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ISOTONIC VELOCITY TRANSIENTS IN FROG MUSCLE FIBRES FOLLOWING QUICK CHANGES IN LOAD

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SUMMARY

1. The isotonic velocity transients following quick changes in load were studied on tetanized frog skeletal muscle fibres with special reference to those following quick increases in load.

2. When the load was increased quickly from the maximum isometric force P_0 to 1·05–1·3 P_0, the fibres exhibited marked oscillatory length changes with distinct reversal in the direction of movement before starting to lengthen with a nearly constant velocity.

3. The period of the oscillatory length changes increased with increasing magnitude of the load step, and decreased with increasing temperature. The amplitude of oscillatory length changes never exceeded 0·5 % of the slack length L_0, i.e. about 50 Å per half-sarcomere.

4. If the load was increased quickly from P_0 to 1·3–1·6 P_0, the fibres lengthened continuously with velocities decreasing with time.

5. The response of fibres, shortening isotonically under a large load (about 0·8 P_0), to quick increases in load was qualitatively similar to that of isometrically contracting fibres.

6. When quick increases in load were applied during isotonic shortening under a moderate or small load (0·1–0·6 P_0), the fibres showed initial transient lengthening before starting to shorten against a new load, indicating a decrease in the ability of the fibres to sustain a load after a period of isotonic shortening and its restoration during the transient lengthening.

7. The extent of decrease in load-sustaining ability as well as its subsequent restoration process was dependent on both the amount of load and the duration of preceding isotonic shortening.

8. The decrease in the load-sustaining ability during the course of isotonic shortening appeared to be complete within 30–50 msec after the beginning of shortening.

9. These results are discussed in relation to the kinetic properties of the cross-bridges responsible for muscle contraction.

INTRODUCTION

It is generally believed that contraction in striated muscle results from the alternate formation and breaking of the cross-links between the projections on the thick filaments, i.e. the cross-bridges, and the sites on the thin filaments, (e.g. A. F. Huxley, 1957; H. E. Huxley, 1960). A. F. Huxley first formulated a contraction hypothesis based on the turnover of the cross-bridges to explain the empirical force-velocity and force-energy relations found by Hill (1938) on frog skeletal muscle. Though Hill assumed that the shortening velocity of the contractile component is instantaneously determined by the amount of load on it, Podolsky (1960) showed that, when the amount of load on an active muscle is suddenly reduced to a new value, the resulting steady isotonic shortening is preceded by non-steady motions which are called *isotonic velocity transients*. Civan & Podolsky (1966) examined the isotonic velocity transients in more detail, and presented evidence that the transient responses reflect the kinetics of turnover of the cross-bridges following quick decreases in load. Similar isotonic velocity transients have also been observed by Armstrong, Huxley & Julian (1966) and Huxley & Simmons (1973).

To account for the isotonic velocity transients which are not readily explained by the contraction model of A. F. Huxley (1957), Podolsky and his co-workers (Podolsky, Nolan & Zaveler, 1969; Podolsky & Nolan, 1973) modified the Huxley model by changing its boundary conditions. In the Huxley model, the formation of the cross-links has a moderate rate constant so that the number of the cross-bridges attached to the thin filaments at any one moment is less the larger the shortening velocity. In the Podolsky model, on the other hand, the rate constant for the formation of the cross-links is so large that the number of attached cross-bridges is greater the larger the shortening velocity.

In the case of the isotonic velocity transients following quick increases in load, no systematic work has hitherto been performed, though Armstrong *et al.* (1966) briefly reported that sudden small increases in load produce damped oscillations of the fibre length. The present experiments were undertaken to study the properties of the isotonic velocity transients in single muscle fibres or small fibre bundles with special reference to the velocity transients following quick increases in load. The quick changes in load were applied not only on isometrically contracting fibres but also on isotonically shortening or lengthening fibres. Some of these results have been reported previously (Tsuchiya, Sugi & Kometani, 1979).

METHODS

Preparation. All experiments were performed with single fast muscle fibres (diameter 50–100 μm) or small bundles consisting of two to four muscle fibres isolated from the semitendinosus muscles of the frog (*Rana japonica*). The preparations were prepared in a glass dissecting chamber filled with Ringer solution, and when the dissection was completed the relation between fibre length and sarcomere length was examined by measuring the length of ten adjacent sarcomeres at several different parts along the length of the preparation with an ordinary light microscope (Nikon screw micrometer: Leitz UMK50X objective, N.A. 0·6, working distance 6·5 mm). The Ringer solution had the following composition (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8 (pH adjusted to 7·2 by $NaHCO_3$). A pair of stainless-steel wire connectors (0·1 mm in diameter and 2–3 mm in length, Civan & Podolsky, 1966; Sugi, 1972) were tied to both tendons with braided silk thread. The connectors were attached close to the fibre insertions, so that the length of tendinous material between the connectors was less than 0·5 mm.

Stimulation chamber. The preparation (0·8–1 cm in slack length L_0) was mounted horizontally in an acrylic plastic chamber (3 ml.) by hooking the connectors to the force and displacement transducers carried on micromanipulators. The chamber contained a multi-electrode assembly consisting of eight platinum wire electrodes connected as alternate anodes and cathodes. In most experiments, cooled Ringer solution (2–3 °C) was constantly circulated through the chamber at a rate of about 20 ml./min with a peristaltic pump, the temperature of Ringer solution being controlled by a thermoelectric device (Coolnix, Yamato Kagaku Co.) with an accuracy of $\pm 0{\cdot}1$ °C.

Displacement transducer. The length changes of the preparation were recorded by a light beam–photodiode system (Civan & Podolsky, 1966; Sugi, 1972). The moving element was a magnesium lever (0·2 mm in thickness) pivoted on bearings. To prevent reaction of the lever with the surrounding fluid, the lever surface was treated with NaF and $Na_2Cr_2O_7$. The long arm of the lever dipped into Ringer solution, and the preparation was attached at a point 1·8 cm from the pivot (*A*, Fig. 1). Movement of the lever varied the amount of light transmitted to the photodiode, which had a sensitivity of 0·5–1 V/mm movement of the lever at the point of attachment of the preparation. The transducer was linear over a range of 1·2 mm. The compliance of the lever at the point of attachment of the preparation was 6 μm/g, while its equivalent mass was about 3 mg. The short arm of the lever was loaded with a stretched stainless-steel wire spring connected at a point 1 mm from the pivot.

Force transducer. The changes in contractile force were recorded by a capacitance-gauge type transducer (Photocon Research Products, Inc.). The force applied to its moving plate caused a change of capacitance which was converted to DC signals with a tuned radio frequency circuit (tuning range, 570–890 KHz). The force exerted by the preparation was transmitted to the moving plate through an angle-shaped extension made of stainless-steel wire (0·5 mm in diameter). The compliance of the force transducer was 2 μm/g, while its natural frequency of oscillation was about 3 KHz.

General procedure. As shown in Fig. 1, the preparation was first kept isometric by fixing the lever L of the displacement transducer in position by stops S_1 and S_2, the fibre length being varied by a micromanipulator carrying the force transducer T. The resting tension was recorded as a base line for the force trace. The preparation was then stimulated for 1 sec with 2 msec rectangular current pulses at 15–30 Hz with a strength of about two times the threshold value. After the maximum isometric force P_0 had been developed (at 0·4–0·6 sec after the beginning of stimulation), the load on the preparation was changed in various sequences as illustrated in Fig. 2.

One end of the loading spring F_1 was connected to the short arm of the lever L, while the other end was hooked to another magnesium lever K (0·2 mm in thickness) at a point 2 cm from the pivot B. The lever K was also fixed in position by stops S_3 and S_4, so that the amount of isotonic load could be varied by changing the length of F_1 with a micromanipulator G_1 carrying the lever K and the stops. By withdrawing S_1 and S_2 electromagnetically, the load on the preparation was quickly changed from P_0 to $P_1 < P_0$ to produce isotonic shortening (Fig. 2*A*), or from P_0 to $P_1' > P_0$ to produce isotonic lengthening (Fig. 2*B*) depending on the predetermined length of F_1.

A pair of additional springs F_2 and F_3 were also connected at both sides of the lever K at the point 0·5 cm from the pivot B, the lengths of F_2 and F_3 being varied independently with micromanipulators G_2 and G_3. Thus, when S_3 and S_4 were further removed electromagnetically, the lever K quickly moved either to the right or to the left, according to the inbalance of force exerted by F_2 and F_3, to change the length of F_1 quickly. By the above procedure, the load on the preparation could further be changed quickly from P_1 to $P_2 \lesseqgtr P_1$ after a period t of isotonic shortening (Fig. 2*A*), or from P_1' to $P_2' \lesseqgtr P_1'$ after a period t' of isotonic lengthening (Fig. 2*B*). The inertial oscillations of the lever K were damped by use of Y-shaped dashpot device H (inset) filled with silicon oil. Thus, the oscillations (period 3–5 msec) following quick changes in load almost disappeared within 3–6 msec after the beginning of the load changes. In both the first and the second load steps, quick changes in load were complete in 1–2·5 msec.

All experiments were made within the range of fibre lengths where the resting tension was negligible (sarcomere length, less than 2·6–2·8 μm) to avoid complications arising from the development of resting tension during the course of isotonic lengthening; in most cases, the initial sarcomere length of the fibres was 2·0–2·2 μm. The length and force changes were simultaneously displayed on a dual-beam oscilloscope (Tektronix, type 565) and recorded with a 35 mm camera,

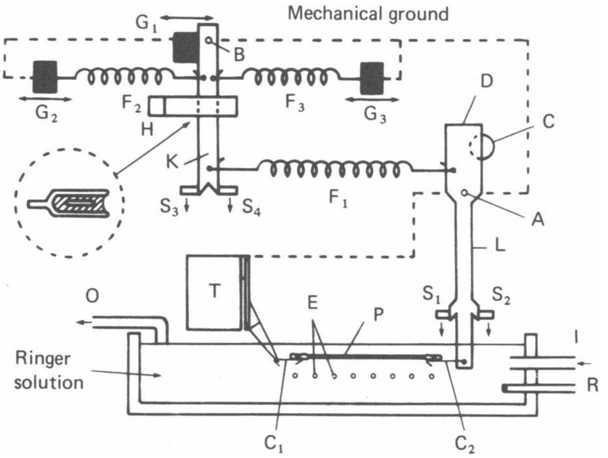

Fig. 1. Schematic drawing of experimental arrangement. The preparation P was hooked to the force transducer T and to the lever L of the displacement transducer with wire connectors C_1 and C_2, and stimulated with eight platinum wire electrodes E fixed to the stimulation chamber. The lever was pivoted at A, and loaded by a spring F_1, which was hooked to the lever L and another lever K, the length of F_1 being changed by a micromanipulator G_1 carrying K. The long arms of L and K were restrained by pairs of electromagnetically controlled stops S_1, S_2 and S_3, S_4 respectively. The short arm of L formed a vane D which interrupted part of a light beam C directed towards a photodiode (not shown). By means of a pair of additional springs F_2 and F_3, the lengths of which were adjusted by manipulators G_2 and G_3, the length of F_1 could be changed quickly when S_3 and S_4 were removed to cause the movement of K. The oscillation of K was damped with a Y-shaped dashpot device H. Mechanical ground (dashed line) linked the elements with a common base. Ringer solution entered into the chamber at I, and flowed past the preparation to the exit O, temperature being measured with a thermistor probe R.

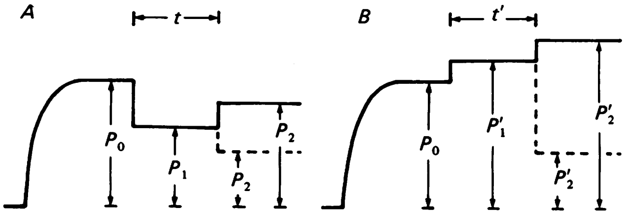

Fig. 2. Schematic diagram showing the experimental procedure. The fibres were first stimulated to produce the maximum isometric tension P_0, and then the load on the fibres was changed in various sequences. In *A*, the load was reduced from P_0 to $P_1 < P_0$ to produce isotonic shortening for a period *t*, and then further changed from P_1 to $P_2 \lessgtr P_1$. In *B*, the load was increased from P_0 to $P'_1 > P_0$ to produce isotonic lengthening for a period *t'*, and then further changed from P'_1 to $P'_2 \lessgtr P'_1$.

or memorized in an eight-bit 2 K-word transient recorder (Kawasaki electronica, TM 1410) with various clock rates of 50–250 μsec/word and subsequently plotted using a DC pen recorder (Rikadenki, B-26). Unless otherwise stated, more than ten preparations were used for each type of experiment described in this paper. The data were discarded when the magnitude of steady isometric force was reduced by more than 5–10 % of the initial value during the experiments.

The stray compliance of the whole recording system including ties was estimated by substituting a length of stainless-steel wire for the preparation, and was found to be about 9 μm/g. Since the maximum isometric force of the preparation ranged from 50 to 400 mg (1·8–3·0 kg/cm^2), the stray compliance was not more than 0·1 % of the fibre length even under a load of 2 P_0.

RESULTS

General features of the isotonic velocity transients following quick changes in load

Fig. 3 shows examples of the length and tension changes when the load on isometrically contracting muscle fibres was suddenly changed to produce isotonic motion. When the lever was released, the force exerted by the fibres quickly changed from the isometric value P_0 to a new steady value P, which was equal to the force exerted by the load (Civan & Podolsky, 1966). The initial rapid shortening (Fig. 3*A*) and lengthening (Fig. 3*B*) coincident with the quick changes in load can be explained as being due to elastic recoil and extension of the series elasticity (or the series elastic component, e.g. Jewell & Wilkie, 1958) respectively, and will be studied in detail in the following paper (Sugi & Tsuchiya, 1981). Although the contractile force reached a new steady value shortly after a step change in load, the velocity of subsequent isotonic movement (upper traces) exhibited a long transient phase before approaching a steady value. These isotonic velocity transients can be recognized by the difference between the actual length trace and the back extrapolation of the steady phase of isotonic shortening or lengthening (dashed lines).

In agreement with previous reports (Civan & Podolsky, 1966; Armstrong *et al.* 1966; Huxley & Simmons, 1973), the velocity of isotonic shortening immediately after a step decrease in load was several times larger than the steady shortening velocity appropriate for the new load; it then declined to a low value and finally reached a steady value (Fig. 3*A*), sometimes with a damped oscillation which was most marked following small step decreases in load (see Fig. 4*A*). During the course of these isotonic velocity transients following small step decreases in load, the shortening velocity was temporarily reduced almost to zero. In a few cases, a reversal in the direction of movement was barely perceptible.

In the case of the velocity transients following step increases in load, on the other hand, oscillatory length changes with distinct reversal in the direction of movement were observed when the load on the fibres was increased from P_0 to less than 1·3 P_0; as can be seen in Fig. 3*B*, the fibres showed an initial lengthening followed by a transient shortening before starting to lengthen with a nearly steady velocity. In many cases, the final steady lengthening was reached after a marked damped oscillation of fibre length (see Fig. 4*B*).

Fig. 4 shows the records of the velocity transients under various isotonic loads P expressed relative to P_0. The velocity transients following quick decreases in load (Fig. 4*A*) were very similar to those reported by Civan & Podolsky (1966), always giving the appearance of damped oscillation irrespective of the magnitude of load steps. The

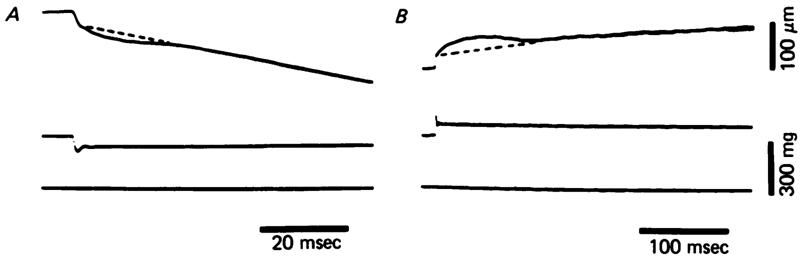

Fig. 3. Examples of records showing the isotonic velocity transients in frog muscle fibres following quick changes in load. In this and subsequent Figures, the upper trace shows the change in fibre length, while the middle and lower traces show the change in force and the base line for the force trace respectively. Bundle of three fibres. Temperature, 2·5 °C. The load on the fibres was changed from P_0 to 0·83 P_0 in *A*, and from P_0 to 1·25 P_0 in *B*. Dashed lines indicate back extrapolation of the steady phase of isotonic movement.

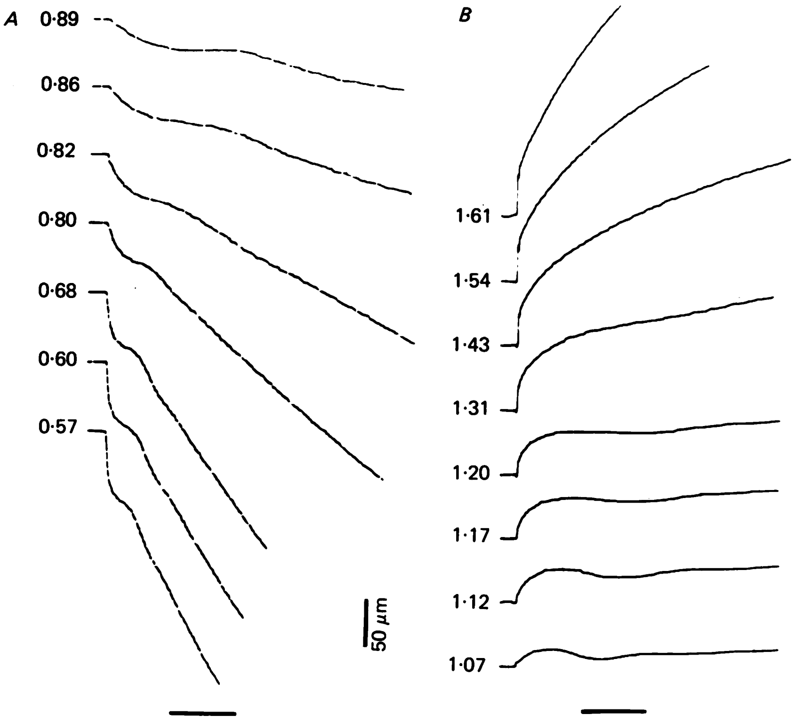

Fig. 4. Records of the isotonic velocity transients following quick decreases in load (*A*) and following quick increases in load (*B*). The load on the fibres was changed from P_0 to $P < P_0$ in *A*, and to $P > P_0$ in *B*. The values of *P* expressed relative to P_0 are shown on the left of each record. Minute steps in each record in this Figure (and also Figs. 5, 10 and 11) are due to the limited precision of the transient recorder used. Bundle of three fibres. P_0, 230–245 mg. Temperature, 2·3 °C.

appearance of the velocity transients following quick increases in load was found to change according to the magnitude of the load step. When the load on a fibre was increased from P_0 to 1·05–1·3 P_0, marked oscillatory length changes with distinct reversal in the direction of movement was observed to take place. If the load was increased from P_0 to 1·3–1·6 P_0, the oscillatory length changes were no longer observable; the fibres lengthened continuously with velocities decreasing with time, and there was no definite phase of steady isotonic lengthening. With further increase in the isotonic load above 1·6–1·7 P_0, the resulting isotonic lengthening proceeded with increasing velocity, indicating 'give' of the fibres (Katz, 1939; Tsuchiya *et al.* 1979; Sugi & Tsuchiya, 1981).

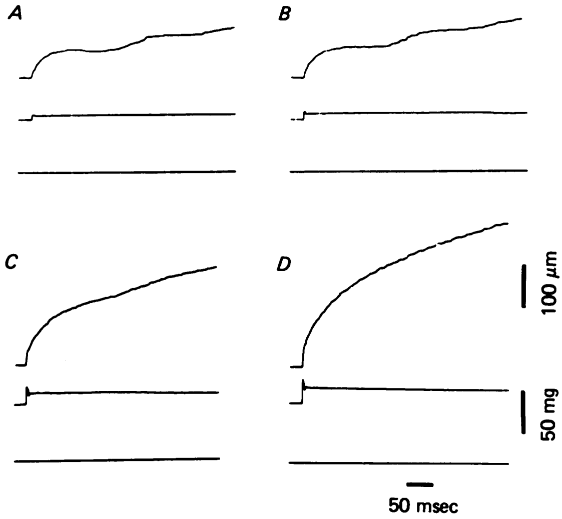

Fig. 5. Responses of a single muscle fibre to quick increases in load. Temperature, 2·8 °C. The load on the fibre was increased from P_0 to 1·08 P_0 in *A*, 1·12 P_0 in *B*, 1·20 P_0 in *C*, and 1·28 P_0 in *D*.

The velocity transients in a single muscle fibre following quick increases in load are shown in Fig. 5. The features of the velocity transients in single fibres were essentially the same as those in the fibre bundles.

The amplitude of the oscillatory length changes observed in the present study never exceeded 0·5 % of L_0, i.e. about 50 Å per half-sarcomere.

Factors affecting the isotonic velocity transients following quick increases in load

Amount of isotonic load. The relation between the amount of isotonic load and the half-period of oscillation of the velocity transients is shown in Fig. 6. Similar results were obtained on five other preparations examined. As illustrated in the inset, the half-periods of oscillation in the length traces under various isotonic loads were determined; in the case of step decreases in load ($P < P_0$), the half-period was measured by drawing a tangential line parallel to the steady phase of isotonic shortening, while it could be measured directly on the length trace in the case of step increases in load ($P > P_0$).

It can be seen that the half-period of the velocity transients increases with increasing amount of isotonic load attained after the load step. It should be noted that, in spite of the difference in features between the velocity transients after step decreases in load and those after step increases in load (Fig. 4), the curve for $P < P_0$ (step decreases in load) is continuous with that for $P > P_0$ (step increases in load). Since there is no oscillation with $P > 1\cdot3\ P_0$ (Fig. 4*B*), the above relation holds only for isotonic loads of less than $1\cdot3\ P_0$.

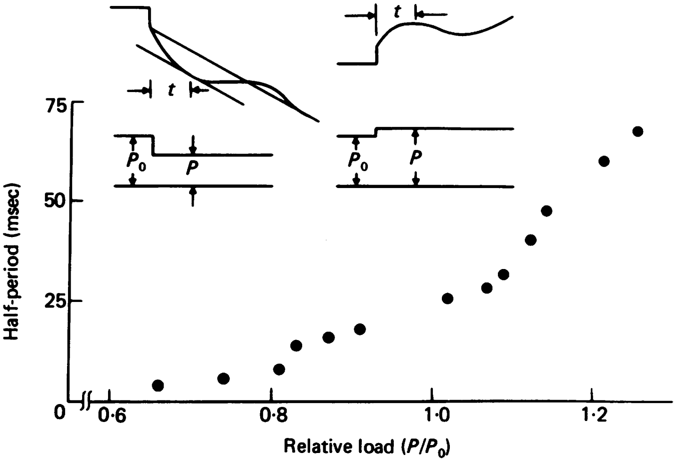

Fig. 6. Relation between the amount of isotonic load P attained after a load step from P_0 and the half-period of oscillatory velocity transients. Inset illustrates method of measurement of the half-period of oscillation of the velocity transients. Bundle of three fibres. P_0, 310–325 mg. Temperature, 2·5 °C.

Temperature. The effect of temperature on the velocity transients for two different step increases in load is shown in Fig. 7. It will be seen that the period of oscillatory length changes decreases with increasing temperature. In Fig. 8, the half-periods of oscillatory length changes are plotted against the isotonic loads ($P_0 < P < 1\cdot15\ P_0$) at different temperatures from 0·8 to 8·8 °C. The half-period of oscillation of the velocity transients for a given load step decreased to about one fourth by raising the temperature from 0·8 to 8·8 °C. The regression lines do not converge into one point at small loads, indicating that these lines do not lie on top of one another by vertical scaling; the dependence of the half-period on the load was more marked as the temperature was decreased. Similar results were obtained on three other preparations.

According to Civan & Podolsky (1966), the period of the velocity transients following step decreases in load also decreases with increasing temperature, with a temperature coefficient similar to that of the maximum velocity of steady isotonic shortening, though they used null time (i.e. the time when the velocity transients intersects the back extrapolation of steady shortening) as a measure of the oscillation period. The effect of temperatute on the velocity of isotonic lengthening was not examined quantitatively in the present study because (1) there was no definite phase of steady lengthening except for relatively small load steps and (2) repeated lengthening under large loads tended to damage the fibres, thought it was noticed that the velocity of lengthening increased with increasing temperature. The temperature dependence of the velocity transients following step increases in load, however, may be taken to indicate that they reflect the turnover of the cross-bridges as has

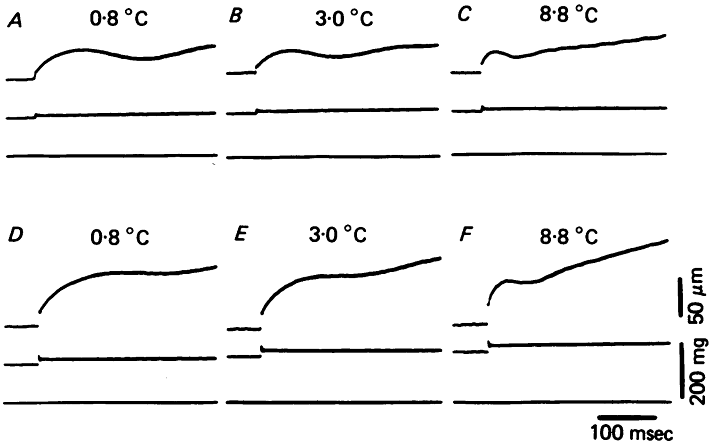

Fig. 7. Effect of temperature on the isotonic velocity transients following quick increases in load. The load was increased from P_0 to 1·06 P_0 (*A–C*), and to 1·13 P_0 (*D–F*). Bundle of two fibres. Note that the period of velocity transients decreases with increasing temperature.

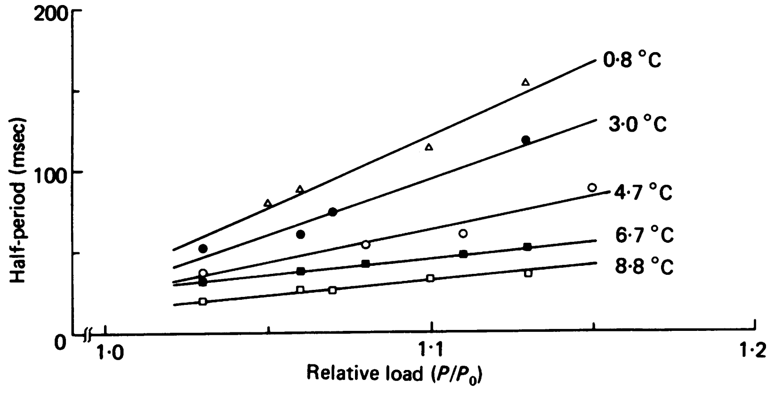

Fig. 8. Effect of temperature on the relation between the amount of isotonic load P attained after a step increase in load from P_0 and the half-period of velocity transients. Each regression line represents a least-square fit. The preparation was the same as that in Fig. 7.

been supposed for the velocity transients following step decreases in load (Civan & Podolsky, 1966).

Sarcomere length. No marked change in the time course of the velocity transients were observed when the initial sarcomere length of the fibres was decreased from 2·0–2·2 to 1·7–1·8 μm.

The isotonic velocity transients following quick increases in load applied during isotonic shortening

General features. In order to obtain information about the kinetic behaviour of the cross-bridges during isotonic shortening, quick increases in load were also applied to the fibres during the course of isotonic shortening under various loads by the procedure illustrated in Fig. 2*A*.

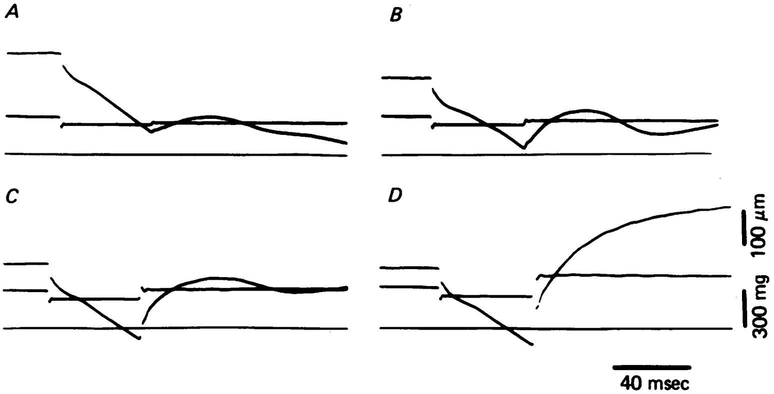

Fig. 9. Records of the experiments in which the load on the fibres was increased quickly after a period (50 msec) of isotonic shortening under a load of about 0·8 P_0. Note marked oscillatory length changes with distinct reversal in the direction of movement following small load steps. Bundle of three fibres. Temperature, 2·5 °C.

Fig. 9 shows examples of the response to quick increases in load applied after a period (30–50 msec) of isotonic shortening under a large load of about 0·8 P_0. As can be seen in Fig. 9*A–C*, the fibres also showed marked oscillatory length changes in response to small load steps ($P_2 < 1{\cdot}3\ P_1$, Fig. 2*A*) before starting to shorten isotonically, and the period of oscillation was also longer as the load step was increased. With larger load steps ($P_2 > 1{\cdot}3\ P_1$), the fibres lengthened continuously with velocities decreasing with time (Fig. 9*D*). Thus, the response of the fibres, shortening isotonically under a large load, was qualitatively similar to that of isometrically contracting fibres.

The response of the fibres, however, differed from that of isometrically contracting ones if the amount of isotonic load, under which the fibres shortened, was moderate or small. When quick increases in load were applied to the fibres shortening under a moderate load of about 0·6 P_0, they first lengthened before starting to shorten provided the new load P_2 was less than P_0 (Fig. 10); the larger P_2, the longer was the time between the onset of a step increase in load and the beginning of isotonic shortening. With loads above P_0, the fibres were always lengthened continuously.

If quick increases in load were applied to the fibres shortening under a small load of about 0·1 P_0 (Fig. 11), they could barely shorten against a new load of about 0·7 P_0 (Fig. 11*C*), and were lengthened continuously under a load of about 0·9 P_0 (Fig. 11*D*) at least for 150 msec after the step increase in load.

Quantitative aspects of the velocity transients. Some quantitative aspects of the

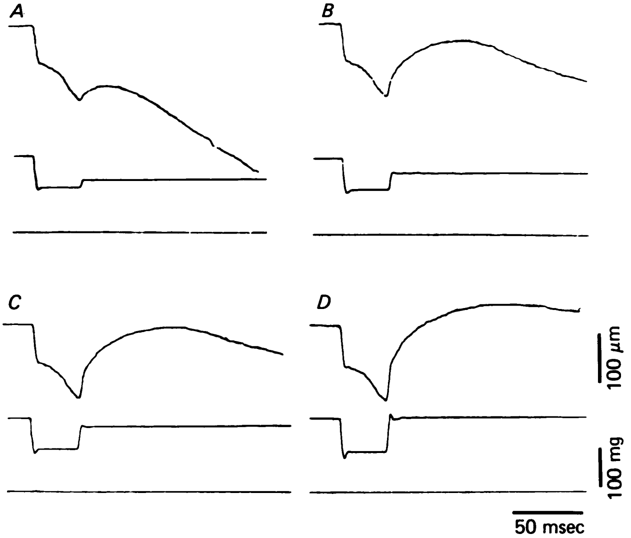

Fig. 10. Records of the experiments in which the load on the fibres was increased quickly after a period (30 msec) of isotonic shortening under a load of about 0·6 P_0. Note the initial transient isotonic lengthening in response to load steps. Bundle of two fibres. Temperature, 2·5 °C.

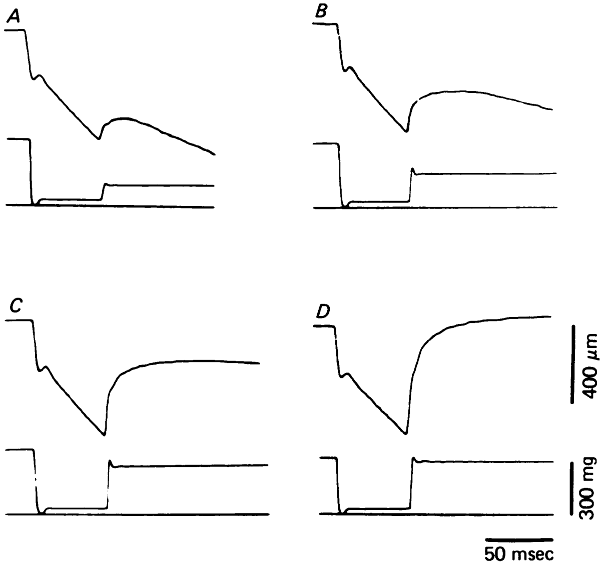

Fig. 11. Records of experiments in which the load on the fibres was increased quickly after a period (50 msec) of isotonic shortening under a load of about 0·1 P_0. Note that the fibres can scarcely shorten against a load of 0·7 P_0 (*C*), and are lengthened continuously under a load of 0·9 P_0 (*D*). Bundle of two fibres. Temperature, 2·3 °C.

velocity transients in relation to the magnitude of load steps and the preceding isotonic shortening were studied in seven preparations with similar results. In Fig. 12, the velocity of initial isotonic lengthening, which may serve as a measure of the load-sustaining ability at the time of a step increase in load from P_1 to P_2 (inset), is plotted against the value of P_2; prior to the load steps from P_1 to P_2 the fibres were allowed to shorten for 50 msec under two different values of P_1 (about 0·55 and 0·75 P_0). The values of initial isotonic lengthening velocity following load steps from P_0 to P_2 are also shown. The initial lengthening velocities were measured at 10–20 msec after load steps from P_1 to P_2.

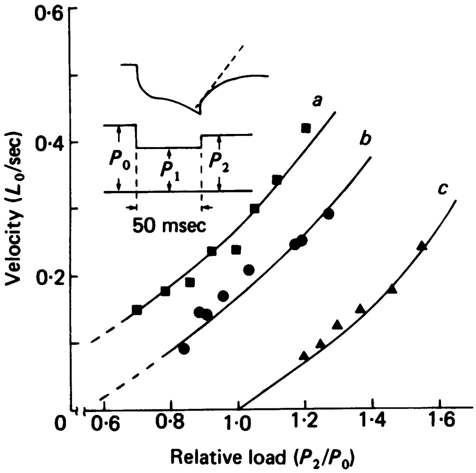

Fig. 12. Relation between the amount of isotonic load and the initial velocity of isotonic lengthening. The fibres were first allowed to shorten isotonically under a load P_1 for 50 msec, and then the load was increased quickly from P_1 to P_2 to produce transient isotonic lengthening (inset). The initial lengthening velocity was measured at 10–20 msec after step increases in load. Curves *a* and *b* were obtained by applying step increases in load during isotonic shortening under P_1 of about 0·55 P_0 and 0·75P_0 respectively, while curve *c* was obtained by applying step increases in load at the maximum isometric tension P_0. Bundle of three fibres. P_0, 350–360 mg. Temperature, 2·5 °C.

The load–velocity curves obtained were approximately parallel to each other, and the lengthening velocity for a given value of P_2 was larger the smaller the value of P_1. While the load–velocity curve obtained at P_0 (curve *c*) intersected the load axis at P_0, the extension of the load–velocity curves obtained during isotonic shortening (curves *a* and *b*) intersected the load axis at points definitely smaller than the corresponding P_1 values, indicating a discontinuity of the load–velocity curves at P_2 values close to P_1.

In Fig. 13, the magnitude and duration of initial transient isotonic lengthening are plotted against the amount of P_2 for two different values of P_1 (about 0·55 and 0·75 P_0) under which the fibres shortened for 50 msec before the application of step increases in load from P_1 to P_2. Both the magnitude and duration of initial lengthening increased with increasing magnitude of P_2 at a constant value of P_1. For a given magnitude of

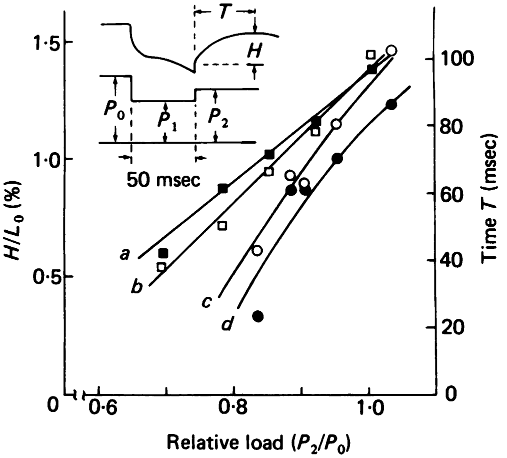

Fig. 13. Relation between the amount of isotonic load and the magnitude (curves *a* and *d*) or the duration (curves *b* and *c*) of the transient isotonic lengthening. The fibres were made to shorten isotonically under a load P_1 for 50 msec before applying step increases in load from P_1 to P_2 to produce transient isotonic lengthening (inset). For curves *a* and *d*, the ordinate is the magnitude of the transient lengthening (*H* in the inset) as a percentage of L_0. This figure shows the results of two experiments; curves *a* and *b* are the magnitude and the duration of the transient lengthening (*H* and *T* in the inset) for an initial load P_1 of about 0·55 P_0, and curves *b* and *c* are the corresponding curves for an initial load P_1 of about 0·75 P_0. The preparation was the same as that in Fig. 12.

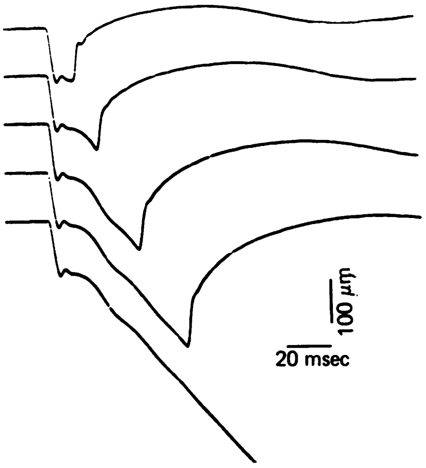

Fig. 14. Records of change in fibre length when the load was increased quickly to P_0 at various times after the beginning of isotonic shortening under a load of 0·53 P_0. Note that the velocity of initial isotonic lengthening is larger the longer the period of preceding isotonic shortening. Bottom record shows isotonic shortening without subsequent step increase in load. Bundle of two fibres. P_0, 240–250 mg. Temperature, 2·5 °C.

P_2 both values were larger the smaller the value of P_1, though the dependence on P_1 was less marked in the case of the duration than in the case of the magnitude of transient lengthening.

The dependence of initial isotonic lengthening following a step increase in load on the duration of preceding isotonic shortening was also examined by applying step increases in load of a constant magnitude at various times after the beginning of isotonic shortening (Fig. 14). As shown in Fig. 15, the velocity of initial lengthening increased with increasing duration of preceding shortening, reaching a maximum value within 30–50 msec after the beginning of isotonic shortening. The magnitude of initial lengthening exhibited a similar dependence on the duration of preceding isotonic shortening (Fig. 16). Similar results were also obtained in the case of the duration of transient lengthening except that its range of variation was much smaller than that of the magnitude of initial lengthening.

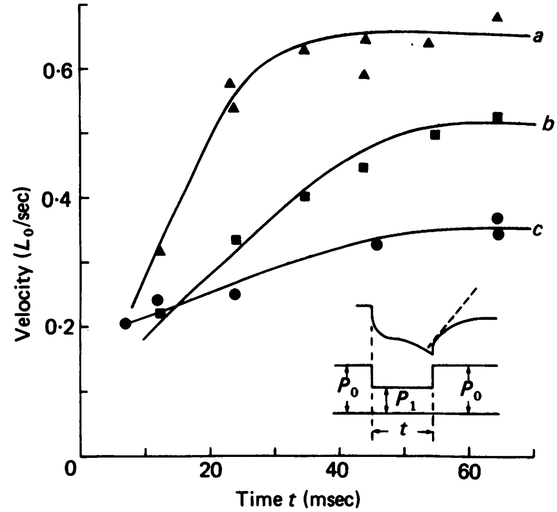

Fig. 15. Relation between the duration of preceding isotonic shortening and the initial velocity of isotonic lengthening. The load on the fibres was increased quickly from P_1 to P_0 at various times t after the beginning of isotonic shortening under a load P_1 (inset). Curves a, b and c were obtained by applying step increases in load to P_0 during isotonic shortening under a load of about 0·4 P_0, 0·55 P_0 *and* 0·75 P_0 respectively. Bundle of three fibres. P_0, 320–335 mg. Temperature, 2·3 °C.

Effect of quick decreases in load applied during isotonic shortening

Experiments were also performed in which the load on the fibres was further decreased during isotonic shortening. Fig. 17 shows typical records of change in fibre length when the load on isotonically shortening fibres was quickly reduced from 0·8 to 0·65 P_0 at various times after the beginning of isotonic shortening. Though the step decreases in load applied during isotonic shortening were also followed by velocity transients with the appearance of damped oscillations (Podolsky & Nolan, 1973), the velocity of the steady isotonic shortening finally attained was the same irrespective of the time of application of the step decreases in load. This result indicates that the velocity of steady isotonic shortening is only determined by the amount of load, but not by the time sequence with which the final load is attained, in contrast to the variable time course of isotonic movements when quick increases in load of a constant magnitude were applied at various times during the course of preceding isotonic shortening (Figs. 14–16).

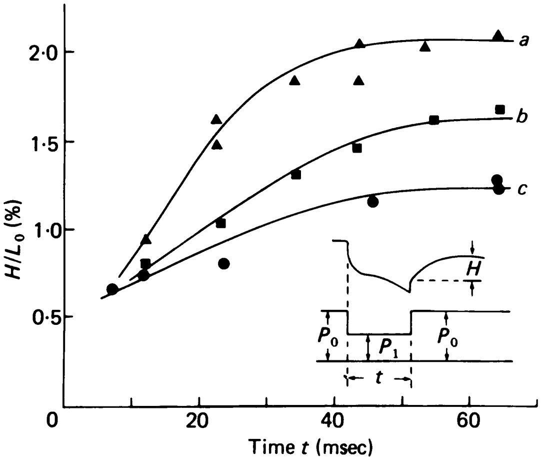

Fig. 16. Relation between the duration of preceding isotonic shortening and the magnitude of the initial transient lengthening (H in the inset). The preparation and the experiments were the same as those in Fig. 15. Curves a, b and c were obtained during isotonic shortening under a load of about 0·4 P_0, 0·55 P_0 and 0·75 P_0 respectively.

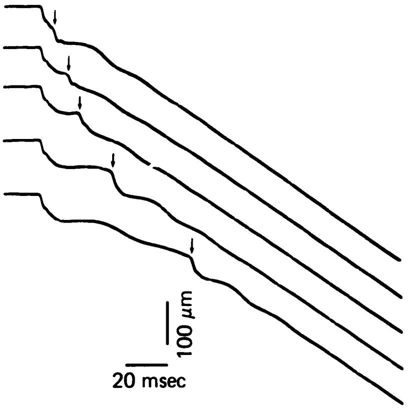

Fig. 17. Records of change in fibre length when the load was further decreased to 0·65 P_0 at various times (↓) after the beginning of isotonic shortening under a load of 0·8 P_0. Note that, in all records, the final steady velocity of shortening is the same, irrespective of the time of application of the second step decreases in load. Bundle of two fibres. P_0, 210–220 mg. Temperature, 2·5 °C.

Podolsky & Nolan (1973) claimed that the null times of the transients remained almost unchanged irrespective of whether the final load was attained by two steps or by a single step, suggesting that the null time mainly depends on the final load. Their observation was not, however, confirmed in the present study; the half-period of oscillation (and also the null time) of the transients under a given final load varied greatly according to the sequence with which the final load was attained, though this point was not studied quantitatively.

DISCUSSION

Possible interpretation of the oscillatory velocity transients following quick increases in load

The present experiments have shown the similarities and the differences between the isotonic velocity transients following quick decreases in load from P_0 to $P < P_0$ and those following quick increases in load from P_0 to $P > P_0$. In the former, the transients exhibited the appearance of heavily damped oscillations irrespective of the value of P (Fig. 4*A*), while the time course of the latter changed greatly depending on the value of P (Fig. 4*B*), the marked oscillatory length changes with distinct reversal in the direction of movement being observed only for small step increases in load (Figs. 3–5 and 7). In both cases, the period of oscillation in the transients was dependent on the size of P (Fig. 6) and on temperature (Figs. 7 and 8), indicating that the underlying mechanism of the oscillatory transients may be the same irrespective of the size of P. After large step increases in load, in which no oscillatory transients were observable, there was no definite phase of steady lengthening (Fig. 4*B*), in contrast to the steady isotonic shortening after step decreases in load (Fig. 17).

We shall interpret the velocity transients following quick increases in load on the basis of a two-state contraction model, in which rate constants for making and breaking the cross-links, f and g, are given as functions of distance x between the site on the thin filament and the equilibrium position of a cross-bridge (A. F. Huxley, 1957). By modifying f and g functions in the Huxley model, Podolsky & his co-workers (Podolsky *et al.* 1969; Podolsky & Nolan, 1973) calculated the oscillatory motions following quick decreases in load. In the Podolsky model there was a gap between f and the major part of g in the negative x region (Fig. 18), which effectively introduced a delay between the effects of formation and breaking of the cross-links to produce the oscillatory velocity transients. Since Podolsky did not consider motions after step increases in load and gave g function only in the negative x region, an attempt has been made in our laboratory to simulate the isotonic motions after step increases in load by choosing appropriate f and g functions in the positive x region. Following step increases in load, a gap between f and the major part of g in the positive x region (Fig. 18) could produce the oscillatory changes of lengthening velocity, but not the length changes with distinct reversal in the direction of movement, indicating the difficulty in simulating this kind of motion with fixed f and g functions. If, however, it was assumed that g increases instantaneously by a step increase in load and returns to the initial value exponentially with time, so that the time course of change in g after a step increase in load was expressed as $g(t) = g_0 + A_e^{-Bt}$, the above contraction model produced oscillatory transients with distinct reversal in the direction of movement for small step increases in load, and produced only continuous lengthening for larger step increases in load; with appropriate values for the constants A and B, the calculated motions were close to the actual responses of the fibres shown in Fig. 4*B* (Tsuchiya *et al.* 1979; S. Chaen, H. Sugi & T. Tsuchiya, unpublished).

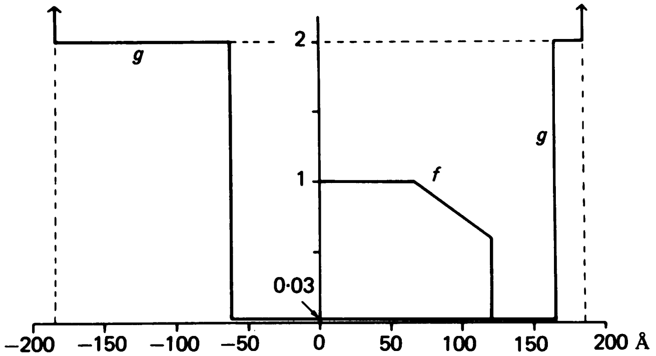

Fig. 18. Dependence of f, the rate constant for making a cross-link, and g, the rate constant for breaking a cross-link, on x, denoting the distance between the site on the thin filament and the equilibrium position at which an attached cross-bridge exerts zero force, in the contraction model used to simulate the velocity transients following quick changes in load. The unit on the ordinate is the value of f at $0 < x < 60$ Å. The value of g is small for -65 Å $< x < 165$ Å, so that there is a gap between f and the major part of g in both positive and negative x regions. g is infinitely large for $x > 185$ Å and $x < -185$ Å. It is further assumed that the value of g increases instantaneously in response to step increases in load, and returns to the initial value exponentially with time, so that the time course of change in g after a step increase in load is expressed as $g = g_0 + A_e{}^{-Bt}$, where A and B are constants determining the magnitude of instantaneous increase in g and the time constant of subsequent exponential recovery respectively. Each attached cross-bridge is assumed to exert positive or negative forces proportional to positive or negative values of x.

These results suggest the possibility that, when attached cross-bridges are suddenly displaced in the direction opposite to muscle shortening, they tend to detach from the thin filaments more or less synchronously as a result of abrupt increase in g; the resulting decrease in the number of attached cross-bridges reduces the load-sustaining ability of the fibres to result in the initial rapid lengthening. Then the detached cross-bridges start to re-attach to the thin filaments more or less synchronously, while the value of g decreases towards the initial value with time; the resulting increase in the number of attached cross-bridges restores the load-sustaining ability to cause the subsequent shortening when P is not much larger than P_0, or to decrease the subsequent lengthening velocity when P is much larger than P_0 (Fig. 4*B*). If this kind of explanation is correct, the oscillatory length changes with distinct reversal in the direction of movement is due to fairly synchronized breaking and re-formation of the cross-links. The possibility of an increase in g in distorted cross-bridges as a result of quick length changes has also been considered concerning the isometric tension transients to insect muscle (Julian, 1969; Thorson & White, 1969; Abbot & Steiger, 1977).

Decrease in load-sustaining ability during isotonic shortening

The present study also provides information about the decrease in the ability of the fibres to sustain a load during isotonic shortening. When quick increases in load were applied after a period of isotonic shortening under a large load (about $0{\cdot}8\ P_0$) the resulting velocity transients were qualitatively similar to those in response to load steps applied during isometric contraction; the fibres showed marked oscillatory length changes with distinct reversal in the direction of movement following small load steps (Fig. 9*A*–*C*), while they lengthened continuously with larger load steps (Fig. 9*D*).

If, on the other hand, quick increases in load were applied during isotonic shortening under a moderate or a small load ($0{\cdot}1$–$0{\cdot}6\ P_0$), the damped oscillation was no longer observable; the fibres always exhibited initial isotonic lengthening before starting to shorten against a new load $P < P_0$ (Figs. 10 and 11), indicating that the ability of the fibres to sustain a load is decreased appreciably during the preceding isotonic shortening and is restored during the initial isotonic lengthening.

The velocity of initial isotonic lengthening for a given amount of load, which may serve as a measure of the load-sustaining ability at the time of load steps, was found to be greater the smaller the isotonic load of preceding shortening (Fig. 12), indicating that the extent of decrease in the load-sustaining ability increased with decreasing isotonic load of previous shortening. In this connexion, it is of interest that the extension of load–velocity curves obtained during isotonic shortening (curves *a* and *b* in Fig. 12) intersected with the load axis at points much smaller than the corresponding values of isotonic load during the preceding shortening. This may be taken to indicate that the extent of decrease in the load-sustaining ability during shortening is not in proportion, to but larger than, the difference between P_0 and the isotonic load. The velocity of initial isotonic lengthening for a given amount of load was also dependent on the duration of preceding isotonic shortening, reaching a finite maximum value within 30–50 msec after the beginning of isotonic shortening (Figs. 14 and 15), suggesting that the decrease in the load-sustaining ability during isotonic shortening is complete within 30–50 msec.

When step increases in load were applied at a fixed time after the beginning of isotonic shortening, both the initial velocity and the magnitude and duration of transient isotonic lengthening increased with increasing magnitude of load steps (Fig. 13). If, on the other hand, step increases in load of a constant magnitude were applied at various times after the beginning of isotonic shortening (Fig. 14), all these values of transient isotonic lengthening increased in parallel with each other with increasing duration of preceding shortening (Figs. 15 and 16). These results indicate that the larger the initial lengthening velocity following a load step, the more distance of lengthening (or the more time) is required until the restoration of load-sustaining ability takes place. Thus, when large step increases in load were applied after a sufficiently long period of isotonic shortening under a small load, the resulting very rapid initial lengthening was followed by very slow development of the recovery process, so that no shortening took place for 150 msec after load steps (Fig. 11*D*).

The contraction model in Fig. 18 can also produce the transient isotonic lengthening in response to load steps applied during isotonic lengthening (Figs. 9–11) if it is assumed that the magnitude of instantaneous increase in g followed by exponential recovery is greater the smaller the amount of load of preceding isotonic shortening, i.e. the value of A increases with decreasing load of preceding isotonic shortening (S. Chaen *et al.* unpublished). In this model, the shape of g function in the negative x region is made essentially similar to that in the Podolsky model so as to produce the oscillatory velocity transients following step decreases in load (Fig. 4*A*). Consequently the number of attached cross-bridges at any one moment is larger during isotonic shortening than during isometric contraction due to the gap between f and the major part of g in the negative x region. On this basis, the initial isotonic lengthening, which is more marked when load steps are applied during shortening under a small load than under a large load, results because the initial rate of breaking of cross-links caused by the instantaneous increase in g is greater the smaller the load of preceding isotonic shortening, but not because the number of attached cross-bridges decreases as the shortening velocity is increased (A. F. Huxley, 1957). The subsequent restoration of load-sustaining ability can then be explained as being due to the re-formation of cross-links as the value of g returns to the initial value.

On the other hand, it is also possible that the decrease in load-sustaining ability during isotonic shortening is related to the decrease in the number of attached cross-bridges at any one moment as expected from the Huxley model, though additional assumptions of change in rate functions may also be necessary to account for the features of transient isotonic lengthening.

The stiffness of muscle fibres, which is generally regarded to serve as a measure of the number of attached cross-bridges at any moment (e.g. Huxley & Simmons, 1971, 1973), is known to decrease with decreasing isotonic load, approaching a finite minimum value as the load tends to zero (Julian & Sollins, 1975; Sugi & Tsuchiya, 1981). Though this seems to favour the Huxley model, the stiffness does not necessarily represent the number of cross-links if there is significant elasticity in series with the contractile component in each sarcomere (e.g. Podolsky & Nolan, 1973). To settle the problem, it may be necessary to determine the degree of contribution to the stiffness of the sarcomere structures other than the cross-bridges. In this connexion, Sugi & Suzuki (1980) have recently demonstrated that both the thick and thin filaments are actually extensible, suggesting possible sources of the muscle fibre stiffness other than the cross-bridges.

In contrast with the decrease in the load-sustaining ability during isotonic shortening, an enhancement of mechanical performance of muscle fibres takes place during the oscillatory length changes or continuous isotonic lengthening after quick increases in load, and is described in the following paper (Sugi & Tsuchiya, 1981).

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