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TEMPERATURE AND AMPLITUDE DEPENDENCE OF TENSION TRANSIENTS IN GLYCERINATED SKELETAL AND INSECT FIBRILLAR MUSCLE

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SUMMARY

1. Quick stretches and releases were applied to small bundles of glycerinated fibres of rabbit psoas and insect fibrillar flight muscle. The resulting tension changes were recorded at various temperatures and amplitudes of length change. The results from the two preparations had many features in common. At temperatures near 0° C the asymmetry of the initial tension recovery after stretch and release originally reported in living frog fibres by Huxley & Simmons (1971*a*) was very obvious.

2. The complete tension course could be described as an elastic change occurring simultaneously with the length change followed by recovery consisting of the sum of a number of exponential terms. These terms usually corresponded to the phases discernible without curve fitting, but in some cases a monotonic rise or fall of tension was seen to consist of two components only after curve fitting.

3. After either stretch or release there was a phase of rapid tension recovery towards the value before the length change. The rate constant of this phase increased as the amplitude of stretch or release was increased to about 2 nm/half sarcomere. At higher amplitudes it remained nearly constant.

4. At temperatures near 0° C there was a second and much slower continuation of the recovery after stretch. The rate constant of this second phase was much more sensitive to temperature than that of the first phase and it became slower with increasing amplitude of stretch. As

The work was carried out in Oxford.

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the temperature was raised the speed of the second phase approached the speed of the first phase so that at room temperatures the initial tension recovery after stretch and release was nearly symmetrical.

5. Under many conditions these processes were followed by a change in the opposite direction, the 'delayed tension' described by earlier workers. This third phase of tension change had about the same temperature sensitivity as the second phase of the recovery seen after stretch. The tension due to stretch activation was not maintained in rabbit muscle, resulting in a fourth possible phase, a recovery of tension towards the value before the length change. This was absent or of low amplitude in insect flight muscle.

6. We interpret these tension changes on the basis of an extension of the non-linear model described by White & Thorson (1972). The elastic tension change and the initial fast recovery are both supposed to be properties of the attached cross-bridges, whilst the slower recovery is considered to be due to the detachment of cross-bridges which happened to be attached at the instant the length change was applied. The delayed tension reflects the approach to equilibrium of the number of attached bridges, changed by an effect of muscle length on the attachment rate. The fact that the delayed tension is not maintained in rabbit psoas muscle may be due to the effect of length on attachment rate being transitory.

INTRODUCTION

The fastest of the tension changes which are observed when muscle fibres are suddenly stretched or shortened have been shown to be due to properties of the cross-bridges (Huxley & Simmons, 1971*b*; Huxley, 1974). The amplitude of these changes varies according to the amount of overlap between the thick and thin filaments, although the sudden length decrease required to reduce the tension to zero is the same regardless of the initial muscle length. Huxley & Simmons (1971*a*) have shown that at 3° C the tension fall after stretch takes longer to reach a minimum than the rise after release takes to reach a maximum or a plateau. They have developed a theory to account for this asymmetry, in which the force in the cross-bridges influences the rate constants between two or more attached cross-bridge states, and thus influences the rate of relaxation of the bridges to a new equilibrium when this is disturbed by a length change. Abbott (1972*b*) criticized this interpretation on the grounds that the transient seen after stretch appeared to consist of two distinct phases, one of about the same speed as the transient seen after release and another much slower one, and that the rate constant of the recovery did not increase nearly as sharply with amplitude of release as is

required by the theory. In reply, Huxley & Simmons (1972) published further details of their experimental records in which the transient after stretch was no longer obviously a two phase process on the time scale shown.

In an attempt to clarify the situation, we have observed the tension changes following quick stretch and release at various temperatures and amplitudes of length change, using glycerinated fibres of insect fibrillar flight muscle and rabbit psoas muscle. Glycerinated muscle has several advantages for this type of study. It is easy to attach fibres to the apparatus without damaging them or introducing a significant amount of series elasticity, the chemical conditions and temperature can be changed at will and comparisons are more reliable because many observations can be made on one fibre bundle.

At temperatures near 0° C the results of this study are similar to those originally reported by Huxley & Simmons (1971*a*). The transient tension fall after quick stretch was always accomplished in two distinct phases. The slower of these phases was temperature dependent whilst the faster one was not correlated significantly with temperature in the range 2–20° C. The transients became steadily more symmetrical as the temperature was raised. Our results are not consistent with the theory put forward by Huxley & Simmons (1971*a*) and we offer an alternative explanation based on the attachment and detachment of cross-bridges, together with a visco elastic element within the bridges.

METHODS

Dorsal longitudinal muscles of the giant water bug *Lethocerus cordofanus* and rabbit psoas muscles were glycerinated in a solution containing 50% glycerol, 100 mM-KCl, 20 mM phosphate buffer at pH 7·0, 5 mM-$MgCl_2$ and 2 nM dithiothreitol (Rüegg, Steiger & Schädler, 1970; Abbott, 1973*a*). The activating solution contained 2 mg/ml each of phosphocreatine and creatine kinase (approx. 50 u.), 10 mM-MgATP, 70 mM-KCl, 20 mM phosphate buffer at pH 7·0, 4 mM-EGTA and $CaCl_2$ to give a calcium ion concentration of 10^{-6} M. This solution and a salt solution (as above without the MgATP) for washing the glycerol out of the fibres were held in a chamber whose temperature was controlled by a jacket containing circulating water at a controlled temperature. A third chamber contained water whose temperature was measured by a thermocouple. In this way the temperature of the incubation medium could be controlled to within a fraction of a degree.

The mechanical apparatus was the same as that described by Abbott (1973*a*). A bundle of up to five fibres (maximum of two for rabbit muscle) was dissected from the glycerinated muscle and glued to the apparatus with cellulose acetate dissolved in acetone. The free length between the vibrator pin and the transducer pin was 5 mm. The pins were coated with glue which was allowed to dry. The muscle fibres were placed under the ends of the pins and wrapped upwards along their outside edges. A small amount of glue was applied and this coalesced with that already on the pins to give a rigid connexion. The compliance of the apparatus was about 2 orders of

magnitude less than the compliance of the fibres. The rabbit muscle had been glycerinated at a sarcomere length of 2·5 μm and this was not changed during the course of the experiment. The fibres were first immersed in the salt solution for a few minutes and were then transferred to the activating solution where they remained for the whole of the experiment, which usually lasted 1–2 hr. The insect flight muscle was set at slack length plus 1·5% in this solution before any measurements were made. The sarcomere length of the insect muscle is about 2·5 μm. The order in which the various conditions were used was varied from experiment to experiment. In some, a complete amplitude series was measured at a few temperatures and in others more temperatures were tested at one or two amplitudes of length change. In all cases the smaller amplitudes were applied first but the temperature was sometimes raised and sometimes lowered. We could not detect any change in the results which was correlated with the use of different sequences of conditions.

A PDP8 computer was programmed to act as a signal averager and ramp generator. Several features of the program made the computer more powerful than hardware averagers. In particular, the sweep was divided into two equal intervals, one for stretch and one for release, and within each half sweep the time base speed could be altered at the mid point. In this way it was possible to record the tension changes up to 275 ms after the length change, while at the same time gathering enough information in the first few ms to estimate the time constants of the fastest transients. A further refinement was that during the sweep the newly averaged signal was held in a temporary store. This was only transferred to the result store at the end of the sweep if no point caused overload of the analogue to digital converter. In this way distortion of the averaged signal due to temporary overload of the converter was avoided.

Each sweep consisted of a stretch at controlled speed, a period of data accumulation, a pause of 450 ms, a release at the same speed, another period of data accumulation and a final pause of 450 ms. During each period of data accumulation 128 points were recorded at an interval of 0·2 ms followed by a further 128 at intervals of 2·0 ms. Thus, the time between each length change was 731·6 ms. When sufficient sweeps had been averaged to give a clean looking result (the number varied between 10 and 200 depending on the amplitude of length change) the numbers were punched on paper tape for analysis later.

The rise time of the length servo was under 1 ms, the exact time depending on the amplitude of movement. There was a small overshoot which was eliminated by placing a condenser across the signal input. The tension transducer was a glass capacitance type similar to that described by Huxley & Simmons (1968) but with a glass tripod. The transducer did not give a measurable response when the vibrator was moved but there were no fibres glued to the apparatus. The free response was a lightly damped oscillation of 4 kHz. At each amplitude of movement to be used in the experiments the ramp velocity of the input to the vibrator servo amplifier was chosen by increasing it until this resonance was excited. This test was made with the muscle fibres in the rigor inducing salt solution. The frequency of the oscillation observed after averaging was 1 kHz, which is the difference between the actual resonant frequency and the 5 kHz measurement rate. This frequency is visible on a few of the experimental records. Up to an amplitude of 6 nm/half sarcomere the optimum ramp duration was found to be a constant 0·75 ms, thereafter it was increased up to a maximum of 1·15 ms for a length change of 10 nm/half sarcomere. This rise time is nearly the same as the rise time of the servo system controlling the fibre length so the length change was completed in just over 1 ms. The first point plotted in the figures was measured 1·0 ms after the end of the ramp, which is therefore about 0·6–0·7 ms after the end of the length change and exactly 1·75 ms after the start of the length change.

There are several reasons why the observed tension transients cannot be an artifact introduced by the transducer. Firstly, the free response of the transducer was a lightly damped resonance of several kHz which cannot be construed as an exponential of time constant around 1 ms. Secondly, the extreme values of the fastest rate constant of the tension response varied by a factor of 5 to 1 and if it were an artifact it would always have had the same speed. Thirdly, after very large releases the fastest component had a small amplitude and was sometimes not visible at all. These releases produced a correspondingly large elastic tension change and if the fastest rate constant had been within the apparatus it would have been observed under these conditions at a high amplitude.

Unfortunately, the tension transducer was not stable enough in the long term for it to be possible to measure the absolute tension. It was particularly sensitive to changes in humidity and these occurred when the temperature was altered. In the extreme case condensation formed on the still cold transducer when the temperature of the incubation medium was being raised. A large dish of silica gel was kept in the box enclosing the whole apparatus which reduced the tendency for condensation to form. We could have applied fast releases large enough to reduce the tension to zero to obtain the base-line value but this would have extended each measurement considerably and there is no guarantee that the fibres would be in the same state after re-stretching. Most of the releases applied were much less than that required to reduce the tension to zero and such large amplitudes were generally left to the end of the experiment as they tended to damage the preparation. The elastic tension change was estimated and plotted against the length change for each experiment. This plot was always straight on the stretch side, indicating that the fibres had probably not been damaged by the experimental procedure, because such damage reveals itself as a decreased magnitude of the response. The absolute mean tension was at least as great as the tension drop caused by the largest quick release applied.

Exponential curves were fitted to the tension responses by least squares analysis. A method in which the rate constants are found by trial and error is necessary because the equation to be fitted is not linear in the rate constants. The commonly used method of linearizing by taking logarithms is not sound for various reasons. Firstly, the 'rate constants' found by this procedure depend critically on the base line chosen and this choice cannot be accurate unless the infinite time value is actually measured. Secondly, much greater weight is given to points near the base line than those away from it whereas the points should be weighted equally. Thirdly, if the base line is not chosen correctly then even truly exponential data will give a curved semilogarithmic plot giving the impression of more components than are in fact present. The trial and error method requires the use of a digital computer. All programmes were written in Algol using the system for the PDP8 developed by one of us (Abbott, 1973*b*).

Initial trial values of the rate constants were found with the aid of a routine which calculated the error and displayed on an oscilloscope the data and the best fit, using given rate constants which were typed directly into the computer. The data and fit were plotted against ln (time) to make it easier to judge the quality of the fit. The points measured at 2 ms intervals were weighted 10 times more heavily than those measured at 0·2 ms intervals. When a reasonable fit had been found the computer refined the values of the rate constants by the following method. The current estimates were taken together with values 5% higher and lower to make three trial values of each rate constant. The error in the fit was worked out for all the possible combinations of the trial values and the minimum was noted. If the previous best estimate was improved upon during this process the trial was repeated using the new best values of the rate constants, except that to save time combinations already

calculated were not tested again. When no further improvement could be obtained the interval between the trial values was reduced to 2% and the whole calculation was repeated.

RESULTS

General description of the tension transients

In both rabbit psoas and insect flight muscle the time course of the tension changes could be resolved into a maximum of five phases, an initial change occurring during the length change followed by up to four which could be described adequately by the exponential function. It was possible to observe all five phases after stretch and release only in the case of rabbit psoas muscle at temperatures around 10° C. Fig. 1*A* illustrates a complete measurement made under such conditions. Actual recording ends at the arrows. The projection to the next stretch or release is a continuation of the fitted curve and is included only to show that it reaches equilibrium shortly after the end of the period of observation.

Phase 0 was an elastic tension change which occurred during the length change. This could not be measured accurately because of the limitations of our apparatus, but an estimate was plotted against the length change for each experiment. The minimum value of the isometric tension of each preparation was the tension drop resulting from the largest quick release applied. This value and the slope of elastic tension–length change curve were variable. In rabbit psoas muscle the estimated tension was usually in the range 100–200 μN/fibre and the slope of the curve for small length changes was about 30 μN/fibre tension for each 1 nm/half sarcomere length change. The tension value, which must be an underestimate, is equivalent to about 0·5–1 kg/cm^2, rather less than the

Legend to Fig. 1.

Fig. 1. Response of rabbit fibres to quick stretch and release of 2 nm/half sarcomere at 10° C. The measured points are indicated by dots. *A* shows the whole of the response. The tension difference between the stretch and release curves is the true measured value. Recording ended at the times indicated by the arrows, the rest of the plot being a continuation of the fitted curve which is shown as a continuous line in the region in which data were measured. The phases discussed in the text have been numbered. In *B* the first 25 ms of the same response is shown. The vertical separation between the traces is chosen for clarity and does not represent the true absolute tension difference between the stretch and release curves. *C* and *D* show how the stretch and release transients respectively are made up of four components. The time scales of *B*, *C* and *D* are the same. Note that the amplitudes and rate constants of the K_1 components are similar after stretch and release, also that the tension recovery after stretch, seen best in *B*, is made up of two components whose rate constants differ by almost an order of magnitude. The remainder of the figures showing tension responses are laid out as in *B*, but scales are not necessarily the same.

values usually reported for fully active muscle. This is probably due to the ATP backup system because when it was removed much higher tensions were observed (see next section). The slope indicates that the tension would be reduced to zero by a release in the range 5–10 nm, agreeing with Huxley & Simmons (1971*a*) and Huxley (1974). The variability was

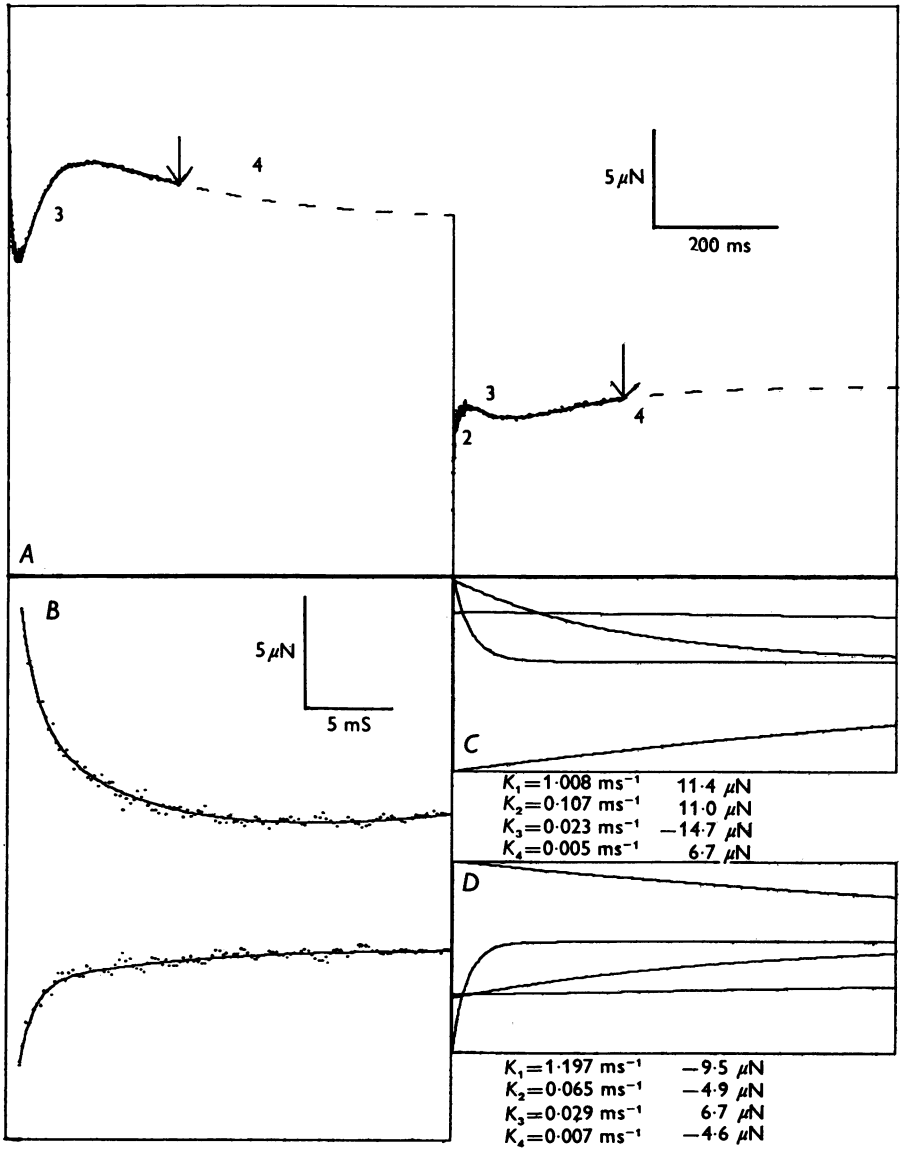

Fig. 1. For legend see opposite page.

presumably due to some deficiency in our experimental technique, with a contribution from the unequal cross-sectional areas of the various preparations. Comparison of experiments made under the same conditions showed that although the amplitudes of the various exponential components changed with the size of the elastic tension change, their rate constants were not affected.

The first phase of tension change at constant length was phase 1, a rapid recovery of tension towards its value before the length change. This can be seen best in Fig. 1*B*, which shows the first 25 ms of the response of Fig. 1*A*. The exponential components which make up the fitted curve are shown in Fig. 1*C* (stretch) and Fig. 1*D* (release). It is seen that the rate constant of the rapid recovery, which we call K_1, is similar after stretch and release as are the magnitudes of this component. This is a consistent feature of our records, except that at the very largest releases the amplitude is smaller than after a stretch of the same amount.

Phase 2 is always a further fall of tension after stretch, but after release it may be a rise or fall of tension. In the record of Fig. 1 it is also a further recovery of tension (i.e. rise) after release. We call the rate constant of this phase K_2. Although it may not be obvious from Fig. 1*B* that the recovery after stretch is a two phase process, it can be seen from Fig. 1*C* that the rate constants K_1 and K_2 differ by an order of magnitude and the two components have nearly the same amplitude, so there is no question of the recovery being a single exponential decay. (The reason why it cannot be a single non-exponential decay will be discussed later). After the release of Fig. 1 the division of the recovery into two components is obvious without curve fitting.

Phase 3 is the same direction as the initial elastic change (except after some large releases) and is the 'delayed tension' due to stretch activation. We call the rate constant of the development of the delayed tension component K_3. In insect flight muscle this component is responsible for the oscillatory activity and we find that its amplitude is much greater than in rabbit psoas muscle (Figs. 3–6). It cannot be seen in rabbit muscle after release at temperatures near 0° C. A further difference between the two types of muscle is that in rabbit psoas muscle the delayed tension is not maintained. It decays slowly towards its value before the length change with a rate constant K_4, which is usually outside the range which can be measured accurately by recording for 275 ms.

The high tension state in rabbit psoas muscle

It has been known for some time that insect flight muscle enters a state known as the high tension state if it is over activated (Jewell & Rüegg, 1966). In this state the mean tension is high but changes resulting from

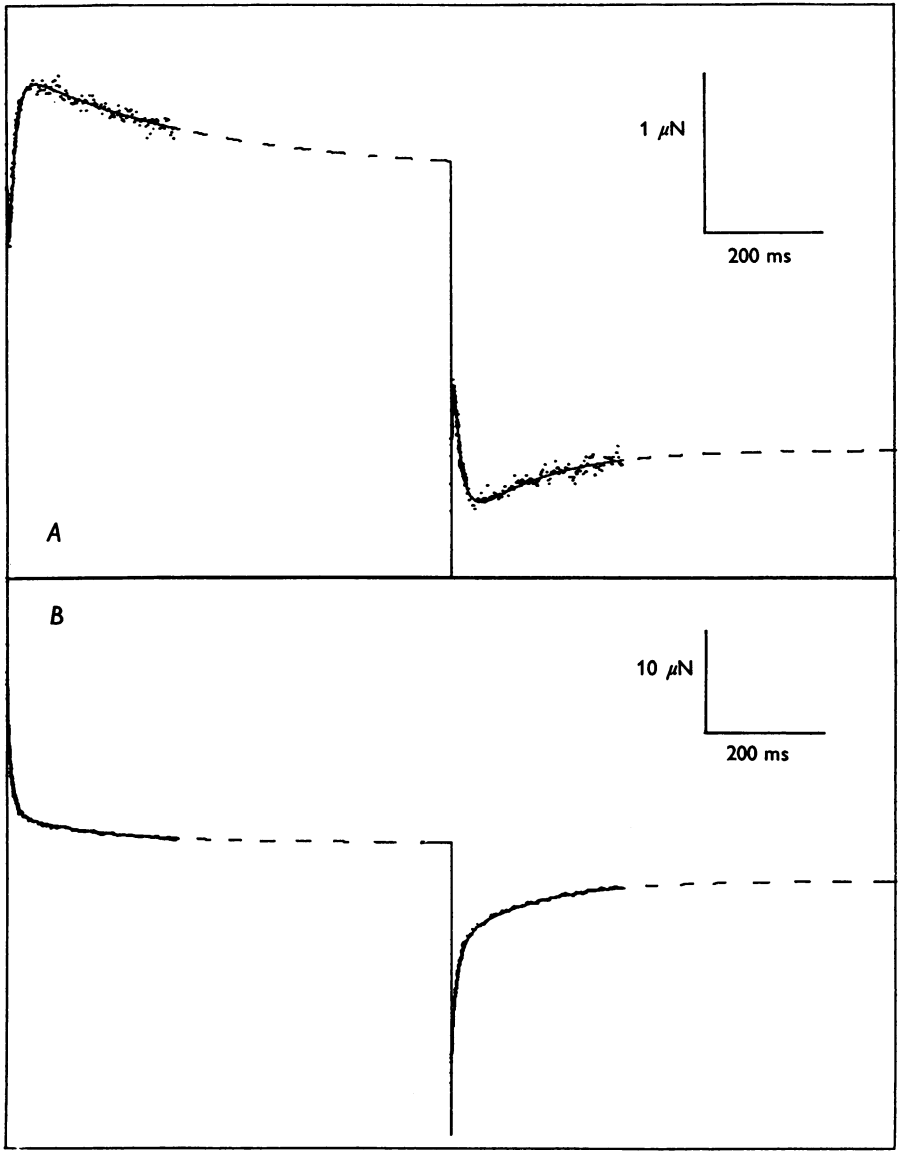

Fig. 2. The effect of omitting the ATP regenerating system from the incubation medium on rabbit psoas fibres at 22° C. In *A* the response to length changes of 0·5 nm/half sarcomere are shown, in the normal activating solution. The fibres were transferred immediately after measuring this response to a solution without creatine phosphate or kinase, with the result shown in *B*. The size of the elastic tension change is increased by about an order of magnitude. The mean tension also rose considerably.

the imposition of length changes occur much more slowly (except the elastic change occurring during the length change). It is thought that this state is due to an excess of ADP relative to ATP in the region of the myofibrils (Pringle, 1967; Abbott & Mannherz, 1970). The same phenomenon is observed in rabbit psoas muscle at room temperature if the ATP regenerating system is omitted from the incubation medium, as is illustrated in Fig. 2. The transient tension recovery is so slow that the stretch activation is completely masked. The effect is reversible, although the magnitude of the tension changes is less after the high tension state, presumably because of structural damage caused by the high forces generated. The result is similar to some of the active responses of Heinl, Kuhn & Rüegg (1974). It is definitely not due to partial rigor because in this state there is very little tension recovery after quick stretch or release (our own unpublished results; Heinl *et al.* 1974). It was to avoid the high tension state that all experiments on rabbit muscle were carried out in the presence of creatine phosphate and creatine kinase.

The effect of amplitude of length change on the shape of the response

The form of the tension–time curves depends on the temperature. In this section we shall consider separately temperatures near 0° C and temperatures around 20° C. The next section will deal with the transition between these extremes. Fig. 3 shows *rabbit psoas muscle at 3° C* and three different amplitudes of length change. Each part of the Figure is laid out as in Fig. 1 *B* except for the scales. After low amplitude stretch and release (Fig. 3*A*) the response is symmetrical and dominated by the K_1 component. No delayed tension component can be seen. At intermediate amplitudes (Fig. 3*B*) the response to release is not much changed, but after stretch the K_2 component is relatively much larger, giving rise to the asymmetry noted by Huxley & Simmons (1971*a*). There is also a trace

Legend to Fig. 3.

Fig. 3. Response of rabbit psoas fibres at 3° C. In each case the response to stretch is above the response to release. In this Figure and in Figs. 4–7 the vertical separation of the stretch and release responses does not represent the true difference in tension, although this was measured and is shown in Figs. 1 and 2. The results are presented in this way so that the relationship between the stretch and release responses is seen to best advantage. The amplitudes of length step are 0·2 nm/half sarcomere in *A*, 1·5 nm/half sarcomere in *B* and 3 nm/half sarcomere in *C*. *D* shows the same result as *B* on a faster time scale. In *B* and *D* the rate constant of the fast component of the recovery after stretch is 0·6 ms^{-1} and its amplitude is 22·5 μN/fibre. After release the corresponding values are 0·65 ms^{-1} and $-19{\cdot}7$ μN/fibre. In *B* and *D* the phases are numbered as described in the text.

of the K_3 delayed tension component. At larger amplitudes of stretch the K_3 component disappears again (Fig. 3*C*). The speed of the tension recovery after release is hardly affected at all by the amplitude of the release.

Fig. 3 *D* illustrates how the appearance of the response varies according to the scaling used. Fig. 3 *D* is identical to Fig. 3 *B* except that the time

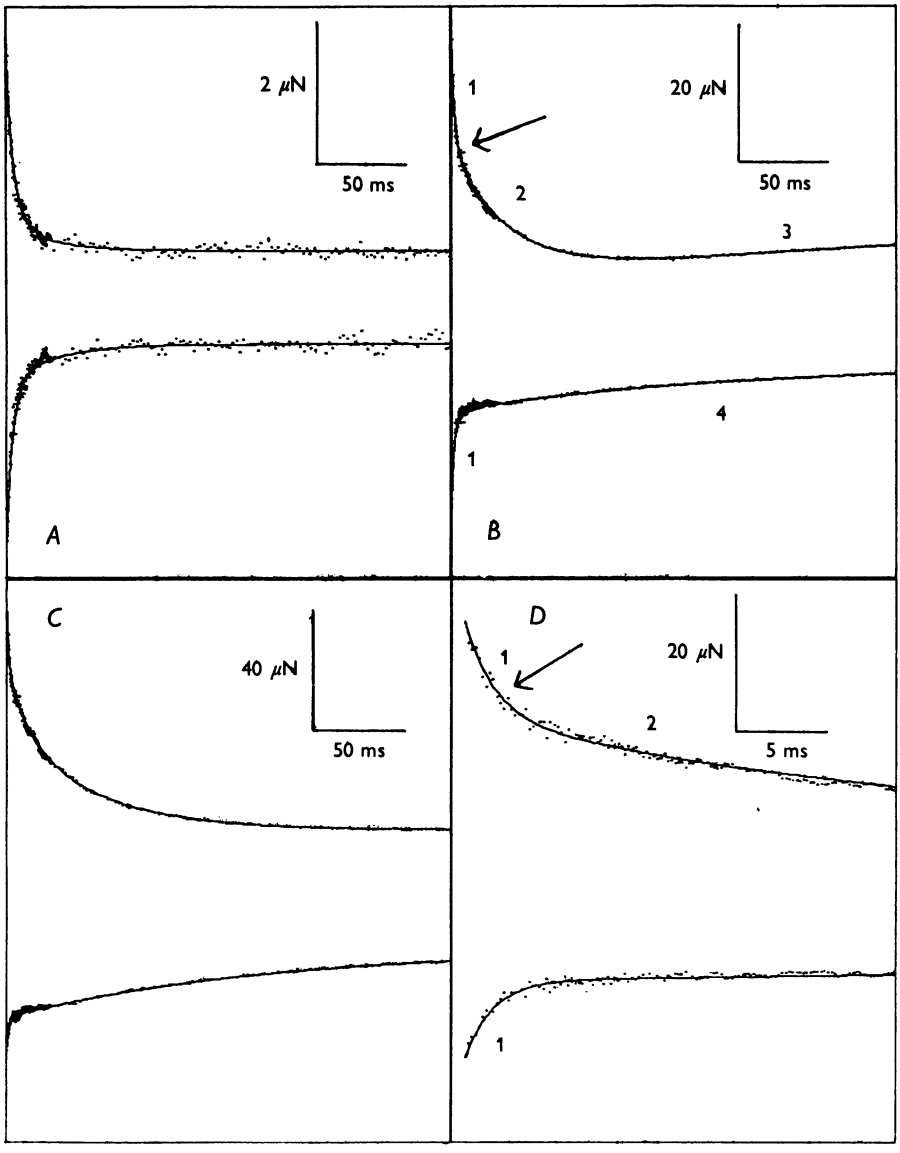

Fig. 3. For legend see opposite page.

scale is ten times faster. The K_1 and K_2 components after stretch are very obvious in Fig. 3*D*, but in Fig. 3*B* the kink (indicated by an arrow) can only be seen on careful inspection. It appears as a sudden change in the

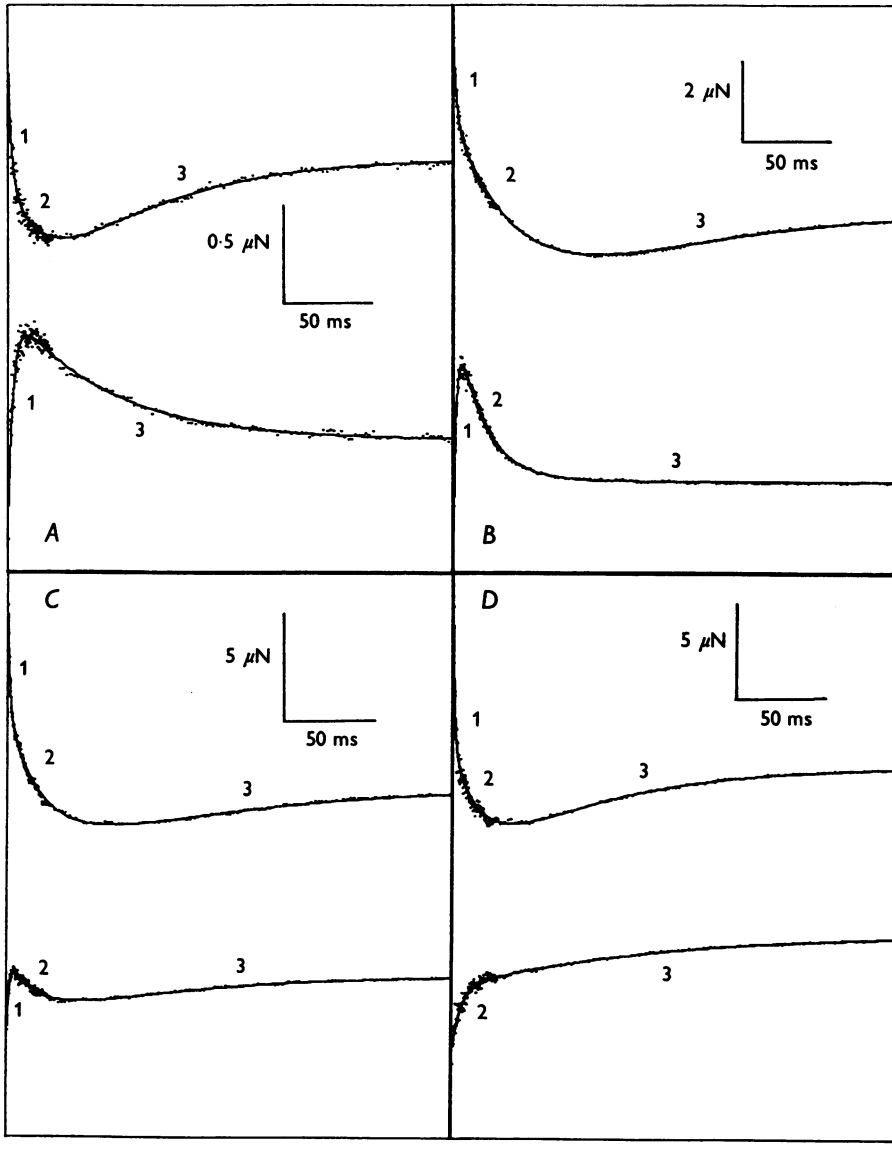

Fig. 4. The response of insect flight muscle at 2° C. Four amplitudes of length change are shown, all to the same time scale. The amplitudes are: *A*, 0·5 nm/half sarcomere; *B*, 3 nm/half sarcomere; *C*, 6 nm/half sarcomere; *D*, 10 nm/half sarcomere.

spacing of the experimental points which were, in this portion of the response, measured at equal time intervals. It is also clear in Fig. 3*D* that the K_1 components after stretch and release have about the same rate constant and magnitude, and that the asymmetry in Fig. 3*B* is due to the additional K_2 component after stretch.

The response of *insect flight muscle at 2° C* and four amplitudes of length change is shown in Fig. 4. At amplitudes lower than any of those shown the stretch and release curves are symmetrical. The behaviour of the initial tension recovery (phases 1 and 2) is similar to rabbit psoas muscle. A plot of the first 25 ms of the response looks like Fig. 3*D*: In contrast to rabbit muscle, the delayed tension (K_3 component) is visible after release as well as after stretch. In Fig. 4*B* the falling tension after 3 nm release is split into two phases which are identified with the components K_2 and K_3 after stretch because of their similar rate constants. After 6 nm release (Fig. 4*C*) the K_2 component after release is still seen as a fall in tension but the K_3 component has changed its sign and becomes a rise in tension. After 10 nm release both components are seen as a rise of tension.

Fig. 5 shows the response of *rabbit psoas muscle at 20° C*. The initial tension recovery has the same rate constant at all amplitudes and is not much faster than at 3° C (Fig. 3). It is not possible in this experiment to resolve the initial recovery into the two components K_1 and K_2. The delayed tension (K_3 component) is seen clearly, as in Fig. 2. As the amplitude of release is increased the K_3 component becomes smaller until in Fig. 5*C* it causes a plateau of tension rather than a fall. After even larger releases the K_3 component becomes a rise in tension, as in insect flight muscle at 2° C. Usually, greater amplitudes than 0·75 nm/half sarcomere were required to produce a plateau of tension, for example K_3 is seen as a fall of tension after 2 nm/half sarcomere release in Fig. 7*D*. As the amplitude of stretch is increased the K_3 component becomes smaller relative to the others.

Fig. 6 illustrates the effect of changing the amplitude of the length step on *insect flight muscle at 23° C*. As at low temperature (Fig. 4), the main difference from rabbit psoas muscle is that the delayed tension (K_3 component) is relatively much greater. The main effect of increasing the amplitude of length change is that the delayed tension component after release becomes progressively smaller relative to that component after stretch. This type of non-linearity was reported and discussed by White & Thorson (1972). It appears from Fig. 6*D* that the initial tension recovery (K_1 component) is more extensive and slower after release than after stretch. This is an illusion caused by the fact that the eye does not compensate for the much greater initial velocity of the delayed tension after stretch. Analysis shows that the K_1 component is nearly identical with

respect to rate constant and magnitude after stretch and release. The real difference is in the amplitude and hence the initial velocity of the K_3 component. Similarly, measurement shows that the initial tension recovery is not more rapid in insect flight muscle than in rabbit psoas muscle, as might be thought from an uncritical comparison of Fig. 5 and Fig. 6. The K_2 component is not visible in these records.

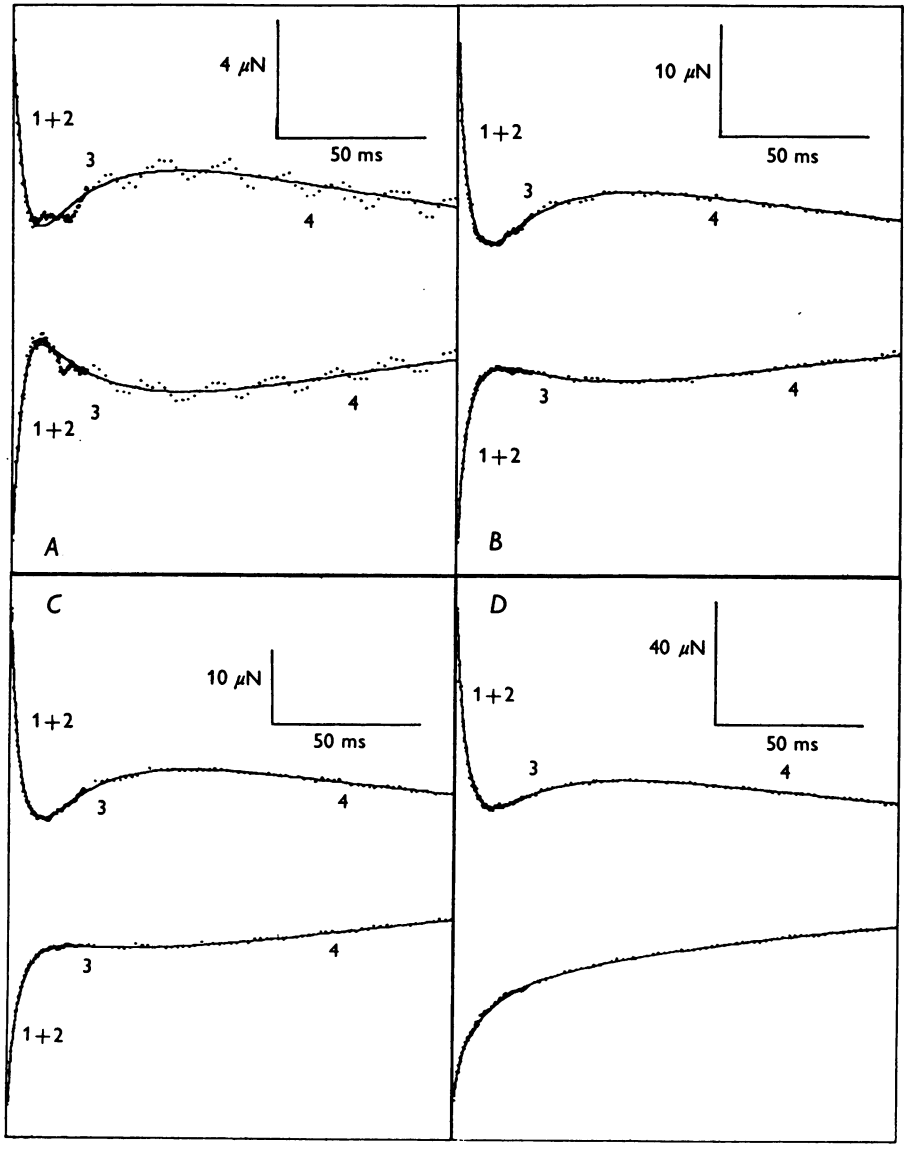

Fig. 5. For legend see opposite page.

The effect of temperature on the shape of the response

Comparing Figs. 3 and 4 with Figs. 5 and 6 it is seen that the initial tension recovery has lost its asymmetry at the higher temperature, also that in rabbit psoas muscle the delayed tension component has become more prominent. The stages in between these extremes are shown in Fig. 7. It illustrates how the K_2 component of the recovery after stretch becomes much faster as the temperature is raised, while the K_1 component hardly becomes faster at all. The kink in the stretch recovery curve (indicated by an arrow) is marked at 2·5° C (Fig. 7*A*), still visible at 7·5° C (Fig. 7*B*), can be resolved by curve fitting but not easily by eye at 14° C (Fig. 7*C*) and at 19° C (Figs. 7*D*) the K_1 and K_2 components cannot be resolved at all. These effects are seen much more clearly when that data are plotted on a faster time scale. They are not shown in this way here to reduce the number of Figures. The speed of the recovery after release (K_1 component) appears to be unaffected by temperature. The speed of the delayed tension component (phase 3) is increased when the temperature is raised.

Insect flight muscle displays identical behaviour with respect to the initial tension recovery, and the delayed tension component is similarly temperature sensitive. However, the shape of the response does not change as much because the delayed tension is prominent at all temperatures.

The effect of temperature and amplitude of length change on the rate constants

We have pooled the rate constant data at all amplitudes of length change to obtain the temperature effects shown in Fig. 8, because examination of the individual experiments (of which Figs. 3–7 are a representative selection) leads to a strong impression that the effect of temperature on the rate constants is very much greater than the effect of step amplitude. The results from insect flight muscle and rabbit psoas muscle are

Legend to Fig. 5.

Fig. 5. The response of rabbit psoas muscle at 20° C. The amplitudes of length step applied were as follows: *A*, 0·2 nm/half sarcomere; *B*, 0·5 nm/half sarcomere; *C*, 0·75 nm/half sarcomere; *D*, 2 nm/half sarcomere. The superimposed oscillation in *A* is 50 Hz mains interference. After stretch the initial tension recovery (phase 1) has a rate constant close to 0·3 ms^{-1} in all the records. After release the rate constants of the initial recovery (phase 1) are: *A*, 0·36 ms^{-1}; *B*, 0·28 ms^{-1}; *C*, 0·29 ms^{-1}. The tension recovery after release in *D* is divided into three components whose rate constants and amplitudes are: 0·52 ms^{-1}, −19·1 μN/fibre; 0·082 ms^{-1}, −24·2 μN/fibre; 0·007 ms^{-1}, −35·1 μN/fibre. A fitted curve with fewer components is not adequate to describe the result. A delayed fall of tension after 2 nm/half sarcomere release could often be seen at 20° C, as in Fig. 7*D*.

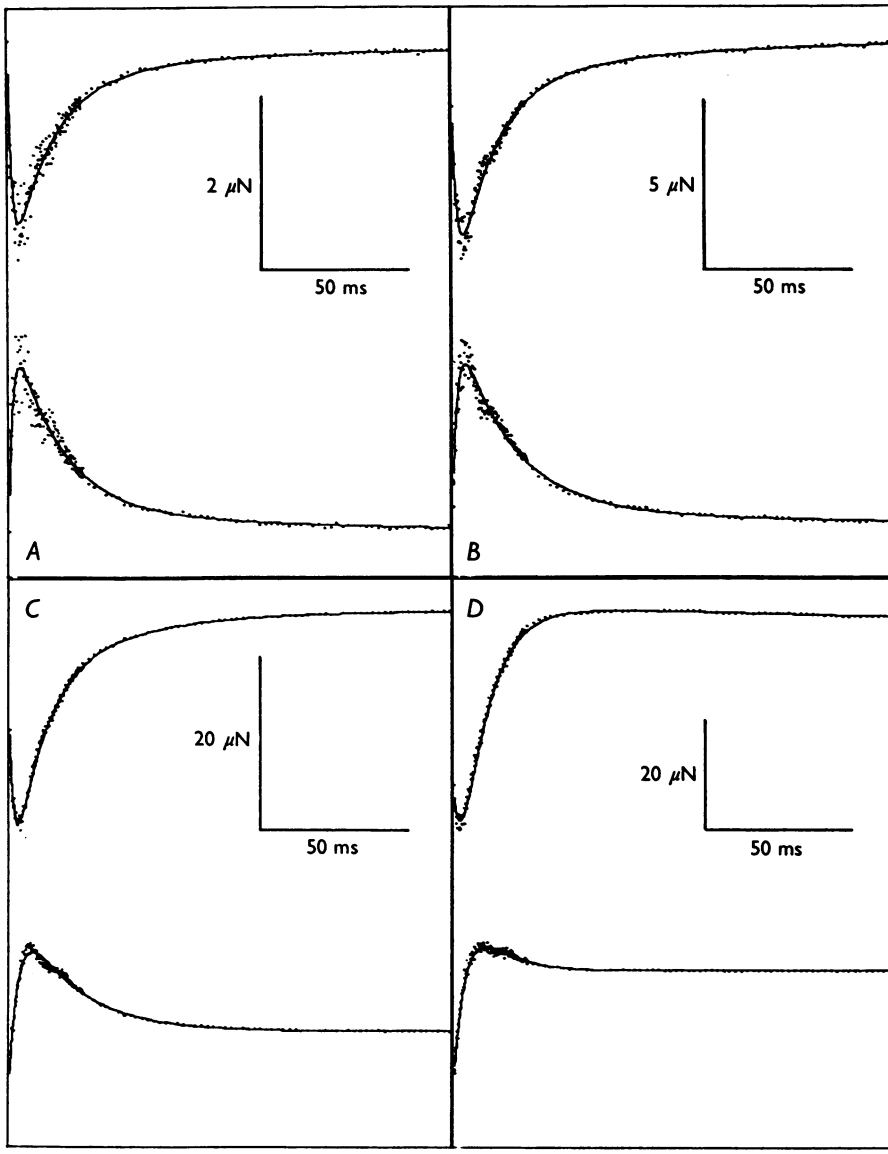

Fig. 6. The response of insect flight muscle at 23° C. The amplitudes of length change are: *A*, 0·2 nm/half sarcomere; *B*, 0·5 nm/half sarcomere; *C*, 2 nm/half sarcomere; *D*, 4 nm/half sarcomere. In *C* and *D* the initial tension recovery after release is slightly slower than after stretch, but the effect is exaggerated by the very different initial velocity of the delayed tension component. In *D* the rate constant and amplitude of the initial recovery after stretch are 0·35 ms^{-1} and 51·8 μN/fibre. The corresponding values after release are 0·25 ms^{-1} and −48·2 μN/fibre.

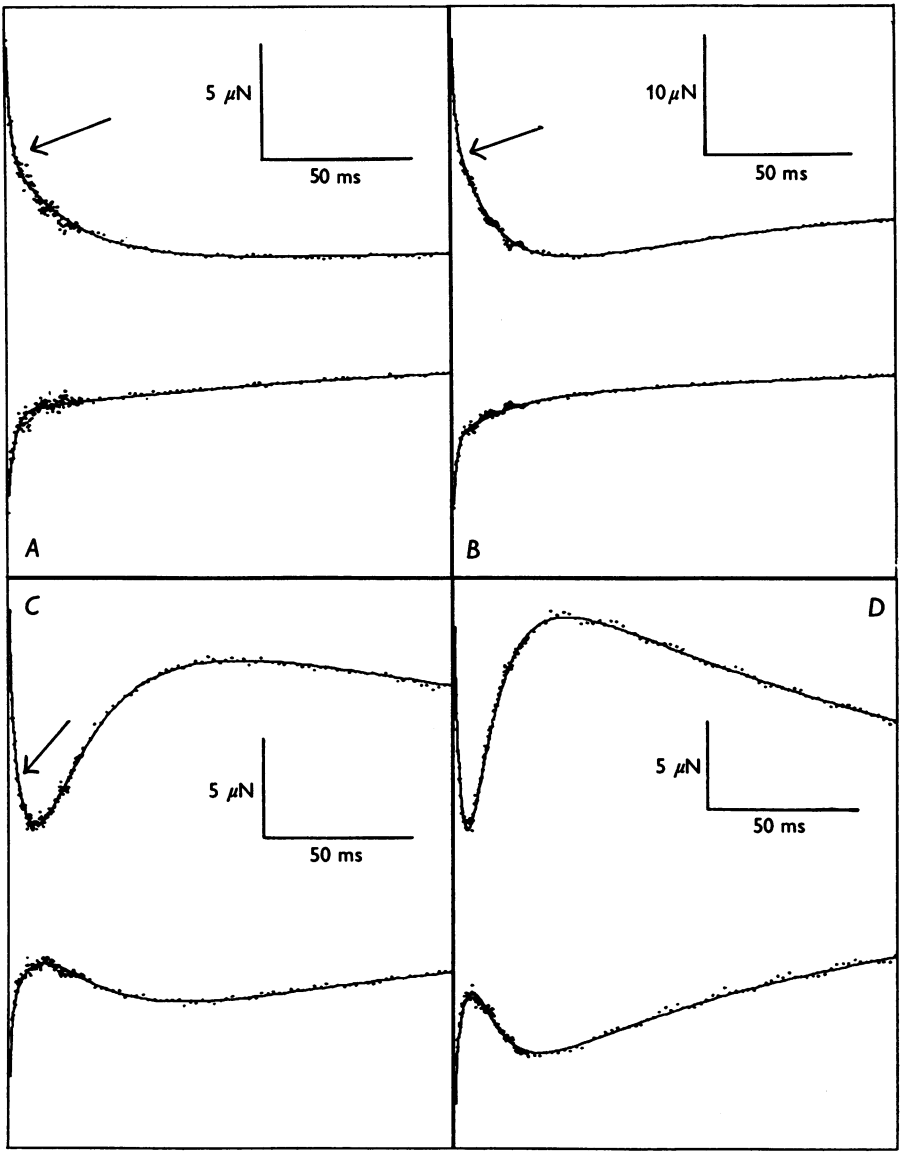

Fig. 7. The effect of changing the temperature on the step response of rabbit psoas muscle at an amplitude of 2 nm/half sarcomere. The temperatures are: *A*, 2·5° C; *B*, 7·5° C; *C*, 14° C; *D*, 19° C. It can be seen that the initial tension recovery is hardly affected by the change in temperature although the other components become much faster as the temperature is raised. The division of the tension fall after stretch into two components is clearly seen in *A* and is still visible at 7·5° C(*B*) even on this slow time scale. The arrows indicate the approximate location of the transition between phase 1 and phase 2.

remarkably similar, rabbit muscle being marginally slower. No values for K_3 in rabbit muscle are shown below 5° C because the rate constant was too slow to be measured in recordings lasting 275 ms. The rate constants K_1 and K_2 are well separated and have quite different Q_{10} values. The slope of the K_1 lines are not significantly different from zero but better experiments might prove that there is an effect of temperature on this rate constant.

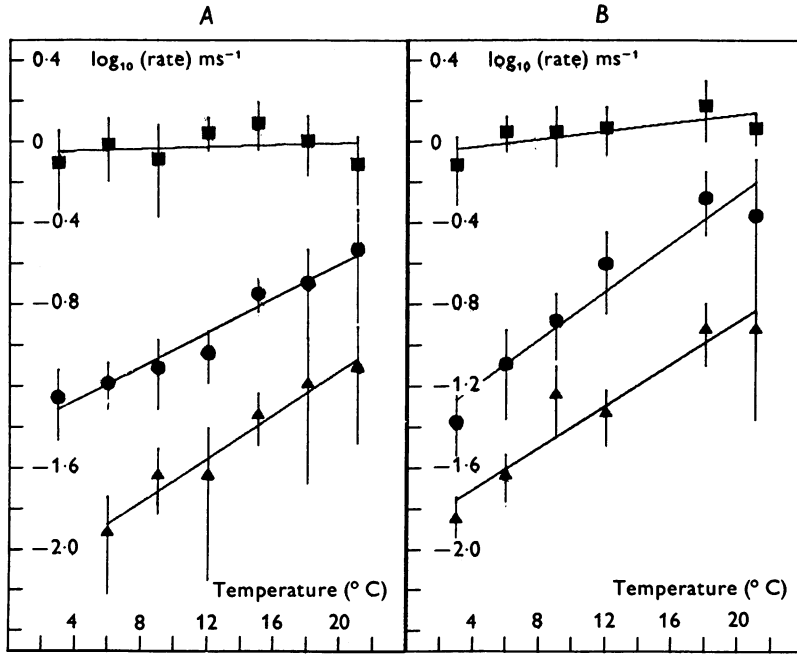

Fig. 8. The effect of temperature on the rate constants in (*A*) rabbit psoas and (*B*) insect flight muscle. The Q_{10} values for rabbit muscle are: K_1 (■), 1·0; K_2 (stretch only, ●), 2·6; K_3 (▲), 3·5. For insect flight muscle they are: K_1 (■), 1·3; K_2 (stretch only, ●), 3·9; K_3 (▲), 3·2. The error bars represent plus and minus 1 standard deviation. Q_{10} may be underestimated, as discussed in the text.

In lethocerus muscle (Fig. 8*B*) it appears that the rate constants are rather less at 21 than at 18° C. This is probably due to the fibres being partially in the high tension state. If the 21° C point is not taken into account the Q_{10} values become 5·4 for K_2 and 3·9 for K_3.

Because K_1 is insensitive to temperature, it was possible to average the measurements of the speed of this component at each amplitude of length change. The result for rabbit psoas muscle is shown in Fig. 9*A*. After either stretch or release the rate constant approximately doubled

between zero and 2 nm/sarcomere length change. At higher amplitudes there is little further change. The time required to impose the length step was significant in relation to the time constant of phase 1, so Fig. 9*A* cannot do more than indicate the direction of the effect.

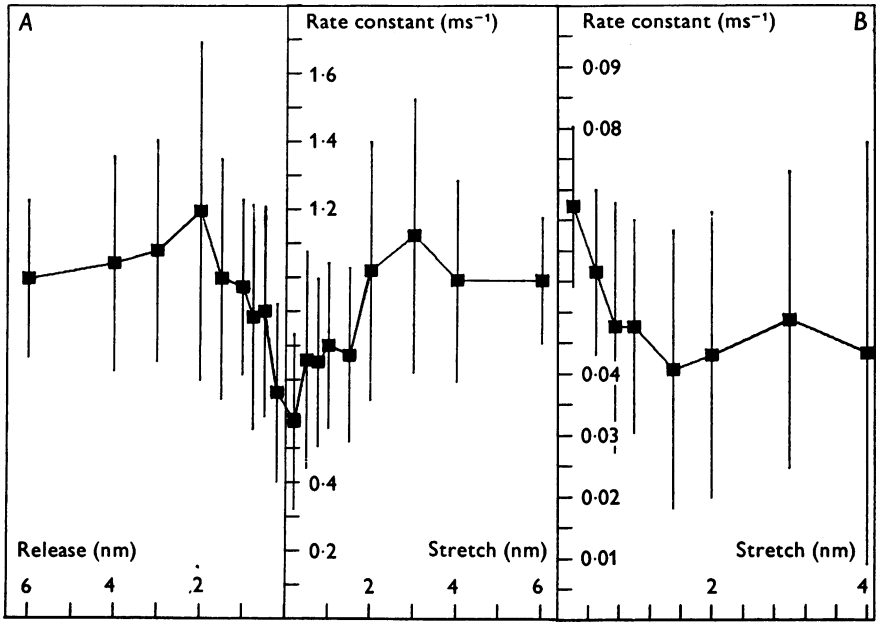

Fig. 9. The effect of amplitude of length change on the rate constants of the tension recovery. *A* shows the averaged values of K_1 in rabbit psoas muscle and *B* the averaged values of K_2 after stretch at 2–4° C for both muscle types.

The only other rate constant which showed any consistent change with the mechanical conditions was K_2 after stretch at temperatures near 0° C. The averaged results for both types of muscle are shown in Fig. 9*B*. It can be shown that the drop in the rate constant between 0·2 and 1·6 nm/half sarcomere length change is significant. If the measured values are normalized by adjusting within each experiment so that the value at 2 nm/half sarcomere is equal to the average value at this length change, then the error bar at that point has zero length and the other ones become shorter.

DISCUSSION

We have chosen to analyse our results in terms of exponential components because this is what most models, including the biochemical schemes now being worked out for the actomyosin system, will predict.

The results have justified this choice because (*a*) a good fit is obtained to the whole of the response using no more exponentials than the number of phases of tension change which are obvious to the eye when the results are viewed as a whole, (*b*) the rate constants so found behave independently and regularly when the temperature is changed and in two cases when the amplitude is changed.

The interpretation depends upon a sound method of extracting exponential components. The usual method of estimating rate constants is to subtract from a guessed base line and linearize by taking logarithms. This method is basically unsound for several reasons. Unless the base line is guessed correctly even truly exponential data will give rise to a curved semilogarithmic plot. The choice of base line has a crucial effect on the estimated rate constants. Points near the base line are weighted much too heavily by taking logarithms and if the data is noisy some of the points may be on the base line or below it. These points cannot be included in the analysis at all. In a sound procedure all the points are weighted as the experiment requires (normally equally) and the base line is calculated and not guessed. For the exponential function such a method requires a trial and error search such as has been used in this work.

We can describe our results adequately as the sum of a maximum of four exponential terms. In most cases the rate constants are readily separated because adjacent terms either have opposite signs or are separated by an order of magnitude. Analysed in this way the tension recovery after stretch is divided into two components whose rate constants we have called K_1 and K_2. The evidence that these derive from different physical processes and are not simply a mathematical description of a non-exponential response is twofold. Firstly, the two components have very different temperature sensitivities and secondly, phase 1 becomes faster as the amplitude of the applied stretch is increased while phase 2 becomes slower. It should be emphasized that this reasoning does not depend on the components being exponential, because we have demonstrated that the shape of the decay curve varies with temperature and amplitude of length change applied. Although the stretch velocity was slow enough to affect the measured values of K_1, this does not alter the argument because only the direction of the change caused by varying the amplitude is important. The fact that measurements did not begin immediately after the end of the length change does not affect the rate constant but the amplitude of the component. Similar reasoning can be used to show that the K_1 component after stretch is correlated with the K_1 component after release. They both have a negligible temperature coefficient and have a similar rate constant and magnitude at any given step size, despite the marked effect of step amplitude on the rate constant

(Fig. 9*A*). It follows that the K_1 component after release does not result from a disturbance of the same equilibrium as the K_2 component after stretch. There is now independent evidence (Pybus & Tregear, 1975) for the suggestion made by Thorson & White (1969) that the delayed tension (K_3 component) is a reflexion of the detachment rate of the cross bridges. Since K_2 and K_3 have similar temperature dependencies we suggest that K_2 is also due to the attachment/detachment cycle. K_1 is much less sensitive to temperature and so is probably due to a different process.

Stretch activation

The results presented here add to the growing body of evidence that stretch activation is a common property of muscle tissue and is not confined to insect fibrillar flight muscle. Other muscles in which the delayed tension has been observed include frog semitendinosus (Armstrong, Huxley & Julian, 1966), synchronous cicada tymbal muscle (Aidley & White, 1969), rabbit heart muscle (Steiger, 1971; Steiger *et al.* 1972), frog sartorius muscle (Heinl, 1972), tortoise ileofibularis muscle (Heinl *et al.* 1974) and frog anterior tibial muscle (Julian & Sollins, 1975). A depressing effect of shortening lasting about 800 ms on the tension developed by frog semitendinosus has been described by Edman (1975) which might be due to stretch activation. All these preparations display an initial fast tension relaxation. It is now reasonable to suggest that the cross-bridge properties of all muscles may be similar, and that their differences lie in the mechanical arrangement of the filaments, the method and speed of activation and relaxation and the rate constants of the transitions between the various states of the actomyosin complex.

The difference between rabbit psoas and insect flight muscle is principally that in rabbit muscle the delayed tension is smaller and is not maintained. The fact that the stretch activation is not maintained in rabbit muscle may be correlated with its difference in structure from insect flight muscle, as was suggested by Chaplain (1967). Insect muscle has a short I-band and a high resting stiffness which is probably due to a connexion between the Z-line and the myosin filaments (White & Thorson, 1973). This arrangement would allow an overall length change to exert a permanent stress on the filament structure. On the other hand, in rabbit muscle there do not seem to be any mechanically significant connexions between the Z-lines and the myosin filaments, so over-all muscle length can only exert a stress on the filaments through the cross-bridges. Since the bridges are being continually broken and remade such an effect cannot be permanent. The idea is supported by the observation of Chaplain (1967) that a transient increase in the ATPase activity of frog sartorius muscle is seen when the preparation is stretched.

We have uncovered a new similarity between rabbit psoas and insect fibrillar muscle, which is that both muscles enter the high tension state (Jewell & Rüegg, 1966) when the ADP concentration rises above a certain level. There is evidence that the high tension state is one in which the cross-bridges remain attached for longer than normal, leading to a higher mean tension and a slower rate constant for changes of tension (Pringle, 1967; Abbott & Mannherz, 1970).

A possible interpretation based on a cross-bridge model

We shall interpret our results in terms of established ideas about possible properties of the cross-bridges. They are supposed to attach to the actin filament, generate a force and then detach again. Some time during this cycle one or possibly two molecules of ATP are split, and the energy derived from this hydrolysis appears as strain energy in some part of the myosin molecule (Huxley, 1957; Huxley & Simmons, 1971*a*). The energy can then be transferred to the load if the filaments are allowed to move relative to one another. The attachment rate constant is denoted by f and the detachment rate by g. Many models derived from the original formulation of Huxley (1957) have been put forward (for a review see White & Thorson, 1973). In most cases an assumption is made about the way in which f and g are determined by the relative position of the actin and myosin filaments. The force–velocity curve and the response to step changes of length and force can then be calculated. The situation is simplified in the case of step length changes because if the filaments are more or less rigid, as has been shown by Huxley & Simmons (1971*b*), there may be only two populations of attached bridges. The differential equations can be solved analytically and the way in which f and g depend on filament displacement does not have to be guessed. It can in principle be measured directly. This is the only real novelty in our analysis.

The delayed tension changes in insect flight muscle at small amplitudes of length change can be explained if stretching the fibres causes an instantaneous increase in f but little change in g, and if f is much smaller than g (Thorson & White, 1969; Julian, 1969; White & Thorson, 1972; Abbott, 1972*a*, 1973*a*). The consequence of these assumptions is that the number of attached cross-bridges follows the muscle length with an exponential delay of rate constant $f+g$. If it is further assumed that all the attached bridges generate the same tension (i.e. are in the β state as defined by Julian, Sollins & Sollins, 1974) and that the filament movement is small then the tension will be related to the number of attached bridges and so an exponentially delayed tension is predicted, the stretch activation phenomenon.

When larger length changes are applied significant relative filament

movement can occur during the lifetime of an attached bridge, thus distorting it, so it is no longer justifiable to assume that g and cross-bridge force will be constant. Julian (1969) suggested that the initial tension recovery after a quick length change might be caused by a large increase in the detachment rate g. White & Thorson (1972) showed by numerical integration that non-linearities of the type we observed in Fig. 6 are produced if the cross-bridge force is increased by overall stretch and reduced by release during the period of attachment. The size of the delayed tension fall after release is reduced because there is a force reduction during the shortening due to the elasticity of the bridges, although the final equilibrium tension after the release is the same. We have extended the differential equation given by White & Thorson (1972) to include an effect of cross-bridge movement on g. This equation can be solved analytically in the case of step changes of length. The result is remarkably simple, a double exponential. The working is given in the Appendix. The exponentially delayed tension of rate constant $f+g(0)$ remains, where $g(0)$ is the detachment rate of undistorted bridges, that is bridges in their normal isometric tension generating configuration. There is an additional component of rate constant $g(x)$, the detachment rate of the bridges which were attached and therefore distorted while the length change was being applied. The solution is:

$$T(t) = N_a + (s(x) - D)\,N_b \exp(-g(x)\,t) - (N_a - DN_b) \exp(-(f(l)+g(0))\,t),$$

$T(t)$ is the tension as a fraction of isometric with all bridges attached, N_a is the fraction of bridges attached at equilibrium after the length change, N_b is the fraction of bridges, assumed undistorted, attached before the step, D is $f(l)/(f(l)+g(0)-g(x))$, $f(l)$ is the attachment rate after the length change l, $s(x)$ is the tension in the distorted bridges as a fraction of the tension in the undistorted bridges, $g(x)$ is the detachment rate of bridges distorted by x.

In the model given here l and x will always have the same value, but we have used separate symbols as it is possible for them to be different under more complicated assumptions. At infinite time, both exponential terms are zero and the tension is determined by the equilibrium number of attached bridges. If x is small so that $s(x) = 1$ and $g(0) = g(x)$, then $D = 1$ and the equation reduces to the small signal case $T(t) = N_a - (N_a - N_b) \exp(-(f+g)\,t)$. Except for positive values less than N_a, D can reasonably take any value between plus and minus infinity, because of the singularity when $g(x) = f(l)+g(0)$. Therefore, the shape of the tension response will depend critically on the way in which cross-bridge distortion affects the detachment rate and bridge force. This is unfortunate because it makes it more difficult to test the model.

A simple case is when $g(x)$ is substantially greater than $g(0)$. D is then negative. The first exponential term with rate constant $g(x)$ will have a positive coefficient, that is, will be observed as a fall in tension, unless the bridges can push $s(x)$ negative. The second exponential term must have a negative coefficient, that is be seen as a rise in tension, as the N's must be positive. In words, this means that after the length change most of the bridges detach quickly, before new undistorted ones can be formed. There is a fall of tension with rate constant $g(x)$ caused by the detachment of the distorted bridges, followed by a rise with rate constant $f(l)+g(0)$ as new bridges are made. This applies equally to stretch and release. If the bridges can push they generate a negative component of tension and there will be a rise in tension as they detach provided that $s(x)$ is less than D.

It is evident that these predictions of the model are closely parallel to the behaviour of the components K_2 and K_3. As can be seen from Fig. 8, K_2 is substantially greater than K_3 at all the amplitudes used, and K_2 is the rate constant which depends on the amount of quick stretch. We are therefore suggesting that K_3 is the rate constant $f(l)+g(0)$, as in the original model of Thorson & White (1969). The additional component K_2 is the detachment rate constant of the distorted bridges which happened to be attached at the time the length step was applied as was proposed by Julian (1969). In Fig. 8 the K_2 values refer to stretch only, because it was frequently impossible to separate this component after release. In rabbit muscle the tension often rose monotonically after release at temperatures below about 10° C and in insect muscle usually two components only (fast tension recovery followed by stretch activation) could be seen except at temperatures below 10° C. The components were clearly separable after releasing insect flight muscle at 2° C as is seen in Fig. 4, and in this case the results follow a pattern consistent with the model. At the lowest amplitude (Fig. 4*A*) the fall of tension after release cannot be resolved into two components. In Fig. 4*B* the fall can be resolved into two components but these rate constants are not very different so the model cannot make any precise predictions about the signs of the components. In Fig. 4*C* after stretch and release K_2 is much faster than K_3 and K_2 is seen as a fall whilst K_3 is seen as a rise, which is as predicted by the model. In Fig. 4*D* K_2 is seen as a rise in tension after release and this means that the bridges must be able to push if our interpretation is correct. They would not have to push very much because D is approximately $-f(l)/g(x)$, a small negative number.

We should point out that the response of rabbit muscle to release at room temperature (Figs. 5 and 7) is not related to the result of Fig. 4*C* just discussed, although in both the tension first rises, then falls and

finally rises again. In insect muscle at low temperature the falling phase has a rate constant similar to that of the second phase of tension fall after quick stretch, and the subsequent redevelopment has a rate constant similar to the delayed tension rise after stretch. In rabbit muscle at room temperature the fall of tension after release has the same rate constant as the delayed tension rise after stretch and must therefore be the K_3 component.

The theory of Huxley & Simmons (1971*a*)

The asymmetry of the tension response to quick stretch and release at low temperature was first described by Huxley & Simmons (1971*a*), who proposed a mechanism based on a multiplicity of attached states in rapid equilibrium with each other. Abbott (1972*b*) challenged the experimental basis of this interpretation on the grounds that in the published records the transient tension recovery seen after stretch was divided into two phases. In reply, Huxley & Simmons (1972) published further details of the experiments actually used in formulating the theory showing that in these records the division into two components was not obvious. Huxley & Simmons demonstrated convincingly that they could not be interpreted best in terms of fixed rate constants in the way suggested by Abbott (1972*b*). We have now found in our results that the rate constants are changed by varying the amplitude of quick length change applied, so this aspect of Abbott's (1972*b*) suggestion must be withdrawn.

The essence of the mechanism proposed by Huxley & Simmons (1971*a*) is that there is an equilibrium between various attached cross-bridge states which is changed when the over-all muscle length is forcibly altered because some of the potential energy of the bridge is stored in an elastic element. Since the various attached states are associated with different bridge forces, the change in equilibrium caused by a forced length change will be manifest as a change in over-all tension. The rate constant of the approach to the new equilibrium will be affected by the amount of the length change because it is actually the rate constants determining the equilibrium which are affected. The model predicts that the rate should become slower as the amplitude of stretch is increased and faster as increasing amplitudes of release are applied. Huxley & Simmons (1971*a*) showed that at least three attached states would be necessary for energetic reasons. They suggested that the two transitions might have similar rate constants so that the predicted tension responses would still have only one component, which is exponential in the non-distributed case which they considered.

If this were the explanation of the asymmetry of our results, then K_1 after release would have to be correlated with K_2 after stretch, which we

have shown is not the case. Even if it were so the cross-bridge force should reduce to zero as the temperature is raised towards 30° C, because at about this temperature the asymmetry disappears. This is contrary to observation. A further possibility is that the rate constants of the two (or more) equilibria might not be similar, because such a system could in principle give rise to a double exponential such as we have observed. In that case K_1 after stretch would be correlated with a faster recovery after release which we were unable to observe because of the limitations of our apparatus. This suggestion is ruled out by the fact that K_1 becomes faster and not slower as the amplitude of quick stretch is increased. We can therefore conclude that the mechanism proposed by Huxley & Simmons (1971*a*) is unable to account for any of the features of our results.

There are a number of differences between our experimental method and that used by Huxley & Simmons (1971*a*). For example, Huxley & Simmons normally used the 'spot-follower' device to control the length of a few sarcomeres in the middle of the preparation, although in some cases they controlled over-all muscle length as in the work described here. We have used glycerol extracted muscle whereas Huxley and Simmons used fresh muscle and different animals were used. The method of recording was not the same. Our results are therefore not strictly comparable. It might be argued that because of this the mechanism proposed by Huxley & Simmons (1971*a*) could be consistent with the results obtained by Huxley & Simmons themselves, but inconsistent with our results. This argument involves the assumption that the mechanism of contraction was quite different in the two sets of experiments despite the great similarity of the results. Unless this assumption is true it can be deduced that the mechanism proposed by Huxley & Simmons (1971*a*) is unable to account for the results obtained by them. The apparent fit between theory and results must in that case have been due to the over-simplified method of analysis used by Huxley & Simmons (1971*a*).

Conclusions

The fastest component of tension recovery has a rate constant (K_1) which is different from the other components in its lack of sensitivity to temperature. It is therefore very unlikely to be a reflexion of cross-bridge attachment and detachment. We have shown that it cannot be due to multiple attachment states as proposed by Huxley & Simmons (1971*a*) and so by a process of elimination we are led to suggest that it is due to some visco-elastic property of the proteins themselves. At the moment there is no evidence or consensus concerning the localization of the visco-elastic part of the cross bridge, because it is not obvious how such a small structure can be deformed to the extent required. This

difficulty is common to all cross-bridge models, because the work of Huxley & Simmons (1971*b*) has established the existence of the elastic component beyond any reasonable doubt.

The behaviour of K_2 and K_3 is explained reasonably well in terms of cross-bridge attachment and detachment, but there is one feature of the results which cannot be explained by the simple model proposed. In Fig. 1 the response to stretch and release is fairly symmetrical, including a two-stage recovery of tension after release. Nonetheless, K_2 is greater than K_3 by such a margin that the model would predict that the K_2 component should be seen as a fall in tension. The record is clean and there is no doubt about the reality of this particular result, so either some additional assumptions will be necessary or further work may render the model no longer useful. A potentially valuable use of this analysis is that it provides, in principle, a direct method of measuring the effect of cross-bridge distortion on the detachment rate. This is a relationship which must be assumed in theories explaining the force–velocity curve and a direct check would be of great value in model building along these lines.

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APPENDIX

It is assumed in this analysis that the filaments are rigid and that in the isometric steady state all attached cross-bridges are in the same state. There may be other attached states such as one with zero tension, the α state in the model of Julian *et al.* (1974), but we assume that these are sparsely populated. There are thus three mechanically significant cross-bridge states: (i) not attached, (ii) attached in the steady-state isometric configuration, (iii) attached and distorted from state (ii) by forced displacement of the filaments. In state (iii) the detachment rate and bridge force are different from their values in state (ii). Muscle length controls the attachment rate f. It will be shown that the tension response of such a system to a step change of length is a double exponential. All the measured tension responses are shown with a superimposed multiple exponential fit, so they may be seen as examples of some of the shapes which the model can generate, when a K_1 and possibly a K_4 component is added.

Let $f(l)$ be the attachment probability after the length change.
Let $g(0)$ be the detachment probability of undistorted bridges.
Let $g(x)$ be the detachment probability of bridges distorted by x.
Let $N(0, t)$ be the fraction of attached undistorted bridges at time t.
Let $N(x, t)$ be the fraction of bridges distorted by x at time t.

The length change is imposed at time $t = 0$. The bridges which are attached are distorted from their isometric configuration by x so there are $N(x, 0)$ distorted bridges and no attached undistorted ones. The distorted bridges will detach with a rate constant $g(x)$, the number remaining attached being given by a simple exponential

$$N(x, t) = N(x, 0).\mathrm{e}^{-g(x)t}. \tag{1}$$

The fraction of unattached bridges is given by $(1-N(0,t)-N(x,t))$. The rate of change of the fraction of attached undistorted bridges is given by

$$\frac{\mathrm{d}N(0,t)}{\mathrm{d}t} = f(l).(1-N(0,t)-N(x,t))-g(0).N(0,t). \tag{2}$$

Substituting (1) into (2) and multiplying the bracket we get

$$\frac{\mathrm{d}N(0,t)}{\mathrm{d}t} = f(l)-N(x,0).f(l).\mathrm{e}^{-g(x)t}-(f(l)+g(0)).N(0,t). \tag{3}$$

This is a standard equation with an exact solution found by multiplying both sides by $\mathrm{e}^{(f(l)+g(0))t}$, which with the initial condition $N(0,0) = 0$ is

$$\begin{aligned} N(0,t) = N(0,\infty)-D.N(x,0).\mathrm{e}^{-g(x)t} \\ +[D.N(x,0)-N(0,\infty)].\ \mathrm{e}^{-(f(l)+g(0))t}, \end{aligned} \tag{4}$$

where $D = f(l)/(f(l)+g(0)-g(x))$. If we let the tension in distorted bridges be $s(x)$ times the tension in undistorted bridges, then the total tension $T(t)$, relative to the tension when all bridges are attached but undistorted, is given by

$$\left.\begin{aligned} T(t) &= N(0,t)+s(x).N(x,t), \\ T(t) &= N(0,\infty)-[N(0,\infty)-DN(x,0)].\mathrm{e}^{-(f(l)+g(0))t} \\ &\quad +(s(x)-D).N(x,0).\mathrm{e}^{-g(x)t}. \end{aligned}\right\} \tag{5}$$

The tension changes predicted by the model are thus described by the sum of two exponentials. One of them (with rate constant $g(x)$) will depend on the amount of the length change, the other will not as long as $f(l) \ll g(0)$. Our interpretation is that K_2 is $g(x)$ and K_3 is $f(l)+g(0)$.

There is a discontinuity in the solution when $f(l)+g(0) = g(x)$ and D is infinite. In that case the two exponential terms have the same rate constant and are of infinite magnitude and opposite sign. There is an exact solution for the number of undistorted bridges at this point which is also an approximate solution in its vicinity. It is

$$N(0,t) = N(0,\infty)-[N(x,0).f(l).t+N(0,\infty)].\mathrm{e}^{-(f(l)+g(0))t}. \tag{6}$$

There is no term containing $\mathrm{e}^{-g(x)t}$ and in the other exponential term $D.N(x,0)$ is replaced by $-f(l).t.N(x,0)$. The expression for the number

of distorted bridges remains the same, so the solution for the total tension is

$$T(t) = N(0,\infty) - [N(0,\infty) + N(x,0).f(l).t].e^{-(f(l)+g(0))t} + s(x).N(x,0)e^{-g(x)t}. \quad (7)$$

These solutions allow us to say something about the expected signs of the exponential terms. When $g(x)$ is greater than $f(l)+g(0)$, D is negative. Under these circumstances the sign of the $f(l)+g(0)$ exponential will be negative, that is K_3 will be seen as a rise of tension. The sign of the $g(x)$ exponential will be positive, so that K_2 should be revealed as a fall in tension unless the bridges can push.

When $g(x)$ is less than $f(l)+g(0)$, D is positive. Both exponential components can take either sign, depending on the relationship between $N(0,\infty)$ and $N(x,0)$ for K_3 and the relationship between $s(x)$ and D for K_2. After very small releases $g(x)$ would be expected to be close to $g(0)$ and D would be close to unity, as would $s(x)$. A positive sign is expected for K_3, i.e. a fall of tension, since $N(0,\infty)$ is less than $N(x,0)$. The amplitude of K_2 will be small but again of either sign, depending on the relative magnitude of $s(x)$ and D.

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