STORAGE AND RELEASE OF MECHANICAL ENERGY BY ACTIVE MUSCLE: A NON-ELASTIC MECHANISM?

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SUMMARY

In frog muscle fibres, tetanically stimulated at a sarcomere length of about $2\,\mu m$, stretched at a velocity of 1 length s^{-1} and released against a force equal to the maximum isometric, P_0, a phase of rapid isotonic shortening takes place after release. As the amplitude of the stretch is increased from 1·5 to 9 % of the initial length: (1) the amount of rapid isotonic shortening increases up to 9–10 nm per half sarcomere and (2) the stiffness of the fibre (an indication of the number of bridges attached) decreases to a value about equal to that measured during an isometric contraction. If a 5–10 ms delay is left between the end of stretch and release, the amount of rapid isotonic shortening increases to about $12\,nm\,hs^{-1}$. A 300–500 ms delay, however, results in a decrease in rapid isotonic shortening to about $5\,nm\,hs^{-1}$ and also results in velocity transients against P_0 that are similar to those described during release from a state of isometric contraction. It is concluded that the force attained after large, fast stretches is due to a greater force developed by each bridge and not to a greater number of bridges. After the elastic recoil (when the force is suddenly reduced to P_0), these strained bridges are able to shorten by about $12\,nm\,hs^{-1}$, suggesting that, during and immediately after stretching, they are charged to levels of potential energy greater than those attained in an isometric contraction.

INTRODUCTION

Muscles are usually considered as sources of mechanical power and this is indeed their main function during such exercises as flying, swimming, pumping blood, cycling, climbing or accelerating at the start of a sprint. In these activities the muscular force performs almost solely positive work, which is dissipated by external friction, or increases the average potential or kinetic energy of the body. However, another equally important functional requirement of muscle is its ability to absorb mechanical energy (i.e. to be stretched, while active, by some external force).

During exercises such as walking or running, on the level at a constant speed, the average increase of gravitational potential energy and of kinetic energy is nil, and the work done against external friction is negligible. The positive work performed by the muscles is used momentarily to increase the potential and the kinetic energy of the whole body, or of the limbs, and is then immediately reabsorbed by the muscles themselves. In these conditions the muscular force carries out an almost equal amount of positive and negative work (i.e. the muscles destroy in one phase of the step what they created in a preceding one). This decreases the economy of locomotion, but provides the opportunity of storing and recovering some of the work.

After a short review of the subject, experiments will be described which suggest the possibility of 'non-elastic' storage and recovery of mechanical energy by active muscle. An 'elastic' relationship between length and force is defined here as a monotonic function between the two variables: elastic structures involved in storage and recovery of mechanical energy by active muscle are the tendons and part of the structure of the cross-bridges between the myosin and actin filaments. The force-length relationship of the elastic elements within the cross-bridges is described by the T1 curve of Huxley & Simmons (1971). A 'non-elastic' relationship between force and length is defined here as a relationship in which the length can change independently from the force, notably against a constant force. A visco-elastic system is, therefore, defined as 'non-elastic'. Such structures are responsible for the recovery of tension, as described by the T2 curve of Huxley & Simmons (1971), ascribed by the authors to rotation of the head of the myosin, as initially proposed by H. E. Huxley (1969). In the experiments of Huxley & Simmons (1971) these structures release mechanical energy when the force is reduced below the isometric force, whereas they absorb mechanical energy when the force is increased above the isometric force. The reverse of the latter process has never been shown (i.e. the rotation of the head of the myosin in the direction of shortening when the muscle is released after stretching against a force greater than or equal to the isometric force).

MECHANICAL FACTORS AFFECTING A POSSIBLE STORAGE OF MECHANICAL ENERGY BY ACTIVE MUSCLE

The factors involved are: (1) length change and (2) force during stretching (which both determine the work done on muscle, i.e. the amount of energy which can possibly be stored), (3) the rate of decay of force after stretching (which may involve the loss of some of the stored energy).

Amount, speed of stretching and average muscle length

Under laboratory conditions the amplitude of lengthening steps is often very small (e.g. 0·15 % of the fibre length in the experiments of Ford, Huxley & Simmons, 1977). In life, however, muscle fibres can be subjected to stretches of the order of 10 % of their length (e.g. Biewener, Alexander & Heglund, 1981). These large, fast stretches are necessary to achieve maximum gain of mechanical energy in isolated frog

muscles and fibres (Cavagna, Dusman & Margaria, 1968; Bergel, Brown, Butler & Zacks, 1972; Edman, Elzinga & Noble, 1978; Cavagna, Citterio & Jacini, 1981). The enhancement due to stretching is larger the greater the average muscle length (Cavagna *et al.* 1968, 1981; Edman *et al.* 1978).

Force developed during stretching

Active muscle resists stretching with a force P' which is greater than the isometric force P_0. The difference $P'-P_0$ increases with the average muscle length and, within limits, with the amplitude and the velocity of lengthening (Abbott & Aubert, 1952; Edman *et al.* 1978).

Why does active muscle exert, during and after stretching, a force greater than the isometric force? The following mechanisms have been proposed: (1) stretching increases the number of attached bridges between actin and myosin (Sugi & Tsuchiya, 1981); (2) each cross-bridge resists stretching with a force which is greater than the force it is able to exert during an isometric contraction (Cavagna & Citterio, 1974; Edman *et al.* 1978; Sugi & Tsuchiya, 1981); and (3) an additional force-bearing mechanism is recruited in parallel with the bridges (Edman *et al.* 1978).

Since the stiffness of an active fibre increases with the number of attached cross-bridges (Ford, Huxley & Simmons, 1981), mechanisms (1) and (3) should result in an increase in stiffness (as found, above P_0, by Sugi & Tsuchiya, 1981). However, an increase in stiffness with force could also be due to the non-linear, force-length relationship of tendons and bridges. A decreased or unchanged stiffness necessarily favours mechanism (2). Julian & Morgan (1979) found no appreciable change of the stiffness during and after stretching.

Decay of force after stretching

If the active muscle is prevented from shortening at the end of stretching, but is maintained in isometric conditions, its force falls rapidly at first and then more slowly. The kinetics of this decay of force have been studied by several authors on whole muscle and on isolated fibres after stretching at different amplitudes and durations (Gasser & Hill, 1924; Abbott & Aubert, 1952; Ford *et al.* 1977; Edman *et al.* 1978). According to the experimental conditions, the kinetics of the decay of force are likely to be affected in different measure by the following mechanisms.

(1) Distortion of bridges (possibly by 'backwards' rotation of the myosin head, Huxley & Simmons, 1971). As a result the force falls (as in a viscous-elastic Voight element). The stiffness, however, would not change unless the force-length relationship of the elastic elements is non-linear. This mechanism, if reversible, may lead to storage and recovery of mechanical energy (see below).

(2) Breaking of most strained bridges (Cavagna & Citterio, 1974). This mechanism requires large stretches and will lead to a decrease of the force and of the stiffness.

(3) Attachment of bridges after the end of stretching. This will lead to an increase in both force and stiffness.

Possible sources of increased work after stretching

The effect could be achieved by an enhancement of the same mechanisms involved in the release of mechanical energy during shortening from a state of isometric contractions, namely: (1) elastic recoil of stretched tendons and bridges; (2) rotation of the heads of the myosin from positions of greater potential energy attained during stretching (this would explain the capability of the previously stretched muscle to shorten against a constant force equal to the maximum isometric P_0) and (3) cycling of bridges allowing a steady shortening against a force greater than or equal to P_0, and a greater velocity of shortening when the force is reduced below P_0 [this mechanism is demonstrated by a shift of the force-velocity curve along the velocity axis; the shift is maximal when the force applied after stretching is near to P_0 and decreases to zero when shortening occurs under zero load (Cavagna & Citterio, 1974; Edman *et al.* 1978; Sugi & Tsuchiya, 1981)].

Both mechanisms (2) and (3) allow muscle shortening against a constant force. However, mechanism (2) can operate only over a short distance which must be a fraction of the stroke of a bridge. Mechanism (3) on the other hand can operate over a greater distance due to 'rowing' of the bridges.

Unfortunately, it is not easy to isolate these three mechanisms experimentally. In fact, it is likely that most of the existing experimental evidence of muscle enhancement due to stretch results from a combination of all three. For example: (a) mechanism (2) would be underestimated and mechanism (1) would be overestimated if the time resolution of the apparatus is too small; (b) the force-shortening curve describing the apparent elastic behaviour of muscle includes mechanisms (1) and (2) (Cavagna *et al.* 1981); (c) the shift of the force-velocity relationship was determined during muscle shortening due to both mechanisms (2) and (3) (Cavagna & Citterio, 1974).

EXPERIMENTAL EVIDENCE OF A TRANSIENT RESPONSE TO SUDDEN
REDUCTION OF LOAD FROM THE VALUE ATTAINED AFTER STRETCHING DOWN
TO THE MAXIMAL ISOMETRIC VALUE P_0

This response has been extensively studied by unloading the fibre from a state of isometric contraction either by a sudden reduction of length ('tension transient': Huxley & Simmons, 1971; Ford *et al.* 1977, 1981) or of load ('velocity transient': Podolsky, 1960; Civan & Podolsky, 1966). In the experiments described below this response has been measured by unloading the fibre from the force reached after stretches of different amplitude (with or without an interval of time after the stretches) down to the maximal isometric force P_0. Sudden unloading of an isometrically contracting muscle gives information about the early transient mechanisms which release the potential energy that the contractile component of muscle was able to build up during the isometric contraction. Sudden unloading immediately after stretching down to the maximal isometric force gives the same information, but in a range of potential energy reached only momentarily as a result of an input of external energy. In contrast to isometric contraction, the state reached at

the end of stretching is not steady, and the release after different intervals of time against P_0 gives information about an eventual attainment of a new steady state.

In the present experiments, fibres of tibialis anterior of *Rana temporaria* (5·5–7 mm long) were stretched, while active, by 0·1, 0·25 and 0·5 mm (i.e. 1·5–9 % of l_0) at a velocity of about 1 length s^{-1}, at a temperature of 2–3 °C. Except for the smallest stretches, lengthening of the active fibre was continued well beyond the length at which the force 'gives' (Fig. 1), indicating that some cross-bridges are forcibly detached (Rack & Westbury, 1974; Flitney & Hirst, 1978).

The active fibre was stretched from the length (l_0) at which it exerted the maximal isometric force (sarcomere length about 2·0 μm). During the state of isometric contraction (before stretching), during the stretch at a constant speed and during an eventual predetermined time interval at the stretched length, the fibre was under length control, whereas after release it was under force control (i.e. the length of the fibre changed in such a way as to maintain the force constant and equal to P_0). The mechanical events occurring after release were studied by recording force and length
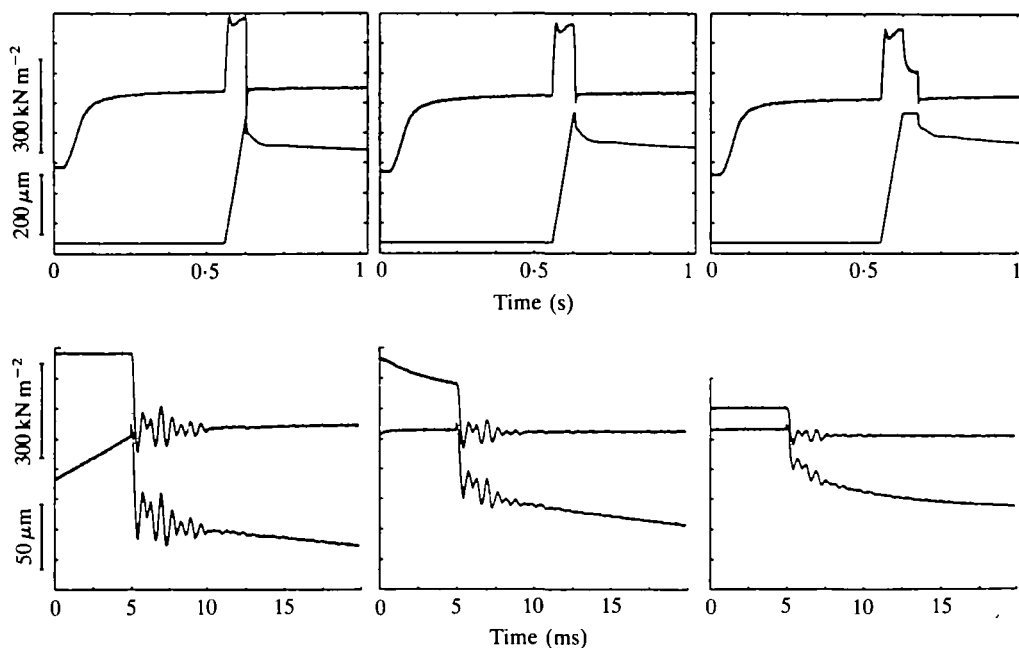

Fig. 1. Top: tension and length changes in an isolated muscle fibre during an isometric contraction, a 0·5 mm stretch and release against a force about equal to that developed isometrically before stretching. On the lefthand tracing, release immediately follows stretching, whereas on the middle and righthand tracings, an interval of 5 ms and 50 ms, respectively, was left between the end of stretching and release. Below: same as above, but on a faster time scale, to determine the average stiffness during elastic recoil and the start of the rapid isotonic shortening (arrow heads in Fig. 2). (Fibre of tibialis anterior of *Rana temporaria*, slack length 6·5 mm, sarcomere length before stretching 2·04 μm, section 12500 μm^2, temperature 2·7 °C.)

of the fibre during the elastic recoil (lasting on the average 270 μs) on a fast time base, and recording the isotonic 'velocity transients' (10–90 ms) on a slower time base (Fig. 1). The 'fast' records were used to determine the average stiffness. In our experimental conditions, this value gives an indication of the number of attached bridges between actin and myosin, but is also affected by the amount of tendon (0·5 mm) left between fibre insertion and the connecting clips (Ford *et al.* 1977). The 'slow' records were used to study the isotonic length changes taking place against a force equal to P_0 (Fig. 2). The amplitude of these length changes was related to one half sarcomere by assuming that over the range of sarcomere lengths studied (2·0–2·3 μm) the change measured at one extreme is uniformly distributed along the whole fibre (Cleworth & Edman, 1972; Edman *et al.* 1978; Cecchi, Colomo & Lombardi, 1983).

Effect of stretch amplitude

In these experiments, the fibre was released after stretches of differing amplitude without a time interval between the end of stretch and the release. After small
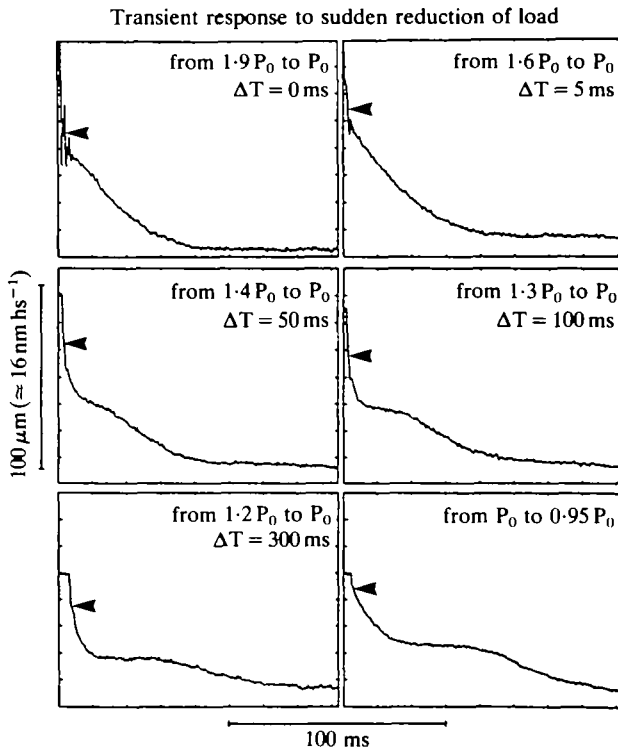

Transient response to sudden reduction of load

from 1·9 P_0 to P_0
$\Delta T = 0$ ms

from 1·6 P_0 to P_0
$\Delta T = 5$ ms

from 1·4 P_0 to P_0
$\Delta T = 50$ ms

from 1·3 P_0 to P_0
$\Delta T = 100$ ms

from 1·2 P_0 to P_0
$\Delta T = 300$ ms

from P_0 to 0·95 P_0

100 μm ($\approx$ 16 nm hs^{-1})

100 ms

Fig. 2. The effect on the isotonic velocity transients of an interval between the end of stretch and release against the maximal isometric force P_0. The tracing in the bottom righthand corner was measured, for comparison, during release from isometric contraction. (Same experiment as shown in Fig. 1.)

stretches the stiffness was always greater than that measured from a state of isometric contraction. Increasing the amplitude of the stretch, however, caused a decrease in stiffness (to values equal to or smaller than those measured from a state of isometric contraction, Fig. 3).

After elastic recoil, the active fibre continues to shorten against a force equal to the maximal isometric force. Shortening takes place in two phases which are often divided by an inflection of the curve where the velocity approaches zero (see Fig. 1). During the first phase, which lasts up to 90 ms, the velocity of shortening is much greater than during the second phase. The amplitude of the first phase increases to 9–10 nm per half-sarcomere when the amplitude of stretching is increased. As mentioned above, this is accompanied by a decrease in the stiffness.

These findings indicate that the greater force P′ (up to $2 \times P_0$) attained at the end of large stretches, and the limited isotonic shortening taking place against P_0, cannot be due to an increase in the number of bridges. In fact, the stiffness measured above P_0 is equal to, or smaller than, that measured from a state of isometric contraction, in spite
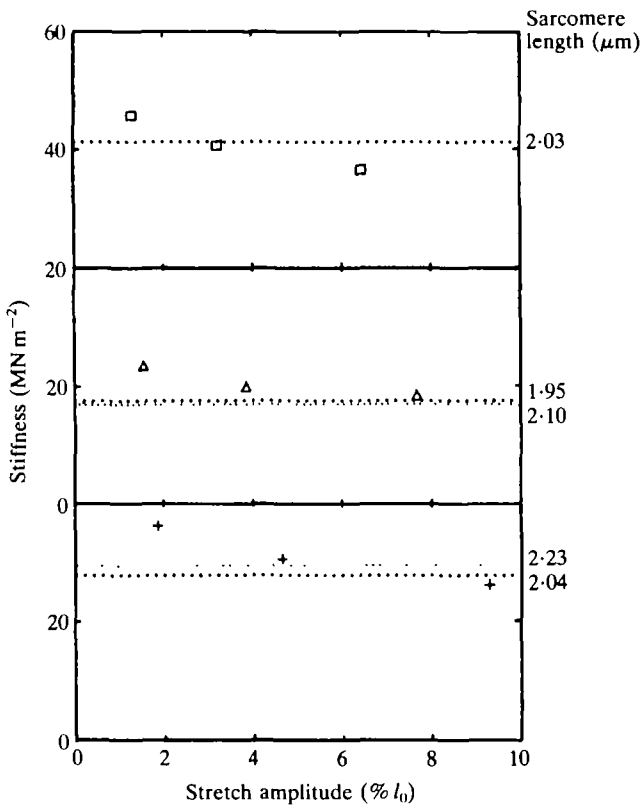

Fig. 3. Effect of the amplitude of the stretch on the average stiffness during elastic recoil. The dotted lines indicate the stiffness measured during recoil from a state of isometric contraction at the sarcomere length before (thicker) and after (thinner) stretching.

of the non-linearity of the force-length relationship of bridges and tendons. The difference $P'-P_0$ must, therefore, be due to bridges which are individually exerting a greater force than during isometric contraction. The increase in the amplitude of the isotonic shortening with the amplitude of stretch suggests a progressive recruitment of strained bridges in series.

Effect of a time interval between end of stretching and release against P_0

In these experiments we studied the effect of a variable time interval, after stretches of 7–8 % of l_0, on stiffness and on the isotonic velocity transients. A delay of 5–10 ms after the end of the stretch results in: (1) a marked decrease of the difference $P'-P_0$ (to 2/3–1/2); (2) an increase in the amplitude of isotonic shortening from 9–10 to about 12 nm per half-sarcomere; and (3) a decrease in the stiffness of about 7 %.

As mentioned above, findings (1) and (2) could be due to completion of 'backwards' rotation of the head of the myosin which was already in progress during stretching. The 'charging up' of this structure will thus decrease the tension in the elastic elements (finding 1), but will increase the capability of the fibre to shorten against P_0 (finding 2). Finding (3) could be due to breaking of bridges (also responsible for part of the fall of the force) and non-linearity of the force-length relationship of tendons and bridges.

A delay of 50–500 ms after the end of stretching results in: (1) a continued decrease in the force, at a more moderate rate, towards P_0; (2) little change in the stiffness; and (3) a modification of the isotonic velocity transients (Fig. 2). As the delay is increased from 5 to 500 ms, the fast isotonic shortening is divided into two parts by an inflection of the curve which then becomes a plateau of increasing duration. The first part becomes steeper and the second flattens into a slow oscillation which is hardly distinguishable from the subsequent slow shortening phase. After 300–500 ms the velocity transients are similar to those recorded after release from a state of isometric contraction except for a smaller duration of the early shortening and a shortening which occurs against a force equal to P_0.

A decrease of the force with an approximately constant stiffness indicates that the force sustained by each bridge becomes, on the average, smaller. This could be due to the substitution of bridges attached after stretching, and exerting a smaller force, for bridges which were strained during stretching and thus generating a greater force. The similarity of the transient response recorded 300–500 ms after the end of stretching to that recorded from a state of isometric contraction suggests a common origin and makes it reasonable to ascribe both to the rotation of the myosin head. These results suggest that after large, fast stretches the head of the myosin attains values of potential energy which allow a shortening against P_0 of about 12 nm hs^{-1}. This is similar to the shortening capability of the same structure after its attachment to actin during a state of isometric contraction (Ford *et al.* 1977).

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