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The maximum velocity of shortening during the early phases of the contraction in frog single muscle fibres

V. LOMBARDI and G. MENCHETTI*

Istituto di Fisiologia, Università di Firenze, Viale G.B. Morgagni 63, 50134 Firenze, Italy

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Summary

The maximum velocity of shortening (V_{max}) was determined at preset times during the development and the plateau of isometric tetani in single fibres isolated from the tibialis anterior muscle of the frog. Experiments were performed at low temperature (3.6–6° C) and at about 2.25 μm sarcomere length. The controlled velocity release method was used. V_{max} was measured by determining the lowest velocity of release required to keep the tension at zero. Extreme care was taken in dissection and mounting of the fibres in order to make the passive series compliance very small.

The value of V_{max} at the end of the latent period for the development of isometric tension (at 4.5° C about 10 ms after the beginning of the stimulus volley) was already the same as later during either the tension rise or at the plateau of isometric tetani. These results show that the value of V_{max} of intact fibres is independent of time and activation subsequent to the latent period, and suggest that the cycling rate of the crossbridges may thus attain its steady-state value just at the end of the isometric latent period.

Introduction

There is in the literature a great deal of work devoted to ascertaining whether the velocity of shortening under zero load of frog skeletal muscle (the maximum velocity of shortening, V_{max}) depends on the concentration of Ca^{2+} at the level of myofilaments and therefore on the degree of activation of the contractile machinery. This question assumes a particular interest in the view of a crossbridge mechanism of contraction (Huxley, 1957), where V_{max} represents a measure of the rate of cycling of crossbridges. In skinned fibres, depending on the experimental conditions (Podolin & Ford, 1983), V_{max} has been found either to be independent of the concentration of Ca^{2+} in the activating solution (Podolsky & Teichholz, 1970; Thames *et al.*, 1974; Gulati & Podolsky, 1981) or to

**Permanent address:* Istituto di Biologia cellulare, Università degli Studi di Perugia, 06100 Perugia, Italy.

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decrease when the Ca^{2+} concentration was reduced to submaximal activating levels (Julian, 1971; Julian & Moss, 1981; Stephenson & Julian, 1982). In intact muscle, most of the previous work on this subject, apart from a few exceptions (Sandow & Seaman, 1964; Julian & Sollins, 1973, indicates that V_{max} is independent both of the isometric tension developed and the level of activation (Fenn & Marsh, 1935; Abbott & Ritchie, 1951; Hill, 1951; Jewell & Wilkie, 1958; Cecchi *et al.*, 1978; Edman, 1979; Gulati & Babu, 1983). The experiments of Cecchi *et al.* (1978), performed on single muscle fibres of the frog using the controlled-velocity release method, have shown that in an isometric tetanus the value of V_{max} at short times after the beginning of the stimulus volley or at low initial tensions (about 20% of the plateau tension) is the same both at higher tensions and at the tension plateau. However, the degree of activation, measured by the characteristics of the instantaneous force–velocity relation, is widely time-dependent.

The present paper extends the previous work (Cecchi *et al.*, 1978) so that the passive series compliance of the muscle fibres used here was considerably smaller and measurements of V_{max} were extended to shorter times. The results are in accordance with the findings of Cecchi *et al.* (1978), such that in a tetanus, while the force–velocity relation takes a significant time to attain its steady-state characteristics, the value of V_{max} at the end of the latent period for the development of isometric tension is the same as at the tetanus plateau.

These results suggest that in intact muscle fibres the cycling rate of the crossbridge is fully developed soon after the start of stimulation and that it is then independent of the degree of activation.

Methods

Preparation and mounting

Experiments were performed on single fibres isolated from either medial or lateral head of the muscle tibialis anterior of the frog (*Rana esculenta*). Special care was taken in dissecting and mounting the fibres in order to minimize the amount of passive series compliance. The total length of both tendons of the muscle fibres used in these experiments was in no case greater than 380 μm. Fibres without firm attachments to tendons were discarded; the connections between fibre tendons and the levers of both the force and length transducers were made by means of aluminium foil clips (Ford *et al.*, 1977). Lateral movements of the fibres at the level of the tendon attachments occurring during the contractions or during the imposed length changes were minimized, by exercising extreme care in the alignment of the clips compared to the longitudinal axis of the fibres. The experimental chamber was a glass trough fastened with a thermoconductive paste to a metal block cooled by a thermoelectric module (Thermagotrons, MCP Electronics Ltd, U.K.). The temperature ranged from 3.6° to 6° C in different experiments, but in a single experiment it remained constant to ± 0.1° C. The bathing solution had the following composition: (mM) 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 3 phosphate buffer (pH 7.1).

Stimulation

Stimuli of alternating polarity were applied transversely to the muscle fibre by means of a pair of stainless-steel plate electrodes (8 mm long, 4 mm apart), across which up to 25 V could be applied with a constant voltage pulse generator. Stimuli of 0.5 ms duration and 1.5 times the threshold

strength were used. Tetanic stimulation was applied in brief tetanic volleys (0.4–0.6 s duration) of even numbers of pulses, at 4 min intervals. The optimal stimulation frequency (the frequency sufficient to produce a fully fused tetanus when judged from the first derivative of the tension) was determined for each fibre and ranged in different fibres from 14 to 25 pulses s^{-1}.

Tension and length transducers

Tension was measured by means of a capacitance-gauge transducer similar to that described by Huxley & Lombardi (1980). The resonance frequency of the various transducers used in this work ranged from 40 to 60 kHz, the sensitivity from 80 to 250 mV mN^{-1} and the noise from 0.1 to 0.5 mV peak-to-peak. Ramp and step length changes were imposed to the muscle fibre by means of a loudspeaker length-transducer servo-system already described (Cecchi *et al.*, 1976; Ambrogi-Lorenzini *et al.*, 1983). Step length changes were complete in about 150 μs.

Determination of fibre length, cross-sectional area and sarcomere spacing

All the measurements were made by means of a microscope mounted on a moveable micrometer slide using a dry objective (Zeiss, West Germany: 40×, N.A. 0.6, working distance 4.9 mm) and 10× or 25× micrometer eyepieces. Striation spacings were determined at rest by averaging the measurements of sequences of sarcomeres at different points of the fibre. The fibre length was measured by the distance between the insertions. The cross-sectional area was computed, at about 2.25 μm sarcomere length, as if the section of the fibre were elliptical, from measurements of the major and minor diameters.

Recording and measurement of the responses

The outputs from the tension and length transducers were recorded on a digital oscilloscope (Nicolet, Explorer III) and stored on floppy disc memory. The sampling rate of the Explorer was triggered externally at different frequencies so as to obtain an adequate temporal resolution for the various events during a given response. Measurements of the responses were made directly with the Explorer oscilloscope by means of its internal reading system.

Definitions

l_0 is the length of the fibre at rest and at a sarcomere length of about 2.25 μm. The number of sarcomeres in a fibre was determined at l_0 by dividing the fibre length by the sarcomere length. The amount of shortening per half-sarcomere was calculated by dividing the length change imposed on the fibre by the number of half-sarcomeres. V_{max} (μm s^{-1} half-sarcomere^{-1}) is the smallest velocity of release required to keep at zero the tension of a stimulated fibre or, conversely, the velocity of unloaded shortening. T is the steady force (kN m^{-2}) exerted during shortening at any velocity (V) lower than V_{max}. T_0 is the plateau tetanic tension. T_0^* is the intercept on the load axis of the Hill's hyperbolic equation (Hill, 1938). T_1 is the extreme tension attained in response to a step length change. Y_0 (nm half-sarcomere^{-1}) is the amount of step release necessary to decrease the plateau tetanic tension to zero.

Procedure for determining V_{max}

The controlled-velocity release method was used. Releases were imposed first at the tetanus plateau and then at different times after the beginning of stimulation throughout the tetanus rise. Determinations were made by several trials to find the lowest velocity of ramp-release which maintained the tension at zero. When in fact the muscle fibre was allowed to shorten at a velocity lower than V_{max} the tension dropped at a steady value above the zero line according to the force–velocity relation. If the velocity of the ramp release was higher than V_{max} the tension dropped to zero, but there was a delay between the end of the release and the redevelopment of

isometric tension. Finally when the velocity of the ramp corresponded to V_{max} the tension dropped to zero and redeveloped quickly just in correspondence with the end of the release. Indications about the sensitivity of the method are given in frames *a* and *b* of Fig. 2, in which the tension responses to two releases of constant amplitude are shown. It can be seen that when the velocity of the ramp is only 13% greater than V_{max}, then the redevelopment of isometric tension is significantly delayed after the end of the release.

Releases imposed at high isometric tensions were made by a combination of both step and ramp. This reduced the amplitude of tension transients (Huxley & Simmons, 1971) as well as the time during the ramp before the tension became steady. Releases imposed at low initial tensions ($< 2\% T_0$) were not preceded by steps, so that, during the first part of the ramp, force records were complicated by the presence of some transients.

An alternative method for determining V_{max} at high levels of isometric tension is to estimate the intercept on the V axis of the hyperbola fitted to $T-V$ data points. This method is not so useful at low tensions because only a few $T-V$ points can be collected for the fitting of the hyperbola.

In no case was the total amount of shortening imposed on the fibres greater than $6\% l_0$, so that the sarcomere spacings, at the end of the shortening, were not smaller than about 2.1 μm.

Computer analysis

$T-V$ data were fitted by means of Hill's hyperbolic equation (Hill, 1938), using the computer program Minuit (CERN Computer, series 6000) to find T_0^*, a and b by direct searching. T_1 data were fitted by means of a parabola using the same computer program.

Results

Figs. 1–3 refer to an experiment performed in a muscle fibre in which the rise of activation, measured by the development of the $T-V$ relation (Cecchi *et al.*, 1978), was relatively fast. Comparable results were however consistently obtained in all of the five fibres used for the present work, independent of their activation time (Fig. 5, Table 1). It can be seen that the value of V_{max} at the end of the isometric latent period was already the same as at the tetanus plateau, while in accordance with previous work of Cecchi *et al.* (1978) the other $T-V$ data points attained their steady-state values between 40 and 80 ms after the beginning of the stimulus volley.

Frames a–d in Fig. 2 show V_{max} records obtained during a tetanus at a time between 11 and 220 ms after the beginning of the stimulation, i.e. at increasing isometric tensions from $0.02 T_0$ to T_0. It can be seen that at 11 ms or $0.02 T_0$ the value of V_{max} was the same as later at higher isometric tensions and at the plateau.

In an attempt to determine the precise moment at which the muscle fibre became able to shorten at V_{max}, the interval between the beginning of the stimulus volley and the start of the V_{max} release was further reduced and the results are shown in Fig. 3. If a V_{max} release was applied at time intervals between 4 and 9 ms, the muscle fibre developed a small negative force of about 2 kN m^{-2} which was slightly less than $1\% T_0$. This was found to be independent of the release time and constantly recovered to zero at about 10 ms after the beginning of the stimulus volley, i.e. at the end of the isometric latent period.

It was also observed that the same kind of response could be obtained from the unstimulated fibre in both release and stretch ramps (Figs. 3, 4). The possibility that the

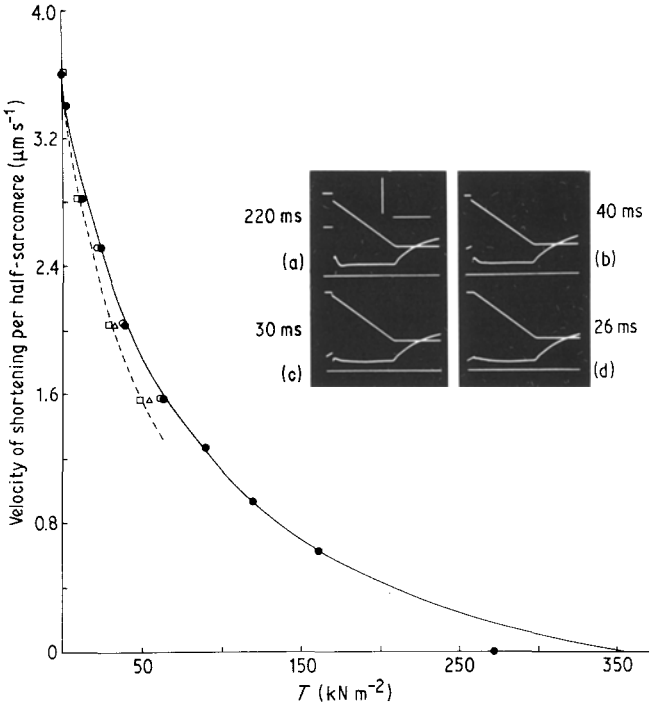

Fig. 1. Force–velocity relations at various times and isometric tensions during a tetanus. Filled circles refer to points obtained at the tetanus plateau, 220 ms after the beginning of stimulation. Open symbols refer to points obtained at 26 ms or 0.27 T_0 (squares), at 30 ms or 0.38 T_0 (triangles) and at 40 ms or 0.58 T_0 (circles). The continuous line was fitted to filled circle data points by means of Hill's hyperbolic equation, according to the procedure indicated in the Methods section. Because of the nonhyperbolic behaviour of the force–velocity relation in the high load region (Edman *et al.*, 1976), the T values greater than 0.7 T_0 were not considered for the computation. The estimated Hill's parameters are: a, 77.04 kN m^{-2}; b, 0.78 μm s^{-1} half-sarcomere^{-1}; T_0^*, 352.93 kN m^{-2}. Note that the intercept of the curve on the V axis is 3.57 μm s^{-1} half sarcomere^{-1} whereas the value of V_{max} measured from the records of Fig. 2 was 3.62 μm s^{-1} half-sarcomere^{-1}. The inset refers to force–velocity points obtained at a velocity of 1.57 μm s^{-1} half-sarcomere^{-1}. Figures at the side of the records indicate the time after the start of stimulation when the release was imposed. In each record the upper trace measures the length change, the middle trace is the tension response, the lower trace is zero tension. Horizontal calibration: 20 ms; vertical calibration: 200 kN m^{-2} or 40 nm half-sarcomere^{-1}. Temperature, 4.7° C; stimulation frequency, 25 s^{-1}; cross-sectional area, 10 014 μm^2; sarcomere length, 2.20 μm; l_0, 6.58 mm.

negative force response was determined by the discharge of a parallel elastic element was excluded because its amplitude was not related to the amplitude of the length change. Moreover, apart from the sign, the initial parts of the passive force responses to release or stretch ramps at the same speeds were comparable. Finally, the viscous coefficient, calculated from the exponential part of the passive response to stretch ramps, ranged in different fibres from 4.1 to 6.1 $\times$ 10^8 Nsm^{-3} half-sarcomere^{-1}, and these values are comparable to those obtained by Ford *et al.* (1977) with stretches 7–8 times faster.

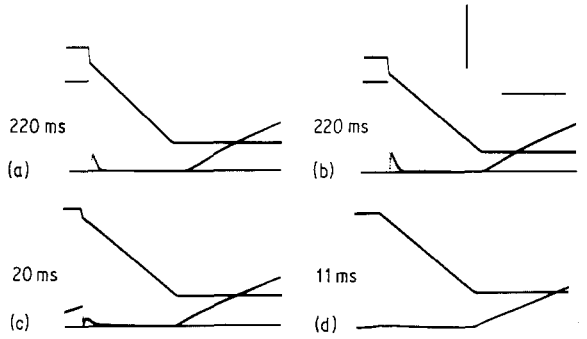

Fig. 2. Same muscle fibre as Fig. 1. Responses to releases imposed at the tetanus plateau (frames a and b) and at different times after the beginning of stimulation when the isometric tension had attained respectively 0.22 T_0 (frame c) and 0.02 T_0 (frame d). In each frame the upper trace measures the length change, the middle trace represents the active tension response and the lower trace (where present) is the resting tension. The velocity of the ramps was 4.08 μm s^{-1} half-sarcomere^{-1} in frame a and 3.62 μm s^{-1} half-sarcomere^{-1} (V_{max}) in frames b–d. Note in frame a the lag between the end of the ramp release and the redevelopment of isometric tension. Figures close to the records indicate the time after the start of stimulation when releases were imposed. Horizontal calibration, 10 ms; vertical calibration, 200 kN m^{-2} or 40 nm half-sarcomere^{-1}.

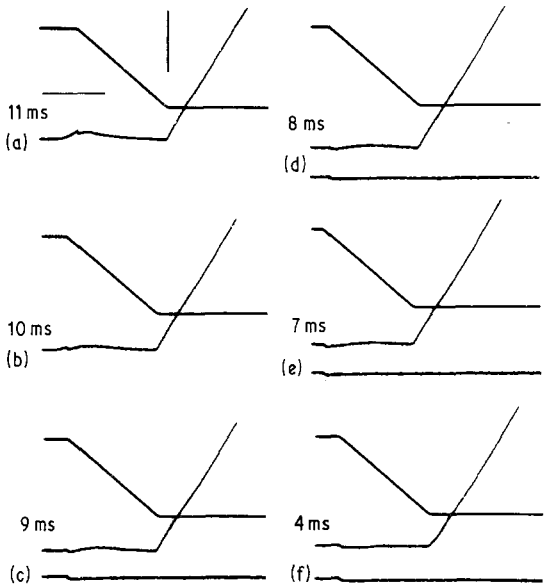

Fig. 3. Same muscle fibre as Fig. 1. In the frames from top to bottom the first trace measures the fibre length, the second trace represents the tension response of the stimulated fibre and the third trace (where present) is the passive tension response of the resting fibre. The vertical sensitivity of the tension traces was four times higher than in Fig. 2. The passive tension response of the resting fibre was determined one minute after the response of the stimulated fibre and was displaced downwards for reasons of clarity. Records refer to ramp releases at the velocity of 3.62 μm s^{-1} half-sarcomere^{-1} imposed at the times after the start of stimulation indicated by figures close to tension records. Record a corresponds to record d in Fig. 2. In record c the release was imposed just before the end of the isometric latent period. Horizontal calibration, 10 ms; vertical calibration, 50 kN m^{-2} or 40 nm half-sarcomere^{-1}.

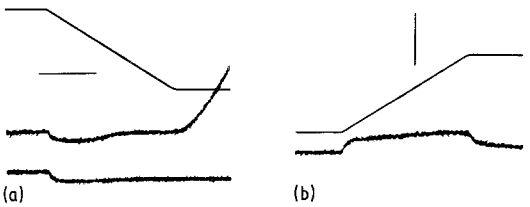

Fig. 4. Tension responses to release (a) and stretch (b) ramps at a velocity of 3.38 μm s^{-1} half-sarcomere^{-1} (V_{max}) in another muscle fibre at 4° C. In either (a) or (b) the top traces measure the fibre length change and the bottom traces measure the passive force response of the unstimulated fibre. In (a) the middle trace measures the tension response to a release starting 5 ms after the beginning of the stimulus volley. Horizontal calibration, 5 ms; vertical calibration, 8 kN m^{-2} or 25 nm half-sarcomere^{-1}; cross-sectional area, 7677 μm^2; sarcomere length, 2.25 μm; l_0, 6.98 mm; T_0, 220 kN m^{-2}. The isometric latent period in this fibre was about 10.6 ms.

The total series compliance of the fibres was measured determining the T_1 relation (Huxley & Simmons, 1971) at the tetanus plateau. Fig. 6 illustrates the results obtained in the same fibre as Figs 1–3, whereas the average values of Y_0 (observed and extrapolated from the linear part of the T_1 relations) for the five fibres used in the course of this work are listed in Table 1. Since the amounts of step release required to drop the plateau tension to zero (8.69 nm half-sarcomere^{-1}, observed, and 5.98 nm half-sarcomere^{-1},

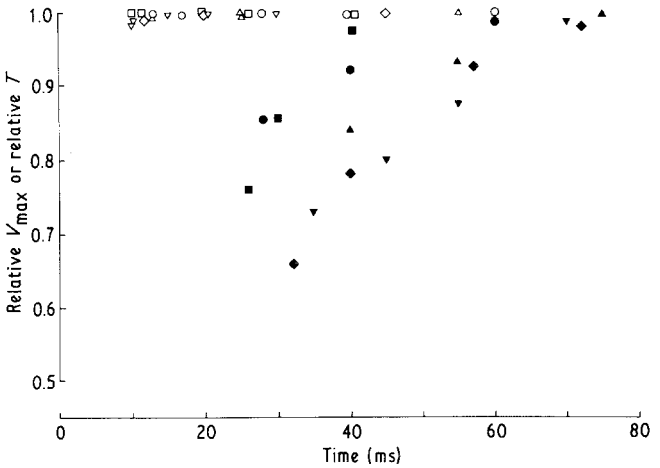

Fig. 5. Time course of V_{max} (open symbols) and of the force–velocity relation (filled symbols) during a tetanic contraction in five muscle fibres. Each symbol refers to a different fibre; squares to the fibre of Figs 1–3 and triangles to the fibre of Fig. 4. The abscissa measures the time after the first stimulus. The ordinate measures (1) the ratio of V_{max} determined at various times during the tetanus rise to V_{max} determined at the plateau (open symbols) and (2) the ratio of the value of T (the steady force exerted during active shortening at the velocity of 1.53 ± 0.03 μm s^{-1} half-sarcomere^{-1}, mean and S.E.M. for the five fibres) determined at various times during the tetanus rise to the value of T determined at the plateau (filled symbols).

Table 1. Means and S.E.M.S for V_{max} at the plateau of the isometric tetanus and for other mechanical characteristics in five muscle fibres at 4.68 ± 0.37° C and at sarcomere length 2.24 ± 0.01 μm.

V_{max} (μm s^{-1} half-sarcomere^{-1})	T_0 (kN m^{-2})	*Isometric latent period* (ms)	*Observed* Y_0 (nm half-sarcomere^{-1})	*Extrapolated* Y_0 (nm half-sarcomere^{-1})
3.30 ±0.09	242.40 ±9.99	10.26 ±0.37	8.69 ±0.14	5.98 ±0.08

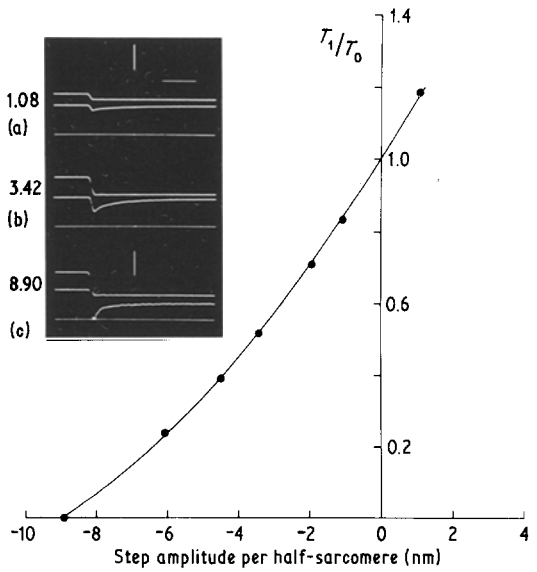

Fig. 6. T_1 relation determined at the tetanus plateau in the same muscle fibre as Figs. 1–3. The curve fitted to the data points was drawn according to the procedure described in the Methods section. In the inset the upper trace measures the length change, the middle trace is the tension response and the lower trace is the resting tension. Figures close to the records indicate the amount of step length change in nm half-sarcomere^{-1}. In this fibre the amount of step release required to drop the isometric tension to zero was 8.9 nm half-sarcomere^{-1} (observed) or 6.2 nm half-sarcomere^{-1} (extrapolated from the linear part of the T_1 relation). Horizontal calibration, 1 ms; vertical calibration, tension, 240 kN m^{-2}; length, (a and b) 5 nm half-sarcomere^{-1}, (c) 10 nm half-sarcomere^{-1}.

extrapolated) are comparable to those reported by Ford *et al.* (1977) for fibres at about the same temperature and under length clamp conditions, it can be concluded that the passive series compliance of the fibres used here was very small.

Discussion

The main result of the present work is that at the end of the isometric latent period (at 4.5° C about 10 ms after the beginning of the stimulus volley) the value of V_{max} is already the same as later at higher isometric tensions during the tetanus rise or at the plateau. In other words the latency time of tension development is the same as that required by the muscle fibre to become able to shorten at V_{max}. An obvious consequence of this result is that different segments along a muscle fibre begin to shorten simultaneously. Any difference in the latency times for the capability of various fibre segments to shorten at V_{max} should in fact result in an appreciable delay of the recovery of isometric tension with respect to the end of the ramp release, but this is not the case. The finding described above, moreover, confirms and extends results of previous work on this subject (Hill, 1951; Abbott & Ritchie, 1951; Cecchi *et al.*, 1978, 1981; Edman, 1979) and agrees with the view that in intact fibres the value of V_{max}, at the end of the latent period, is independent both of the concentration attained by activating Ca^{2+} at the level of myofilaments (assuming no high local Ca^{2+} concentrations) and of the degree of activation of the contractile substratum. In terms of a crossbridge model of contraction (Huxley, 1957), this suggests that while the concentration of activating Ca^{2+} ions at the level of myofilaments and consequently the number of actin sites available for crossbridge formation require a significant time to attain their steady-state values (Podolsky & Teichholz, 1970; Thames *et al.*, 1974), crossbridge turnover is independent of time.

Further indirect evidence that in intact fibres V_{max} is not critically dependent on myoplasmic Ca^{2+} concentration is given by the following observation. If allowance is made for the differences in experimental temperatures, the interval of 10 ms, which is the isometric latent period at the end of which the muscle fibres is already able to shorten at V_{max}, is substantially shorter than the time required by Ca^{2+} concentration to attain its peak value in a twitch (Jöbsis & O'Connor, 1966; Rüdel & Taylor, 1973; Taylor *et al.*, 1975; Miledi *et al.*, 1977; Baylor *et al.*, 1982; Miledi *et al.*, 1982).

Another point concerns the development of a negative passive force by the muscle fibre, in response to V_{max} releases. It is very likely that this force is a viscous phenomenon. Certainly the observation that the value of the coefficient of viscosity of the resting fibre is comparable to that reported in the literature (Ford *et al.*, 1977) agrees with this view, whereas the absence of recovery to zero force at the end of the V_{max} release is probably attributable to the fact that releases were imposed on the fibres near slack length. The mechanism that underlies the viscous behaviour could be due to a fraction of crossbridges attached at rest (Hill, 1968), which are responsible for a frictional resistance to sliding between the thick and thin filaments. If this view is correct, the

moment at which the negative passive force recovers to zero during a V_{max} release starting before the end of the isometric latent period, should indicate the moment at which the crossbridges become able to cycle. The fact that this moment corresponds to the end of the isometric latent period is not readily explained by the possiblity that the negative force is balanced by a positive force developed by the fibre as soon as it becomes active. The negative force disappears while a shortening at V_{max} is imposed on the fibre, i.e. under conditions that should keep at zero the force developed at the tetanus plateau. Thus under these conditions, the recovery to zero represents a genuine fall of resistance to shortening. Moreover, the finding that V_{max} is the same at very low levels of isometric tension as at the tetanus plateau confirms that, in the active fibre, the load becomes very small.

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