

ELASTIC ENERGY STORAGE AND RELEASE IN WHITE MUSCLE FROM DOGFISH *SCYLIORHINUS CANICULA*

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Summary

The production of work by the contractile component (CC) and the storage and release of work in the elastic structures that act in series (the series elastic component, SEC) with the contractile component were measured using white muscle fibres from the dogfish *Scyliorhinus canicula*. Heat production was also measured because the sum of work and heat is equivalent to the energy cost of the contraction (ATP used). These energy fluxes were evaluated in contractions with constant-velocity shortening either during stimulation or during relaxation. The muscle preparation was tetanized for 0.6 s and shortened by 1 mm (approximately 15% of L_0) at 3.5 or 7.0 mm s⁻¹ (approximately 15 or 30% of V_0), where L_0 is the muscle length at which isometric force is greatest and V_0 is the maximum velocity of shortening. In separate experiments, the stiffness of the SEC was characterized from

measurements of force responses to step changes in the length of contracting muscle. Using the values of SEC stiffness, we evaluated separately the work and heat associated with the CC and with the SEC. The major findings were (1) that work stored in the SEC could be completely recovered as external work when shortening occurred during relaxation (none of the stored work being degraded into heat) and (2) that, when shortening occurred progressively later during the contraction, the total energy cost of the contraction declined towards that of an isometric contraction.

Key words: muscle, contraction, energetics, work, elastic energy, series elasticity, dogfish, *Scyliorhinus canicula*, heat production, power.

Introduction

It is well established that, for some muscle–tendon complexes, the existence of the elasticity in series (SEC) with the muscle fibres is a major factor influencing how much work the contractile component (CC) actually does and when it is done (Alexander, 1988). In some cases, most of the work during external shortening is done by the SEC, rather than by the CC. We have used a myotomal preparation which, although isolated from the fish, retains the structures that function together *in vivo* (muscle fibres attached to myosepta at each end). Our aim was to determine how much work is done by the SEC and how much by the CC, and also when each of these elements does work.

Recordings of electromyographic (EMG) activity and kinematics during locomotion have shown in many cases (but not all) that muscle activation starts while the muscle is lengthening (see, for example, Williams et al., 1989; Rome et al., 1993; Marsh and Olson, 1994; Franklin and Johnston, 1997; James and Johnston, 1998; for a review, see Wardle et al., 1995). Also, experiments on isolated muscles have shown that maximum power is produced when stimulation starts

while the muscle preparation is being stretched and ends well before shortening is complete (see, for example, Altringham and Johnston, 1990; Curtin and Woledge, 1993a,b, 1996; James et al., 1995; Altringham and Block, 1997). It has been suggested that enhanced performance may be due to the stretch having changed the properties of the cross bridges. However, another factor that may contribute to improving performance is that some of the shortening occurs during relaxation rather than during stimulation.

The experiments reported here were designed to compare work performance and total ATP use during contractions with shortening during stimulation with those of contractions with shortening during relaxation. In our protocol, the muscle preparation was not stretched before shortening; instead, force developed under isometric conditions before shortening. This is not intended to correspond to the situation during locomotion; it has been used to allow an investigation of the effects on work and total energy cost of the timing of shortening, independent of other factors.

We evaluated the ‘useful work fraction’, which we have

defined as the fraction of the work done by the contractile elements that appears as work done on the external environment (the motor in our *in vitro* experiments). The remainder of the contractile element work is degraded into heat. The useful work fraction was higher when shortening occurred during relaxation than during stimulation.

Materials and methods

The experiments were carried out at 12 °C on bundles of white myotomal muscle fibres from the dogfish *Scyliorhinus canicula* (L.) using methods described fully elsewhere (Woledge et al., 1985; Curtin and Woledge, 1996; Lou et al., 1997). The bundles were dissected under saline, which contained (in mmol l⁻¹): NaCl, 292; KCl, 3.2; CaCl₂, 5.0; MgSO₄, 1.0; Na₂SO₄, 1.6; NaHCO₃, 5.9; urea, 483; and tubocurarine, 1.5 mg l⁻¹, 12 °C. The myoseptum at each end of the fibre bundle was held in a platinum foil clip. The clip was placed so as to minimize the length of myoseptum between the clip and the muscle fibres. The length of myoseptum is not as small as could be achieved using a conventional single fibre preparation because the bundles of fibres usually contained two layers of fibres and these are staggered by up to 0.5 mm, the normal *in vivo* arrangement. The preparation was mounted between a force transducer (Cambridge Technology, Inc., model 400A) and a motor (Cambridge Technology, Inc., model 300B) that controlled muscle length. The preparation was in contact with a thermopile, which measured temperature change. The heat production by the muscle preparation was determined from the temperature change.

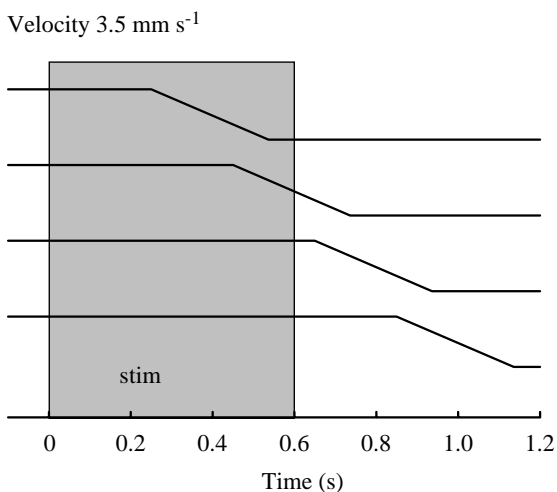


Fig. 1. Diagram illustrating the patterns of stimulation and movement that were used in the experiments with shortening at 3.5 mm s⁻¹. The shaded area indicates the period of stimulation (stim). The lines show the movement of the motor arm. Downward movement indicates muscle shortening. The distance shortened was 1.0 mm. The delays between the start of tetanic stimulation at time 0 and movement were 0.25, 0.45, 0.65 or 0.85 s. Experiments were also made with shortening at 7.0 mm s⁻¹ (not shown); the distance shortened and delays were as shown here.

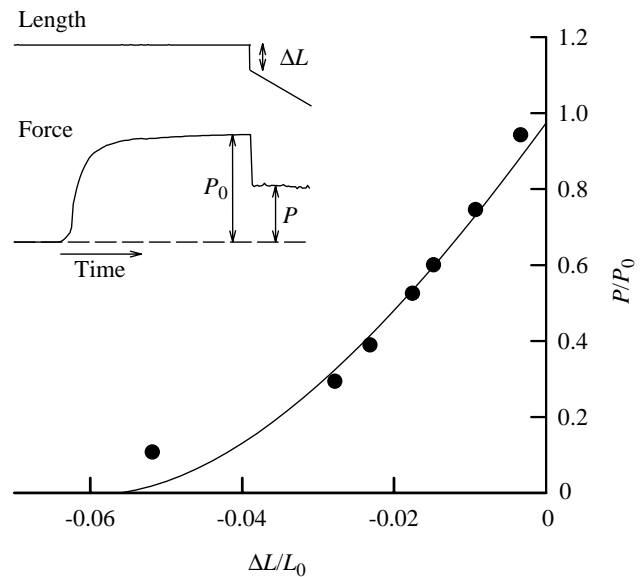


Fig. 2. The relationship between the change in length during the step and force during ramp shortening. Change in length (ΔL) is expressed relative to L_0 , the length at which isometric force is greatest, and force (P) is expressed relative to P_0 , where P_0 is maximum isometric force. The line is $P/P_0 = 40[(\Delta L/L_0) - 0.064] - 2.2375 \{1 - \exp[-(\Delta L/L_0) - 0.064]/0.05187\}$. The inset is an illustration of length and force recordings and shows how they were measured.

Ramp shortening

The muscle fibres were electrically stimulated every 5 min to produce a 0.6 s tetanus. During the tetanus, the frequency of stimulation was approximately 30 Hz (near the fusion frequency). The preparation was either held at L_0 (the length at which isometric tetanic force was maximal) or shortened by 1 mm, starting from a muscle length 0.5 mm longer than L_0 , at a velocity of 3.5 or 7.0 mm s⁻¹ (approximately 15% or 30% of V_0 , where V_0 is the maximal velocity of shortening). Shortening started at different times (0.25, 0.45, 0.65 or 0.85 s) after the start of stimulation (see Fig. 1). Shortening starting at 0.25 s was completed during stimulation, whereas shortening starting at 0.65 s and 0.85 s occurred entirely after the end of stimulation. Results were obtained from ten muscle fibre preparations (six at each velocity) from nine fish.

In these experiments, the work done on the motor by the muscle preparation as a whole (CC+SEC) is referred to as external work (W_E) and was calculated as the integral of force and movement of the motor arm. The preparation consists of muscle fibres (the contractile component, CC) acting in series with elastic structures (myosepta, etc., collectively referred to as the series elastic component, SEC). As explained below, if the stress-strain characteristics of the SEC are known, the CC work can be evaluated from the recordings of force and motor arm position.

Series elasticity

The method we used to characterize the stress-strain curve

of the SEC is based on the following principles (Curtin et al., 1998): (a) forces in the CC and the SEC are equal, and the same as the measured force; (b) the movement of the motor arm, which is referred to as the external shortening, is the sum of the length changes in the CC and the SEC; (c) the SEC behaves like a spring in that its length depends only on force; and (d) the SEC can shorten very much faster than the CC.

In the experiment to characterize the SEC, the muscle preparation was shortened by a rapid step followed by a ramp shortening as shown in the inset of Fig. 2. The size of the step was adjusted so that the force in the SEC at the end of the step matched that produced by the CC during shortening at the velocity of the ramp.

Control experiments were performed to measure the compliance outside the muscle preparation. In these control experiments, a piece of platinum foil was mounted between the motor and force transducer in place of the muscle preparation, and the stress-strain relationship was recorded. The results

showed that approximately 25% of the total compliance resided outside the muscle preparation. The values shown in Fig. 2 and used in the calculation reported here are based on total compliance.

Work and heat

During muscle contraction, ATP is broken down during the cyclic interaction between actin and myosin, and the chemical energy released from ATP is converted into work and heat. This work is referred to here as the CC work. As explained below, the CC work may be done on the external environment (the motor in this case) or on the SEC, or it may be degraded into heat. In contrast, the SEC is a passive, elastic structure that can transfer work to and from the CC and the external environment, but cannot convert energy from coupled chemical reactions into work.

The total heat produced during a contraction has two components: metabolic heat and degraded work. Metabolic

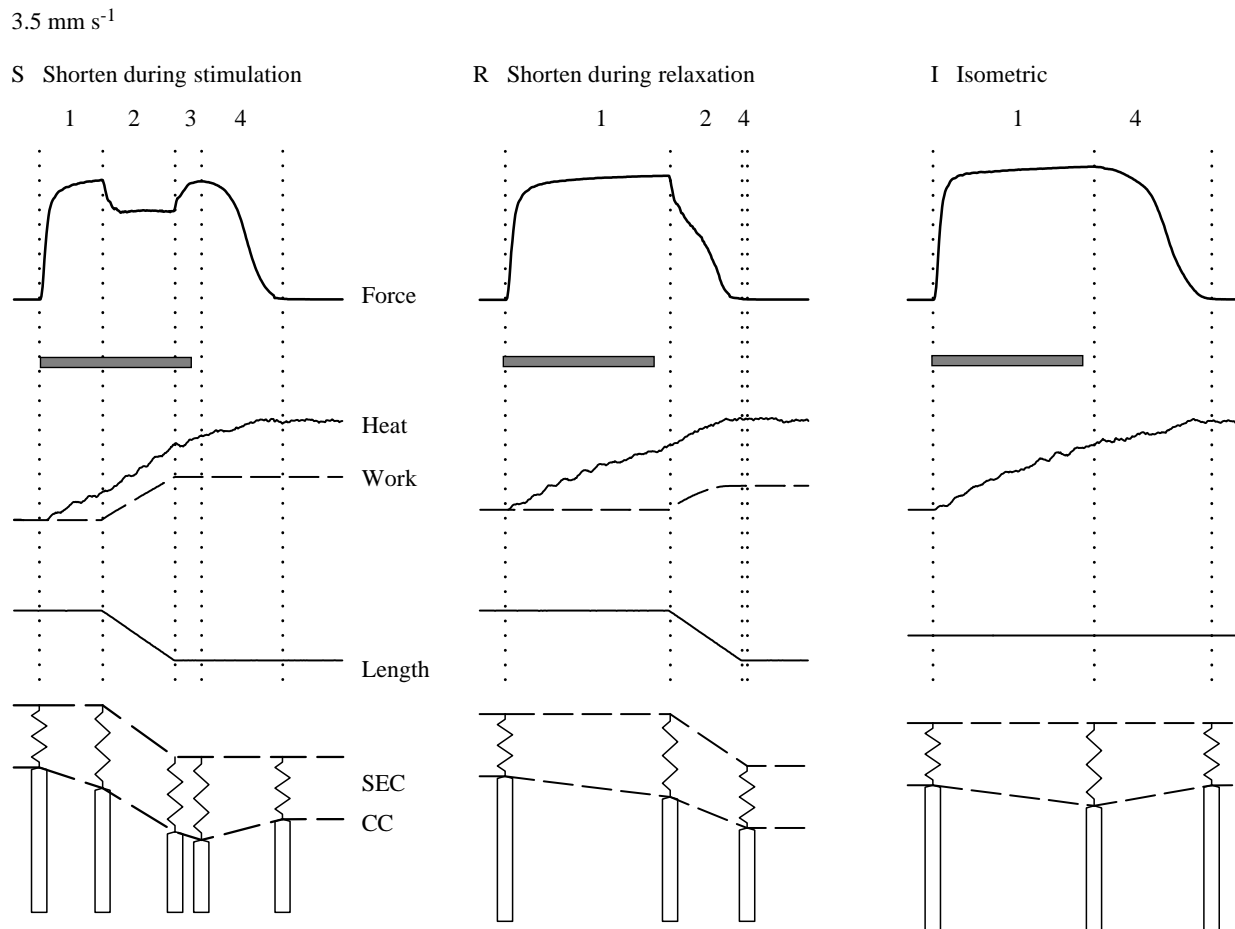


Fig. 3. Example recordings of force, heat production, work and muscle length (downwards indicates shortening). The horizontal bar under the force record shows the 0.6 s tetanic stimulation. Broken vertical lines divide the recordings into sections for which the energy fluxes are described in the text. S, shortening at 3.5 mm s^{-1} starts 0.25 s after the start of stimulation and is complete before the end of stimulation. R, shortening at 3.5 mm s^{-1} starts 0.65 s after the start of stimulation and occurs entirely during relaxation. I, isometric tetanus at L_0 , the length at which isometric force is greatest. The diagrams below the recordings illustrate the lengths of the series elastic component (SEC) and contractile component (CC) at the times indicated by the vertical lines. These diagrams are not drawn to scale. The lower broken line shows how the length of the CC changes, and the upper broken line shows how the length of the CC+SEC changes.

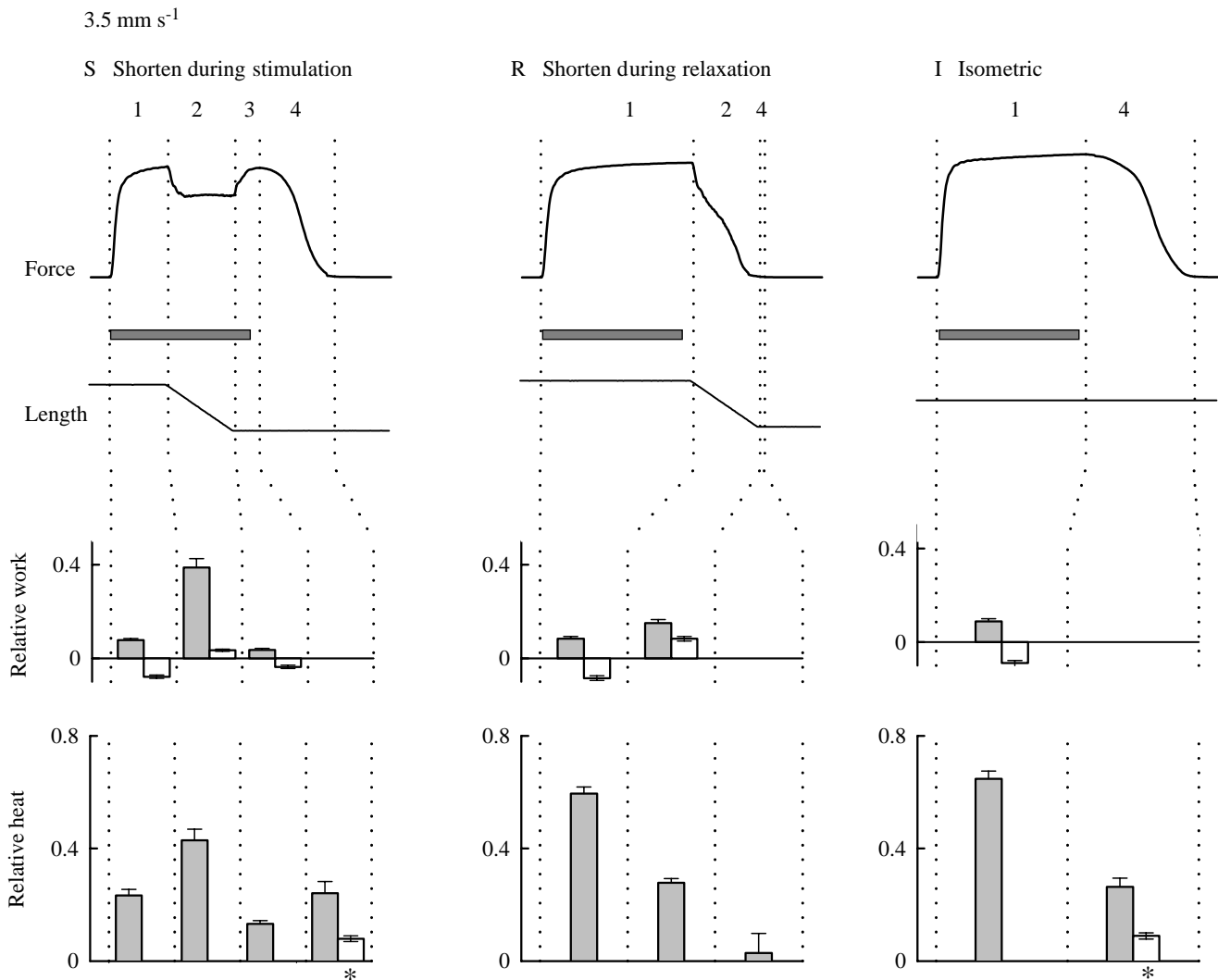


Fig. 4. Example force, stimulus and length recordings (shortening at 3.5 mm s^{-1}) as in Fig. 3 with bar graphs showing the means (\pm S.E.M., $N=6$) of the work and heat produced during the marked time periods. The horizontal bar under the force record shows the 0.6 s tetanic stimulation. The filled bars are work and heat produced by the contractile component, and the open bars are work and heat from the series elastic component (SEC). The asterisks indicate the amount of heat produced by the dissipation of work stored in the SEC.

heat is the heat from the splitting of ATP during the interaction between actin and myosin and also from the activity of ion pumps (and associated buffer reactions, etc.; see Woledge et al., 1985). Degraded work is the heat produced when the CC is stretched.

Evaluating CC work and SEC work

The sign convention used here is that mechanical work done by the CC or SEC on an element external to itself is treated as a positive quantity. Mechanical work done on the CC or SEC by an element external to it is treated as a negative quantity.

The CC work was evaluated from the external work and the characteristics of the SEC on the basis of the principles given above and the following. (a) Work done on the SEC can be stored in it, the amount of stored SEC work being a function of the force and strain in the SEC. Stored SEC work can be

collected as external work if the SEC shortens while there is external shortening. (b) The CC cannot store work. Therefore, stored SEC work is released as heat when the SEC shortens and stretches the CC. This occurs, for example, during relaxation when force decreases under isometric conditions (no external shortening).

The following procedure was used to evaluate the work:

$$W_E = \int P dL_M, \quad (1)$$

where W_E is external work, P is force and dL_M is the movement of the motor arm.

$$W_{SEC} = \int P dL_{SEC}, \quad (2)$$

where W_{SEC} is work done by the SEC and dL_{SEC} is the change in length of the SEC.

For each section of the recording (see Figs 4, 5), W_{SEC} was

calculated by integrating the stress–strain relationship for the SEC (Fig. 2) between the values of force at the start and at the end of that section of the recording.

Thus, the CC work (W_{CC}) can be calculated as:

$$W_{CC} = W_E - W_{SEC}. \quad (3)$$

Evaluating metabolic heat and SEC heat

Heat released from the CC or SEC is treated as a positive quantity. Any heat absorbed by the CC or SEC would be treated as a negative quantity, but no heat absorption was detected in any of these experiments. The total heat, H_T , is calculated from the thermopile recordings of temperature. H_{SEC} is the work done by the SEC as it stretches the CC (this occurs when force is decreasing, which means that the SEC is shortening, and the motor arm is not moving).

Thus, the metabolic heat from ATP splitting (H_{MET}) can be calculated as:

$$H_{MET} = H_T - H_{SEC}. \quad (4)$$

Values of heat and work for each muscle preparation are

expressed relative to the mean value of its isometric heat production.

Results

Example recordings from tetani with shortening at 3.5 mm s^{-1} either during stimulation or during relaxation after the end of stimulation, along with recordings from an isometric tetanus, are shown in Fig. 3. The figure includes the force, heat production and work done on the motor by the muscle preparation as a whole (CC+SEC). These results show that the muscle does external work when shortening occurs either during stimulation (S) or during relaxation (R), but no external work is done in the isometric case (I) because the overall length of the muscle preparation is constant. Heat is produced during the entire time that force is being produced in all the contractions.

Energy from the CC and SEC

The vertical dotted lines in Fig. 3 divide the recordings into sections with different patterns of work and heat production by

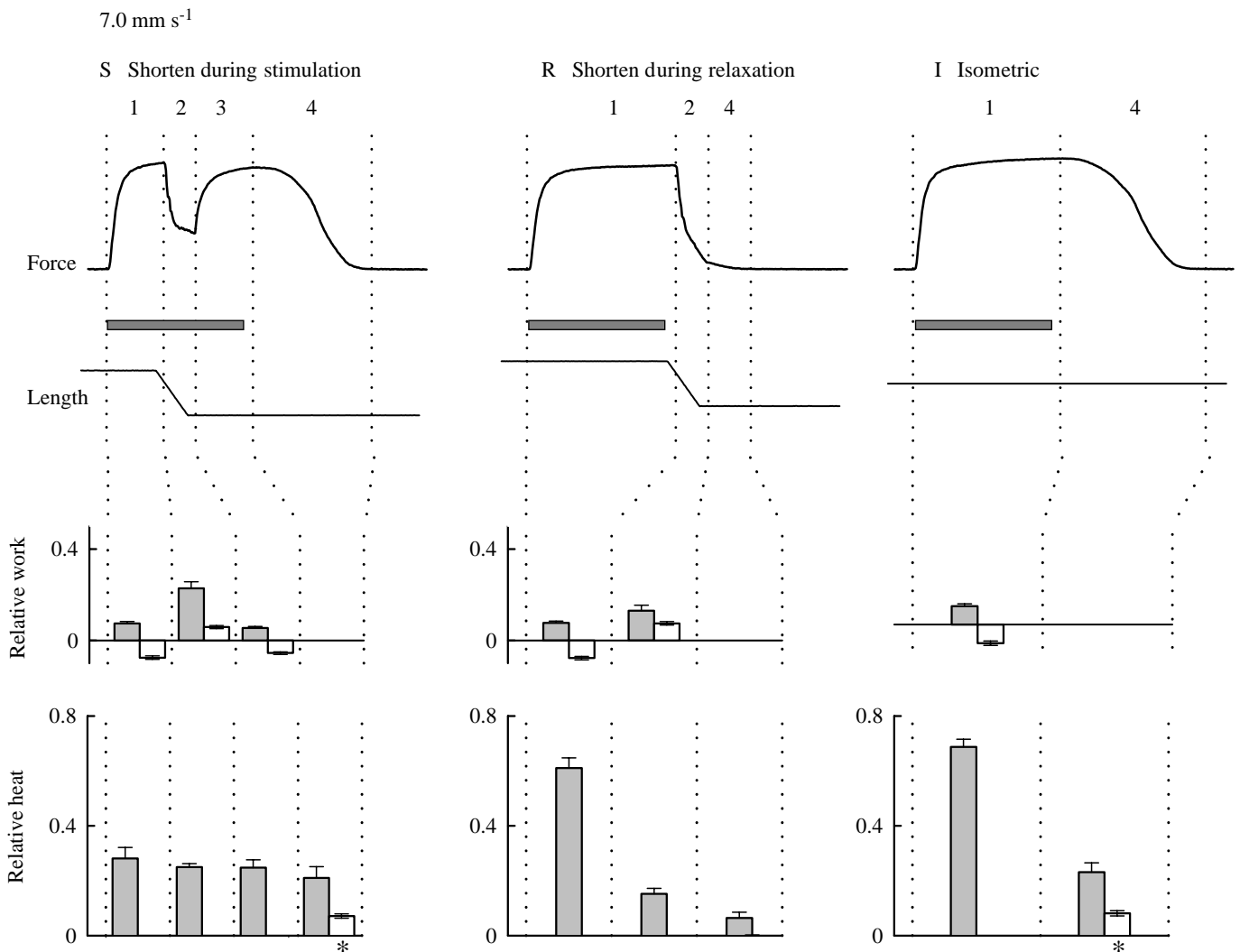


Fig. 5. As Fig. 4 except that the velocity of shortening is 7.0 mm s^{-1} .

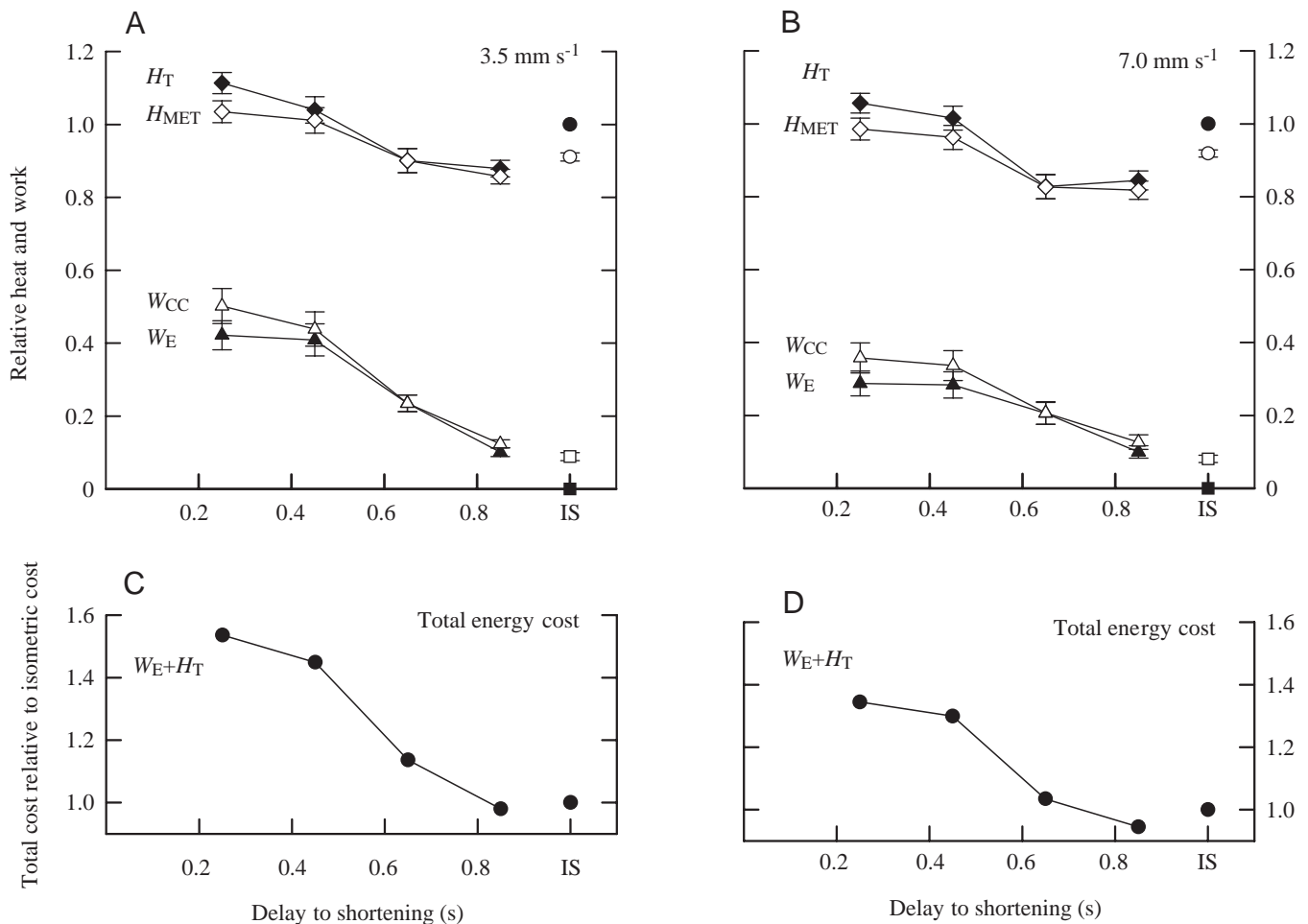


Fig. 6. (A,B) Mean values (\pm S.E.M., $N=6$) of the energy output during the entire period of stimulation plus relaxation for isometric tetani and for tetani with shortening. W_E is work done on the external environment (motor); W_{CC} is work done by the contractile component; H_T is the total observed heat and H_{MET} is the heat from metabolic processes. Results for tetani with shortening are plotted against the delays from the start of stimulation to the start of shortening. Work and heat are expressed relative to the isometric heat for the entire period of stimulation plus relaxation. (A) Shortening at 3.5 mm s⁻¹; (B) shortening at 7.0 mm s⁻¹. (C,D) The sum of W_E and H_T , which is a measure of the total cost of contraction for (C) shortening at 3.5 mm s⁻¹ and (D) shortening at 7 mm s⁻¹. IS, isometric contraction.

the CC and by the SEC, and the diagrams below the recordings illustrate (not to scale) the length changes in each component. Figs 4 and 5 show separately the amounts of work done by or on the CC and SEC in each of these time sections, as well as the corresponding heat production.

Section 1 is the isometric period at the start of stimulation; during this time, there is no external movement and, therefore, no external work. Force is increasing, and therefore the CC shortens and thus lengthens the SEC as shown in the diagrams in Fig. 3; the CC does work on the SEC. None of the work is degraded into heat, so all the observed heat is metabolic heat.

During section 2, there is external shortening. While force is declining, the SEC shortens and does external work, thus using some of the work stored in it. However, none of the work is degraded into heat. The CC also does external work. As in section 1, all the observed heat is metabolic heat.

Section 3 is a period of redevelopment of isometric force

after shortening. This only occurs if shortening starts early enough to be completed while the muscle is still active enough for force to increase. Note that this occurs in recordings labelled S but not in those labelled R in Figs 3–5. The sources of work and heat are the same as in section 1.

Section 4 is the period of isometric relaxation. There is no external movement and, therefore, no external work. Force is declining, so the SEC is shortening and is stretching the CC (see diagrams in Fig. 3). Since the CC cannot store work, the work is dissipated into heat. During section 4, the metabolic heat is the observed heat minus the heat due to dissipation of stored SEC work.

The quantities of work and of heat produced by the CC and by the SEC are shown as bar graphs below each section of the recordings in Figs 4 and 5. Comparisons of these quantities for the different contractions show that, in section 1, the isometric period at the start of stimulation, the work done by the CC on the SEC is the same in all cases. All the heat is metabolic heat,

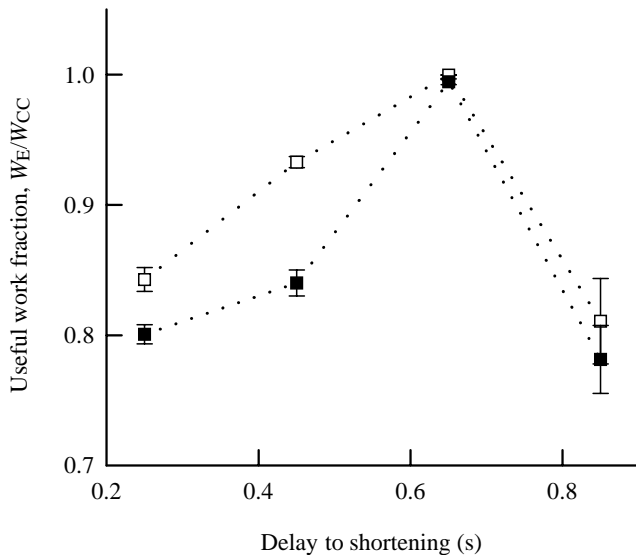


Fig. 7. Relationship between the useful work fraction and the delay between the start of stimulation and the start of shortening. The useful work fraction is work done on the external environment/work done by the contractile component (W_E/W_{CC}). Values are means ± 1 S.E.M., $N=6$. Open symbols, shortening at 3.5 mm s^{-1} ; filled symbols shortening at 7.0 mm s^{-1} .

and the amount is proportional to the duration of this period. During section 2, the period with external shortening, CC work is greater for shortening during stimulation than for shortening during relaxation, whereas the work done by the SEC is smaller for shortening during stimulation than for shortening during relaxation. Consequently, the fraction of the total work done in section 2 by the SEC is much greater when shortening occurs during relaxation than during stimulation. All the heat during this period is metabolic heat and, like CC work, it decreases as shortening occurs later during the contraction. During section 4, isometric relaxation, metabolic heat continues to be produced and there is also heat from dissipation of the work stored in the strained SEC. The amount of heat produced by the SEC at this time is marked by an asterisk in Figs 4 and 5, and it represents the remaining part of the work that was done on the SEC during the isometric phase(s) and that is left after external shortening has finished. The important point here is the fate of the work done on the SEC during the isometric period(s): when shortening occurs during relaxation, all this stored energy is eventually converted to external work and none is converted to heat. In the other two cases, shortening during stimulation and isometric contraction, some of the stored elastic work is degraded into heat (bars marked with an asterisk).

Total quantities of energy for the whole contraction

The amount of total heat and the amount of external work produced from the start of stimulation to full relaxation of force back to the baseline level are shown in Fig. 6A,B (filled symbols). Values are given for all four patterns of shortening, i.e. shortening starting 0.25, 0.45, 0.65 and 0.85 s after the start

of stimulation. All quantities are expressed relative to the isometric heat produced by the same muscle. These results show that more external work was done when shortening occurred during stimulation than during relaxation, but that some work was done even when shortening started after the end of stimulation (delays of 0.65 and 0.85 s). The total heat produced during the contraction also depended on when the shortening occurred. It is noteworthy that heat production fell to values below the isometric value when shortening occurred during relaxation. As shortening occurs progressively later, the total heat plus external work, which is a measure of the total energetic cost of contraction, declines towards the isometric value (Fig. 6C,D).

In Fig. 6A,B, the open symbols show the quantities of CC work (W_{CC}) and of metabolic heat (H_{MET}) for the entire period of contraction plus relaxation. It is instructive to compare these values with the external work (W_E) and the total heat (H_T) respectively (filled symbols). When shortening occurs during stimulation, W_{CC} exceeds W_E because work is done by the CC on the SEC in addition to that done on the external environment. Only part of the work stored in the SEC appears later as external work during shortening; the rest is dissipated as heat during relaxation with no external shortening. As shortening occurs later, a larger fraction of the stored work appears as useful work and less as heat. The heat results show a corresponding difference between early and late shortening.

The 'useful work fraction', which we have defined as the work done on the motor (W_E) expressed as a fraction of the work done by the CC (W_{CC}), is shown in Fig. 7. For both velocities of shortening, the useful work fraction is between 0.80 and 0.85 when shortening occurs entirely during stimulation. The fraction can be higher when shortening occurs partly or wholly during relaxation, and reaches 1.00 when shortening starts soon after the end of stimulation while force is still high (see 0.65 s delay and R in Fig. 3). The useful work fraction is lower, only approximately 0.80, when shortening is delayed to 0.85 s after the start of stimulation; in these cases, by the time that shortening started, the force was very low and the work stored in the SEC had already been dissipated as heat.

Discussion

SEC and work

A significant amount of work was done during contractions with shortening starting after the end of stimulation. Is the source of this work done during relaxation the same as that done during stimulation? Under the conditions used here, all the work must ultimately come from the conversion of energy from ATP splitting by the CC, but it is of interest to know the immediate source of the work while it is being done.

As the bar graphs in Figs 4 and 5 show, both the CC and the SEC make a significant contribution to the total work done during external shortening. The results show that a substantial fraction of the external work is done by the SEC when shortening occurs during relaxation. The results presented here show that storage and release of SEC work was significant in

our isolated fish muscle preparations, even though we deliberately made the pieces of myosepta as short as possible at the ends of the muscle fibres to minimize their effects. Thus, although the mechanical properties of our isolated preparations are not exactly the same as those of intact fish, they do indicate that the elasticity of the myosepta may be a factor that significantly affects swimming performance.

Relevance to in vivo patterns of stimulation and movement and to optimum power

As described in the Introduction, *in vivo* recordings show that muscle activation commonly starts during stretch and ends well before the end of shortening. This pattern also commonly gives maximum power in experiments *in vitro*. While stimulation during stretch is probably important, our results indicate that the fact that shortening occurs during relaxation is also relevant. We found that work stored in the SEC was most effectively recovered as external work done on the environment (the motor in this case) when shortening occurred during relaxation (see Fig. 7). External work matched CC work best when external shortening started after the end of stimulation and included the entire period when force was declining. Thus, we conclude that, to recover all the work done by the CC during sinusoidal movement, shortening should occur during the decline in force during relaxation. This would contribute to high power output independent of any enhancement due to stretch of the muscle.

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