (1956), Homsher, Mommaerts, Ricchiuti & Wallner (1972), and Curtin & Woledge (1981). It thus seems possible that labile heat has a force-dependent component. To investigate this in more detail we measured labile heat production at different force levels in order to estimate the force-independent labile heat.

The stable heat is the result of a number of heat-producing processes of which the two main contributors are: heat production from high energy phosphate splitting by the contractile proteins, and the 'activation heat', i.e. that part of the stable (maintenance) heat that is unrelated to the contractile apparatus, and is predominantly the result of

continuous calcium sequestration during contraction by the sarcoplasmic reticulum (SR) (e.g. Curtin & Woledge, 1981; Burchfield & Rall, 1985*a*). It has been found in several studies that the contribution of the activation heat to the stable heat is variable (e.g. Curtin & Woledge, 1981; Baker, Brandes, Schendel, Trocha, Miller & Weiner, 1994). These variations may play an important role in the differences in efficiency of dynamic contractions (i.e. the ratio of work to total energy output), and the economy of isometric contractions (i.e. the ratio between the maximum force development and the steady rate of energy liberation) as found in several studies (e.g. Woledge, 1968; Barclay, Constable & Gibbs, 1993; Potma, Stienen, Barends & Elzinga, 1994). Part of this variation, however, may be related to differences in species or muscle type (Barclay *et al.* 1993). Therefore, to determine the efficiency and/or economy of *cross-bridge* cycling, the energy liberated needs to be corrected for activation heat. To achieve this in the present study the contribution of energy turnover by activation processes to stable maintenance energy utilization (i.e. the force-independent stable heat) has been determined.

The classical method to determine force-independent heat is to study heat production and force production at different muscle lengths and extrapolate the results to zero force production (Homsher *et al.* 1972; Smith, 1972). However, this approach fails when single muscle fibres are used during tetanic contractions, because the sarcomeres close to the tendons are relatively short (e.g. Huxley & Peachey, 1961; Julian, Sollins & Moss, 1978). As a result, forces at long muscle fibre lengths are higher than expected on the basis of the sliding filament theory. To overcome this problem, we determined the relationship between labile heat and sarcomere length, and between stable heat and sarcomere length, which are hardly affected by sarcomere length inhomogeneity along the length of the fibre. Force-independent labile heat and heat due to activation processes can then be estimated by extrapolation to zero filament overlap.

Obviously, this approach is based on the assumption that energy liberation is independent of filament overlap. Although in previous experiments on whole muscles (e.g. Burchfield & Rall, 1985*a*) it has been concluded that calcium release is not affected at long muscle length, the study by Blinks, Rüdel & Taylor (1978) suggests that this may not be the case in single muscle fibres. Therefore, we also determined force-independent heat at optimal sarcomere length by inhibiting force production with 2,3-butanedione 2-monoxime (BDM) (Higuchi & Takemori, 1989; Hui & Maylie, 1991), a substance that reduces force production in a dose-dependent manner by acting on the contractile machinery (Alpert, Blanchard & Mulieri, 1989; Higuchi & Takemori, 1989). In the present study BDM was administered at different concentrations and force-independent heat was estimated by extrapolation to zero force the relationships between labile heat and force and between stable heat and force.

BDM-induced variations in force occur without any major changes in the amount of calcium released by SR (e.g. Horiuti, Higuchi, Umazume, Konishi, Okazaki & Kurihara, 1988). Hence these interventions most likely do not affect the activation heat or the labile heat component associated with calcium binding to PA. To allow an unambiguous discrimination between these force-independent heat components and the force-dependent heat components, the experiments were performed both on fast-twitch (type 1) and on slow-twitch (type 3) muscle fibres from the iliofibularis muscle of *Xenopus*, which differ in PA content (Simonides & van Hardeveld, 1989; Lännergren *et al.* 1993), and the rate of stable maintenance heat production. Because, *in vivo*, muscles usually work at low levels of activation (Hennig & Lømo, 1985), and thus Ca^{2+} saturation of PA may be incomplete, labile heat and stable heat were also studied during unfused tetanic contractions.

METHODS

Preparation

Single muscle fibres were dissected from the iliofibularis muscle of adult female *Xenopus laevis*. The animals were kept in tap water at room temperature and fed every other day Amphibia diet 3 (Special Diet Services, Witham, UK). They were killed by decapitation, pithed, and the two iliofibularis muscles were excised and put in oxygenated Ringer solution at 0–1 °C, which contained (mM): NaCl, 116·5; KCl, 2·0; $CaCl_2$, 1·9; NaH_2PO_4, 2·0; EGTA, 0·1; adjusted to pH 7·2 with NaOH. Type 1 (fast twitch) and type 3 (slow twitch) fibres were dissected under a microscope fitted with dark-field illumination. The fibre type was identified by its position in the muscle and its microscopic appearance according to the criteria developed by Lännergren & Smith (1966).

After dissection the tendons were trimmed down and small platinum hooks (50 μm in diameter) were tied on, close to the fibre endings. Before the experiment the sarcomere length of the fibre was set to 2·3 μm as determined by He–Ne laser diffraction, and the fibre length was measured using an ocular scale of a microscope. The mean fibre length was 16·9 mm in type 1 fibres and 12·0 mm in type 3 fibres.

Experimental protocol

The fibre was transferred to the experimental chamber and positioned on the thermopile. The Ringer solution in the experimental chamber was maintained at 20 °C by means of a water-jacket through which a temperature-controlled medium circulated. The fibre was mounted between a platinum electrode attached to a force transducer (AE 801, SensoNor, Horten, Norway) and a platinum electrode attached to a stainless steel rod. Both ends could be moved by a micrometer in such a way that the fibre could be positioned accurately over the active region of the thermopile.

For stimulation, 0·4–0·6 ms current pulses, 1·25 times threshold, were applied via the ends of the platinum wires. The stimulation frequency used to achieve a fused tetanus was 40 Hz for type 1 fibres and 35 Hz for type 3 fibres (cf. Nagesser, van der Laarse & Elzinga, 1992). An unfused contraction was obtained by 10 Hz stimulation in both fibre types. Tetanus duration in type 1 fibres was 0·5 s at 40 Hz stimulation, and 1·5 s at 10 Hz stimulation. Type 3 fibres were stimulated for 1·5 s at both stimulation frequencies. The time between tetani was about 40 min to allow for the repriming of labile heat production (Lännergren *et al.* 1993).

Sarcomere length variation experiments

Sarcomere length was increased by stretching the fibre when submerged in Ringer solution. Fibre lengths at the various sarcomere lengths above 2·3 μm were calculated assuming that stretch in the tendons with increasing fibre length was negligible (Edman & Flitney, 1982; iliofibularis muscle fibres are favourable in this respect because they are relatively long: 0·7–2·0 cm). Mean sarcomere length was increased in steps of 0·2 μm. Before adjusting the fibre length to a new sarcomere length care was taken to free the fibre completely from the thermopile.

In four experiments the sarcomere length distribution along the length of the fibre was investigated in the dissection chamber by laser diffraction. These measurements showed that about 10% of the sarcomeres, all situated near the tendon ends, were shorter than the remaining sarcomeres in the central part of the fibre. This result is similar to that found by Huxley & Peachey (1961). They showed that the short sarcomeres shorten, stretching the longer sarcomeres during fixed-end contractions. It can be calculated (cf. Results) that the contribution of this effect to the total energy turnover is negligible. This agrees with Abbott, Aubert & Hill (1951), who have found that the rate of heat production at slow or moderate isovelocity stretch (10% of resting fibre length) is not different from the isometric rate of heat production.

It was found that the striation pattern changed irreversibly when the mean sarcomere length was higher than 3·3 μm. Therefore we decided not to stretch the fibres above this sarcomere length.

[BDM] variation experiments

2,3-Butanedione 2-monoxime (BDM; Sigma) was added to the Ringer solution in different concentrations (0·5, 1, 2, 5 and 10 mM). After 1–2 min in Ringer solution, and after a few short tetani, excess solution was removed. At the end of the experiment the fibre was stimulated in Ringer solution without BDM to check whether the tetanic force and heat production were the same as at the start of the experiment. This was the case in all experiments described here.

Heat measurements

Two (bismuth–antimony) thermopiles were used for these experiments. Electrical connections were made as described by Mulieri, Luhr, Trefry & Alpert (1977). The maximal length of the active thermopile was 15·5 mm. The sensitivity of the thermopiles amounted to 93·8 and 85·2 μV °C^{-1} per junction. The thermopile records were amplified by a low noise amplifier (ANCOM Ltd, Cheltenham, UK), and filtered at 50 Hz. After analog-to-digital conversion, force and temperature signals were stored in an Olivetti M290S computer at a sample rate of 100 Hz. The temperature change during stimulation was measured from the central region of the fibre, i.e. about 70–80% of the fibre length at 2·3 μm sarcomere length. At a sarcomere length of 3·1 μm, the active region was about 50–60% of the stretched fibre length. Correction of heat loss using the Peltier method, and calculation of the heat produced by the fibre segment, was done as described by Elzinga, Lännergren & Stienen (1987). Heat records were then corrected for that part of the fibre segment that did not lie over the active region of the pile, by multiplying by the fibre length to active region ratio. In this calculation it is assumed that heat is produced uniformly along the whole fibre.

Determination of heat and force

After the experiment the fibres were washed in distilled water and dried in air. The tendons were removed and the fibres weighed on an electrobalance (Model 29, Cahn Instruments, Cerritos, CA, USA). Force was normalized on dry weight per unit length (i.e. fibre length at 2·3 μm sarcomere length; L_0), heat on dry weight of the fibre. The stable rate of heat production was determined from the slope of the regression line fitted to the total heat record from 0·35–0·5 s for type 1 fibres at 40 Hz stimulation, and from 1·0–1·5 s for all other conditions. When the regression line was extended back to the beginning of stimulation it had an intercept on the heat axis which was taken as a measure of the labile heat. We compared this method for determining labile heat with the one based on the empirical equation developed by Aubert (1956), i.e. by fitting the total heat record to:

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

where h_a is labile rate of heat production, h_b is stable rate of heat production, α is the rate constant in s^{-1}, and t the time in seconds. The results of six type 1 and seven type 3 fibres are shown in Table 1. It can be seen that both ways of analysing the data are equivalent.

Table 1. Stable rate of heat production (h_b) and total labile heat ($H_a = h_a/\alpha$)

Fibre type		Aubert's fit	Linear fit
1	H_a	0·084 ± 0·015	0·082 ± 0·012
	h_b	0·560 ± 0·090	0·580 ± 0·110
3	H_a	0·051 ± 0·012	0·050 ± 0·021
	h_b	0·250 ± 0·060	0·250 ± 0·080

H_a is expressed as joules per gram dry weight; h_b is expressed as watts per gram dry weight. The data (means ± S.E.M.) are from type 1 (n=6) and type 3 (n=7) fibres, determined by linear regression of the stable rate of heat production, and by using Aubert's empirical equation (cf. Results). The data presented are from the total heat records of fused contractions.

Assuming the lengths of the thick and thin filaments in *Xenopus* to be the same as in *Rana temporaria*, the force-independent heat (i.e. both labile and stable heat) was determined by extrapolation of the heat to 3·65 μm sarcomere length (zero overlap of the contractile proteins; Page & Huxley, 1963). Mean force was determined from the same period as that in which the stable rate of heat production was determined. In unfused contractions the time-averaged force and rate of heat production were calculated over an integer number of contraction–relaxation cycles.

Part of the heat produced might be due to series elasticity in the tendon ends of the fibre. The magnitude of this contribution can be estimated using the results of Cleworth & Edman (1972), who found the internal shortening (ΔL), due to series elasticity in single fibres, to be about 0·4 mm, and the shortening heat coefficients (α/P_0; see equation below) for type 1 and 3 fibres to be, respectively, 0·43 (H. P. J. Buschman, M. Linari, G. Elzinga & R. C. Woledge, unpublished results) and 0·13 (H. P. J. Buschman, G. Elzinga & R. C. Woledge, unpublished results). Shortening heat (SH) is calculated as:

$$SH = (\alpha/P_0) \times \Delta L \times P_0,$$

in which P_0 is maximal isometric force during fused contraction (cf. Woledge, Curtin & Homsher, 1985).

Parvalbumin extraction and electrophoretic analysis

To determine the mean parvalbumin (PA) content in type 1 and 3 fibres, fibre bundles of about ten fibres each were dissected from the iliofibularis muscle. In these bundles every single fibre could be

inspected under the microscope in order to verify that they contained fibres of the same type. These bundles were then rapidly frozen, freeze-dried and weighed. Determination of PA content was carried out as described by Simonides & van Hardeveld (1989). In short, the freeze-dried bundles were homogenized on ice for 1 min in a glass Potter tube with a motor-driven pestle in 0·5 M Tris buffer (pH 6·8) with 14% glycerol, 2 mM dithiothreitol, and afterwards supplemented with 2% sodium dodecyl sulphate (SDS) and 0·25% Bromophenol Blue. The homogenate was heated at 80 °C for 15 min and subsequently centrifuged at 10000 *g* at 0 °C for 5 min. The supernatant, or 'crude extract', containing all soluble proteins was collected and used for analysis without further purification. The supernatant was loaded on two 8–18% SDS polyacrylamide gradient gels (Excelgel Pharmacia, Milwaukee, WI, USA). Frog PA (1·5 μg, unknown isoform) and rabbit PA (0·75, 1·0, 1·25 and 1·5 μg; 12·1 kDa; Blum, Lehky, Kohler, Stein & Fischer, 1977), obtained from Sigma, were run on the same gels for calibration. Rabbit PA was used for constructing calibration curves. After electrophoresis, the gels were fixed with acetic acid, stained with Coomassie Brilliant Blue R-250 and dried. Absorbance profiles were obtained using an LKB 2202 Ultroscan laser densitometer combined with the LKB 2109-001 gelscan software program. With the use of the relation between the optical density on the gels and the added rabbit PA ($Y = 1{\cdot}02X - 0{\cdot}08$ and $Y = 0{\cdot}87X + 0{\cdot}14$, correlation coefficient $r = 0{\cdot}97$ in both cases; in which Y is the amount of rabbit PA (μg), and X is the measured density), the PA content of the fibre bundles was calculated. For conversion of milligrams protein per gram dry weight to a millimolar concentration we assumed a mean molecular mass for PA of 13 kDa and a fibre dry weight to volume ratio of 0·28 g cm^{-3} (Elzinga & van der Laarse, 1988).

Statistics

Student's *t* test was used to test for equality of means between different groups. In all cases the test was performed at a 95 % level of significance.

RESULTS

Effect of sarcomere length on force and heat production

The effect of sarcomere length on force and heat production is illustrated in Fig. 1. This figure shows examples of both a type 1 and a type 3 fibre for fused isometric contractions. At the onset of stimulation force production increases rapidly and reaches its maximum value early in the tetanus, after

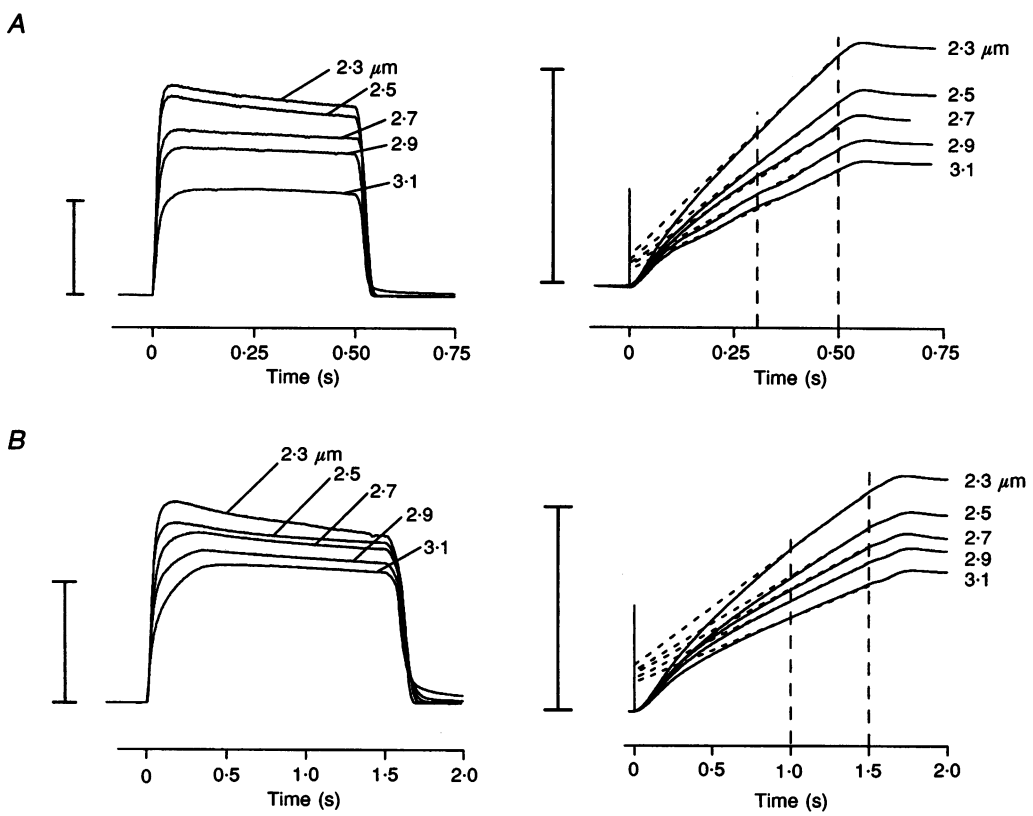

Figure 1. Examples of force and total heat production of a type 1 and a type 3 fibre during fused isometric contractions at different sarcomere lengths

Type 1 fibres (*A*) were stimulated for 0·5 s and type 3 fibres (*B*) for 1·5 s. Stable rate of heat production was reached at about 0·35 s in type 1 fibres and 1·0 s in type 3 fibres. Labile heat was determined from the total heat records (right panels) by linear extrapolation of the stable heat regression line (dashed), indicated by the period between the vertical dashed lines, to the onset of stimulation. The numbers against the total heat records refer to the different sarcomere lengths. These recordings clearly show the decrease in labile heat and stable rate of heat production with increasing sarcomere length. Fibre dimensions: type 1, 19·2 mm (control), dry weight 66·4 μg; type 3, 10·6 mm (control), dry weight 14·0 μg. Calibration bars: force, 0·30 N m (g dry wt)$^{-1}$; heat, 0·25 J (g dry wt)$^{-1}$.

which it slowly decreases to a more stable level. This decrease in force production probably results from inorganic phosphate accumulation that comes from net splitting of phosphocreatinine (PCr), (Cooke & Pate, 1985; Elzinga, Stienen & Wilson, 1989), but which has little effect (maximally about 10%) on the rate of ATP splitting by the contractile apparatus (Kawai, Güth, Winnikes, Haist & Rüegg, 1985). It can be seen that with increasing sarcomere length force production decreases in both type 1 and 3 fibres. In this example force decreases more rapidly with increasing sarcomere length in the type 1 fibre than in the type 3 fibre. However, the averaged results of type 1 and 3 fibres did not show a significant difference. In all fibres it was found that the maximum tetanic force at short sarcomere lengths was reached earlier than at long sarcomere lengths. Force relaxation was also faster at the shorter sarcomere lengths.

The rate of heat production at the onset of stimulation was high in both fibre types, after which it levelled off to a steady rate (i.e. the stable rate of heat production). At the end of stimulation the rate of heat production returned to zero. In both types of fibres the total heat produced during the tetanus decreased with increasing sarcomere length. Part of the decrease in total heat is due to a decrease in the stable rate of heat production as is shown by a decrease in the slope of the regression line fitted to the total heat record with increasing sarcomere length. However, the reduction in total heat is also due to a reduction in labile heat produced. This is reflected in the decrease of the heat intercept of the fitted regression line with increasing sarcomere length. These examples also illustrate the consistent finding that labile heat production in type 1 fibres decays more rapidly than in type 3 fibres. Labile heat production is negligible after about 0·3 s in the type 1 fibre and after about 0·8 s in the type 3 fibre (see below).

Examples of force and heat production during unfused contractions of a type 1 and a type 3 fibre at different sarcomere lengths are shown in Fig. 2. At optimal sarcomere

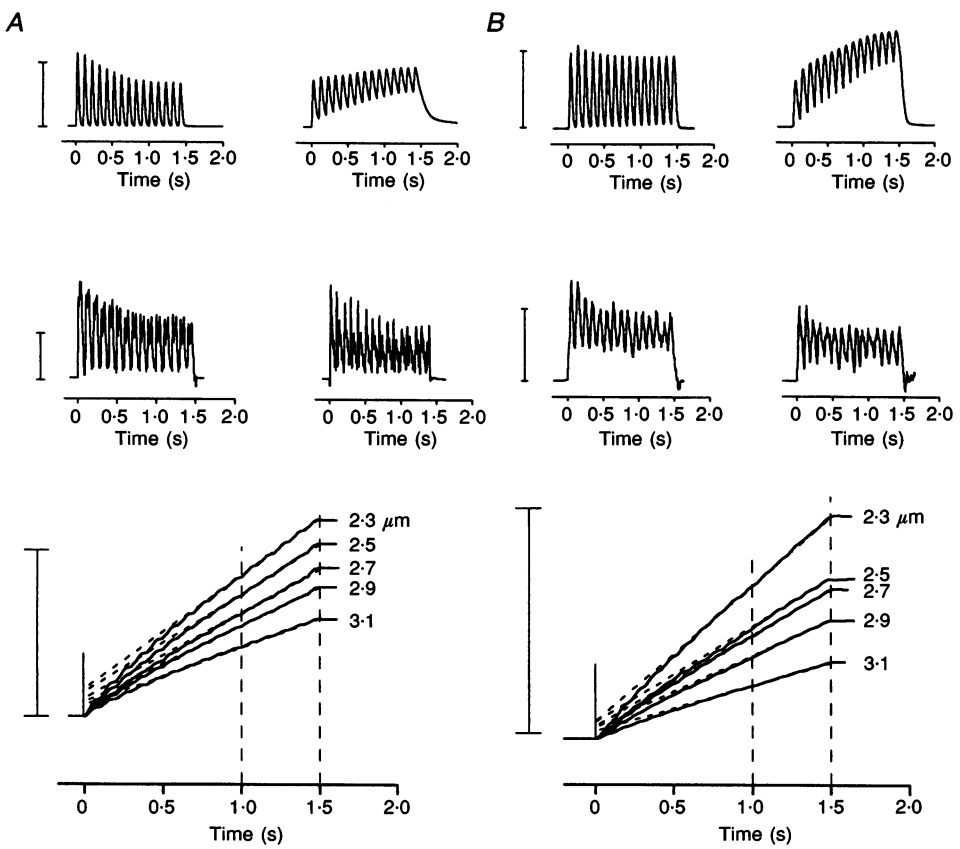

Figure 2. Examples of heat and force production during unfused isometric contractions at different sarcomere lengths

Type 1 (*A*) and type 3 (*B*) fibres were stimulated at a frequency of 10 Hz for 1·5 s. Shown are records of total heat (uppermost panels), the rate of heat production (middle panels), and force production (lowermost panels). In the middle and lowermost recordings the results at 2·3 μm sarcomere length are shown on the left, and at 3·1 μm sarcomere length on the right. At this low stimulation frequency the responses of the fibre to the individual stimuli can be seen. Note the increase in mean force production with time at 3·1 μm sarcomere length. Type 1 fibre: fibre length at 2·3 μm, 19·2 mm; dry weight, 85·6 μg. Type 3 fibre: fibre length at 2·3 μm, 12·8 mm; dry weight, 55·8 μg. Calibration bars: total heat, 0·25 J (g dry wt)$^{-1}$; rate of heat production, 0·20 W (g dry wt)$^{-1}$; force, 0·2 N m (g dry wt)$^{-1}$.

Table 2. Overall results of labile heat production

Fibre type	Freq. (Hz)	Total labile heat at 2·3 μm SL (J (g dry wt)$^{-1}$)	Force-independent labile heat relative to 2·3 μm SL (%)	Force-independent labile heat relative to 0 mM BDM (%)	Mean force-independent labile heat (%)*	Force-independent labile heat (J (g dry wt)$^{-1}$)
1	40	0·086 ± 0·008 (13)	56 ± 9 (7)	54 ± 5 (6)	55 ± 4 (13)	0·047 ± 0·007
	10	0·035 ± 0·006 (13)	72 ± 7 (7)	47 ± 4 (6)	60 ± 5 (13)	0·021 ± 0·006
3	35	0·048 ± 0·005 (15)	23 ± 7 (8)	30 ± 12 (7)	27 ± 8 (15)	0·013 ± 0·006
	10	0·036 ± 0·005 (11)	41 ± 9 (5)	30 ± 10 (6)	36 ± 8 (11)	0·013 ± 0·007

Results from sarcomere length variation and BDM concentration variation experiments for type 1 and 3 fibres, and for fused and unfused isometric contractions. Stimulation frequency, Freq.; sarcomere length, SL. Values are means ± S.E.M. Values in parentheses represent the number of fibres. * Calculated by averaging the individual data by sarcomere length variation and BDM concentration variation.

length (2·3 μm sarcomere length) in both type 1 and 3 fibres force production rises and falls after each stimulus. In between stimuli force production nearly returns to zero. Maximum force production is reached early in the tetanus after which force decreases to a stable level. The recordings of the heat production at this sarcomere length reveal that the time course of the rate of heat production is very similar to the time course of force production.

Force production at a sarcomere length of 3·1 μm has a different time course than at 2·3 μm sarcomere length in both fibre types. Although force production at the onset of stimulation is less, due to reduced overlap of the contractile filaments, the mean force level increases during the tetanus. This steady increase in force is probably caused by 'creep' due to sarcomere non-uniformities along the fibre length. This idea is strengthened by the finding that although the rate of heat production is less at longer sarcomere length the time course of the rate of heat production is not affected by sarcomere length. An additional factor involved may be a slowing in force relaxation during the continuation of stimulation.

From the total heat records (lower panels in Fig. 2) the stable rate of heat production was determined, after labile heat production was negligible, from the slope of the regression line fitted to the total heat record 0·35–0·5 s after the onset of stimulation (type 1, 40 Hz) or 1·0–1·5 s (type 1, 10 Hz; type 3, 35 and 10 Hz). Assuming the filament lengths in iliofibularis muscle from *Xenopus* to be

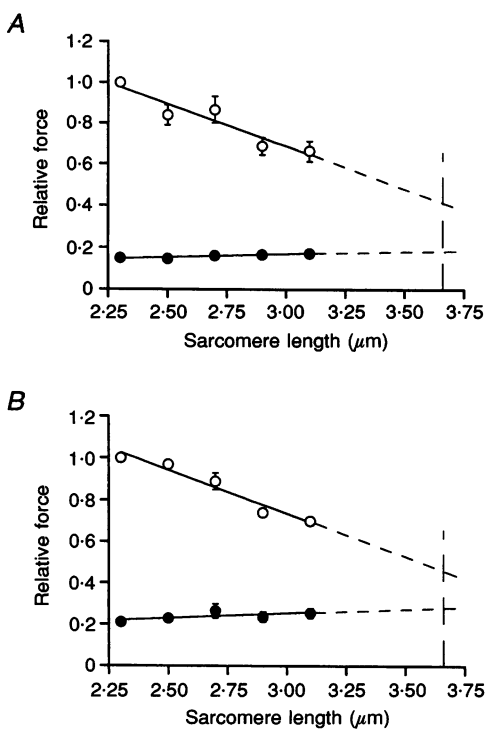

Figure 3. The relation between sarcomere length and relative mean force production.

The results are shown for type 1 (*A*) and 3 fibres (*B*), and both fused (●) and unfused contractions (○). For both fibre types the mean force production was normalized to the value at 2·3 μm. Extrapolation of force to a sarcomere length of 3·65 μm, i.e. the sarcomere length at which the overlap of the contractile proteins is thought to be negligible, shows that for both fibre types and both fused and unfused contractions there is still significant force production present. The error bars represent the S.E.M.

the same as in *Rana*, the activation heat expressed as a fraction of the stable heat was determined by extrapolation of the stable heat to 3·65 μm sarcomere length (zero overlap between the contractile proteins) in the sarcomere length variation experiments.

Figure 3 shows the relation between sarcomere length and mean force production in type 1 and 3 fibres, for both fused and unfused contractions. For fused contractions force production decreases with increasing sarcomere length in both fibre types. However, at 3·65 μm sarcomere length, the length at which average overlap between the thin and thick filaments is assumed to be negligible, there is still considerable force production (about 45% of maximum force in type 1 fibres and 40% in type 3 fibres), which is in agreement with the results of Gordon, Huxley & Julian (1966*a*, *b*). For unfused tetani force production, in fact, tends to increase with decreasing filament overlap. This suggests that also in *Xenopus* muscle fibres force production at long sarcomere lengths is affected by the (shorter) sarcomeres near the fibre ends. This means that in the experiments in which sarcomere length was varied, extrapolation to zero force does not provide an appropriate means to determine the force-independent rate of heat production. To avoid these effects on force production, we plotted labile heat (Fig. 4*A*) and stable rate of heat production (Fig. 4*B*) as a function of sarcomere length. Despite substantial variation between fibres, it is clear that labile heat production gradually decreased with increasing sarcomere length both in type 1 and type 3 fibres. Furthermore, at zero filament overlap (3·65 μm sarcomere length) there was still significant labile heat production in both fibre types (cf. Table 2).

To obtain an estimate for activation heat the stable rate of heat production was plotted as a function of the mean sarcomere length, and extrapolated to zero filament overlap. The results are shown in Fig. 4*B*, and clearly show a linear decrease in stable rate of heat production with increasing sarcomere length at high levels of activation, in both fibre types. For unfused contractions in type 3 fibres, however, the stable rate of heat production decreased non-linearly

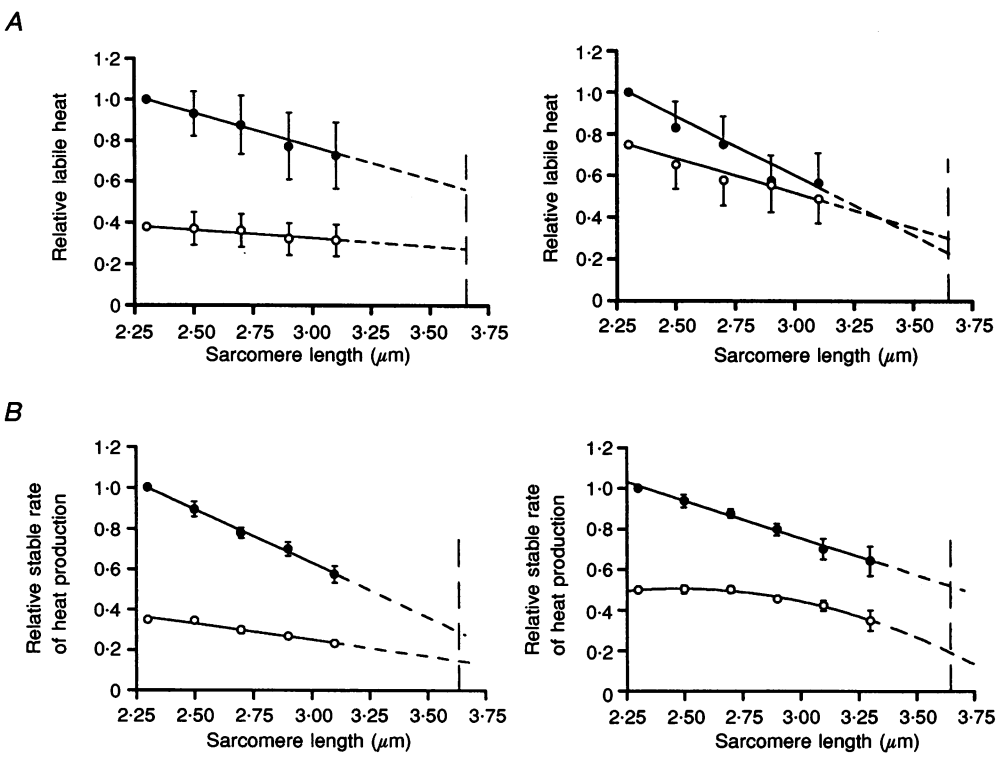

Figure 4. The effect of sarcomere length on heat production in type 1 and 3 fibres

The results are shown for labile heat (*A*) and the stable rate of heat production (*B*), for type 1 (left) and type 3 fibres (right), and both fused (●) and unfused contractions (○). Total labile heat decreases with increasing sarcomere length for both fibre types and both fused and unfused contractions. At zero filament overlap (3·65 μm sarcomere length) there is still significant labile heat production (i.e. force-independent labile heat), see Table 2. Extrapolation of the relative stable rate of heat production to a sarcomere length of 3·65 μm gives the relative rate of activation heat production, see Table 3. In all cases extrapolation was carried out by means of linear regression except for determination of rate of activation heat production for the unfused type 3 fibre results where a 2nd-order polynomial was used ($Y = -0{\cdot}906 + 1{\cdot}14X - 0{\cdot}23\ X^2$), in which Y represents stable rate of heat production (W (g dry wt)$^{-1}$) and X, sarcomere length (μm). The results for the unfused contractions are scaled to the relative mean heat for the fused contractions. The error bars represent the S.E.M.

with increasing sarcomere length. This effect is possibly due to an increase in calcium sensitivity with sarcomere length (cf. Discussion). The type 3 results at 10 Hz stimulation were therefore fitted with a non-linear (2nd-order polynomial) function. The contribution of the activation heat to the stable rate of heat production in type 1 and type 3 fibres, for both fused and unfused tetani, are shown in Table 3.

An effect that could influence heat production in unfused tetani is related to the force oscillations. As a result of these oscillations, work is being done by the fibre on the tendons, which is dissipated as heat when the force drops. However, based on calculations using the length extension experimental results of Cleworth & Edman (1972) we infer that this effect in unfused tetani, i.e. when the extra energy liberation is expected to be highest, is negligible (2–4% of the stable heat rate).

Another part of the heat produced is due to internal movement of the central part of the fibre because of sarcomere non-uniformity. The work involved is dissipated as heat. On the basis of marker movements in experiments of e.g. Gordon, Huxley & Julian (1966*a*), with a 24 μm stretch in a fibre segment of 6·1 mm and a force of 3 mN, it is calculated that the heat produced as a result of this effect is 72 nJ. When we compare this with the total heat produced in a 0·5 s tetanus (1·6 μJ) we conclude that 'creep' is an unimportant source of heat production.

Effect of BDM on force and heat production

Figure 5 shows examples of fused tetanic contractions and the associated heat production at different concentrations of BDM, in a type 1 and and a type 3 fibre. In these examples the depressive effect of BDM on force and total heat production is clearly shown. In contrast to what was seen with sarcomere length variation, the contraction and relaxation times were not different when force decreased with increasing BDM concentration. In type 1 fibres at BDM concentrations of 5 mM or more, force production was not maintained during the tetanus, which may reflect activation failure. Therefore we decided not to use the results obtained with 5 and 10 mM BDM in the fused contractions in type 1 fibres. In contrast, this effect of BDM on the excitation–contraction coupling was not observed in type 3 fibres, and during unfused tetani in both fibre types.

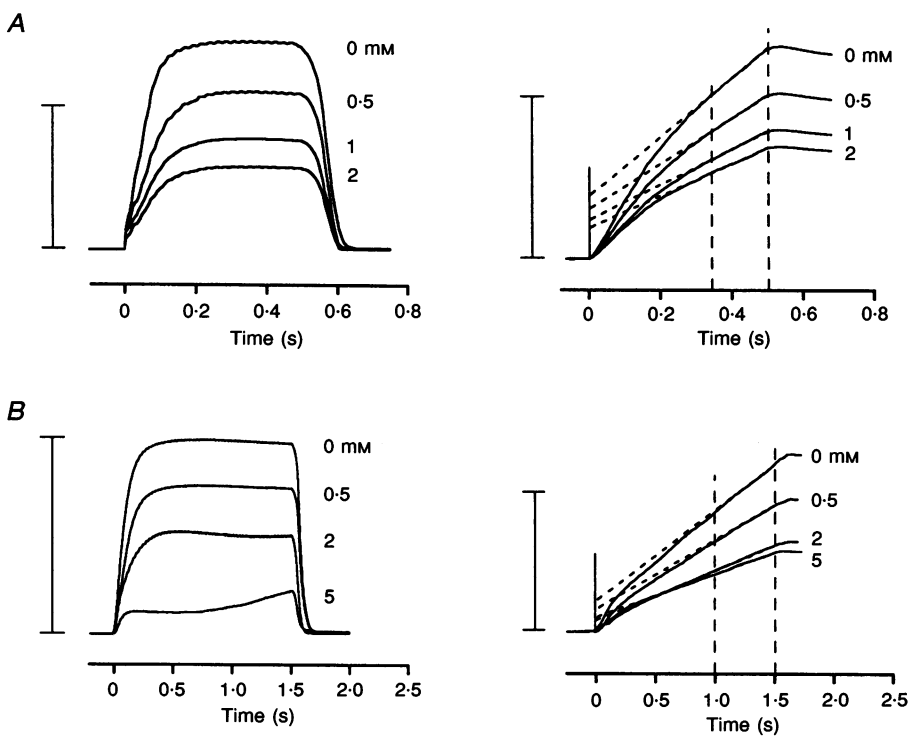

Figure 5. Examples of force and heat production of a type 1 and type 3 fibre during fused isometric contractions at different concentrations of BDM

Records of force production (left) and the rate of heat production (right) are shown for a type 1 fibre (*A*) and a type 3 fibre (*B*). Clearly visible is the depressive effect of the concentration of BDM on tetanic force and heat production. Labile heat was determined by extrapolation of the regression line fitted to the period of stable heat production, indicated by the time between the vertical dashed lines, to the onset of stimulation. Fibre length: type 1, 19·2 mm; type 3, 12·2 mm. Dry weight: type 1, 66·4 μg; type 3, 30·6 μg. Stimulation time: type 1, 0·5 s; type 3, 1·5 s. Calibration: heat, 0·50 J (g dry wt)$^{-1}$; force, 0·50 N m (g dry wt)$^{-1}$. Sarcomere length: 2·3 μm.

From the heat production records it can be seen that, like tetanic force, heat production decreases with increasing BDM concentration. However, as expected, heat production was less sensitive to BDM than force. The decrease in heat production is not only due to a decrease in the stable rate of heat production as described before, but also to an inhibiting effect of BDM on labile heat, as can be seen by the decrease in intercept of the regression line fitted to the total heat record.

Figure 6 shows examples of force and heat production of a type 1 and a type 3 fibre for unfused contractions (10 Hz stimulation) at different concentrations of BDM. At this low activation level the force records show the oscillating response of the fibres in synchrony with the individual stimuli. The recordings of the rate of heat production show that the time course of this rate is very similar to the time course of force production. As was observed in fused contractions, during unfused contractions the decrease in total heat with increasing concentration of BDM is not only due to a decrease in the stable rate of heat production but also a decrease in labile heat. This is again reflected in the decrease in the heat intercept found by extrapolation of the stable heat regression line to the onset of stimulation. These records also show that in unfused contractions the rate of decay of labile heat production is about the same in type 1 and type 3 fibres.

To determine the sensitivity of force production to BDM we fitted the relation between the BDM concentration and mean tetanic force for fused tetanic contractions with a hyperbolic function:

$$F = 1 - (1 - F_{\infty})([\mathrm{BDM}]/([\mathrm{BDM}] + K_{\mathrm{i}}))$$

(Hui & Maylie, 1991). This was done for both type 1 and type 3 fibres, and at both levels of activation. In all cases the mean value of the extrapolated forces at infinite BDM concentration (F_{∞}) was not significantly different from zero. The stimulation frequency had no effect on the BDM sensitivity of force development. The BDM concentrations at which force was decreased to 50% (K_{i}) for fused contractions was 0·72 ± 0·04 mM (mean ± S.E.M.; $n = 6$) for type 1 fibres, and 1·03 ± 0·04 mM for type 3 fibres ($n = 7$).

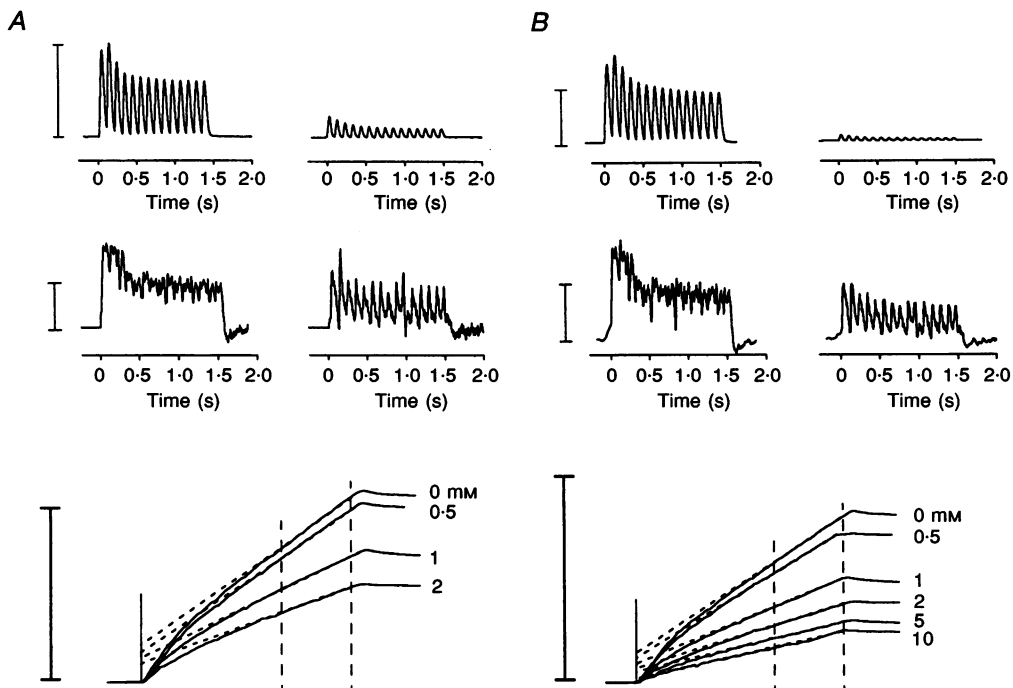

Figure 6. Examples of force and heat production of a type 1 and a type 3 fibre during unfused isometric contractions at different concentrations of BDM

A, examples of a type 1 fibre, and *B*, examples of a type 3 fibre. For unfused contractions (10 Hz stimulation) both fibre types were stimulated for 1·5 s. In these examples the force oscillations can be clearly seen. Labile heat was determined by extrapolation of the stable heat regression line, indicated as the time between the vertical dashed lines, to the onset of stimulation. These records clearly show that with increasing BDM concentration both labile heat and the stable rate of heat production are reduced. Type 1 fibre: length, 14·7 mm; dry weight, 51·7 μg. Type 3 fibre: length, 12·8 mm; dry weight, 34·3 μg. Calibration bars: force, 0·20 N m (g dry wt)$^{-1}$; heat, 0·20 J (g dry wt)$^{-1}$. Sarcomere length: 2·3 μm.

Table 3. Activation heat results from sarcomere length variation and BDM concentration variation experiments

Fibre type	Freq. (Hz)	Stable heat rate (W (g dry wt)$^{-1}$)	Activation heat by SL variation (%)	Activation heat by [BDM] variation (%)	Mean activation heat † (%)	Activation heat rate (W (g dry wt)$^{-1}$)
1	40	0·553 ± 0·022(13)	35 ± 6(7)	34 ± 7(6)	34 ± 4(13)	0·187 ± 0·020
	10	0·162 ± 0·025(13)	40 ± 7(7)	57 ± 4(6)	48 ± 5(13)	0·078 ± 0·008
3	35	0·249 ± 0·012(15)	57 ± 6(8)	48 ± 4(7)	52 ± 4(15)	0·127 ± 0·010
	10	0·140 ± 0·021(11)	38 ± 7(5)*	35 ± 2(6)	‡	0·051 ± 0·003

Results from type 1 and 3 fibres, and fused and unfused isometric contractions. Values are means ± S.E.M. Freq., stimulation frequency. Values in parentheses represent the number of fibres. * Fitted with 2nd-order polynomial (cf. Results). † Obtained by averaging the individual results by sarcomere length and BDM concentration variation. ‡ Not averaged because the activation heat values were obtained by different extrapolation methods.

The relations between force and labile heat and force and the stable rate of heat production at different BDM concentrations are shown in Fig. 7, for both type 1 and type 3 fibres, and for fused and unfused contractions. The mean force production in unfused tetani in the absence of BDM, was not different between fibre types (see below). Labile heat in unfused tetani, however, differed considerably (40% of heat produced in fused contractions in type 1 fibres and 75% in type 3 fibres). Figure 7*A* shows that with increasing BDM concentration labile heat production decreases. Furthermore, in both fibre types and at both levels of activation, the relative decrease in labile heat with increasing BDM concentration is always less than the decrease in relative force, i.e. at zero force there is still labile heat production. For unfused contractions in both fibre types the relation between mean force and labile heat was steeper than for fused contractions. In type 1 fibres, the force-independent labile heat component differed by more than twofold between fused and unfused contractions, while in type 3 fibres the force-independent labile heat in fused and unfused contractions was the same (see Table 2).

Figure 7*B* shows that both force and the stable rate of heat production decreased with increasing BDM concentration, although force production was more affected than the rate of heat production. These relations also show that with increasing concentration of BDM, force and the rate of heat production decrease linearly. This is in agreement with the results of Stienen, Zaremba & Elzinga (1995), who found a proportional relationship between force and ATP turnover in Triton X-100-skinned muscle fibres. Furthermore this indicates that BDM did not influence activation heat. The contribution of the activation heat to the stable heat for the individual fibres was determined by extrapolating to zero force and is shown in Table 3.

Comparison of sarcomere length and BDM experiments

The experiments on (1) fibre stretching, (2) the use of BDM, (3) fused and (4) unfused contractions were each performed on a separate group of fibres. However, no statistically significant differences were found between groups in the control values (i.e. values at 2·3 μm sarcomere length and 0 mM BDM), for force, labile heat or stable rate of heat production in either of type 1 or 3 fibres and high and low levels of activation. For fused contractions mean force production (P_0) was 0·72 ± 0·03 N m (g dry wt)$^{-1}$ (mean ± S.E.M.; $n = 13$) in type 1 fibres and 0·60 ± 0·05 N m (g dry wt)$^{-1}$ ($n = 15$) in type 3 fibres. The relaxation rate (i.e. the slope of the force record until the 'shoulder' is reached) was 5·60 ± 0·42 P_0 s^{-1} in type 1 fibres and 3·64 ± 0·32 P_0 s^{-1} in type 3 fibres. Time-averaged force production for unfused contractions was 0·06 ± 0·02 N m (g dry wt)$^{-1}$ ($n = 13$) and 0·13 ± 0·03 N m (g dry wt)$^{-1}$ ($n = 11$) for type 1 and 3 fibres, respectively. These values are not statistically different. The time-averaged force production in unfused tetani was about 15% of that in fused tetani.

During the first phase of relaxation, which is isometric (cf. Lännergren & Arner, 1992), the rate of relaxation correlated weakly with the activation heat ($r = 0·47$), but only when the data for type 1 and 3 fibres were pooled. This implies that only about 22% (i.e. $r^2 \times 100\%$) of the variation in relaxation rate is explained by variations in activation heat.

In both fibre types the force-independent labile heat component determined by sarcomere length variation and by BDM variation is the same. This is the case in fused as well as in unfused contractions. The average force-independent labile heat values are presented in Table 2. For fused contractions the amount of force-independent labile heat in type 1 fibres is significantly higher than in type 3

fibres, while force-independent labile heat in type 1 and 3 fibres for unfused contractions is not significantly different. Also the absolute, and the fraction activation heat of the total stable rate of heat production between the two groups of fibres of the same type is not significantly different. Both the mean absolute and the activation heat component of the total stable rate of heat production values are presented in Table 3. From this we conclude that the force-independent labile heat and the activation heat results obtained with the different methods agree.

Labile heat time constants

If labile heat originated from two different processes, a force-dependent and a force-independent process, the (composite) decay time constant for labile heat production might be dependent on the amount of force produced. The decay time constant of labile heat production was determined by fitting the results with Aubert's equation (cf. Methods). This was only done for fused contractions because in unfused contractions the labile heat decay time constants could not be determined reliably due to the oscillations in heat production. It was found that the decay time constant did not depend on sarcomere length or BDM concentration in both fibre types. In type 1 fibres the mean time constants for fused contractions were 0·26 ± 0·02 s, (± S.E.M.) and in type 3 fibres, 0·30 ± 0·03 s. These values are not significantly different.

Parvalbumin concentration

In order to determine the fraction of labile heat associated with calcium binding to PA in type 1 and 3 fibres, the concentration of PA was determined in five slow-twitch (type 3) and five fast-twitch (type 1) muscle bundles. Examples of gel electrophoretic separation of samples from type 1 and 3 muscle bundles are shown in Fig. 8. For type 1 two protein bands, the heavier one just separated from a protein band of about 14 kDa (probably myosin light chain 3), can be seen towards the front of the gel, co-migrating with frog and rabbit PA. In type 3 fibres, however, the lighter of these PA proteins seemed to be absent in all samples, while in one sample (lane 3) the heavier PA was also nearly absent. The mean PA content, determined densitometrically, was 0·84 ± 0·08 mM (± S.E.M.; $n = 5$) in type 1 fibres and 0·12 ± 0·02 mM ($n = 5$) in type 3 fibres. Thus the PA content in type 1 fibres was, on average, about 7 times higher than in type 3 fibres.

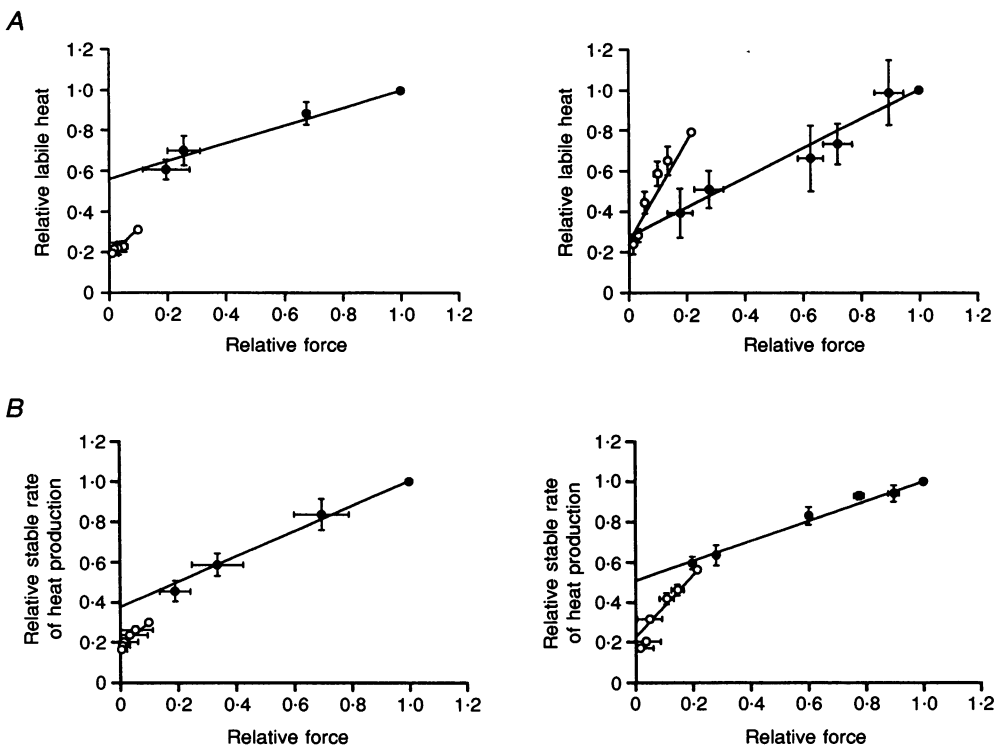

Figure 7. The relation between relative force and relative heat production for type 1 and 3 fibres at different concentrations of BDM

A, labile heat results, and *B*, stable heat results, for both type 1 fibres (left panels) and type 3 fibres (right panels), for fused (●) and unfused contractions (○). Extrapolation of the regression lines to zero force gave the estimate of the heat production that is not inhibited by BDM, i.e. force-independent labile and stable heat (activation heat). BDM concentrations used are: 0·5, 1, 2, 5 and 10 mM (cf. Fig. 6). The results at 5 and 10 mM BDM for fused contractions in type 1 fibres were not included (cf. Results). The results for the unfused contractions are scaled to the relative mean heat for the fused contractions. Means ± S.E.M. are shown.

DISCUSSION

In this study we determined the force-dependent and force-independent labile and stable heat production in type 1 and 3 fibres from *Xenopus laevis* by sarcomere length variation or by using different concentrations of BDM. In general, variation of either sarcomere length or BDM concentration gave similar estimates of force-independent labile heat and stable heat. It was found that the labile heat produced during a tetanic contraction has at least two different origins: (1) a force-dependent process, and (2) a force-independent process, most likely related to Ca^{2+}–M exchange on PA. Furthermore, the force-dependent and force-independent parts of the stable heat production were quantified in both fibre types at fused and unfused tetanic contractions.

Effects of sarcomere length and BDM concentration variation on force and heat production. In this study force production was varied by stretching the fibre to reduce filament overlap and by increasing BDM concentration. Stretching the fibre to long sarcomere length, however, causes the force level to 'creep' to higher values with continuing stimulation, due to the sarcomeres near the myotendinous junction (about 10% of the total number) being shorter than those in the centre of the fibre (Huxley & Peachey, 1961; Gordon, Huxley & Julian, 1966*b*). As Fig. 3 shows, in muscle fibres from *Xenopus laevis*, creep also affected the force level reached in both fused and unfused tetani. This means that extrapolation to zero *force* in sarcomere length variation experiments in muscle fibres from *Xenopus* does not result in accurate activation heat estimates. To overcome the effect of the short sarcomeres near the fibre ends on force production, *heat production* was recorded from the central part of the fibre and related to the mean calculated sarcomere length. Force-independent labile heat and activation heat were then determined by extrapolating heat production to zero filament overlap (Fig. 4). Another factor disturbing force production in unfused contractions was a slowing of relaxation during the stimulation period; this resulted in increased mean force production at increasing sarcomere length. Again effects on heat production due to this were minimized by measuring heat production from the central part of the fibre, i.e. where sarcomere length is much less affected than near the fibre ends. Furthermore, it should be noted that both increasing sarcomere length and the use of BDM were assumed only to affect the interaction of the contractile proteins and not to influence calcium handling. The activation heat determined in fused contractions with both methods gave similar results, which suggests that calcium handling is unaffected, because it is unlikely that both methods would affect calcium handling equally. This view is supported by the results presented by Alpert *et al.* (1989), in which it was found that calcium release was not affected at moderate concentrations of BDM, and by the linear relations between relative force and labile heat and force and relative rate of heat production for the different fibre types and levels of activation. Although both methods gave similar results, force-independent heat determination by BDM concentration variation is more accurate than by sarcomere length variation, because the extrapolation is more accurate in the BDM experiments (the S.E.M. values of BDM experiments tend to be smaller; cf. Tables 2 and 3).

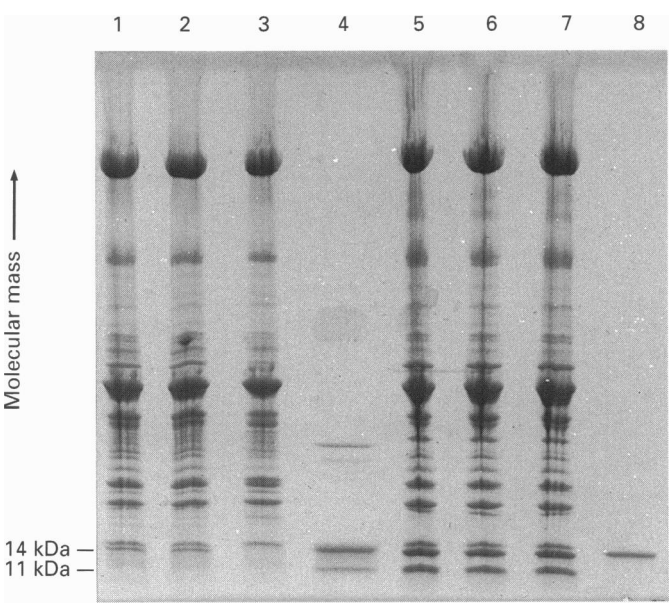

Figure 8. Electrophoresis of equally sized samples from type 3 and type 1 muscle bundle extracts

Type 3: lanes 1 (bundle, 31·5 μg; PA, 0·19 μg), 2 (29·9 μg; 0·14 μg) and 3 (22·4 μg; 0·02 μg). Type 1: lanes 5 (42·9 μg; 1·59 μg), 6 (33·0 μg; 1·39 μg) and 7 (34·7 μg; 1·54 μg). In lane 4, 1·25 μg frog parvalbumin and in lane 8, 0·75 μg rabbit parvalbumin were added as standards. Note that in type 3 fibres the 11 kDa parvalbumin protein was almost absent.

Labile heat

Comparison with previous studies. The mean values of labile heat production and PA concentration in fast type 1 fibres are higher than in slow type 3 fibres. These findings are in general agreement with the results from Lännergren *et al.* (1993), who found labile heat to be a function of the PA concentration; type 2 fibres, which contained, on average, less PA than type 1 fibres, produced less labile heat. In fact, the results presented in this paper fit the relation presented by Lännergren *et al.* (1993), and extend its range. Note that labile heat in the study of Lännergren *et al.* (1993) is erroneously given in joules per gram wet weight, while the values are in joules per gram dry weight (J. Lännergren, G. Elzinga & G. J. M. Steinen, personal communication). The total labile heat in type 1 fibres in the present study is somewhat higher than the total labile heat in the study by Lännergren *et al.* (1993), but this is consistent with the finding that the PA content in type 1 fibres in the present study is also higher.

The time constant of labile heat decay in type 1 fibres ($0{\cdot}26 \pm 0{\cdot}02$ s) is in good agreement with the value found for type 1 fibres ($0{\cdot}27 \pm 0{\cdot}02$ s) in the study by Lännergren *et al.* (1993).

We found that the rate of decay of labile heat production was not affected by fibre stretching or by BDM. Curtin & Woledge (1981) observed on semitendinosus muscle from *Rana* at 0 °C that labile heat was produced more slowly at a sarcomere length of 3·8 μm than at 2·3 μm. This observation would be consistent with the disappearance of a faster force-dependent labile heat component. We did not observe a similar change either with sarcomere length or BDM concentration variation. The reason for this is not clear. The difference could be due to the experimental temperature or the degree of filament overlap, which differed in the study by Curtin & Woledge from the present study.

Force-dependent and force-independent labile heat. Total labile heat decreases linearly with decreasing overlap between the thick and thin filaments and also with a decrease in force by increasing BDM concentration, and thus contains a force-dependent component. The force-independent labile heat component was obtained by linear extrapolation of the labile heat to zero filament overlap in the stretch experiments or to zero force in the BDM experiments. In both cases the force-independent labile heat was a significant fraction of the total labile heat. The results reported in Homsher *et al.* (1972), and in Curtin & Woledge (1977, 1981), in which labile heat was reduced at long sarcomere length, and the results reported in Berquin & Lebacq (1992) and Lännergren *et al.* (1993), who found that not all labile heat could be explained by calcium binding to PA, are compatible with this. The finding that the estimation of force-dependent labile heat production, determined by either variation of sarcomere length or BDM concentration, was similar suggests that it is dependent on the number of active cross-bridges rather than a change in cross-bridge kinetics.

In previous studies it was argued that a likely candidate for labile heat production is the Ca^{2+}–Mg^{2+} exchange on PA (e.g. Smith & Woledge, 1985; Peckham & Woledge, 1986). In a study on *Xenopus* muscle fibres (Lännergren *et al.* 1993), a relation was found between total PA content and labile heat production. Under these conditions PA was probably saturated with calcium due to the relatively high concentrations of calcium in the cytosol. When we combine the results from both studies, statistical analysis shows that there is good linear correlation ($r = 0{\cdot}84$) between the total amount of PA and labile heat. The slope of the regression line corresponds to -78 kJ (mol PA)$^{-1}$. This value (at 1·7 mM free Mg^{2+}; Westerblad & Allen, 1992) agrees well with values reported previous for Ca^{2+}–Mg^{2+} exchange, e.g. -77 kJ (mol PA)$^{-1}$ (at 1 mM Mg^{2+}; Smith & Woledge, 1985) and -65 kJ (mol PA)$^{-1}$ (at 5 mM Mg^{2+}; Tanokura & Yamada, 1987).

In type 3 fibres force-independent labile heat was not different between fused and unfused contractions, while a large difference was found between fused and unfused contractions in type 1 fibres. This is compatible with the notion that at a stimulation frequency of 10 Hz, i.e. at a level of activation that was about the same in both fibre types, the small amount of PA present was saturated in type 3 fibres, while in type 1 fibres, which contain much more PA, the PA was only partly saturated at this level of activation.

In both fused and unfused contractions labile heat decreased with a decrease in mean force production by reducing filament overlap and by an increase in BDM concentration. From these findings it was inferred that part of the labile heat production was related to cross-bridge interaction. However, as was shown in Fig. 7, the relation between labile heat and mean force was steeper in unfused contractions than in fused contractions. Because of the difficulty in interpreting the results at changing cross-bridge activity in unfused contractions, we further analyse force-dependent labile heat production for fused contractions only.

The force-dependent labile heat production in fused contractions may be explained by a shift in cross-bridge population at the onset of stimulation from detached to attached states, and could thus be related to the number of attached cross-bridges. The slope of the relation between force and labile heat was similar for type 1 and type 3 fibres. Force-dependent labile heat in fused contractions in type 1 fibres was 0·039 J (g dry wt)$^{-1}$, while in type 3 fibres this was 0·035 J (g dry wt)$^{-1}$. These values are similar to the value obtained by extrapolation of the relation between PA and labile heat to zero [PA] of the combined results from the present study and the study by Lännergren *et al.* (1993); 0·031 J (g dry wt)$^{-1}$. If we assume a mean exothermic enthalpy change for cross-bridge attachment of -30 kJ mol^{-1} ($A + M{\cdot}ADP{\cdot}P_i \rightarrow A{\cdot}M{\cdot}ADP{\cdot}P_i \rightarrow A{\cdot}M{\cdot}ADP + P_i$, in which

A represents actin, M myosin, ADP adenosine diphosphate, and P_i inorganic phosphate; cf. Kodama, 1985), a dry weight to volume ratio of 0·28 g cm^{-3} (Elzinga & van der Laarse, 1988), 0·28 mmol cross-bridge l^{-1} (Squire, 1981) independent of fibre type, and all cross-bridges to attach during fused contractions, we can calculate the enthalpy change for cross-bridge attachment as 0·033 J (g dry wt)$^{-1}$. This is similar to the measured amount of force-dependent labile heat for fused contractions.

Stable rate of heat production

Comparison of different techniques to estimate activation heat. We found large differences in activation heat between single fibres of one type in both sets of experiments, indicating that the energy consumption associated with calcium uptake by the SR calcium pumps during activity varies considerably between fibres of the same fibre type. This finding is in agreement with the results on single skinned type 1 and 2 iliofibularis fibres reported by Stienen *et al.* (1995), in which the total (SR plus myofibrillar) and myofibrillar–ATPase activity was measured. The values of the stable rate of heat production in intact fibres in the present study can be compared with the absolute values for the two major ATP-consuming processes (myofibrillar and SR ATPase activity). Based on the mean value of the total ATPase activity at 4·3 °C (2·04 μmol s^{-1} (g dry wt)$^{-1}$), an increase in ATPase activity at 20 °C by a factor of 7·6 (temperature coefficient, Q_{10}, between 5 and 10 °C of 4·80; Q_{10} between 10 and 20 °C of 2·97; Stienen *et al.* 1995), and an enthalpy change for creatine phosphate (PCr) splitting of −34 kJ mol^{-1} (Curtin & Woledge, 1978), this would correspond to a stable maintenance rate of heat production of 0·53 W (g dry wt)$^{-1}$. This value is in good agreement with the rate of heat production in fused tetani in intact type 1 fibres (0·553 W (g dry wt)$^{-1}$). The SR ATPase activity in skinned fast fibres (types 1 and 2) at 20 °C was 48% of the total ATPase activity, and is somewhat higher than the value for type 1 fibres found in the present study (34%). A possible reason for the discrepancy between both studies may be related to the fact that in the study by Stienen *et al.* (1995) in the fast-twitch fibres type 2 fibres were included.

The contractile apparatus of the slow-twitch fibres was less sensitive to BDM than that of the fast-twitch fibres (Fig. 6). The K_i values we determined in this study, however, are about twice the values found by Hui & Maylie (1991) in twitch muscle from *Rana temporaria* at 5–6 °C. This difference could be due to the difference in temperature, since Higuchi & Takemori (1989) found the depressive effect of BDM to be higher at low temperatures. We also found the effect of BDM on force to be independent of the level of activation, which again suggests that BDM does not have an effect on the calcium handling during activation, and is in agreement with the results of previous studies on intact frog muscle (e.g. Maylie & Hui, 1988). Also the proportionality between relative force and relative rate of heat production for the different fibre types at different levels of activation supports this. In type 3 fibres, for both fused and unfused contractions, in the range of concentrations used, no activation problems were observed. Only when the BDM concentration was increased to 5 mM or more, and only in fused contractions in type 1 fibres, activation failure occurred. From this we conclude that, taking into account the low K_i values in type 1 and 3 fibres, a strong reduction in force can be achieved with low to moderate concentrations of BDM, without significant effects on calcium handling by the SR or on excitation–contraction coupling.

Activation heat in fused contractions. The fraction of activation heat relative to the stable heat found in previous studies by fibre stretching to zero sarcomere overlap in twitches (e.g. Smith, 1972; Rall, 1982) or in tetanic contractions (Curtin & Woledge, 1981; Elzinga, Peckham & Woledge, 1984; Burchfield & Rall, 1985*a*), and with the use of BDM (Siegman, Mooers, Warren, Warshaw, Ikebe & Butler, 1994), varied between 20 and 61%. The fraction activation heat of the stable rate of heat production found for type 1 fibres in the present study is in the upper half of this range, whereas in the slow type 3 fibres the fraction is close to the maximum reported so far. ATP hydrolysis due to calcium sequestration by the SR is the most important contributor to the activation heat, because the energy turnover by the second most important energy-consuming process, the high energy phosphate splitting associated with restoration of the membrane potential after each action potential (based on 2 nmol ATP (g wet wt)$^{-1}$; Kushmerick, 1983), only accounts for about 5% of the activation heat. Most of the above studies were performed at 0 °C, which could mean that the temperature at which the experiments are done may influence the contribution of the activation heat to the stable rate of heat production. The temperature sensitivity for actomyosin ATPase is less than that for SR ATPase (Q_{10} values of 3·9 and 5·7, respectively, in frog semitendinosus; Burchfield & Rall, 1985*b*; and 4·72 and 7·90 in fast-twitch *Xenopus* iliofibularis fibres in the range between 5 and 20 °C; Stienen *et al.* 1995). This means that the SR ATPase activity increases more with rising temperature than actomyosin ATPase activity, and therefore the contribution of the activation heat to the stable rate of heat production increases with temperature. The results from heat measurements at 20 °C on semitendinosus from frog (Rall, 1982), from high energy phosphate usage on EDL from mouse (Siegman *et al.* 1994), and also from ^{31}P-NMR measurements on intact frog muscle at room temperature (Baker *et al.* 1994), in which 56, 61 and 43%, respectively, of the total ATP turnover was consumed by non-contractile processes, are in agreement with this.

Activation heat in unfused contractions. The time-averaged total stable rate of heat production at 10 Hz stimulation in type 1 and type 3 fibres was two to three times lower than the total stable rate of heat production of fused contractions. Furthermore, the contribution of the activation heat to the stable rate of heat production at 10 Hz in type 1 fibres was higher than in type 3 fibres.

Table 4. Energy turnover and economy of force maintenance of the contractile proteins

Fibre type	Freq. (Hz)	Heat rate (AM)* (W (g dry wt)$^{-1}$)	ATP turnover† (mol ATP (s mol myosin)$^{-1}$)	Economy (pN s (molecule ATP)$^{-1}$)
1	40	0·366 ± 0·031	10·6 ± 0·9	0·094 ± 0·005
	10	0·098 ± 0·020	3·0 ± 0·6	0·035 ± 0·006
3	35	0·122 ± 0·014	3·6 ± 0·4	0·247 ± 0·015
	10	0·083 ± 0·009	2·4 ± 0·3	0·083 ± 0·019

Results are from type 1 and 3 fibres, and fused and unfused isometric contractions. Values are means ± S.E.M. Freq., stimulation frequency. * Heat production by actomysin (AM) interaction. † Assuming an enthalpy change of PCr splitting of −34 kJ mol^{-1} and a myosin concentration of 0·28 mM.

In type 3 fibres we found a sustained high stable rate of heat production in the sarcomere length range of 2·3–2·7 μm, beyond which the rate of heat production decreased. This non-linear effect of sarcomere length on stable rate of heat production, although much less conspicuous, was also visible in type 1 fibres between 2·3 and 2·5 μm sarcomere length (Fig. 4*B*). A possible explanation for these findings is an increase in calcium sensitivity of the contractile apparatus at longer sarcomere lengths. The findings of Stephenson & Wendt (1984), who observed this change in calcium sensitivity at low activation levels in skinned muscle fibres, supports this. The results presented here could therefore be a first indication that a change in calcium sensitivity at longer sarcomere lengths is also present in intact fibres.

The activation heat data with sarcomere length variation for unfused contractions in type 3 fibres were obtained with extrapolation of a non-linear fit, while with BDM concentration variation the activation heat results were obtained by linear extrapolation. The results of both methods agree. From the results in the present study we conclude that in unfused contractions both the absolute activation heat and the fraction activation heat of the stable rate of heat production in type 3 fibres is less than in type 1 fibres at 20 °C.

Comparison of fused and unfused contractions. We found the absolute activation heat for fused contractions in type 1 fibres to be significantly higher (by a factor of 1·44) than in type 3 fibres (Table 3). This means that in fused contractions, type 1 fibres consume 1·44 times more ATP for calcium pumping than type 3 fibres. Relaxation is thought to be at least partly determined by the calcium uptake speed (e.g. Lännergren & Arner, 1992). When we compare the above activation heat ratio with the calculated relaxation rate ratio between type 1 and 3 fibres (5·60/3·64 = 1·54) we find the ratios are about the same. This indicates that the relaxation rate is probably related to the speed of calcium uptake by the SR, but, because the activation heat and the relaxation rate do not correlate among fibres of the same type, other factors must be involved as well. Assuming an equal density of SR calcium pumps in both fibre types, this difference in activation heat between both fibre types may be due to differences in the surface area of the SR, for it is well known from studies on mammals that fast-twitch muscle contains more SR than slow-twitch muscle (for a review see Eisenberg, 1983). The results of Smith & Ovalle (1973) on the sarcotubular system in different muscle types from *Xenopus laevis* are in agreement with the above suggestion.

For unfused contractions the ratio between the activation heat values in type 1 and 3 fibres is similar to that for fused contractions (1·53), whereas force production between the fibre types was not significantly different. This implies that with a decrease in the level of activation in both type 1 and type 3 fibres the energy turnover by the SR decreases in proportion.

Economy of cross-bridge interaction. Using the force-dependent stable rate of heat production, i.e. the stable rate of heat production in the control condition corrected for the fraction activation heat, we can now make an accurate estimate of the economy of cross-bridge interaction in intact type 1 and type 3 muscle fibres. The economy of force maintenance, expressed as the time integral of force production by one myosin head, and resulting from the hydrolysis of one molecule of ATP, was calculated assuming an enthalpy change for PCr splitting of −34 kJ mol^{-1} (Curtin & Woledge, 1978). The results are presented in Table 4. It follows that, for both fused and unfused contractions, the economy of type 3 fibres is about twice the economy of type 1 fibres. Furthermore, in type 1 and 3 fibres for fused contractions the economy is almost a factor of 3 higher than in unfused contractions. This result is different from that in skinned fibres, where the economy was independent of the level of activation (e.g. Potma, Stienen, Barends & Elzinga, 1994). A difference between the present study with intact fibres *in vitro*, and probably also *in vivo* (Hennig & Lømo, 1985), and the above study on skinned fibres is that at low levels of activation in intact fibres the force level oscillates, whereas in skinned fibres force production is smooth. If the energy turnover by the cross-bridges were dependent on the phase of contraction,

the use of mean force for calculating the economy of contraction would not be appropriate. It should be noted, however, that the economy for fused and unfused contractions would be identical only if peak force was to be used in the calculation of economy in unfused contractions. Another explanation for the less economic cross-bridge interaction in unfused contractions could reside in an as yet unidentified heat-producing process. However, two processes, energy storage in the tendons that is dissipated as heat when the fibre relaxes, and shortening heat produced when the centre of the fibre shortens during relaxation after first being stretched by the stronger sarcomeres near the fibre ends during contraction, were excluded on the basis of their calculated effect (cf. Results).

Concluding remarks

This study indicates that for *Xenopus* fibres at 20 °C, labile heat production has a force-dependent and a force-independent labile heat component. The force-independent labile heat can be explained by the enthalpy change of calcium binding to PA in exchange for magnesium, whereas the force-dependent labile heat can be explained by the enthalpy change of cross-bridge attachment. Furthermore, irrespective of the mechanism underlying the difference between fused and unfused contractions, it can be concluded that *in vivo* cross-bridge interaction in fused contractions is more economical than in unfused contractions. Finally, the difference in economy between fast- and slow-twitch muscle fibres does not change when the level of activation is lowered to give about 15% of the force produced at the high level of activation.

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Acknowledgements

The expert assistance of R. Zaremba and M. J. Zuidwijk in the parvalbumin analysis is gratefully acknowledged. This study was supported by the Netherlands Organization for Scientific Research (NWO; grant no. 900-516-092).

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Received 10 February 1996; accepted 7 June 1996.