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THE MAXIMUM SPEED OF SHORTENING IN LIVING AND SKINNED FROG MUSCLE FIBRES

BY F. J. JULIAN, L. C. ROME*, D. G. STEPHENSON† AND S. STRIZ

From the Department of Anesthesia Research Laboratories, Harvard Medical School, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02115, U.S.A.

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SUMMARY

1. This study was performed to determine whether V_{iso} (the maximum speed of shortening extrapolated from force–velocity curves) equalled V_u (the unloaded speed of shortening determined by the slack test) in both living fibres from *Rana temporaria* and mechanically skinned fibres from *Rana pipiens*.

2. In living fibres (*R. temporaria*) we obtained improved estimates of V_{iso} by performing force clamps (isotonic) and length ramps (isovelocity) down to very low loads (0·005 isometric tension, P_0). Force–velocity characteristics determined by force clamps and length ramps were the same. The hyperbolic Hill curves deviated from the force–velocity data at both high and low loads and underestimated V_{iso} by varying degrees. A better estimate of V_{iso} was obtained by linear extrapolation of data at loads from 0·005–0·02 P_0 and the mean V_{iso} at 7·5 °C was 4·08 muscle lengths/s ± 0·11 (mean ± S.E., $n = 14$).

3. Improved estimates of V_u in living fibres were obtained by photographically calibrating the slack test. The mean V_u was 4·05 muscle lengths/s ± 0·13 (mean ± S.E., $n = 14$) and the intercept was 0·0156 fibre lengths (L_0) ± 0·0013 (mean ± S.E., $n = 14$).

4. The step-ramp photographic method, in which the motor speed is matched to V_u, was developed as an independent way to measure V_u in living fibres. V_u measured in this way agreed well with V_u measured by the slack test.

5. In all living fibres, the improved estimates of V_u agreed well with the improved estimates of V_{iso}. $V_u/V_{iso} = 0·99 \pm 0·01$ (mean ± S.E., $n = 14$).

6. In mechanically skinned *R. pipiens* fibres, force clamps were performed down to loads of 0·01 mN. The force–velocity curve of the skinned fibres differed in shape from that of the living fibres. Although there was significant deviation from the Hill equation at low loads, the data at high loads were well fitted by the Hill curve. V_{iso} determined by extrapolating the Hill equation to zero load was 5·87 muscle lengths/s ± 0·38 (mean ± S.E., $n = 9$) at 7·5 °C. In five fibres, the linear extrapolation of low loads (0·01–0·05 P_0) showed that the Hill equation underestimated the true V_{iso} by 6 %.

* Present address: Department of Zoology, University of Tennessee, Knoxville, TN 37996, U.S.A. To whom correspondence should be addressed.

† Present address: Department of Zoology, LaTrobe University, Bundoora 3083, Australia.
The authors' names are in alphabetical order.

7. The slack test with mechanically skinned fibres was calibrated by taking a series of photographic exposures of the fibre at various times following each length step. $V_u = 6{\cdot}12$ muscle lengths/s $\pm 0{\cdot}44$ (mean $\pm$ S.E., $n = 10$) and the intercept was $0{\cdot}0585\ L_0 \pm 0{\cdot}0069$ (mean $\pm$ S.E., $n = 10$). In several fibres the step-ramp photographic method confirmed the values obtained by the slack test.

8. At 80–100% activation (pCa = 5·5–4·4), V_u nearly equalled V_{iso}. $V_u/V_{iso} = 1{\cdot}05 \pm 0{\cdot}03$ (mean $\pm$ S.E., $n = 10$). In fibres where V_{iso} could be determined by linear extrapolation to low loads, $V_u/V_{iso} = 0{\cdot}99 \pm 0{\cdot}018$ (mean $\pm$ S.E., $n = 5$). There also appeared to be good agreement between V_u and V_{iso} in submaximally activated fibres (pCa = 6·0–6·2).

9. We conclude that there is a unique maximum speed of shortening in single fibres. Discrepancies previously observed between V_u and V_{iso} were probably due to inaccuracies in the estimation of these parameters.

INTRODUCTION

An intrinsic property of muscle is its ability to shorten when activated and maximum speed of muscle shortening is thought to be related to the rate of cross-bridge cycling. There are, however, large discrepancies in the values of maximum speed of shortening obtained with different techniques.

One estimate of this important parameter is obtained by measuring the unloaded speed of shortening in which the activated muscle is rapidly released to a slack length where no external force is exerted on the fibre. The velocity at which it shortens, V_u, is typically assessed by the 'slack test' (Hill, 1951; Edman, 1979; Julian & Moss, 1981). Various amounts of imposed slack are plotted against the times required for the muscle to take them up (t_s; determined from the force record) and V_u is estimated from the slope of this representation.

The other, more traditional, method to estimate the maximum speed of shortening is to allow the muscle to shorten against constant loads (isotonic) or at constant velocities (isovelocity) and determine the force–velocity characteristics of the muscle over a range of loads from 0·05–0·8 isometric tension (P_0) (Hill, 1938). The maximum speed of shortening in the limit as the load approaches zero (V_{iso}) is typically estimated by fitting the Hill equation to the force–velocity data by various means and then extrapolating this equation to zero load (Hill, 1938; Edman, Mulieri & Scubon-Mulieri, 1976; Lännergren, Lindblom & Johansson, 1982).

Previous estimates of V_u have exceeded those of V_{iso} by 5–30% in living fibres (Edman, 1979; Julian & Moss, 1981; Lannergren *et al.* 1982) and by up to 50% in skinned fibres (Julian & Moss, 1981; Goldman, 1983). Several possible causes for this discrepancy are: (1) the slack test as described above does not give an accurate estimate of V_u due to uncertainty in determining t_s; (2) extrapolation from the Hill equation does not provide an accurate estimate of V_{iso} because the shortening velocity at very light loads may deviate from the Hill equation; or (3) there is a discontinuity in the force–velocity relationship at zero load and V_u may actually exceed V_{iso}. The resolution of this problem necessitated re-examination of the methodologies used to determine V_u and V_{iso} and more accurate estimations of these parameters.

As a first approach to the problem, we used living *Rana temporaria* fibres because they generate large forces and provide many repeatable contractions which allowed

us to compare various techniques for measuring the maximum speed of shortening on the same fibre. Improved estimates of V_u were obtained by developing new photographic techniques, while improved estimates of V_{iso} were obtained by measuring force–velocity relations during isotonic and isovelocity contractions at very low loads and by using new curve-fitting procedures. No difference was observed between these improved estimates of V_u and V_{iso}.

As a second approach we applied these techniques to mechanically skinned fibres of *Rana pipiens*. Again, we found no differences between V_u and V_{iso}. These results strongly suggest that muscle fibres have a unique maximum speed of steady shortening and that the previous discrepancies noticed between estimates of V_u and V_{iso} were mainly due to limitations in the techniques used.

Preliminary reports of these findings have been presented at meetings of the (American) Biophysical Society (Rome, Striz & Julian, 1984; Rome, Striz, Stephenson & Julian, 1985).

METHODS

Living fibres (R. temporaria)

Preparation

Twitch fibres, together with pieces of tendon at either end, were dissected from anterior tibialis muscles of frogs (*R. temporaria*) which had been stored before use in a moist environment at about 4 °C. The dissection was carried out at room temperature, under a stereomicroscope equipped with a dark-field illuminator, in a Ringer solution, using 27 gauge hypodermic needles (o.d. 0·016 in). The Ringer solution contained (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; Na_2HPO_4, 2·15; NaH_2PO_4, 0·85, pH 7·2. A small hole was cut in each tendon and transfer to the experimental chamber was carried out in a small glass spoon filled with Ringer solution.

Experimental apparatus

The experimental chamber and servo system were the same as previously described (Julian & Morgan, 1979) except for several modifications. First, a Cambridge Technology (Model 400) capacitance force transducer was used that had a resonance frequency of 2 kHz and a sensitivity of 0·2 V/mN. The transducer did not exhibit creep as we demonstrated by exerting tension on it through a fine thread attached to the servomotor and then rapidly dropping the tension to zero by stepping the motor. Secondly, the motor and force transducer were attached to the chamber through shock mountings that eliminated the ringing of the force transducer caused by the transmission of forces of the motor through the chamber during a quick length step. Thirdly, under force control, positive feed-back of the length signal was used to counteract the restoring force generated by the spring in the General Scanning motor.

All force and length signals were recorded on a Nicolet 4099 digital oscilloscope and were stored on floppy disks. The records were analysed using the Nicolet and where indicated, smoothing and differentiation of records were performed with programs supplied by the manufacturer.

Photomicroscopy

Active and passive sarcomeres were photographed at a magnification of 460 × with a Zeiss 40UD objective. The photographs were taken with an open shutter using a triggerable Xenon flash (Zeiss, flash duration, 0·5 ms). Photographs of the entire fibre length were taken with a 5 × eyepiece, 2·5 × objective, a shortened microscope tube, and with the condenser removed. The photographs were taken with either Polaroid 107 or Polaroid 665 film.

Measurement of V_{iso}

Both force clamps (isotonic shortening) and length ramps (isovelocity shortening) were used to define the force–velocity characteristics of the fibre. To improve the extrapolation of V_{iso}, many contractions were made at low loads 0·005–0·05 P_0. The experiments were conducted at sarcomere

lengths from 2·2–2·0 μm where the resting tension was extremely low (approx. 0·002 P_0) and its subtraction from the force records was usually not necessary. In length ramps where the tension was below 0·005 P_0, the force record from a passive contraction was digitally subtracted from that during an active contraction to give a more accurate record of active tension. In both types of contraction the fibre was released during the plateau of the tetanus to either a new force level or a fixed velocity. During isovelocity releases the ramp was preceded by a short length step to unload the series elastic component. We found that at speeds corresponding to loads above 0·4 P_0 it was difficult to adjust the initial length step accurately so length ramps were generally not done over this range of speeds.

V_{iso} was estimated from the force–velocity data by four different methods. First, the linearized version of the Hill equation (1938) $(P/P_0+a/P_0)(V+b) = (1+a/P_0)b$, was used, where V is velocity and the Hill constants, a/P_0 and b, were determined by linear regression of $(1-P/P_0)/V$ *versus* P/P_0. V_{iso}, the speed of shortening at $P = 0$, was equal to $b(P_0/a)$. In the second approach, the data were fitted by the Hill equation in an unbiased fashion by means of a computer program (written in collaboration with D. L. Morgan) in which the mean square perpendicular distance between data points and the curve was minimized. The curve was defined by two parameters, V_{max} and a/P_0. In the third approach, the same program was used except the constraint that the curve pass through the measured isometric tension was removed and the data were fitted by the Hill equation determined by three parameters P_0^*, a/P_0^*, and V_{iso}, where P_0^* was the intercept on the load axis of the curve extrapolated to $V = 0$. Fourthly, velocity values over the range of loads of 0·02–0·005 P_0 were fitted by linear regression, and V_{iso} was estimated by extrapolation to $P = 0$.

Measurement of V_u

Slack test. Slack tests were performed as previously described (Hill, 1951; Edman, 1979; Julian & Moss, 1981) except that the fibres were always released to the same length. Thus 1 min prior to the contraction, the fibre was stretched out to various lengths by the motor, and then during the plateau of the tetanus, it was rapidly (<1 ms) shortened down to the original length. This modification enhanced the similarity of the force records from different sized steps and facilitated photographic calibration. Typically, ten different length steps were used for a slack test.

Photographic calibration of slack test. To determine the actual t_s, photographs of the whole fibre were taken (on Polaroid 665 film) at different times following a length step. Since only one photograph was taken after each step, five–seven contractions were needed to calibrate a given length step (Pl. 1). The fibre was released to a length close to the slack length (approx. sarcomere length = 2·05 μm). Comparison of the pictures (enhanced by superposition of 10 × 8·5 cm negatives) of the fibre at various times after a length step to a picture of the passive fibre at that same length, enabled us to determine whether the fibre had taken up the slack.

Step-ramp photographic method. As the value of V_u estimated by the slack test varies with the criteria used for t_s (see Results), V_u was also determined by a completely independent technique. The fibre was given a small step to induce a small amount of slack (unloading the fibre) and then the motor arm was moved at a fixed rate. If the motor arm moved faster than V_u, more slack would be induced. If the fibre shortened faster than the motor arm, then the amount of slack would be decreased. From comparison of the photographs, an upper and lower limit for V_u could be determined (Pl. 2).

Protocol. The fibre was secured to wire from the force transducer and motor by 10–0 nylon monofilament (Ethilon, diameter 23–25 μm). The fibre was orientated, so that when released, it bent in the horizontal plane. The active sarcomere length was calibrated in terms of the micrometer setting as previously described (Rome, Morgan & Julian, 1985). The sarcomere length of the fibre was then set at 2·2 μm, and a number of measurements were made generally starting with a slack test, followed by length ramps, force clamps, slack test calibration, and step-ramp photographic method. Typically three or four of these tests were done on a given fibre. All measurements were done at 7·5 ± 0·2 °C and all speeds were normalized to muscle length measured at sarcomere length of 2·1 μm.

Skinned fibres (R. pipiens)

Preparation

Mechanically skinned fibres from the iliofibularis muscle of *R. pipiens* were used because the *R. temporaria* fibres were of greater diameter, and therefore did not activate quickly (> 5 s), possibly

leading to sarcomere non-homogeneity (Julian & Moss, 1981). The fibres were mechanically skinned under paraffin oil according to the procedure described by Moisescu & Thieleczek (1978). A segment of skinned fibre was initially tied to the titanium wires of the motor arm and the transducer with loops 10–0 monofilament. The segment was then aligned parallel to the wires and finally was securely attached as close as possible to the ends of the wires with two double overhand knots of polyfilament surgical silk 9–0 (Deknatel) so as to provide a wider (0·15–0·20 mm) contact area.

Experimental apparatus

The experimental apparatus used for the skinned fibre experiments differed from that used for the living fibres in several ways. The sensitivity of the force transducer was increased to 2 V/mN. The gain of the servo was altered depending on the level of activation and force level of the contraction. The chamber was similar to that described by Julian & Moss (1981). It contained a wide shallow well which facilitated the mounting of the fibre and four other wells with a capacity of about 1 ml in which the fibre could be immersed.

Solutions

The compositions of the bathing solutions used for the skinned fibres are shown in Table 1. Solutions of type A, B, H were prepared using the methods previously described by Ashley & Moisescu (1977); Moisescu & Thieleczek (1978) and Stephenson & Williams (1981). Free-Mg^{2+} concentration in the solutions was close to 1 mM. Total Mg was calculated using apparent affinity constants of cations to the various ligands measured for a similar set of conditions (Stephenson & Williams, 1981). At low levels of activation, solutions contained caffeine (see Table 1) in order to reduce Ca^{2+} movements associated with sarcoplasmic reticulum (Moisescu & Thieleczek, 1978).

Total and excess free EGTA was determined by titration in all batches of solutions A, B, H (see Stephenson & Williams, 1981). Solutions of various Ca^{2+} concentrations lower than 10^{-5} M were obtained by mixing in different proportions solutions of type A and B. pCa was calculated as in Stephenson & Williams (1981). The fibres were soaked in pre-activating solution for 5 min, then moved into activating solution and at the end of shortening placed in relaxing solution (Fig. 1).

Photomicroscopy

Passive sarcomere length was measured using the same system of lenses as described for the intact fibres. Active sarcomere length was not routinely measured because we felt that the long period needed for focusing after a rapid activation, would lead to sarcomere non-homogeneity and permanent damage to the fibre. A series of superimposed images of the fibre at various times following a length step were produced with an open shuttered camera and multiple flashes from a triggerable strobe (EGG no. PS302) (calculated duration of the flash was 20 μs).

Measurement of V_{iso}

In the skinned-fibre experiments, force clamps were used exclusively because constant force was not maintained during length ramps. The change to a new constant force level was generally attained in under 5 ms, and this performance required adjustment of the gain of the servo system to the load and level of activation. It was not possible to achieve clamps at as low *relative* loads as attained with the intact fibre, since the force generated by the smaller diameter *R. pipiens* fibres during full activation was considerably smaller than that developed by the intact *R. temporaria* fibres. Moreover, P_0 was further reduced during contractions at partial activation. Another problem was the 'zero' force level measured by the force transducer was altered slightly, as the fibre was switched between pre-activating, activating and relaxing solution (Fig. 1); by surface tension (as previously noted by Ferenczi, Goldman & Simmons, 1984). This problem was circumvented by fully unloading the fibre with a quick length step at the end of the force clamp (see Fig. 6*B*) while still in the activating solution, in order to obtain a better estimate of zero tension (see Fig. 6*C*). The effect of resting tension on results was also minimized. At full levels of activation, experiments were conducted over sarcomere lengths between 2·3 μm–1·8 μm where the passive resting tension was less than 0·01 P_0. At partial levels of activation, the experiments were conducted at sarcomere length of 2·1–1·8 μm where resting tension was negligible. We were thus able to clamp and discern force levels of about 0·01 mN (1 mgw) representing 2–5 % P_0 at full activation and 10–20 % P_0 at partial activation.

TABLE 1. Composition of bathing solutions for skinned fibres.

Solution	ATP total (mM)	Mg total (mM)	Mg^{2+} (mM)	CP (mM)	CK (u./ml)	HDTA (mM)	EGTA (mM)	CaEGTA (mM)	TES (mM)	Cl (mM)	Na (mM)	K (mM)	Caf (mM)	pH*
A	8	8·0	1	10	15	—	—	50	70	16	36	123	10†	7·10
B	8	9·2	1	10	15	—	50	—	70	18·5	36	124	10†	7·10
H	8	8·4	1	10	15	49·85	0·15	—	70	17	36	124	10†	7·10

Abbreviations: HDTA, hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate; EGTA, ethyleneglycol bis (β-aminoethylether)-*N*,*N*′-tetraacetate; TES, 2(2-hydroxy-1,1 bis (hydroxymethyl) ethyl) aminoethane sulphonic acid; ATP, adenosine-5′-triphosphate; CP, creatine phosphate; CK, creatine phosphokinase; Caf, caffeine.

A is the high calcium solution. B is the relaxing solution. H is the pre-activating solution. Activating solution was formed by mixing A and B in various proportions.

* pH was carefully adjusted to 7·10 ± 0·01 at 7·5 °C.

† Used only in solution when desired pCa > 5·9.

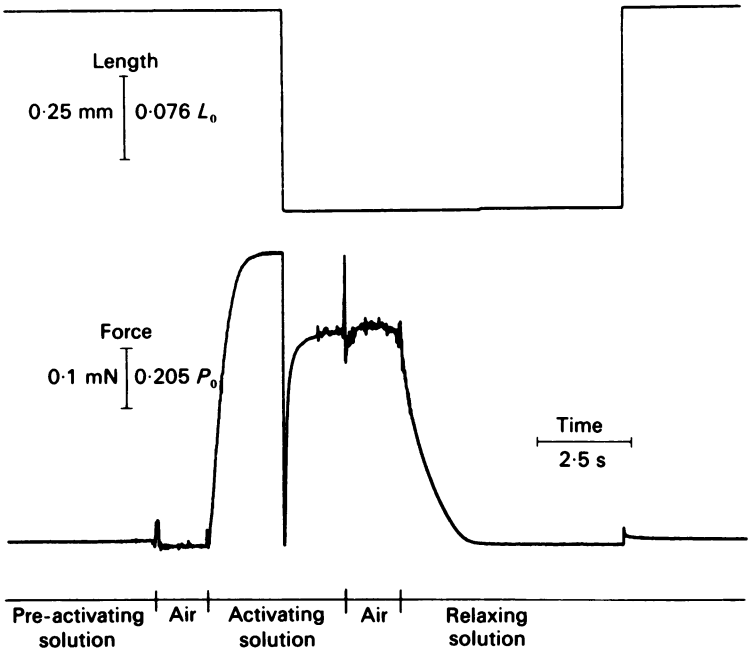

Fig. 1. Activation–relaxation procedure of a skinned fibre (*R. pipiens*, m. iliofibularis). Length and tension records obtained at a slow time scale of the low load force clamp shown in Fig. 6. The fibre was soaked in a pre-activating solution, then transferred to the activating solution, which resulted in a rapid activation (i.e. maximum tension was achieved in approximately 1 s). At the completion of the force clamp, the fibre was transferred to the relaxing solution. The zero force level could be easily determined in the wells containing pre-activating and relaxing solutions, because the fibre was relaxed. The zero force level determined in these wells, however, may differ from that in the activating well due to surface tension differences. It was therefore, necessary to give the fibre a length step at the end of shortening (Fig. 6*B*), so that zero force could be determined in the well containing activating solution. Fibre length was 3·3 mm at 2·1 μm, pCa = 5·2.

Measurements of V_u

Slack test. In the skinned-fibre experiments, the fibres were again released from various lengths down to the same length. Instead of being pre-stretched by the motor arm, the fibres were stretched to various lengths by the micrometer prior to activation. Releasing the fibres down to the same length in each contraction is important, especially at partial activation, because sarcomere length influences the force–pCa relationship (Stephenson & Williams, 1981). Due to the low force redevelopment at partial activation, photography was especially helpful in determining t_s. Since a skinned-fibre preparation gives only a limited number of repeatable contractions, a series of superimposed photographic images of the fibre following a single release were used for calibration (see *Photomicroscopy* and Pl. 3). Due to the limited number of contractions, only three or four different length steps were used for a slack test instead of the ten used in living fibres.

Protocol. The skinned fibre was mounted such that it bent in a horizontal plane when given a rapid length step. After mounting, the fibre was placed in a relaxing solution containing a low concentration of a non-ionic detergent (0·05 % Brij) to wash away remaining paraffin oil. The fibre was contracted at submaximal activation and observed through the microscope to look for non-uniform activation. Passive sarcomere length as well as resting tension were measured as a function of fibre length. The slack test, force clamps at light loads, and force clamps at heavy loads were then performed at 7·5 °C. Experiments were terminated when the fibre deteriorated in any of the following ways: (1) isometric force decreased by more than 20 %; (2) activation became

non-uniform; or (3) the series elastic component increased to a level where slack tests and force clamps could not be performed reproducibly. Twelve to twenty contractions were obtained from fibres during successful experiments.

RESULTS

Living fibres (R. temporaria)

V_{iso}. We were routinely able to obtain constant force and velocity records by both the length ramp and the force clamp methods at loads as low as 0·005 P_0 (Fig. 2). On occasion we obtained isovelocity records at lower loads (0·002 P_0) and digitally subtracted the force record of a passive length ramp from that of an active one. At all loads studied from near 0 to about 0·4 P_0, the force–velocity characteristics measured from isovelocity and isotonic contractions were the same (Figs. 2 and 3).

The force–velocity data were hyperbolic between loads of about 0·04–0·8 P_0. At high loads, as previously described by Edman *et al.* (1976), the velocities deviated from the classical Hill hyperbola (see Fig. 3*A*). When a hyperbola was fitted to the data that was not constrained to go through P_0 (continuous curve, loads above 0·8 P_0 and below 0·05 P_0 were not included in the regression) the extrapolation to the load axis averaged 1·39 $P_0 \pm$ S.E. = 0·02 ($n = 6$) agreeing well with the value of 1·32 P_0 determined by Edman *et al.* (1976). The a/P_0^* value (see Methods) of this curve was 0·216 ± S.E. = 0·02 ($n = 6$), also fairly typical of that found by others (Edman *et al.* 1976). When the hyperbola was constrained to pass through P_0 (dashed curve), then it did not fit the data well over any region of the curve. It fell below the data points at loads less than 0·11 P_0 and between 0·5 P_0 and 0·85 P_0 and was significantly above the data points at the other loads. The a/P_0 of this classical Hill curve was on average 0·52 ± S.E. = 0·06 ($n = 7$) which is considerably higher than usually reported. The poorness of the hyperbolic fit is further demonstrated when the data is plotted in linearized form of the Hill equation (Fig. 3*C*). The plot indicates a very non-linear relationship which resembles Fig. 3 in Edman *et al.* (1976). This curve fitting procedure also gave very high values for a/P_0 (0·60 ± S.E. = 0·06, $n = 7$).

At low loads (Fig. 3*B*) the data deviated from both hyperbolas. At loads below 0·04 P_0, velocities exceeded those predicted by the curve not constrained to pass through P_0. By constraining the curve to go through P_0, the fit at low loads was worse, and data deviated significantly from the unbiased Hill curve at an average value of about 0·07 P_0, and from the linearized Hill curve ($0{\cdot}05 < P/P_0 < 0{\cdot}6$ included) at even higher loads. Since we were able to obtain repeatable data at very low loads, the most accurate measure of V_{iso} was attained by simple linear extrapolation of the data ($0{\cdot}005 < P/P_0 < 0{\cdot}02$) to zero load (continuous line Fig. 3*B*). Using this technique we found that in living *R. temporaria* fibres, V_{iso} was 4·08 muscle lengths (m.l.)/s ± S.E. = 0·11 ($n = 14$). When high loads (i.e. $> 0{\cdot}8$ P_0) and low loads ($< 0{\cdot}05$ P_0) were excluded, the hyperbola not constrained to go through P_0 underestimated V_{iso} by about 7% (V_{iso} estimated unconstrained hyperbola)/V_{iso} = 0·93 ± S.E. = 0·01, $n = 6$).

V_u. A basic problem that we encountered trying to analyse the slack test was determining which point on the force record corresponded to the time at which the imposed slack was taken up (t_s). t_s is usually taken as the point at which force increases

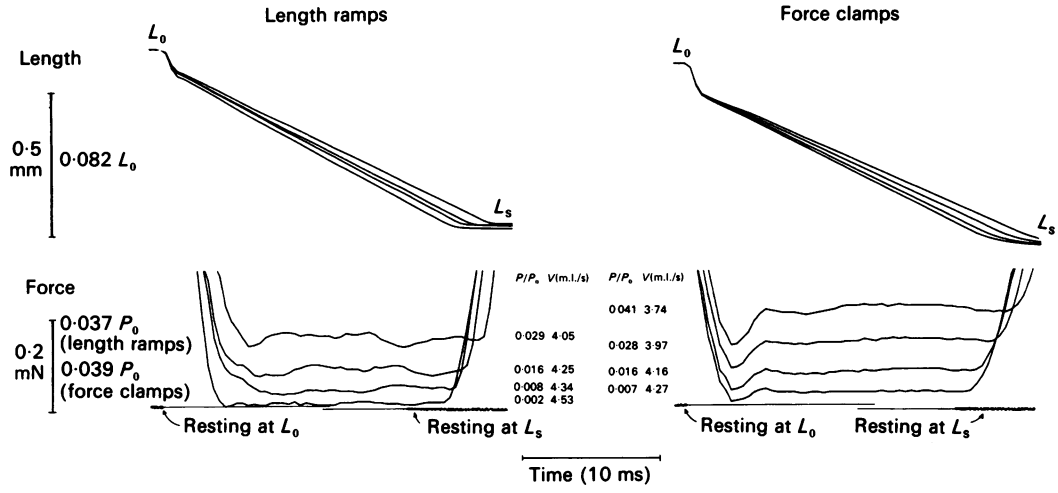

Fig. 2. Length ramps and force clamps at low loads. Length (top) and tension (bottom) records obtained from a single living fibre (*R. temporaria* m. tibialis anterior) during the length ramps (left) and the force clamps (right). The fibre was released about 300 ms into the contraction after steady isometric force had been reached. The relative force (P/P_0) and velocity (V, in muscle lengths/s, m.l./s) for each contraction is tabulated. As can be seen, these two techniques agree very well. The fibre was released from an initial sarcomere length (s.l.) of 2·2 μm (L_0) to a final s.l. of 2·0 μm (L_s). The resting tensions at L_0 and at L_s are shown. The resting force was about 0·001 mN at L_0. Fibre length was 6·1 mm at 2·1 μm. Maximum isometric tension was 5·4 mN.

above the base line, but this criterion can be ambiguous. Record *A* (Pl. 1) shows a length step and *B* shows the force record. As can be seen, the force appears to remain constant for some time after the step and then increases at a point between arrows 3 and 4. In *C* and *D* this same force record is shown at a greater sensitivity and it appears that the point at which the force increases above the base line is now between arrows 1 and 3 depending on the amplification. Taking the different estimates for t_s in a series of records resulted in quite different (approximately 50 %) estimates of V_u. To determine the real t_s, photographs (Pl. 1) were taken at various times (indicated by arrows) following repeated length steps. In photographs 1 and 2, the fibre is clearly slack. By examining the photographs we determined that the slack is taken up between arrows 3 and 4 (23–25 ms). It should be noted, that although it is relatively easy to exclude times as being too short because the fibre is clearly slack, it is more difficult, from just the pictures, to determine exactly when t_s occurs. This is because fibres can have a natural curvature in them, and they might not become completely straight until a sizeable force is generated. The fibres were thus compared to resting fibres under nearly zero tension. We found that t_s measured by photography corresponded to the time at which the rate of force development was 5–10 P_0/s as can be seen in the derivative of the force record (*E*). This same correlation was found in large and small fibres and after long and short length steps. Using this criteria for t_s, the slack test appeared to be linear (Fig. 4). The mean V_u obtained from fourteen fibres was 4·05 m.l./s ± S.E. = 0·13, and the mean intercept, a measure of the series elasticity, was 1·56 % fibre length (L_0) ± S.E. = 0·13 %.

A step-ramp photographic method further verified our method for performing the slack test. Photographs were taken at 1 ms after the step and 2–3 ms before the end of the ramp (Pl. 2). In Pl. 2*A* and *B*, the photographs show that the slack increased with time and therefore the velocity of the motor arm was greater than V_u. At lower speeds (*C* and *D*), the slack was reduced and tension was generated during the ramp

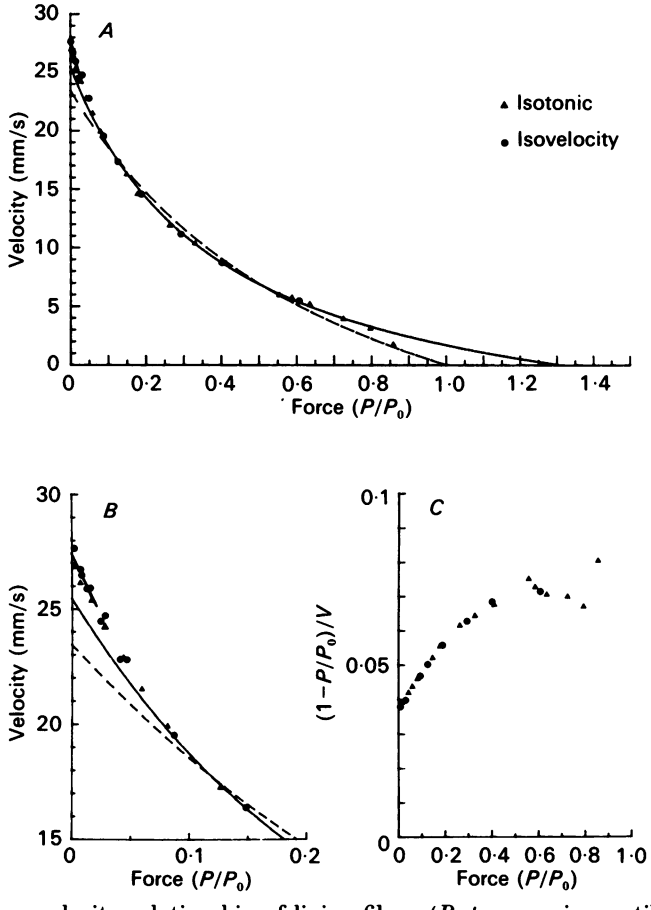

Fig. 3. Force–velocity relationship of living fibres (*R. temporaria*, m. tibialis anterior). *A*, the velocity as a function of normalized force is plotted from both length ramps (●, isovelocity) and force clamps (▲, isotonic). Both techniques provide the same result. Length ramps were not done at loads larger than 0·6 P_0, because it was difficult to adjust the magnitude of the initial step to give constant force generation. The continuous curve represents the unbiased Hill equation not constrained to pass through P_0, the dashed curve represents the unbiased Hill equation constrained to pass through P_0, and the continuous line represents linear extrapolation of loads from 0·005–0·02 P_0 to zero load. Note that many superimposed points at low loads were omitted for clarity. *B* shows an expanded view of the data and the curves at low loads (0–0·2 P_0). Note that the data clearly falls above both Hill curves at low loads, and that the linear extrapolation of loads from (0·005–0·02 P_0) significantly exceeds the values of V_{iso} extrapolated from the Hill curves. *C* shows the linearized Hill equation plot of the data (Hill, 1938). Note that if the data followed the Hill equation constrained to pass through P_0, then the data should fall on a straight line. Initial s.l. was 2·2 μm and final s.l. was 2·0 μm during measurements of V_{iso}. Fibre length was 6·9 mm at 2·1 μm. P_0 was 1·35 mN.

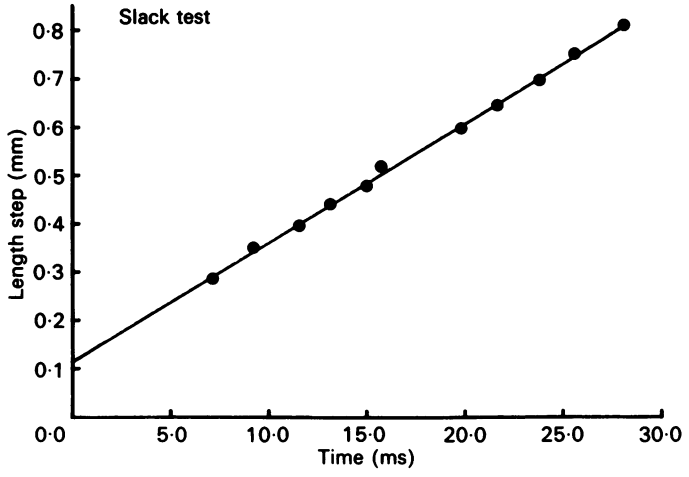

Fig. 4. Slack test of single intact fibre (*R. temporaria*, m. tibialis anterior). Plot of length steps *versus* t_s. t_s was determined at the point where the force was redeveloped at a rate of between 5 and 10 P_0/s. The regression equation for these points is step length (mm) = 24·5 mm/s $\times t_s$ + 0·114 mm, r^2 = 0·999. Fibre length was 6·1 mm at 2·1 μm.

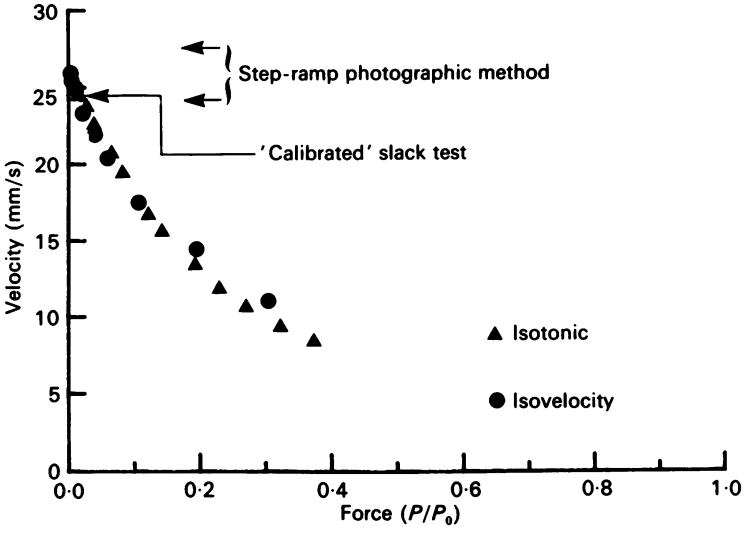

Fig. 5. The maximum speed of shortening of living fibre determined by four methods. The force–velocity characteristics of the fibre (*R. temporaria*, m. tibialis anterior) determined from length ramps (●, isovelocity) and force clamps (▲, isotonic), as well as V_u determined from the photographically calibrated slack test indicated by arrow (see also Fig. 4), and the step-ramp photographic method (range indicated by arrows, see also Pl. 2). As can be seen, estimates of V_{iso} from the length ramps and force clamps, agree well with the estimate of V_u from the slack test, and all are within the range of values of V_u set by the step-ramp photographic method. All contractions were performed between s.l. of 2·2–2·0 μm. Same fibre as Fig. 4 and Pl. 2.

and thus V_u was greater than the motor arm velocity. Note also the rate of tension redevelopment at the end of ramp (signified by vertical line) was much higher in *C* and *D* than in *A* and *B*. The results of this experiment indicate that V_u was between 24·2 and 27·5 mm/s, which bracket the value determined from the slack test (Fig. 5). In all cases, when this comparison was made ($n = 5$), V_u estimated from the slack test fell within the limits defined by the step-ramp photographic method.

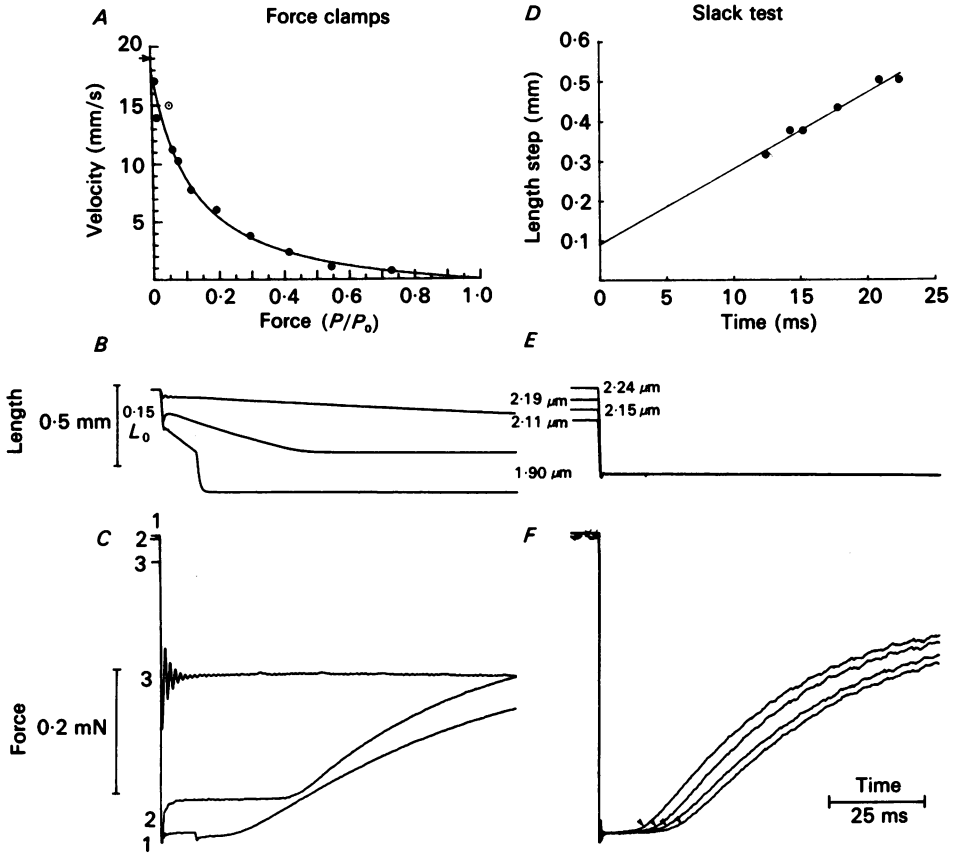

Fig. 6. V_{iso} and V_u of a skinned muscle fibre (*R. pipiens*, m. iliofibularis). The force–velocity curve, *A*, was determined from force clamps. The length and tension records of three of these are shown in *B* and *C* respectively. The fibre was released from an initial s.l. of 2·3 μm after isometric tension had reached a plateau. The isometric tension and the clamped tension of the three contractions in *C* have been identified by 1, 2 and 3. Note that for the lowest load (highest speed), the fibre was given a length step at the end of the shortening, so that the zero-force level could be accurately determined. *D* shows the plot of a slack test and *E* and *F* show the length and force records for four of the individual contractions. The arrows in *F* indicate where t_s was read. The equation for the slack test is length step (mm) = 19·074 (mm/s) × t_s + 0·091 mm, $r^2 = 0·97$. This value for speed agrees very well with that determined by extrapolating the force clamps to zero load and is marked on *A* by an arrow. Note that the skinned fibre is not as stable a preparation as the living fibre. In *A*, contractions were performed in order of increasing loads. The open circle represents the last contraction and its velocity falls above the curve determined for the other points. In *D*, two length steps were repeated, and in each case, the repeated point had a shorter t_s than the first point. Fibre length was 3·3 mm at 2·1 μm. pCa = 5·2.

Comparison of estimates of V_{iso} *and* V_u. We found that estimates of V_{iso} determined by either force clamps or length ramps agreed very well with estimates of V_u determined by either the 'calibrated slack test' or the step-ramp photographic method (Fig. 5). Fig. 5 shows all four estimates made on a single fibre. As can be seen, all values fell within the range of values set by the step-ramp photographic method. Comparison of V_{iso} determined by linear extrapolation of loads of (0·005–0·02 P_0) to zero load and V_u derived from the calibrated slack test, show that the average ratio (V_u/V_{iso}) was 0·99 ± S.E. = 0·01.

Skinned fibres (*R. pipiens*)

V_{iso}. Several factors made determination of V_{iso} in skinned fibres less accurate than in living fibres. First, we found that fibres do not shorten at a constant velocity during the entire force clamp. The curvature of the length record was significant but modest at high levels of activation (Fig. 6), and it became more pronounced at low levels of activation and higher relative loads. We therefore tried to read the velocity as early in the contraction as possible as has been previously done (Ferenczi *et al.* 1984). A constant force was generally achieved in 3–5 ms, and the velocity was measured from the change in length over the subsequent 3–10 ms time period (shorter time periods for low loads, longer ones for large loads). In some fibres at large loads, oscillations prevented reading for some 10 ms.

Another problem was that velocity records for a given load were more variable in the skinned-fibre experiments than in living fibres. This was due to some extent to the uncertainty in determining actual load and the fact that velocity did not remain constant during shortening. There also appeared to be a trend, as the experiment progressed, towards faster speeds when measured at light loads ($P < 0·05\ P_0$). In Fig. 6, the light loads were done first, then progressively heavier loads, and then we returned to light loads (open circle). Even though the isometric force had decreased, the fibre now appeared faster. Further experimentation must be performed, however, to test whether or not this trend is significant, and if so, what is the underlying mechanism.

The shape of the force–velocity curve in skinned fibres differed from that of living fibres. We found that the force–velocity data of the skinned fibre did not deviate from the hyperbola at high loads, as in the living fibres, and the Hill curve constrained to pass through P_0 could be used to fit the data. This was further verified by the analysis in which the curve was not constrained to pass through P_0. In three fibres, P_0^*/P_0 fell in range of 0·93–0·98. As in the living fibres, the points at low loads did deviate from the curve, no matter which technique was used to fit the curve. To get the best extrapolation to V_{iso} all points were included in the regression, and an extapolated V_{iso} of 5·87 m.l./s ± S.E. = 0·38 ($n = 9$) was obtained. The mean a/P_0 value (0·11 ± S.E. = 0·01; range 0·08–0·18, $n = 9$) was slightly lower than that observed in skinned *R. temporaria* (0·16) by Ferenczi, Goldman & Simmons (1984) and substantially lower than that observed in living fibres.

On a number of fibres, we also used a linear extrapolation of the points similar to that used in living fibres. This was drawn through points from 0·01 P_0 to 0·05 P_0. In these fibres the linearly extrapolated value of V_{iso} exceeded the value of V_{iso} determined by the Hill equation, by about 6 %.

V_u. The problem in determining t_s in skinned fibres was similar to that encountered in living fibres. The same calibrating procedure was initially used to try to determine the point on the force record signifying t_s. Although successful, this technique was not as useful on skinned fibre as on living fibres because the number of contractions was limited. This limitation was overcome by a different procedure in which superimposed images of the fibre at fixed times following the length step were photographed on film (Pl. 3). Superimposing the images had the added benefit of indicating small changes in the shape of the fibre. One image was produced for each flash following the step. When the fibre became straight, however, all subsequent images were the same and thus indistinguishable. The time at which the fibre took up the slack could be determined by simply counting the number of distinguishable images.

As in the living fibres, the force record rose slowly following the step and then rose quite steeply. We could exclude the period of the slow rise in tension by showing the fibres to be clearly slack during that time. We obtained a clearer indication of t_s by this method than in the living fibres because the fibres became quite straight when generating small forces and then did not further change shape significantly. Although we were able to identify t_s by the shape of the force curve (steep increase of the force redevelopment), we did not quantitate this because records of the force derivative were too noisy to interpret.

t_s was found to increase linearly with the magnitude of the length step (Fig. 6*D*). The intercepts of the lines were considerably higher than for living fibres (5·85 % $L_0 \pm$ S.E. = 0·69 %). Fig. 6*D* shows that as the experiment progressed, the t_s for a given length step became shorter. This decrease of t_s was observed for both long and short steps and was observed to varying extents in almost all fibres. When fibres began to show large decreases in t_s (i.e. $>0{\cdot}25$ ms for each contraction), where the calculation of the speed would be significantly affected, the experiments were terminated. The apparent parallel shift in the line is suggestive of an increase in the series elastic component. In fibres showing large decreases in t_s, the speed derived from the slack test depends on the order in which the steps are given. If one progresses from short to long steps, the slope of the line will overestimate V_u, and conversely, if the step size progresses from long to short, the slope will underestimate V_u. It is therefore, essential to repeat length steps during the experiment. The mean value for V_u obtained by this method in ten fibres was 6·12 m.l./s $\pm$ S.E. = 0·44.

The step-ramp photographic method as described with the living fibres was performed in two cases and agreed with the slack test. It was not used as extensively as in living fibres because each speed required two contractions.

Comparison of V_{iso} and V_u. V_{iso} and V_u were estimated in ten fibres at 80–100 % activation (pCa = 4·4–5·5). In nine, V_{iso} was estimated by the Hill curve, and the ratio of V_u estimated by the slack test, to V_{iso} was 1·05 $\pm$ S.E. = 0·03, although as mentioned, this value of V_{iso} is an underestimate. In five fibres, where a sufficient number of low loads (0·01 to 0·05 P_0) were made, V_{iso} was determined by linear extrapolation to zero load. By this method of analysis, the ratio of V_u to V_{iso} was 0·99 $\pm$ S.E. = 0·018.

The relationship between V_{iso} and V_u was also studied in several fibres at low level

of activation (pCa = 6–6·2, activation 25–50 %). We again found good agreement between V_u and V_{iso}, although our inability to measure velocity at low fractional loads limited the accuracy of this comparison.

DISCUSSION

The major finding of this study is that under our measurement conditions, V_{iso} equals V_u and thus there is a unique maximum speed of shortening which characterizes a muscle fibre. This is reasonable, in fact, it would be difficult to visualize a mechanism that would lead to a discontinuity in force–velocity relationship at zero load. The discrepancy that originally prompted this study was apparently due to inaccuracies in the estimation of V_{iso} and V_u.

V_{iso}

We have shown in this study that in living fibres, at least over the plateau region (sarcomere length = 2·2–2·0 μm), there is a unique relationship between force generation and shortening velocity. This relationship does not depend on the time during shortening when the force and velocity were measured, nor on which parameter was kept constant. At high loads ($P > 0{\cdot}5\ P_0$) isotonic contractions were easier to use, whereas at extremely low loads ($P < 0{\cdot}01\ P_0$), isovelocity contractions were advantageous because it was possible to repeat the exact motor movements with fibres in a passive and in an active state. The passive force record could then be digitally subtracted from the active force record, if need be, to determine the active force generated during shortening.

We demonstrated that the force–velocity relationship in living fibres deviates from the Hill equation at low loads. It has been previously recognized that the Hill equation does not fit force–velocity data for high loads in living fibres (Edman *et al.* 1976), and our results confirm this finding. Edman (1979) briefly mentioned that velocities from contractions at light loads ($P < 0{\cdot}05\ P_0$) tend to fall above the hyperbola, but this was not rigorously tested.

The fact that the curves used to extrapolate the data to V_{iso} do not really fit the force–velocity data, suggest that the estimation of V_{iso} might be very sensitive to the actual curve fitting procedure employed, and which loads are included in the curve fitting program. Therefore, it is likely that V_{iso} has been underestimated by varying degrees in the previous studies. Julian & Moss (1981) used a linearized Hill curve which we found in our experiments to underestimate V_{iso} by almost 19 %. Edman (1979) and Lannergren *et al.* (1978) used an unbiased Hill curve not constrained to pass through P_0, and by a similar technique we found that it underestimated V_{iso} by 7 %. By measuring the force–velocity characteristics at extremely low loads, we were able to avoid problems associated with curve fitting and achieved more accurate estimates of V_{iso} by linear extrapolation to zero load.

In the skinned fibres there were more serious problems (see Methods and Results), associated with the measurement of V_{iso}. Since we did find that velocities at low loads did exceed those predicted by the Hill curve, it is likely that V_{iso} has generally been underestimated by the extrapolation of the Hill curve in skinned fibres as well (Julian

& Moss, 1981; Goldman, 1983). Further, because of the curvature of length records, V_{iso} extrapolated from records measured 15–20 ms following the initiation of shortening appeared to be only 70–80% of V_{iso} extrapolated from records measured at 5–10 ms. Therefore it is also likely that V_{iso} has been underestimated in the past by reading the shortening records later than necessary.

The shape of the force–velocity curve in the skinned fibres differed from that in the living fibres. At large loads, the data points fell above the hyperbola constrained through P_0 in intact fibres, but fell on this hyperbola in skinned fibres. The basis for this shape difference is presently unknown, but it indicates, at least, that the skinning procedure and subsequent treatment may produce significant changes in force–velocity properties.

V_u

It is usually assumed that during a rapid length step, a fraction of the length step is absorbed by the shortening series elastic element contained in the cross-bridges and ends of the fibre and the remainder becomes slack in the fibre. The fibre is then thought to shorten under a constant zero load, thereby removing the slack, until the fibre becomes straight and redevelops tension abruptly. In a series of length steps of different magnitudes, the length of the series elastic component would be expected to remain constant, and thus the amount of slack induced into the fibre would be proportional to the magnitude of the length step. The time to take up the slack should, therefore, be linearly proportional to the step length and the slope of the relation will be V_u.

We found in fact that this was not a totally accurate description of what occurred during a slack test. First, we observed that the force transmitted through the fibre changed slowly throughout the shortening phase. Although this change in force is small in magnitude, it can cause considerable error in determining t_s (see Results). Although it is difficult to be certain, the force exerted through the fibre onto the transducer appears to be slightly negative during much of the shortening phase of the contraction. Even under zero load, the fibre has a finite stiffness due to attached cross-bridges, and thus a negative force is necessary to keep the fibre buckled after the step. As the fibre takes up the slack, the buckling decreases, and the force increases slowly to zero. As the fibre continues to straighten, the force increases at a more rapid rate, and finally there is a steep increase in the rate of force development. As the force increases, the fibre straightens further. This presumptive explanation of events requires further investigation but these forces transmitted through the fibre (whether positive or negative) are too small to influence the speed of shortening. On the other hand they can influence the reading of t_s and consequently the estimate of V_u. Some criteria must be chosen to read the records, but it is somewhat arbitrary unless this criterion is verified by another technique (such as the step-ramp photographic method). We have demonstrated that different criteria give quite different answers. From the criterion determined from our pictures, we were able to find a quantitative correlation with the force record, but the value used (5–10 P_0/s) for the *R. temporaria* fibres might not be universal. For example, this figure was not appropriate for the skinned *R. pipiens* fibres.

The assumption that the series elastic element remains constant during a whole

set of contractions required for the slack test requires some further consideration as well. The decrease of t_s for a given length step observed with skinned fibres is either due to an increase in speed of shortening or an increase in the series elastic element or both.

The step-ramp photographic method provides an estimate of V_u which does not depend on any assumptions other than that V_u is constant over the range of shortening. The method simply tests whether the amount of slack is increasing or decreasing during a length ramp. It is potentially more general than the slack test because it does not depend on force redevelopment which is a function of sarcomere length and fibre type, and it does not depend on the series elastic component remaining constant in a number of contractions. The precision with which V_u can be determined by the step-ramp photographic method of course depends on how well one can determine whether the amount of slack is increasing or decreasing during the length ramp. By interpreting the photographs conservatively, we were able to narrow the range of possible shortening speeds to within 15%.

The step-ramp photographic method for determining V_u differs from that presented by Cecchi, Colomo & Lombardi (1978). In their controlled release experiments, the fibre was given a length step and then ramped at a fixed speed. They defined V_{max} as the speed at which the fibre could not generate tension. This technique provides a lower limit for V_u but does not accurately estimate an upper limit because during ramps in which no tension is generated, one cannot exclude the possibility that the amount of slack is actually decreasing (i.e. V_u exceeds the velocity of the motor arm). A second problem is that the rate of force redevelopment while the fibre shortens and the motor moves at nearly the same speed would be very slow and might go undetected. We believe that photographs are necessary to provide a proper estimate of V_u. Measurement of V_u, made by either the slack test or step-ramp photographic method, will be very sensitive to resting forces and can only be performed over sarcomere lengths where it is negligible.

Comparison of V_{iso} and V_u in whole muscles

The discrepancy between V_{iso} and V_u found in the whole muscle has been attributed to the fact that the whole muscle contains different fibre types which have different maximum speeds of shortening. It was argued that at finite loads, different fibre types contribute, but that in the slack test, only the fastest fibres are involved (Hill, 1970; Claflin & Faulkner, 1985). In the whole frog sartorius muscle where this comparison was first made, Hill (1951) found that the V_u estimated by slack test was 30% higher than estimated V_{iso}. Recently, Claflin & Faulkner (1985) found that in a rat preparation estimated V_u exceeded estimated V_{iso} by 60%.

It is quite reasonable to suspect that the existence of fibres with different force–velocity characteristics would result in a steeper curve at low loads compared to that found in the slower fibres. However, if V_{iso} was determined by extrapolation of the velocities at very light loads to zero load (as we have done in this study), the discrepancy between V_{iso} and V_u might have been considerably smaller than was reported. The apparent discrepancy between V_{iso} and V_u could be partly explained by (1) inappropriate curve fitting methods; (2) the non-hyperbolicity of the force–velocity curve (demonstrated in single fibres); and (3) the actual summation of

contractile properties of different fibre types. To resolve this question, the shape of the force–velocity curve of the whole muscle must be compared to that of a single muscle fibre, especially at very low loads.

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EXPLANATION OF PLATES

PLATE 1

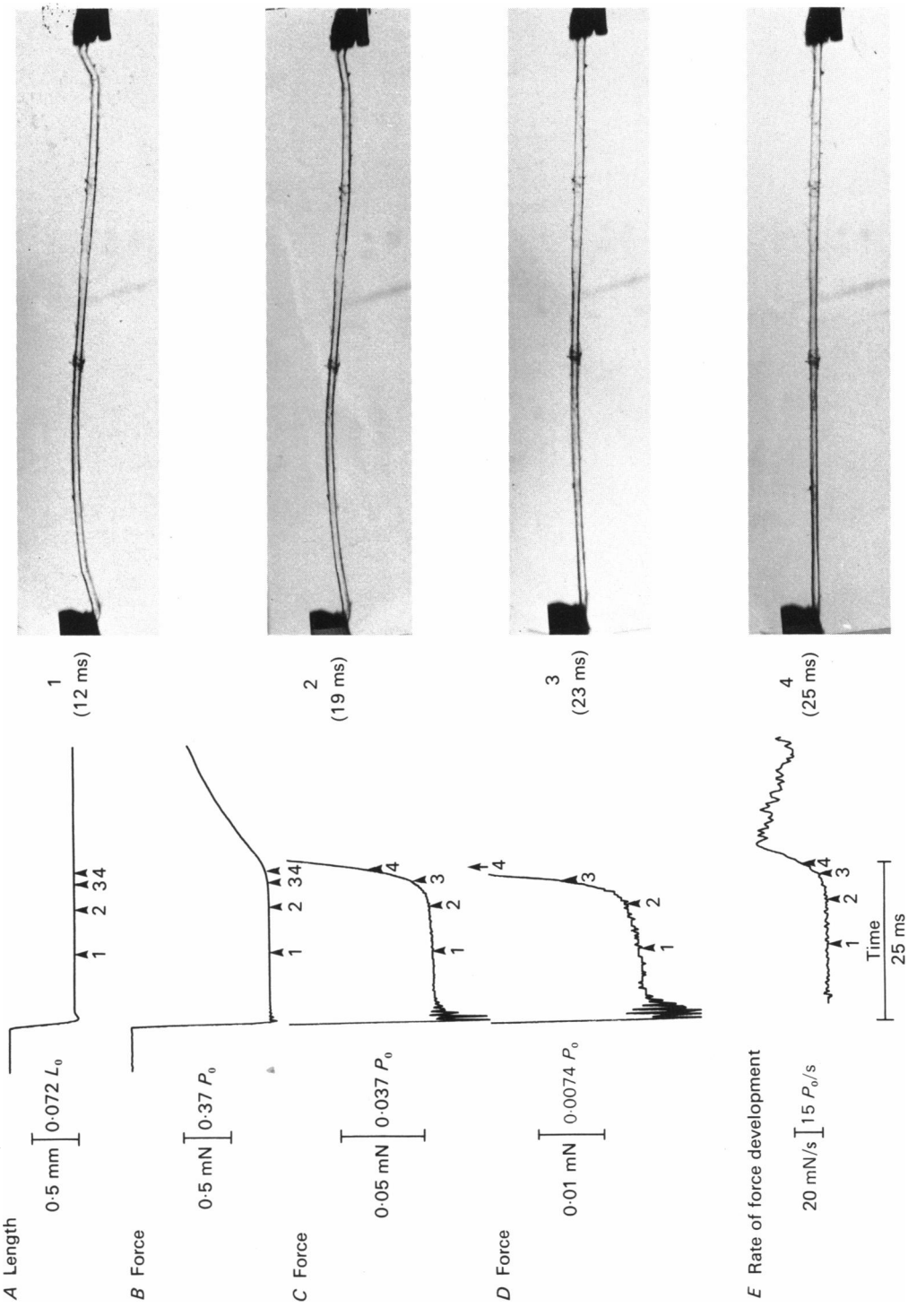
Photographic calibration of the slack test of a living fibre (*R. temporaria*, m. tibialis anterior). *A* shows the length record of a fibre given a rapid length step. *B–D* show the force record at different sensitivities and *E* shows the derivative of the force record (i.e. the rate of force redevelopment). t_s is usually read from the force record as the point at which the force deviates from the base line. Comparison of *B*, *C* and *D*, shows that this criterion is ambiguous, and would lead to different values of t_s depending on the sensitivity of the force record. To determine the real t_s, photographs (right) were taken at various times (indicated on force record by arrows) following repetitions of the same length step. In photographs 1 and 2, the fibre is clearly slacked at times when force record *D* might suggest that the slack has been removed. By examining the photographs we determined that the slack is taken up between arrows 3 and 4 (23–25 ms). We found that t_s measured by photography corresponded to the point at which the rate of force development is 0·5–1 % P_0/ms as can be seen in the derivative of the force record, *E*. t_s was subsequently determined using this quantitative criterion, thus calibrating the slack test. Fibre length was 6·9 mm at 2·1 μm. P_0 was 1·35 mN. Same fibre as in Fig. 3.

PLATE 2

Determination of V_u of living fibre by the step-ramp photographic method. The fibre (*R. temporaria*, m. tibialis anterior) was given a step to impose a small amount of slack, and then the motor was moved at a fixed rate. At each motor speed, the length and force records are shown, as well as photographs taken 1 ms after the end of the step (1) and 2–3 ms prior to the end of the ramp (2). In *A* and *B*, the motor speed was greater than V_u, because the amount of slack in the fibre increased during the ramp. In *C* and *D*, the V_u was clearly faster than the speed of the motor because the fibre became less slacked, and actually generated tension during the ramp. From this analysis, we concluded that V_u of this fibre is between 27·5 and 24·2 mm/s. Same fibre as in Figs. 4 and 5.

PLATE 3

Photographic calibration of the slack test of a skinned muscle fibre (*R. pipiens*, m. iliofibularis). *A* and *B* are length and force records respectively during a length step and subsequent force redevelopment. Subsequent to the length step the fibre was photographed five times by gated flashes (5 ms, 12 ms, 19 ms, 26 ms and 29 ms subsequent to step) which are marked by artifacts 1 to 5 on the force record. On the photograph of the fibre we can distinguish only four images of (marked 1–4) the fibre. Image no. 5 is indistinguishable from image no. 4, which suggests that the fibre became straight before the fourth image was made. Thus, t_s is between points 3 and 4 on the force trace. t_s is taken at the point indicated by the arrow. This point also coincided with where the rate of force redevelopment increased 'sharply' (note the line showing the maximum rate force redeveloped). Length of the fibre was 4·65 mm at 2·1 μm. pCa was 6·2.



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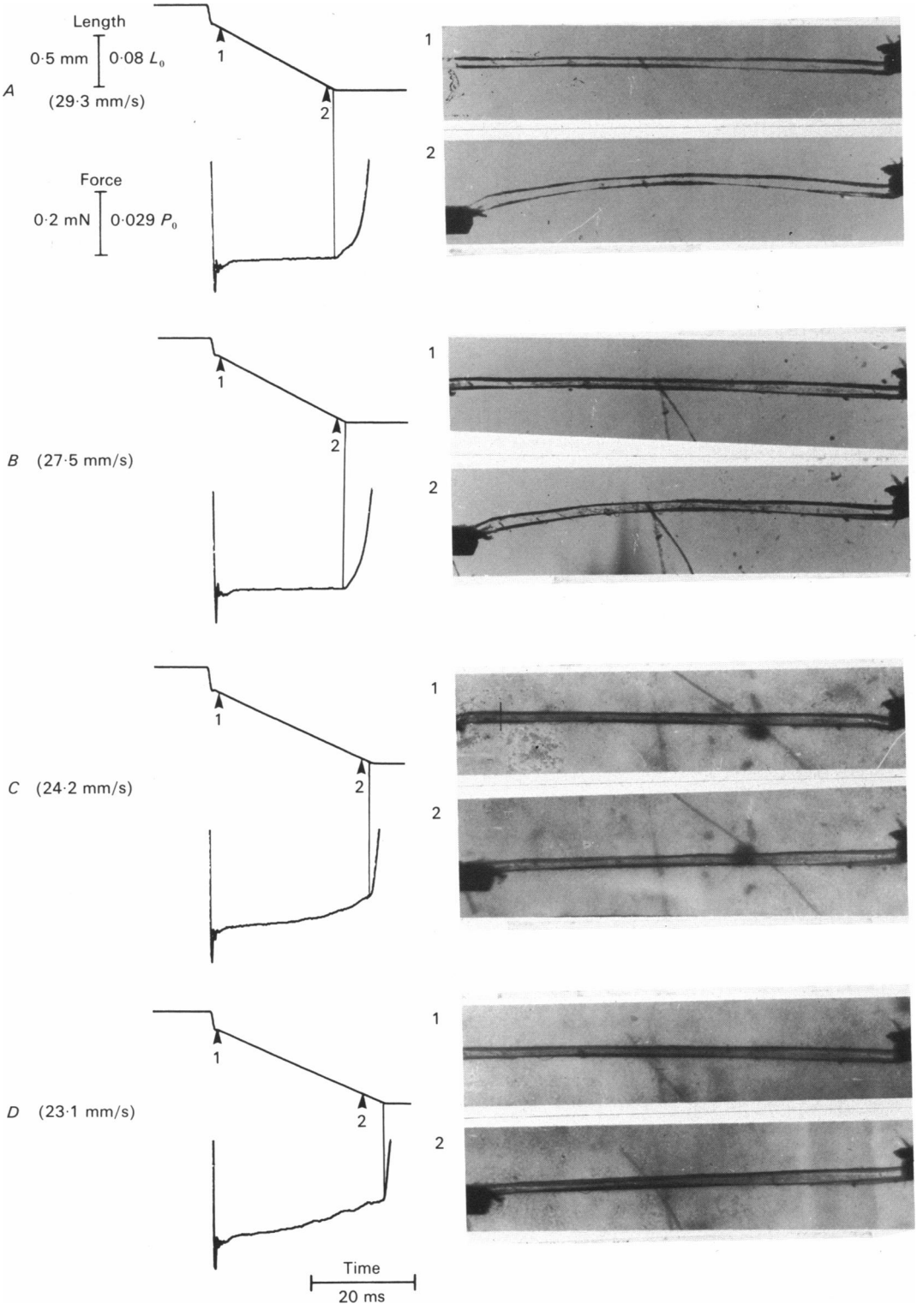
(*Facing p.* 200)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(29·3 mm/s)
Force
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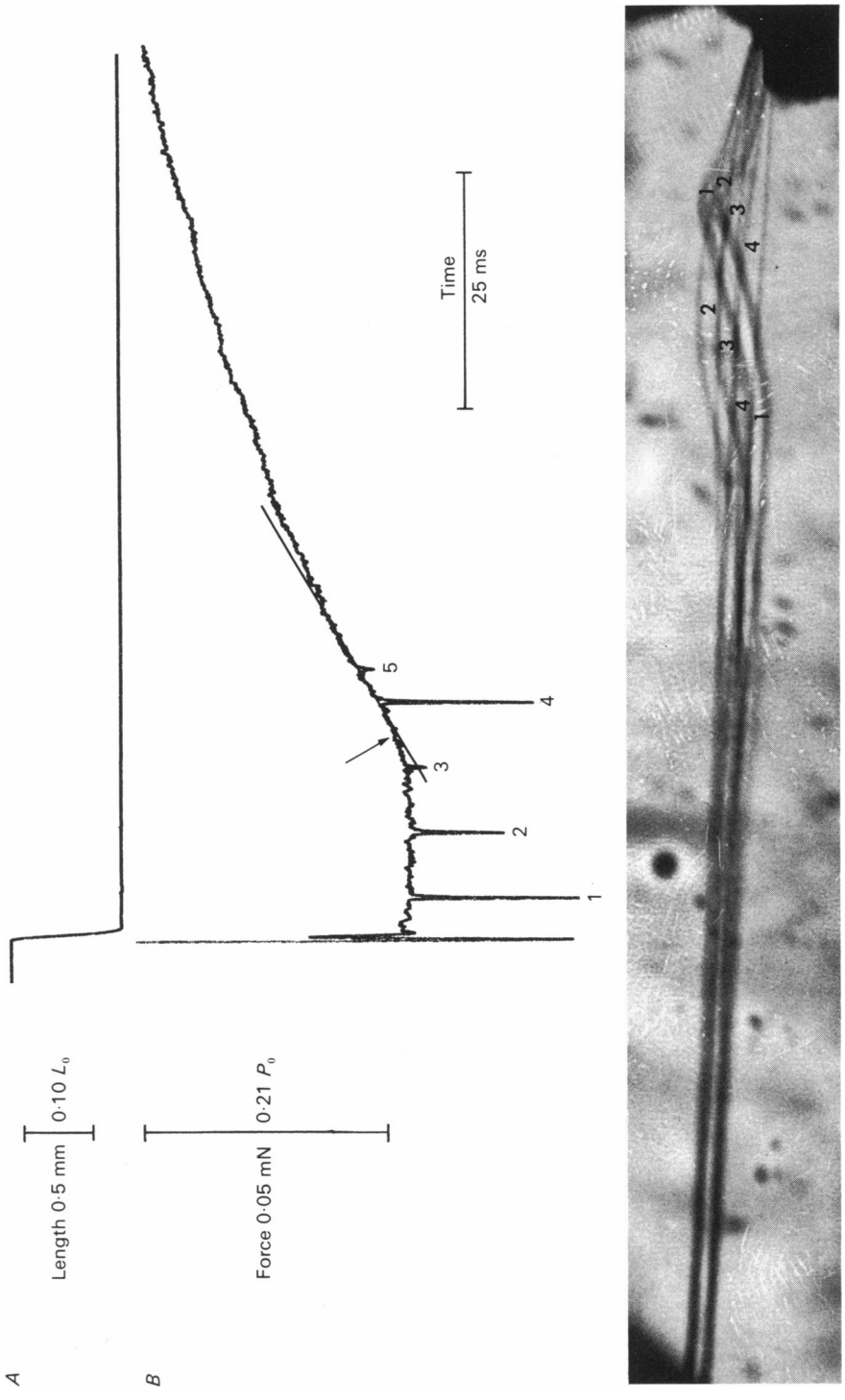
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