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Fast deformation calorimetry on muscle-fibre bundles[1]

S. Tewes*, G.W.H. Höhne

Sektion Kalorimetrie, Universität Ulm, D-89069 Ulm, Germany

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Abstract

A deformation microcalorimeter was built, which allows simultaneous measurements of the very small heat and work contributions of living single muscle fibres on quick-stretch with a temporal resolution of 1 ms. The thermodynamic behaviour of small muscle-fibre bundles of Iliofibularis and Tibialis anterior muscles of *Xenopus laevis* frogs have been investigated in the relaxed and active states. In particular, twitch, tetanus and quick-stretch experiments have been performed. An exothermic heat production is strongly correlated to the changes in force and almost consumed again when the force decreases after stimulation. In the case of active quick-stretch, there are two heat events clearly distinguishable in time, the first exothermic and the second endothermic. The amount of heat is equal in both the events, but with opposite sign, and ≈20 times larger than the work done during stretching. One event is suspected to be an elastic heat, the other may be due to an activated process (i.e. a reversible chemical or folding/unfolding reaction of proteins). © 1998 Elsevier Science B.V.

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1. Introduction

The details of the "molecular motor" inside a muscle fibre are not yet fully understood, though much research has been done on this topic [1]. The thermodynamics (heat and work, and Gibbs energy) of active and passive muscles has been investigated, but often on rather long time scales and mostly with compact muscles [1–4]. Nevertheless, the passive behaviour seems to be well known, and the same is true for single-twitch and tetanus stimulations of the active state [1,5,6]. The behaviour of active muscles on rapid extension (quick-stretch) is, however, very complicated and highly nonlinear. This behaviour is not yet understood, and precise measurements with high temporal resolution are rare [4,7]. In particular, as far as we are aware, there are no published experiments which simultaneously measure both, heat and work on single muscle fibres with high temporal resolution. Therefore, we built a deformation microcalorimeter to measure simultaneously the very small heat and work contributions of living, single muscle fibres on quick-stretch with a resolution of 1 ms.

2. Measuring apparatus

A specific measuring apparatus was constructed, to carry out "quick-stretch" experiments on active single

*Corresponding author.

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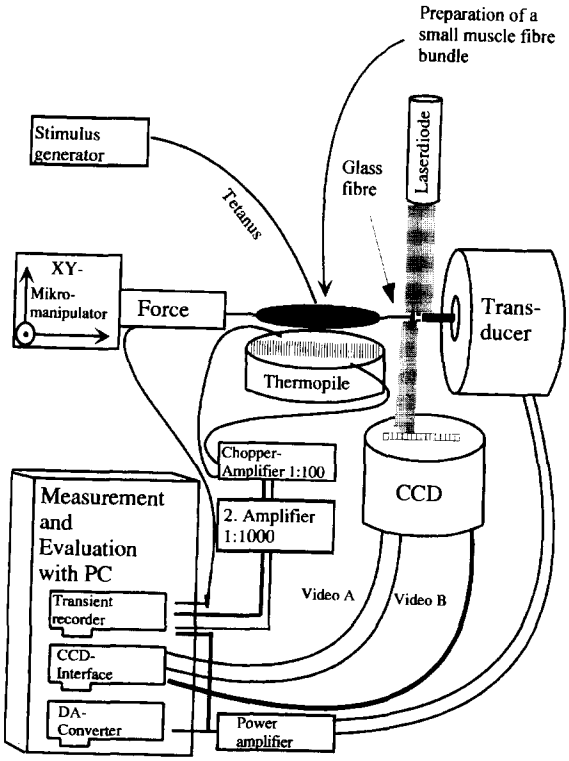

Fig. 1. Scheme of the deformation equipment.

muscle fibres as well as to measure force, length and temperature changes simultaneously with high temporal resolution. From these data, we are able to compute the time-dependent work and heat of a muscle fibre during the process in question. The single fibre (or fibre bundle of few individual fibres) was glued via the tendons to two optical fibres which were attached to a force probe and to the moving part of a vibrator for fast extension. The thermal probe was positioned under the muscle fibre. Electrical stimulation of the muscle was done via two fine platinum wires inserted between the muscle and the optical fibre. Accordingly, this equipment (see Fig. 1) essentially consists of the following components:

2.1. Fast extender

For a quick-stretch, it is necessary to expand the active fibre ca. 5–10% within a very short time (<1 ms). To achieve that, a commercial transducer (Vibrator 101, Ling Dynamics), designed for periodic movements up to a maximum of 12 kHz at amplitudes of a maximum of 2.5 mm, was mechanically modified in such a way as to hold the voice coil, by dc control, in a defined position ($\Delta l = -1$ mm, measured with the aid of the CCD line described below). A triggering signal switched the holding current off and, simultaneously, the maximum permissible operating current on, but with changed polarity. Such a coil of the transducer moved as quickly as possible until it was stopped by a mechanical interruption. Measurements showed that extensions of the fibre of 1 mm occurred linearly within 1 ms.

2.2. Position measurement

To measure the extension quantitatively, a CCD line camera (with a fast interface, Schäfter and Kirchhoff) was mounted such that the edge of the shadow of the moving vibrator part was projected on the CCD line with a laser diode. The position of the edge of the shadow was registered with suitable software every 100 μs and transmitted to data acquisition. In this way, it was possible to measure the position of the moving transducer coil, and with it, the change in length of the muscle fibre with a spatial and temporal resolution of 9.5 μm and 100 μs, respectively.

2.3. Force measurement

We used a silicon chip (Sensonor) for force measurement [7], which was shortened first to increase the resonant frequency. Small deformations of the chip cause a change in resistance, that can be registered with a sensitive bridge amplifier. With this probe it was possible to measure forces down to 0.1 mN with a temporal resolution of 0.1 ms.

2.4. Thermopile

To measure the heat generated during manipulation of muscle fibres a specific thermopile was developed, which is sensitive and fast enough to be able to measure the small amount of heat developed during quick-stretch. This component consists of a bismuth–antimony thermopile deposited on a foil in high vacuum [7,8]. Such a thermopile can be used for fast events like a Tian calorimeter [9,10]. Its function can

be described by a linear second-order differential equation:

$$\phi_{\text{muscle}} = k_1 \frac{d^2\Delta T}{dt^2} + k^2 \frac{d\Delta T}{dt} + k_3 \Delta T \quad (1)$$

which connects the heat flow rate from the sample (ϕ) with the measured signal (the temperature difference of the thermopile: ΔT). The solution of this differential equation for a pulse-like heat event is a sum of two exponential functions with different time constants for increasing and decreasing parts of the peak

$$\Delta T = c(\exp(t/\tau_1) - \exp(t/\tau_2)) \quad (2)$$

which was proved experimentally and by model calculations (see Fig. 2).

First, we used polyimide (Kapton^{TM}) as foil material (thickness: 7.5 μm), the essential physical properties of this thermopile (sensitivity: 6.4 mV/K, time constant: 1.5 ms) are described in detail in Ref. [8]. Unfortunately, this material proved to be unsuitable for measurements on active muscle fibres. The Ringer solution caused a swelling of the foil leading to microcracks of the fairly brittle antimony layer. Therefore, the function of the thermopile was lost nonreversibly after 10–20 min. Also, the electrical pulses from the stimulation disturbed the high-impedance amplifier linked to the thermopile, which resulted in a very noisy signal.

As a consequence, we were forced to place the thermopile on an electrically conducting foil (20 μm of Al), the surfaces of which were isolated by electrochemical oxidation. With this new combination, we succeeded in reducing the stray capacity pickup by grounding the foil. The physical properties of this sensor (sensitivity: 0.12 mV/K, time constant: 1 ms) are, unfortunately, more unfavourable: the thermal conductivity is higher, but the sensitivity considerably decreased. The thermal noise of the thermopile, limiting the sensitivity, is at room temperature (for the given bandwidth of the chopper amplifier used) ca. 400 nV.

2.5. Chopper amplifier

For amplifying the very small emf of the thermopile with a bandwidth of 10 kHz (in order to be able to obtain 1 ms temporal resolution), we built an amplifier with a 9.9 kHz chopper chip. The overall gain was 10^5 with an approximate bandwidth of 3 kHz. To avoid ground loops and other hum pickups, this amplifier

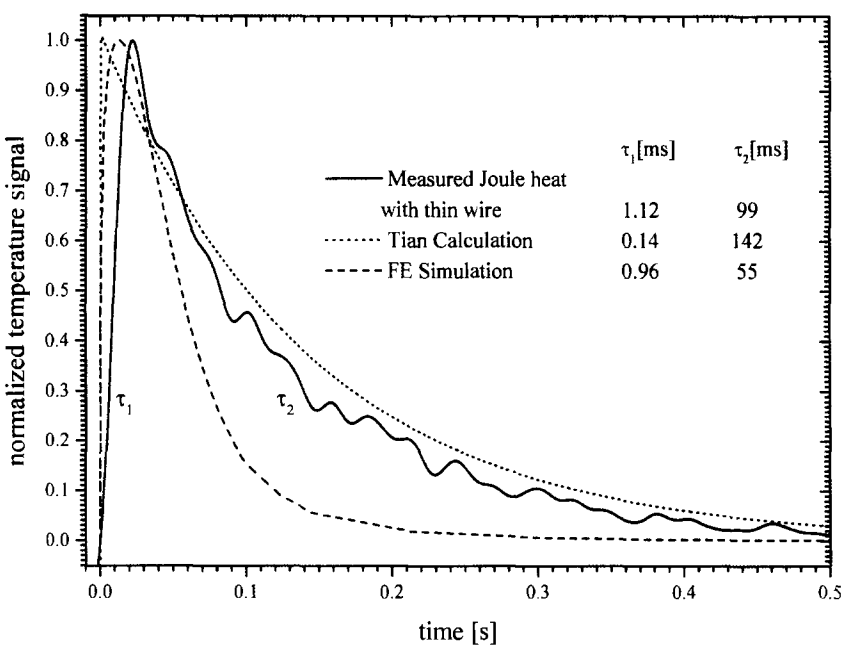

Fig. 2. Calculated and measured pulse-response functions of the thermopile.

was driven with batteries; power supply units led to unsatisfactory signals.

2.6. Data acquisition

A personal computer (with 486 processor) and a transient-recorder plug-in card (Spectrum) with four channels and a sampling rate of 312 kHz was used for data acquisition. The simultaneous registration of length, force and thermal signals was possible with a temporal resolution of 13 μs. However, the amount of data for one period of measurement (3.5 s) was as large as 3.5 MB which caused problems with further evaluation.

2.7. Calibration

Calibration of the force probe was done with the aid of small pieces of wire (of a known weight) hanging in a loop at the end of the glass fibre glued to the silicon chip. The calibration factor of the sensor proved to be 3.7 μV mN^{-1} (at a noise of 400 nV rms) resulting in a signal voltage after amplification of 0.37 V mN^{-1}.

Calibration of the thermopile was more difficult since an influence of the sample shape and properties on the calibration factor could not be, a priori, excluded. To prove this, we used both experimental and theoretical approaches:

(i) Positioning of a fine constantan wire along the hot junctions of the thermopile and thermal link-up of the same with an exactly known amount of water (0.7 μl) and generation of a heat pulse from a well-defined electrical pulse. In this way, we got both the pulse response function (Fig. 2) and the calibration factor. The latter can be determined from the total area of the thermal-response peak compared with the total heat produced in that part of the wire which is on top of the hot junctions.
(ii) Model calculations and simulation of the heat relaxation process with finite element method software (Ansys) which supported the validity of the solution of Eq. (1) and gave almost the same time constants as determined experimentally.

From both the methods, we determined the time constants of the pulse-response function (see Fig. 2) and the caloric sensitivity of the thermopile 57 nV μW^{-1} (at a noise of 350 nV rms) resulting in a signal voltage of 5.7 mV μW^{-1} after amplification.

2.8. Data processing

From raw data, the quantities of interest (work and heat) had to be calculated by corresponding analysis routines. It was necessary to transform the measured voltages into the proper physical units and the thermal signal had to be corrected mathematically due to the rather high noise and hum (from 50 Hz line) as well as the nonnegligible response times of the sensors and of the electronics.

2.9. Filtering and desmearing

The recorded signals are normally delayed compared to the event. This is caused by finite signal propagation both, from electronics (finite signal-rise time because of finite bandwidth) and from the respective physical processes (heat transfer, force propagation, acceleration). It could be shown from the corresponding experiments and computations that the actual evolution of the force and the acceleration of the muscle fibres, coupled with optical fibres to force sensor and vibrator, is practically instantaneous with the measured quantities. Stiffness and sound velocity within the optical fibres is so high that a possible retardation of the recorded signal, compared to the true event, can be neglected due to the temporal resolution of 1 ms, as was desired.

In the case of the thermal signal, however, the finite thermal diffusivity cannot to be neglected. The time constants of the pulse response are, at least, in the same order of magnitude as the temporal resolution of interest. A corresponding correction to the measured heat-flow-rate function is possible within the framework of the theory of linear response. The mathematical relation reads as follows:

$$\phi_{\mathrm{meas}}(t) = \frac{\mathrm{d}}{\mathrm{d}t}\int \phi_{\mathrm{true}}(t')g(t-t')\mathrm{d}t' \qquad (3)$$

In this so-called "convolution product" ($\phi_{\mathrm{meas}}(t)=\phi_{\mathrm{true}}(t)*g(t))\phi(t)$ is the heat flow rate and $g(t)$ the Green's function (apparatus function) of the corresponding apparatus. In our case, $g(t)$ would be

the normalized response signal caused by a pulse-shaped event in the muscle fibre.

Eq. (3) is usually solved in Fourier space (characterized by capitals), where it transforms into a usual product:

$$\Phi_{\text{meas}}(\omega) = \Phi_{\text{true}}(\omega)G(\omega) \tag{4}$$

From this equation, ϕ_{true} can be calculated by division of the (complex) functions Φ_{meas} and $G(\omega)$ in Fourier space and subsequent inverse Fourier transform. This procedure is called "desmearing". However, the Green's function of the device must be known for this purpose. For the thermopile, we tried to get this function in two different ways, first by measuring the response of an electric pulse in the wire of the calibration arrangement described above, and second from computation of the heat relaxation behaviour of muscle fibres on the thermopile foil with a commercial finite-element-programme (for results see Fig. 2). There was no other way to check whether the principally different arrangements of the thermopile for calibration with metallic wires and for measurement with muscle fibres would influence the Green's function, as was expected. Unfortunately, the true apparatus function of the muscle experiment setup could not be measured, as it was not possible to produce a true heat-pulse event inside the fibre. As a result, we found that there were no big differences and that the generated pulse-response function was suitable as a proper Green's function in a first approximation.

A great disadvantage of any mathematical desmearing is a considerable decrease in the signal-to-noise ratio; an improvement of the temporal resolution of about a factor of ten causes an increase in the noise by a factor of ten. The noise can be corrected mathematically by suitable filtering procedures in Fourier space, but this in turn leads to a decreased temporal resolution. Unfortunately, the relatively poor signal-to-noise ratio of original signals from the measurements of muscle fibres made desmearing exceptionally difficult. The desmeared curves were normally so noisy that the actual signal could hardly be separated. In particular, the system hum (50 Hz) appeared to be an unavoidable component of the measurements. This could only be reduced with the aid of a proper band-stop filter in Fourier space.

3. Experimental

We used Iliofibularis and Tibialis anterior muscles of *Xenopus laevis* frogs for our experiments. From these muscles, individual fibres or small fibre bundles (<15 fibres), including tendons were prepared in Ringer solution and fixed to the optical fibres (0.2 mm diameters) with fast-binding glue.

Thereafter, a positioning (±200 μm) along the hot junctions of the thermopile was carried out as precisely as possible. In addition, the initial stress, and the respective strain of the fibre and, thus, the initial sarcomere length was chosen by adjusting the length.

With these preparations, single-twitch, tetanus (1.2 s, 66.7 Hz) and quick-stretch experiments were carried out. The latter ones were performed by fast extension (1 mm in 1 ms) after equilibration of the tetanus (after 0.5 s). The raw data then became filtered with the aid of the method described above. The results of measurement are represented in Figs. 3, 5, 7, 8. If possible, these data were desmeared as required (Figs. 4, 6, 9).

4. Results

4.1. Active isometric muscle fibre

In Figs. 3–6, the results of single-twitch and tetanus experiments are presented. The measurements correspond to expectations. Accordingly, a heat production proportional to force development is "switched on" during stimulation which first causes a constant rise of the temperature of the muscle fibre which, with increasing flowing off of the heat, decreases in time. Finally, in the steady-state stimulation, a constant temperature is reached. The signal measured by the thermopile represents this behavior only in a smeared form due to the limited heat transfer. From the shape of the pulse-response function (see Fig. 2), we can conclude (by integration) that such a steady state will be reached after ca. 200 ms. In the case of twitch experiments (Fig. 3), the time of stimulation is too short to achieve this state. The measured temperature signal is rather like what one would get from the convolution product of the change in force and the pulse-response function (Fig. 4(a)). The desmeared measurement (Fig. 4(b)) confirms this pre-

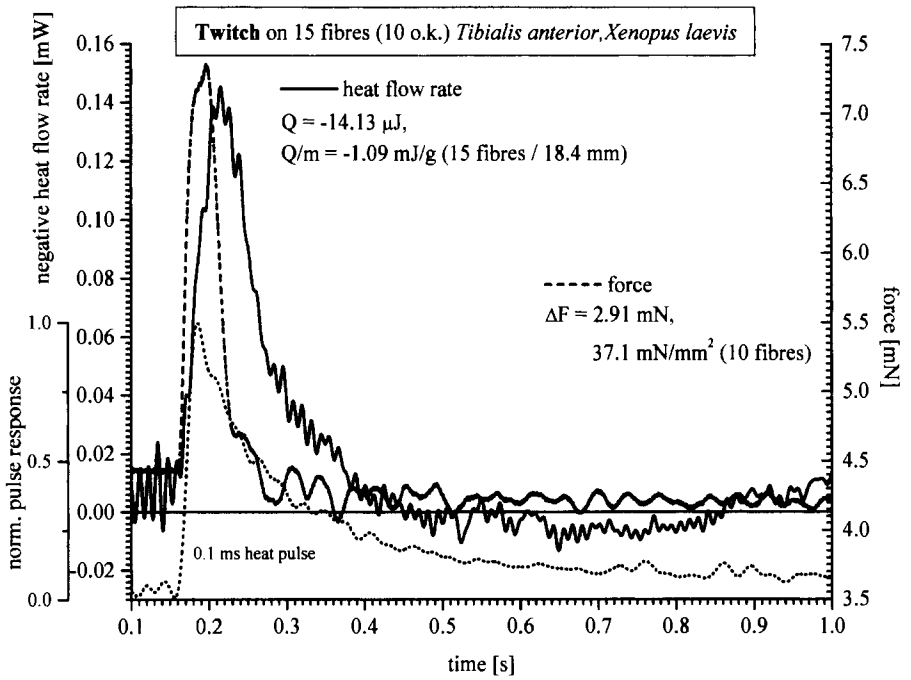

Fig. 3. A twitch experiment. (———) Heat flow rate; (- - -) force; and (· · ·) pulse-response function.

sumption. During positive force change, we find an exothermic heat flow rate; however, during relaxation (negative force change) an endothermic heat flow occurs. Thus, the conclusion can be drawn that the main part of the heat signal is closely (<1 ms) and reversibly correlated in time with the change in force. Such effects have been observed with deformation of rubber-like materials.

In the case of tetanus, the situation is somewhat more complex: in addition to the heat produced on change in force in the very beginning of the stimulation, which is similar to that of the twitch, there is an almost constant heat production during continuance of the tetanus. This is visible in a rather constant signal, which occurs after ca. 1 s of stimulation. Switching the stimulation off causes a fast decrease of the force, and with it a consumption of heat leading to a cooling of the muscle fibre. This, in contrast to the twitch result, is clearly visible in the measured curve (Fig. 5) but becomes more distinct in the desmeared one (Fig. 6). Comparing the heats, correlated to the initial and final changes in force by comparing the areas of the respective parts of the curve (Q_1, Q_2 in Fig. 5), leads to the result that they are almost the same but with opposite signs. We have to draw the conclusion that this part of the heat is elastically stored in the fibre, as is the case in elastic rubbers. These results are known from literature, in principle [1], but not with such a small temporal resolution.

4.2. Quick-stretch

With quick-stretch, the fibre, during isometric stimulation, is stretched fast (1 mm in 1 ms), where work must be achieved against force. In this case, a (exothermic) heat production occurs (Fig. 7, Q_1) which in the beginning looks like that of a twitch (Fig. 3) but decreases slowly in time. A relatively slow endothermic effect Q_2 occurs later on, which fades away after seconds only, leading to a somewhat higher heat flow-rate level as before stretching.

Performing the same experiment (fast extension) with a resting muscle fibre (Figs. 8 and 9), this additional endothermic effect is dropped while the first exothermic heat production, correlated with change in force (see Fig. 9), is found to be of the same size and shows the same temporal behaviour (compare Figs. 7 and 8).

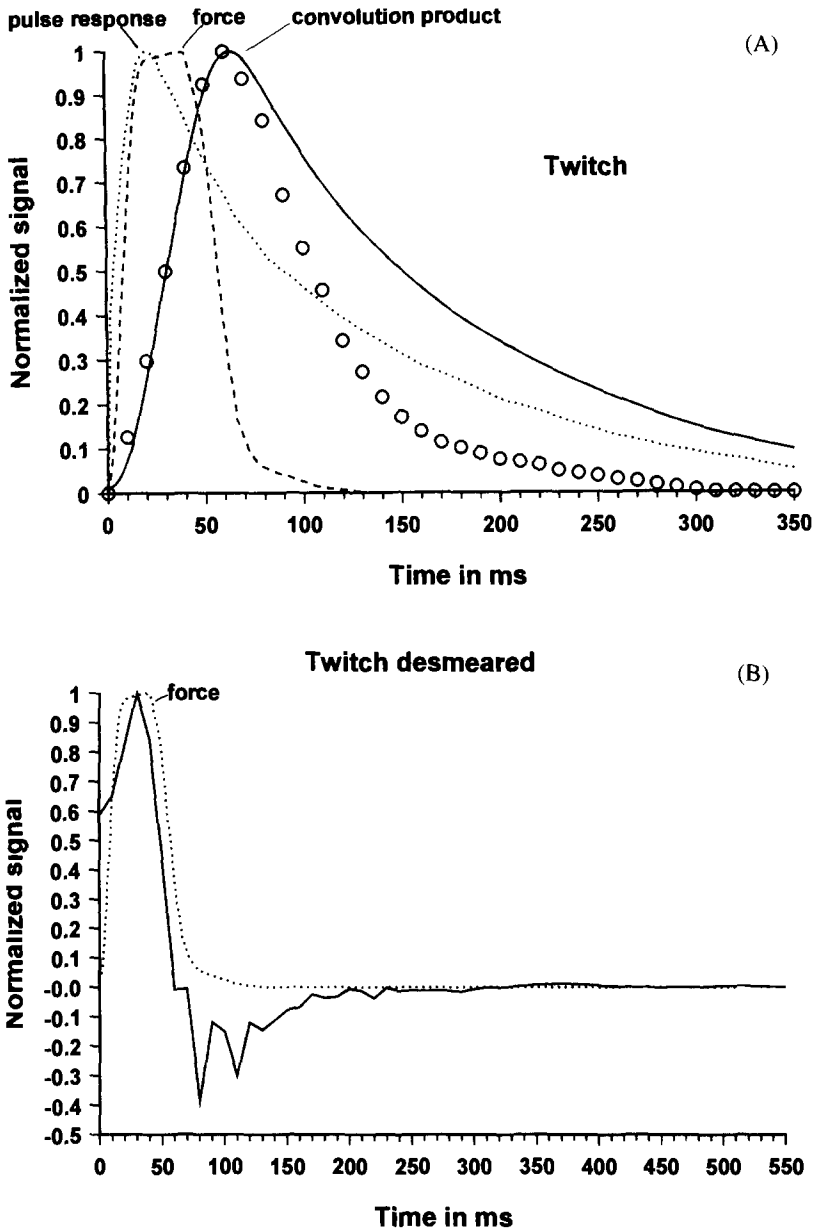

Fig. 4. (A) Normalized heat flow-rate function of a twitch experiment (○) compared with the convolution product of the pulse-response function with the change in force. (B) Normalized desmeared heat flow-rate function of a tetanus experiment together with force function.

Exact quantitative data are not easily received, because of the fairly low signals compared to those of noise and hum and the distortions (stray pickup) from stimulation pulses. However, the exothermic and endothermic parts of the total heat can be evaluated by integration of the raw data without desmearing. As a result, there is a certain dependence on sarcomere length. The first (exothermic) heat seems to increase a

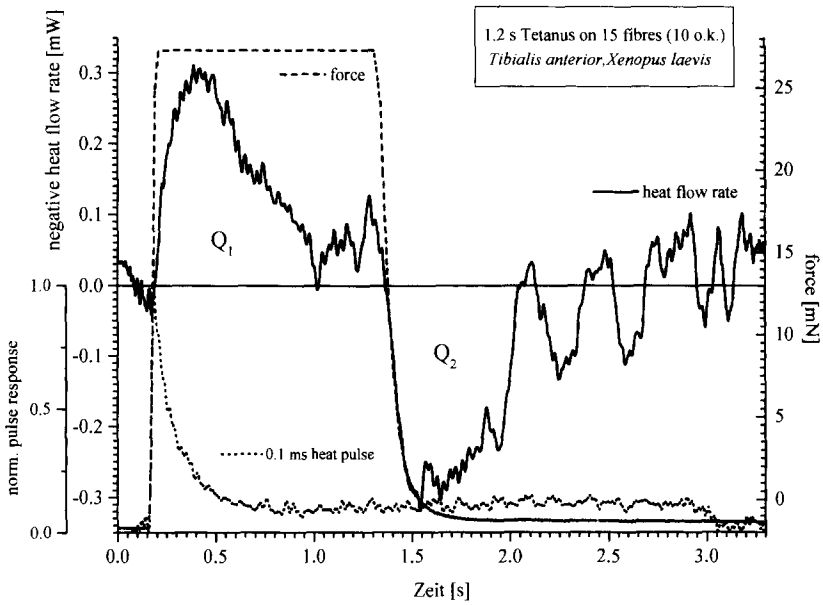

Fig. 5. A tetanus experiment. (———) Heat flow rate; (- - -) force; and (· · ·) pulse response function. Areas Q_1 and Q_2 (ca. 150 μJ) are the heats correlated to the respective changes in force. (The true force is larger as plotted, because the force exceeded the limit of the sensor.)

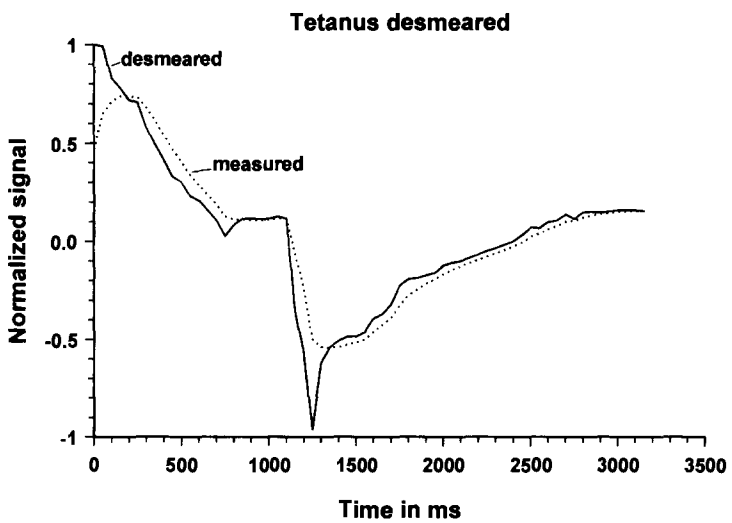

Fig. 6. Normalized desmeared heat flow-rate function of a tetanus experiment together with the measured function (scaled so as to fit in the steady state).

little with sarcomere length, this can be explained with an increasing change in force (and, thus, the respective heat) during the fast extension. The second (endothermic) heat remains almost unchanged except for a clearly visible step-like change at 2.5 μm sarcomere length.

From heat and work, the change of the internal energy can be calculated. The total work done

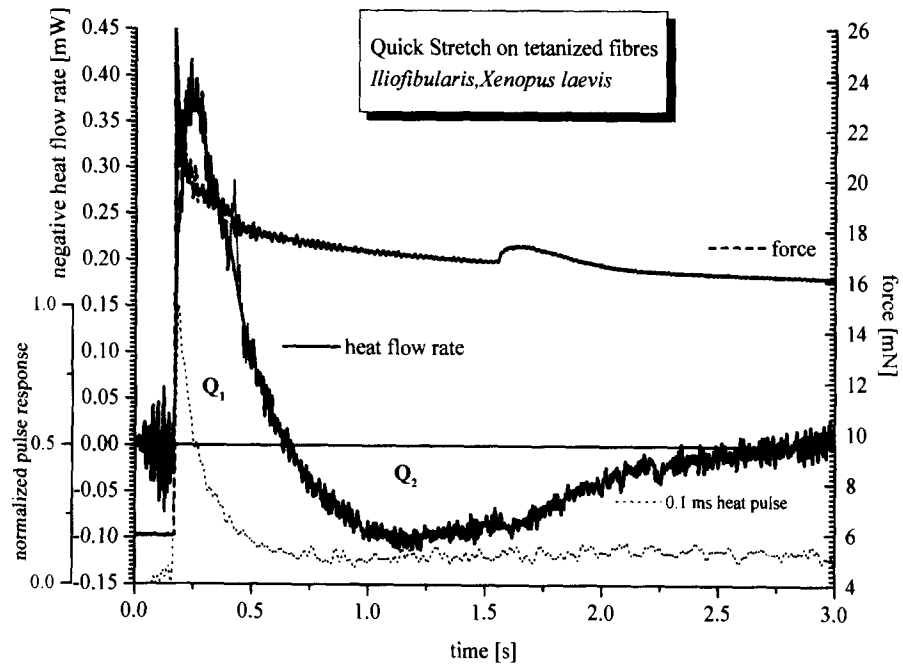

Fig. 7. A quick-stretch experiment of active muscle fibres. (———) Heat flow rate; (- - -) force; and (· · ·) pulse-response function.

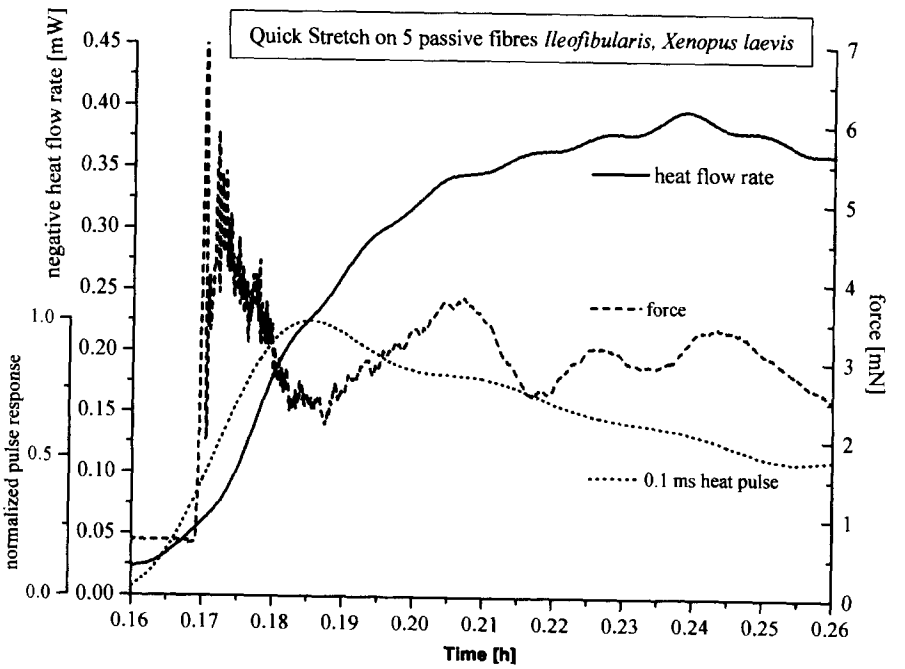

Fig. 8. A quick-stretch experiment of passive muscle fibres. (———) Heat flow rate; (- - -) force; and (· · ·) pulse-response function.

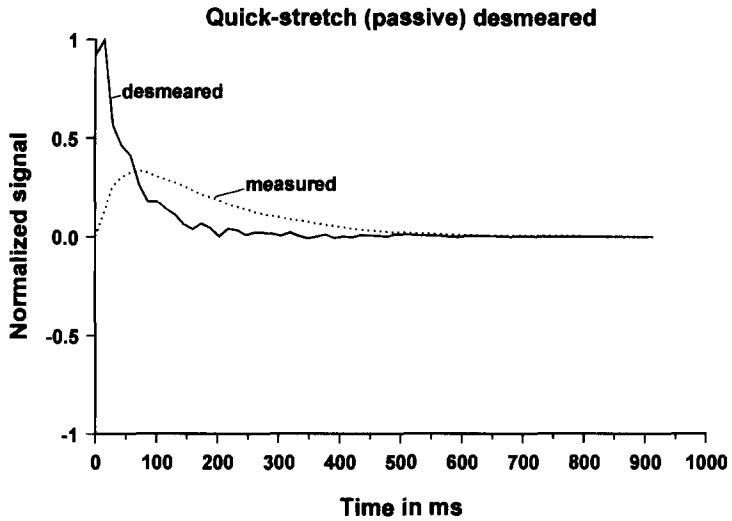

Fig. 9. Normalized desmeared heat flow-rate function of a quick-stretch experiment of passive muscle fibres together with the measured function (scaled so as to fit in the steady state).

($\approx$14 μJ) roughly equals the heat generated during the first millisecond, so that the internal energy remains unchanged during this period. The subsequent heat production however, becomes twenty times larger, but is reduced later by the endothermic effect. These processes are in reality on top of the steady-state heat production during tetanus, so there is only a corresponding change of the exothermic heat flow (which is coupled to the force generating processes in the fibre). However, there must be some kind of control mechanism which almost cancels the strong disturbances of steady state caused by the quick-stretch.

5. Conclusions

The described thermopile calorimeter enables determination of heat and work of muscle fibres with very high temporal resolution. The results seem to have brought some new findings on thermodynamics of the quick-stretch behaviour of an active muscle. There are two different heat producing and consuming processes, one after the other. The first one is strongly correlated to the change in force, as is the case in twitch experiments, but relaxes more slowly. This may be an (visco-)elastic physical process. The second one is delayed in time and seems, thus, to be an activated process (like a reversing chemical process or a folding/unfolding of proteins). It is a process controlled by the active living cell, which looks like a "repairing" of the "harm" caused by the rather nonphysiological fast straining of the muscle fibres. However, further detailed evaluations are necessary to support these preliminary results.

Acknowledgements

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