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# Force–velocity relation for frog muscle fibres: effects of moderate fatigue and of intracellular acidification

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1. Intact frog single fibres were investigated under control conditions (1 s tetanus every 2, 3 or 5 min) and during moderate fatigue (interval between tetani 15 or 30 s).
2. Fatigue reduced isometric force ($P_0$) by 25·8 ± 1·6 % (S.E.M.; $n = 13$) and depressed the maximum velocity of shortening ($V_{max}$) by 10·2 ± 2·2 % ($n = 13$). The force–velocity relation became less curved, $a/P_0^*$ (see Methods) being increased by 29·5 ± 8·8 % ($n = 13$). Thus, power was less affected than isometric force or $V_{max}$.
3. The velocity of unloaded shortening ($V_0$), from slack test measurements, was reduced proportionally more than $V_{max}$ during fatigue. Under control conditions $V_0$ was larger than $V_{max}$, but during fatigue their values were not significantly different.
4. Stiffness during shortening was reduced during fatigue indicating fewer attached cross-bridges in fatigue. Force was reduced more than stiffness indicating that, on average, there is less force per attached cross-bridge.
5. The force–lengthening velocity relation showed that the ability to resist forces greater than isometric was well preserved in fatigue.
6. Compared with fatigue, intracellular acidification with $CO_2$ produced a smaller reduction in isometric force. However, reduction in $V_{max}$ was not significantly different from that in fatigue. These results are consistent with both inorganic phosphate and $H^+$ increasing in fatigue, but only $H^+$ increasing during acidification, and isometric force being reduced by both, $V_{max}$ being sensitive only to $H^+$.

Fatigue of intact muscle fibres is a complex process in which a number of steps in the contractile process may be affected and contribute to the changes in muscle function (Westerblad, Lee, Lännergren & Allen, 1991).

Here we are concerned with moderate fatigue resulting from repeated brief tetani (1 s every 15 or 30 s) which reduces isometric force to about 75 % of its control value. This is a relatively stable state in that many tetani can be performed with little further reduction of force.

In severe fatigue of intact fibres, in which force is less than 40 % of its rested value, the loss of force is largely due to impaired activation and the consequent reduction in the number of attached cross-bridges (Allen, Lee & Westerblad, 1989; Lännergren & Westerblad, 1989; Lee, Westerblad & Allen, 1991; Edman & Lou, 1992).

In moderate fatigue an important factor is that less force, on average, is produced by each attached cross-bridge. This conclusion was reached by Edman & Lou (1990), who found that in moderate fatigue the decline in force in isometric tetani is more than twice the reduction in the number of attached cross-bridges, deduced from stiffness. Measurements of energy production in acidified fibres lead to the same conclusion (Curtin, Kometani & Woledge, 1988). An 'average' reduction in force per attached cross-bridge could be due to a uniform change in every cross-bridge, or a redistribution among attached states, with some cross-bridges shifting to a state that produces less force. Further evidence for a change in cross-bridge function is the slowing of $V_0$, the unloaded velocity of shortening (Edman & Mattiazzi, 1981). Experiments have shown that a reduction in the level of activation of intact muscle fibres (Edman, 1979) and of skinned fibres (Podolin & Ford, 1986) does not cause a slowing of the maximum velocity of shortening. Thus any reduction of activation that may occur during fatigue, either due to lowering of intracellular $Ca^{2+}$ or lowering of the sensitivity of the filaments to $Ca^{2+}$, could not cause the slowing of $V_0$ that occurs in fatigue.

On the basis of the evidence that moderate fatigue affects cross-bridge function, changes in the force–velocity relation would be expected, but have not been reported for intact frog single fibres. We have investigated these changes and have also measured stiffness during shortening for more direct evidence about the number of bridges that are attached.

Inorganic phosphate ($P_i$) and $H^+$ are metabolic factors that have been implicated as causes of the changes in mechanical properties that occur in fatigue. Experiments on skinned fibres show that both the accumulation of $P_i$ and $H^+$ affect the force–velocity relation (for example, Altringham & Johnston, 1985; Chase & Kushmerick, 1988; Cooke, Franks, Luciani & Pate, 1988; Godt & Nosek, 1989; Stienen, Roosemalen, Wilson & Elzinga, 1990; Stienen, Versteeg, Papp & Elzinga, 1992, and references therein). Intracellular acidification with the permeant acid $CO_2$ has been shown to affect both force during shortening and lengthening of frog whole muscle (Curtin, 1990). Isometric force and stiffness of intact frog fibres are known to be affected in a similar, but not identical, way by fatigue and intracellular acidification (Edman & Lou, 1990). Thus we have also investigated the effect on rested fibres of intracellular acidification with $CO_2$.

A preliminary report of some of the results has been presented (Curtin & Edman, 1991).

## METHODS

The experiments were done on single fibres dissected from the anterior tibialis of the frog, *Rana temporaria*. The frogs were killed by decapitation followed by destruction of the spinal cord. The standard Ringer solution contained (mM): NaCl, 115·5; KCl, 1·8; $CaCl_2$, 2·0 and $Na_2HPO_4 + NaH_2PO_4$ (total concentration), 2·0; pH 7·0. In the experiments on intracellular acidification, bicarbonate-buffered solutions were used which contained (mM): $NaHCO_3$, 17·0; NaCl, 101; KCl, 1·8; $CaCl_2$, 2·0 and were equilibrated with mixtures of $O_2$ and $CO_2$ to give pH 7·0 or 6·5 at 0 °C. The proportions of $CO_2$ in the gas mixtures were 4·8 and 15·2 %, respectively (calculated using p$K$ 6·319 and $CO_2$ solubility of 0·0737 mol $l^{-1}$; Edsall & Wyman, 1958). The fibres were normally dissected the day before the experiment and kept in the standard Ringer solution at 4 °C overnight.

For the experiment the fibre was mounted between a force transducer and a servo-controlled motor; in experiments in which stiffness was measured the force transducer was also mounted on a motor. The tendons were held in aluminium clips of the type described previously by Edman & Reggiani (1984). The fibre was continuously superfused with solution at constant temperature.

The temperature varied between 1·0 and 2·5 °C for experiments on different fibres. Sarcomere length of the resting fibre was adjusted to either 2·10 or 2·25 $\mu$m, measured by laser diffraction.

The strength of the electrical stimulus was set approximately 20 % above the threshold by adjusting voltage; pulse duration was 0·2 ms. The frequency was set to give a fused or almost fused tetanus (see Fig. 1). During the fatigue protocol and during intracellular acidification, some fibres do not always respond to every stimulus if the frequency is as high as in the control state. If this occurs force drops suddenly then redevelops at the next stimulus producing a force record that cannot be used; the fibre must be discarded. To avoid this problem a lower frequency of stimulation than in control conditions was generally used. The force remains fused or nearly fused (see Fig. 1) because fibres relax more slowly when fatigued and acidified under the conditions used here (Curtin & Edman, 1989). Stimulation, force and motor position, and in some experiments, stiffness, were recorded on a digital oscilloscope and stored on disk.

At the end of the experiment, fibre length and diameter were measured with a stereomicroscope. The fibre was rotated around its long axis and the largest and smallest diameters were measured. The cross-sectional area was calculated, assuming an elliptical shape.

**Control conditions.** The fibre was tetanized under isometric conditions for 1 s at fixed intervals (of 2, 3 or 5 min; duty cycle 0·0083, 0·0056, 0·0033) until the force in successive tetani was relatively constant.

**Fatigue protocol.** To produce fatigue, the interval between isometric tetani was reduced to 15 or 30 s (duty cycle 0·067 or 0·033).

**Acidification.** To produce intracellular acidification, the normal Ringer solution superfusing the fibre was changed to one containing the permeant acid $CO_2$. The stimulation pattern was kept the same as during the control period.

### Force–velocity relation

After having established a near steady state in the control, fatigued or acidified state, as judged by isometric force production, a series of tetani (the PV series) was given in which load clamp was applied in most tetani (a few were purely isometric). In tetani with shortening, the force was reduced to a pre-set fraction of the isometric force after 0·5 s of stimulation under isometric conditions. In some experiments the fibre was also subjected to forces greater than the isometric force ($P_0$). At the end of the PV series of tetani with movement, purely isometric tetani were given.

In some experiments there was a reduction in force during the PV series, but it was small compared with the changes due to fatigue produced by reduction in the interval between tetani, and due to acidification. Furthermore, it did not significantly affect the changes in maximum shortening velocity ($V_{max}$), $P_0^*$, and $a/P_0^*$ (see below) due to fatigue and acidification. Changes based on absolute force were not significantly different than those based on values of force relative to the isometric force developed in the same tetanus before movement started.

**Data analysis for force–velocity curves.** Measurements were made of the force under isometric conditions just before the load clamp was applied, and during load clamp. Digital records of motor position were differentiated to give a record of the velocity of movement. The velocity was measured just after the transient at the start of movement (see Fig. 1).

A hyperbola of the form:

$$(P+a)(V+b)=(P_0^*+a)/b,$$

was fitted to the experimental points for each fibre for forces less than 78 % of $P_0$ by the method of Wohlfart & Edman (1994).

The fitting procedure minimized the squares of the deviations about power (force × velocity). Analytical results derived by this method agree closely with those obtained by the interactive computer routine used previously (Edman, Mulieri & Scubon-Mulieri, 1976). Three variables were fitted: $V_{max}$, the intercept on the velocity axis; $P_0^*$, the intercept on the force axis and $a$ (since $b=aV_{max}/P_0^*$, $b$ is not an independent variable). Fitted values of $P_0^*$ and $V_{max}$ for each fibre were used to normalize the results for reasons described later.

The relative force at which maximum power was produced and the maximum power were calculated as follows using the $a/P_0^*$ values found in fitting the force–velocity curve:

$$P/P_0^* \text{ for maximum power} = (\sqrt{(1+G)}-1)/G,$$

where $G = P_0^*/a$.

$$\text{Maximum power} = (P/P_0^*)(V/V_{\max}),$$

at a point where $P/P_0^* = V/V_{\max}$ (see Woledge, Curtin & Homsher, 1985, p. 49).

### Velocity of unloaded shortening ($V_0$)

In these experiments the velocity of unloaded shortening was measured under control conditions and in the fatigued state by the method of Edman (1979). The fibre was released after 0·5 s of stimulation under isometric conditions. Three different amplitudes of release from the same starting length were used, and each was repeated three times. In some experiments $V_0$ was determined before, and in others after, the series of tetani for the force–velocity relation.

### Measurement of fibre stiffness

Full details of methods and apparatus used to measure fibre stiffness are given by Edman & Lou (1990); only an outline is given here. In these experiments the fibre was mounted between two electromagnetic pullers. One of the pullers had a force transducer mounted on its shaft and was used to produce a ramp movement, or a step and ramp. The other puller produced a sinusoidal length oscillation of constant amplitude throughout the tetanus period. The frequency of the length

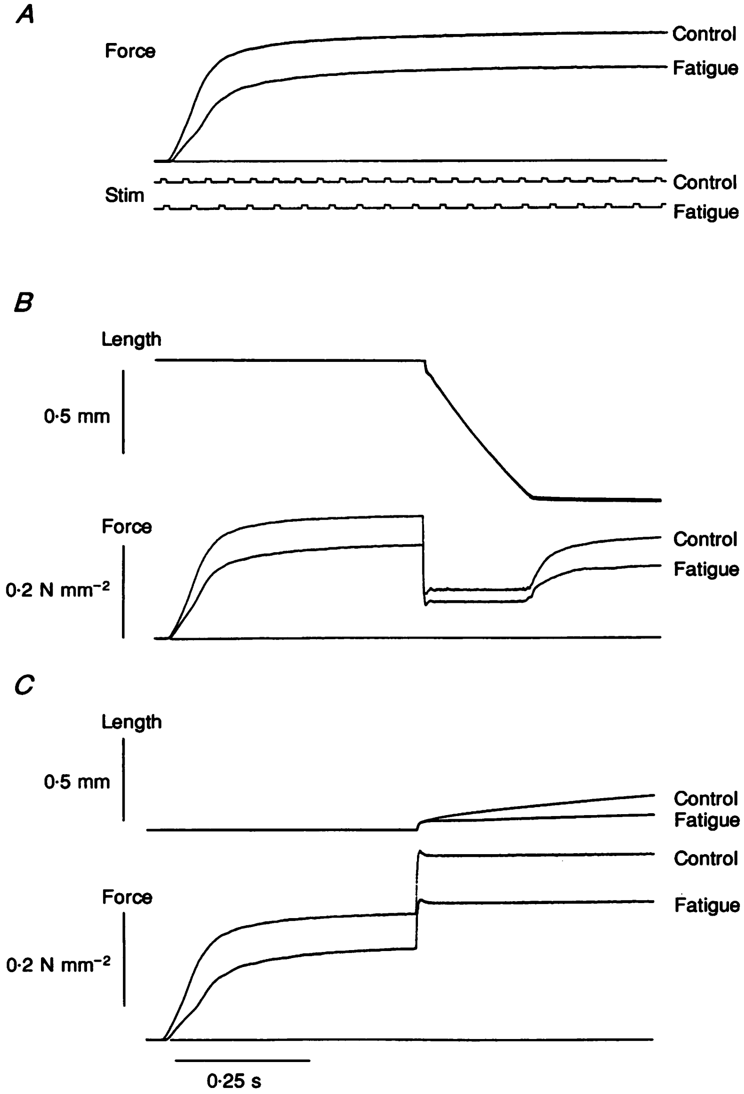


**Figure 1. Examples of records for control state and moderately fatigued state**

Force, stimulation (labelled Stim) and motor position (labelled Length) in the control state (1 s tetanus at 5 min intervals) and when fatigued (1 s tetanus at 15 s intervals). *A*, isometric conditions. *B*, tetani with shortening during a period of force clamp to about 40 % of the isometric force under control and fatiguing conditions. *C*, tetani with lengthening during a period of force clamp to a force about 40 % greater than the isometric force under control and fatiguing conditions. All records from the same fibre.

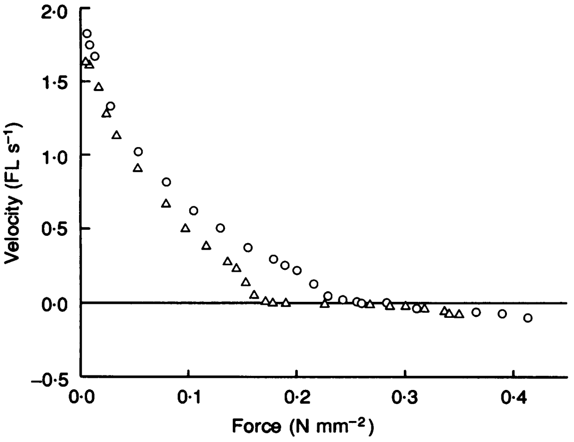


**Figure 2. Relation between velocity and force for control state and moderately fatigued state**

Force and velocity (positive velocities for shortening, negative velocities for lengthening) for the same fibre as used in Fig. 1. FL is the fibre length at resting sarcomere length of 2·1 μm. Control conditions (○), fatigued (△).

oscillation was 4 kHz and the peak-to-peak amplitude was 5·3 μm, corresponding to approximately 1·2 nm per half-sarcomere. Stiffness was measured during tetanic stimulation by recording the changes in force that occurred in response to 4 kHz length oscillations. A stiffness signal was formed by first passing the signal from the force transducer through a narrow bandpass filter (Q-value 5·5), the optimum frequency of which was set to 4·0 kHz. The filtered signal was thereafter rectified by means of a precision rectifier circuit to provide a direct read-out of the stiffness during the course of the tetanus. The bandwidth of the rectified signal was 2·3 kHz DC. The force signal was recorded without the superimposed force oscillations by using a notch filter which produced maximum damping at 4·0 kHz. The signal from the position transducer of the oscillating puller was treated in the same way to provide a measurement of the peak-to-peak amplitude of the length oscillation.

### Statistics

Unless stated otherwise, the probability values are based on Student's $t$ test (paired). Probability of 0·05 was taken as statistically significant.

## RESULTS

### Force–velocity relation during shortening

Figure 1*A* shows examples of records of force during isometric tetani under control conditions and in the fatigued state. In this experiment the steady level of force under isometric (fixed-end) conditions was about 25 % less in the fatigued state than in the control state. The values summarized in Table 1A show that the mean reduction in isometric force for the fatigue experiments was 25·8 ± 1·6 % ($n = 13$).

Figure 1*B* shows the corresponding records for tetani in which the load was clamped to about 40 % of the isometric value after the fibre had been stimulated for 0·5 s. The force and velocity of shortening were measured during the load clamp after the initial transient. The measured values from a series of tetani with different load clamps for one experiment are shown in Fig. 2. In Fig. 3*A* the results for shortening were fitted with hyperbolae fitted to force

**Table 1. Values for the isometric force, $P_0$, and force–velocity characteristics, $P_0$*, $V_{max}$, $a/P_0$*, from the fitted curve (see Methods)**

| | $P_0$ (N mm$^{-2}$) | $P_0$* (N mm$^{-2}$) | $V_{max}$ (FL s$^{-1}$) | $a/P_0$* | Max power ($VP/V_{max}P_0$*) | $P/P_0$ for max power |
|---|---|---|---|---|---|---|
| A. Fatigued fibre ($n = 13$) | | | | | | |
| Control | 0·287 ± 0·010 | 0·338 ± 0·014 | 2·26 ± 0·04 | 0·298 ± 0·015 | 0·104 ± 0·0028 | 0·322 ± 0·004 |
| Fatigue | 0·214 ± 0·011 | 0·264 ± 0·015 | 2·03 ± 0·07 | 0·379 ± 0·025 | 0·117 ± 0·0036 | 0·342 ± 0·005 |
| Percentage difference | −25·8 ± 1·6<br>(< 0·001) | −22·0 ± 2·5<br>(< 0·001) | −10·2 ± 2·2<br>(< 0·001) | 29·5 ± 8·8<br>(< 0·01) | 12·9 ± 3·8<br>(< 0·001) | 6·1 ± 1·8<br>(< 0·001) |
| B. Acidified fibre ($n = 7$) | | | | | | |
| Control | 0·295 ± 0·022 | 0·383 ± 0·014 | 2·25 ± 0·07 | 0·265 ± 0·021 | 0·098 ± 0·004 | 0·312 ± 0·007 |
| Fatigue | 0·239 ± 0·016 | 0·303 ± 0·013 | 2·05 ± 0·06 | 0·317 ± 0·022 | 0·108 ± 0·003 | 0·328 ± 0·005 |
| Percentage difference | −18·6 ± 2·4<br>(< 0·001) | −19·6 ± 3·8<br>(< 0·01) | −8·74 ± 1·6<br>(< 0·002) | 24·6 ± 14·3<br>(> 0·10) | 11·6 ± 6·4<br>(> 0·05) | 5·4 ± 2·9<br>(> 0·1) |

The maximum power and the relative force giving maximum power were calculated from the fitted curve. FL is the fibre length at which $P_0$ was measured. Percentage difference = 100 × [($e - c$)/$c$], where $e$ is the value for fatigued or acidified state, and $c$ is the control value. The probability values, shown in parentheses, refer to comparison of the means with 0.

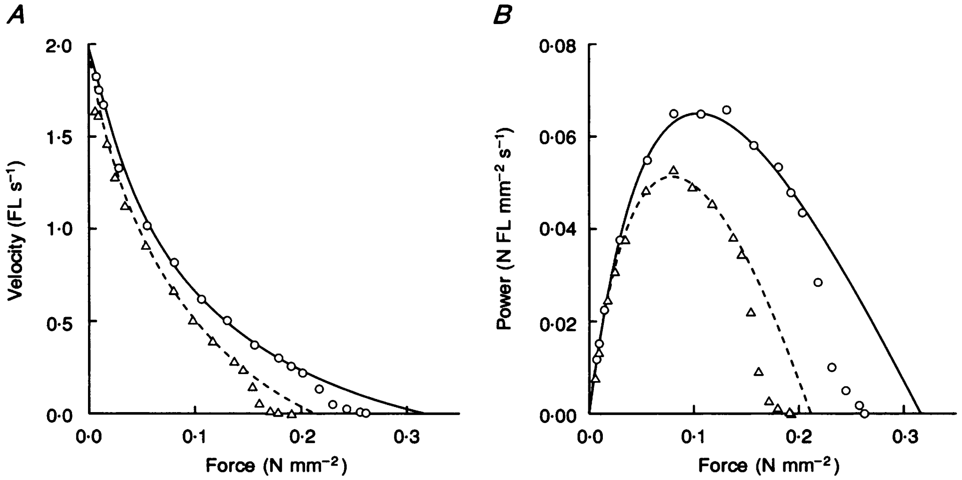


**Figure 3. The velocity–force and power–force relations for shortening in the control state and in the moderately fatigued state**

*A*, force and velocity of shortening for the same fibre as used in Figs 1 and 2. FL is the fibre length at resting sarcomere length of 2·1 μm. The lines were fitted to the data points for forces less than 78 % of $P_0$ as described in Methods. Control and fatigued conditions, respectively: $P_0 = 0{\cdot}260$ and 0·192 N mm$^{-2}$, $V_{max} =$ 1·97 and 1·79 FL s$^{-1}$, $P_0{}^* = 0{\cdot}316$ and 0·211 N mm$^{-2}$, and $a/P_0{}^* =$ 0·296 and 0·522. *B*, the relation between power and force. Power is the product of force and velocity values shown in *A*. Control conditions (○), fatigued (△).

values up to 78 % of $P_0$ (the 'break-point', Edman, 1988). The lines fit the points well in both cases, which shows that in fatigue the force–velocity relation remains hyperbolic.

The mean values of $V_{max}$, $P_0{}^*$ and $a/P_0{}^*$ obtained from the curve fitting are summarized in Table 1A. Fatigue decreased the intercept on the velocity axis, $V_{max}$ (fibre length s$^{-1}$) by 10·2 ± 2·2 %, considerably less than the reduction in the isometric force, 25·8 ± 1·6 %. The intercept on the force axis, $P_0{}^*$ (N mm$^{-2}$) was reduced by 22·0 ± 2·5 %, similar to the change in isometric force. In most fibres the force–velocity relation become straighter during fatigue, that is the value of $a/P_0{}^*$ increased; the mean increase was 29·5 ± 8·8 %.

The change in the curvature of the force–velocity relation is clear in Fig. 4*A* where force and velocity are expressed relative to $P_0{}^*$ and $V_{max}$, respectively. Note that the relative force points for fatigue lie above the controls. The significance to muscle function of this change can be appreciated in plots of mechanical power, calculated as the product of relative force, $P/P_0{}^*$, and relative velocity,

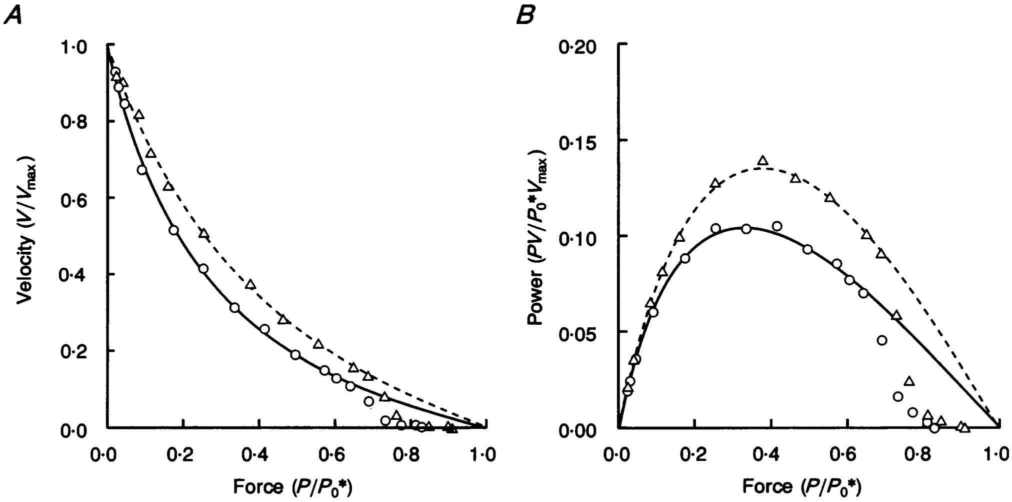


**Figure 4. Normalized form of the velocity–force and power–force relations for shortening**

*A*, force and velocity of shortening for the same fibre as used in Figs 1–3. Force expressed relative to $P_0{}^*$, the intercept on the force axis of the fitted curve shown in Fig. 3, and velocity expressed relative to $V_{max}$, the intercept on the velocity axis. *B*, the relation between power and force. Power is the product of relative force and relative velocity values shown in *A*. Control conditions (○), fatigued (△).

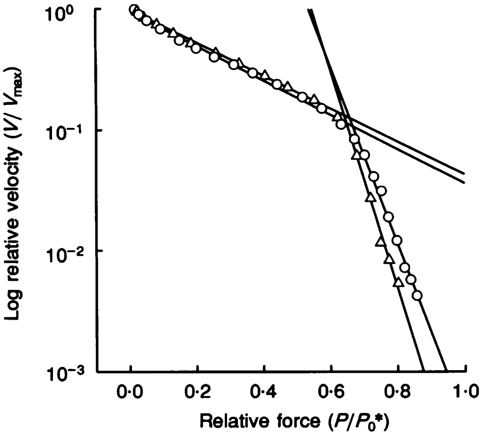


**Figure 5. 'Break point' in the force–velocity relation**
Log relative velocity ($V/V_{max}$) plotted *versus* relative force ($P/P_0$* measured under the same conditions, control or fatigue). The two lines are linear regressions through the points for forces less than and greater than 78 % of the isometric force, $P_0$. Control conditions (○), fatigued (△).

$V/V_{max}$ (Fig. 4*B*). Compared with the control state, peak relative power is greater and is reached at a higher relative force in the fatigued state (Table 1A).

## Acidification

Intracellular acidification with $CO_2$ produced changes in the isometric force and in the force–velocity relation for shortening that were similar in some respects to those produced by fatigue. There were statistically significant reductions in isometric force ($P_0$), $P_0$* and $V_{max}$, and a non-significant increase in $a/P_0$* with acidification (Table 1B). The quantitative comparison with fatigue shows interesting differences. The reduction in isometric force with acidification, 18·6 ± 2·4 % ($n = 7$), was significantly smaller than that due to fatigue (probability < 0·05, unpaired *t* test). In contrast, the changes in $V_{max}$ and $a/P_0$* were not different from those produced by fatigue ($P > 0·05$, unpaired *t* test).

## High force region

Edman (1988) has shown that the force–velocity relation for shortening consists of two hyperbolae, one describing the relation for forces less than 78 % of $P_0$, and another for higher forces. To test whether fatigue or acidification changed the 'break-point' between the two parts of the relation, semi-log plots were made of the results of each experiment. The log of the velocity/$V_{max}$ was plotted against force/$P_0$* as shown in Fig. 5, and two straight lines were fitted to the data. The point of intersection of the two

**Table 2. Force (*P*) expressed relative to $P_0$, required to lengthen the fibre at a velocity of 0·02 FL $s^{-1}$**

| | $P/P_0$ |
|---|---|
| A. Fatigued fibre ($n = 5$) | |
| Control | 1·178 ± 0·028 |
| Fatigue | 1·503 ± 0·034 |
| Difference | 0·325 ± 0·045<br>(0·002) |
| B. Acidified fibre ($n = 3$) | |
| Control | 1·300 ± 0·058 |
| Fatigue | 1·494 ± 0·106 |
| Difference | 0·194 ± 0·062<br>(0·088) |

Values were calculated by linear interpolation between measured values. FL is the fibre length at which $P_0$ was measured. Difference = fatigue or acidified − control. The probability values, shown in parentheses, refer to comparison of the means with 0.

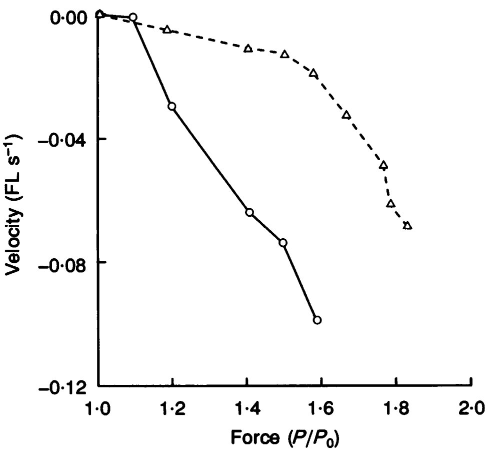


**Figure 6. Normalized form of the velocity–force relation for lengthening**
The relation between force (as a fraction of $P_0$ measured under the same condition, control or fatigue) and velocity of lengthening. FL is the fibre length at resting sarcomere length of 2·1 μm. Same fibre as used in Figs 1–4. Control conditions (○), fatigued (△).

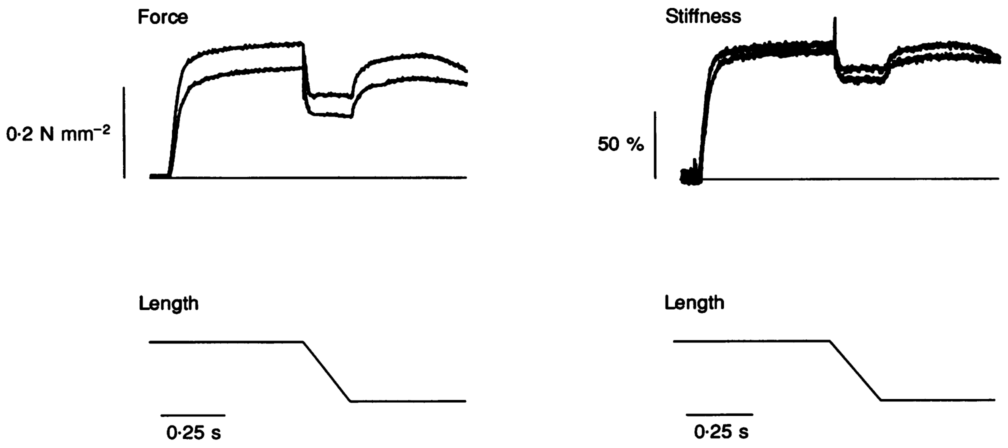


**Figure 7. Example records of force and stiffness**

Force and stiffness during tetani with a period of constant velocity shortening. Records from the same fibre in the control state (upper record in both cases) and during fatigue. The traces labelled length show motor position. Stiffness was measured in arbitrary units and is expressed as a percentage of the value during the plateau of force in the isometric part of the tetanus under control conditions. The spike seen at the onset of clamp in the stiffness records is an artifact, a signal picked up by the stiffness measuring circuit when the clamp is switched on.

lines was calculated for each experiment. The 'break-point' value was not significantly affected by fatigue or acidification. The difference between the control value and that during fatigue was 2·1 ± 2·2 % $P_0$* ($n = 9$) and for acidification −0·4 ± 3·4 % $P_0$* ($n = 7$).

## Fibre lengthening at forces greater than $P_0$

The fibre was subjected to forces greater than $P_0$ in five experiments with fatigue and three with intracellular acidification; this caused lengthening of the fibre. An example record is shown in Fig. 1*C* where force of about 140 % $P_0$ was imposed on the fibre after 0·5 s of stimulation. A summary of the results for this fibre is shown in Fig. 2 along with the results for shortening. Clearly the effect of fatigue on muscle behaviour was different during shortening than lengthening. For every force less than isometric force, velocity of shortening is lower in fatigue than under control conditions, whereas

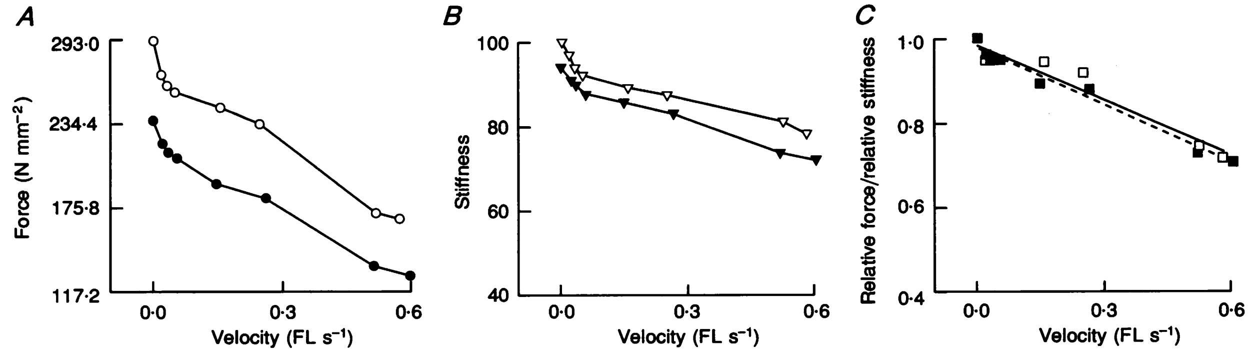


**Figure 8. Effects of fatigue on force, stiffness, and relative force/relative stiffness during shortening**

*A*, the relation between velocity of shortening and force in the control state (open symbols) and during moderate fatigue (filled symbols), for the tetani in which stiffness was measured. Note that only forces greater than 40 % of the control isometric value were used (see text). *B*, the relation between velocity and stiffness. Stiffness was measured in arbitrary units and is expressed as a percentage of the isometric value under control conditions. Control, open symbols; fatigue, filled symbols. *C*, the relation between velocity and relative force/relative stiffness. Control relative value = value during shortening/isometric value, where both values are for control conditions. Fatigue relative value = value during shortening/isometric value, where both values are for fatigue. Control, open symbols; fatigue, filled symbols. The lines are straight line fits through the results. Control, continuous line, slope = −43·7 ± 4·5/(FL s$^{-1}$) (degrees of freedom, d.f. = 7) and $y$-intercept = 98·5 ± 1·3, (d.f. = 7); regression coefficient, 0·969. Fatigue, dashed line, slope = −45·8 ± 2·6/(FL.s$^{-1}$) (d.f. = 7) and $y$-intercept = 97·9 ± 0·8, (d.f. = 7); regression coefficient, 0·990. All results from the same fibre. FL, fibre length.

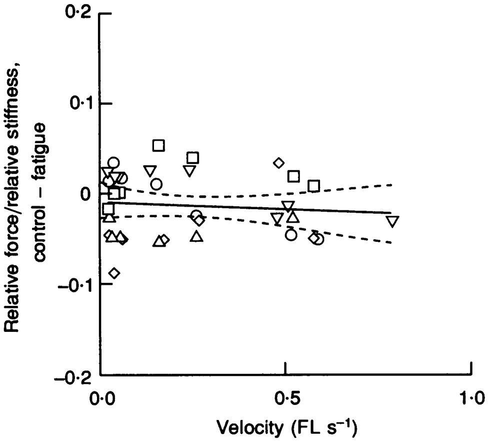


**Figure 9. Difference between control and fatigue values of the ratio of relative force to relative stiffness at various velocities of shortening**

See Fig. 8*C* for values of the ratio of relative force to relative stiffness. FL, fibre length. Different symbols are results from different fibres. Continuous line shows straight line fit, slope = $-0{\cdot}018 \pm 0{\cdot}027$ s FL$^{-1}$ ($n = 34$) and intercept = $-0{\cdot}009 \pm 0{\cdot}009$ ($n = 34$); regression coefficient, 0·115.

there is considerable overlap of the results for lengthening in the control and fatigued state. In Fig. 6 the force is expressed relative to the corresponding isometric value, $P_0$, either control or fatigued. Here we see that when the fibre was subjected to a force greater than its isometric value, it lengthened more slowly in the fatigued state than under control conditions. In other words, when in the fatigued state the fibre was better able to resist a force exceeding the isometric force by a particular fraction.

Table 2 summarizes the results for all the fibres and shows the force required to stretch the fibre at a velocity of 0·02 fibre lengths s$^{-1}$ in the control state, during fatigue, and with intracellular acidification. At this velocity of stretch, the force expressed relative to $P_0$ under the same conditions was significantly greater ($32{\cdot}5 + 4{\cdot}5$ %, $n = 5$, probability = 0·002) in the fatigued state than when the fibre was rested. During acidification, the relative force was $19{\cdot}4 \pm 6{\cdot}2$ % ($n = 3$, probability = 0·088) greater than the relative force under control conditions.

## Fibre stiffness

In experiments on five fibres, force and stiffness were measured in isometric tetani and in tetani which included a period of ramp shortening under control conditions and during fatigue. The isometric force was $20{\cdot}6 \pm 0{\cdot}9$ % ($n = 5$) less and $V_{\max}$ was $6{\cdot}4 \pm 3{\cdot}2$ % ($n = 5$) less in the fatigued state than under control conditions.

We only considered stiffness measurements for velocities giving force of 40 % or more of the control isometric tetanus. Measurements of fast length steps (completed in 200 $\mu$s) of small segments (about 1 mm long) of the fibres have shown that at forces greater than 40 % of isometric the change in segment length reliably matches that expected from the change imposed on the whole preparation; error due to series compliance is negligible (Edman, 1993).

Figure 7 shows example records of force and stiffness during tetani with shortening under control conditions and during moderate fatigue. Fatigue reduced both force and stiffness, but the effect on force was greater than that on stiffness (see Fig. 8*A* and *B*). This was the case at each velocity in all of the five fibres. Under isometric conditions, fatigue reduced force by $20{\cdot}6 \pm 0{\cdot}9$ % ($n = 5$) and stiffness by only $5{\cdot}4 \pm 1{\cdot}0$ % ($n = 5$).

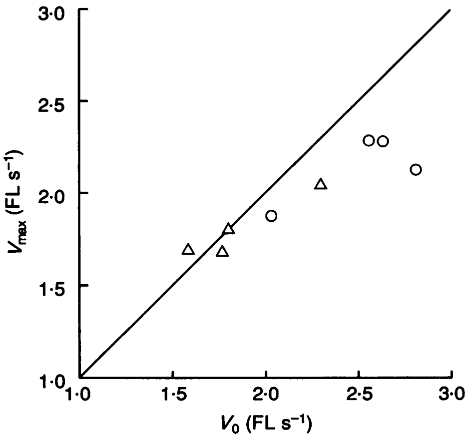
To examine the effect of filament sliding on the change in force and stiffness during fatigue, the ratio of relative force to relative stiffness was calculated for each velocity of shortening (relative force = force during shortening/ isometric force for the same condition, either control or fatigued). Figure 8*C* shows the dependence of this ratio on velocity of shortening for this fibre. As expected (Ford, Huxley & Simmons, 1985), the ratio of force to stiffness decreases as velocity increases. We observed this dependence under both control and fatigue conditions. The relationships between velocity and relative force/relative stiffness were

**Table 3. $V_{\max}$ and $V_0$ measured in the same fibre**

| | $P_0$ (N mm$^{-2}$) | $V_0$ (FL s$^{-1}$) | $V_{\max}$ (FL s$^{-1}$) | Percentage difference $V_0$ – percentage difference $V_{\max}$ |
|---|---|---|---|---|
| Control | $258{\cdot}7 \pm 9{\cdot}7$ | $2{\cdot}50 \pm 0{\cdot}17$ | $2{\cdot}14 \pm 0{\cdot}10$ | |
| Fatigue | $180{\cdot}8 \pm 3{\cdot}7$ | $1{\cdot}86 \pm 0{\cdot}15$ | $1{\cdot}81 \pm 0{\cdot}08$ | |
| Percentage difference | $-30{\cdot}0 \pm 1{\cdot}6$<br>(< 0·01) | $-24{\cdot}6 \pm 7{\cdot}9$<br>(< 0·05) | $-15{\cdot}4 \pm 2{\cdot}9$<br>(< 0·002) | $-9{\cdot}2 \pm 5{\cdot}2$<br>(0·181) |

$V_{\max}$ is the intercept of the force–velocity relation on the velocity axis. $V_0$ is the velocity of unloaded shortening determined by the slack test method. FL is the fibre length at sarcomere length 2·25 $\mu$m. Percentage difference = 100 × ([fatigue − control]/control). $n = 4$. The probability values, shown in parentheses, refer to comparison of the means with 0.

**Figure 10. Comparison of $V_0$ and $V_{max}$**
Values of $V_0$ and $V_{max}$ measured in the same fibres under control conditions (○) and in the fatigued state (△). Results for four fibres. The continuous line is the line of identity.



very similar for the control and fatigue conditions, at least within the velocity range we could examine. For each fibre a straight line was fitted through the data for each condition, and the values of the intercepts and slopes were compared for the control and fatigue conditions. In none of the fibres was there a statistically significant difference between the fatigue and control values. Figure 9 shows the pooled results for all the fibres of the difference between control and fatigue values of relative force/relative stiffness plotted *versus* velocity. The slope was $-0{\cdot}018 \pm 0{\cdot}027$ s FL$^{-1}$ ($n = 34$, probability $= 0{\cdot}51$, not significant) and the intercept at a velocity of zero was $-0{\cdot}009 \pm 0{\cdot}009$ ($n = 34$, probability $= 0{\cdot}29$, not significant). Thus the effect of velocity is similar during fatigue to that in control conditions.

### $V_0$ and $V_{max}$

Edman & Mattiazzi (1981) found that $V_0$, measured by the slack test method (Edman, 1979), was reduced when $P_0$ was reduced by fatigue. Our observations (Table 1A) from the force–velocity relation showed that the reduction in $V_{max}$ of 10·8 % was not as great as the reduction in $V_0$ of 18 % expected for a reduction in $P_0$ of 25·8 % on the basis of Edman & Mattiazzi's results (percentage reduction in $V_0 = [0{\cdot}006 \times$ percentage reduction in $P_0^{2{\cdot}48}] - 1{\cdot}0$).

Therefore in four fibres we measured $V_0$ by the slack test and $V_{max}$ by extrapolation of the force–velocity curve, so the results could be directly compared. In the fatigued state, both $V_0$ and $V_{max}$ were reduced compared with the values found in control state of the fibres (see Table 3). The mean reduction in $P_0$ for these four fibres was 30·0 % for which Edman & Mattiazzi's predicted change in $V_0$ is 26·6 %. Our observed change in $V_0$, 24·6 ± 7·9 %, is close to and not significantly different from this prediction.

Figure 10 shows that under control conditions $V_0$ was in every case larger than $V_{max}$. However, $V_0$ and $V_{max}$ were close to being equal in three out of four cases in the fatigued state. The reduction in $V_0$ during fatigue tended to be larger than the change in $V_{max}$ particularly in fibres with a high velocity of unloaded shortening, $V_0$, under control conditions.

## DISCUSSION

### Force–velocity relation

#### Shortening

In moderate fatigue, the velocity of shortening (fibre length s$^{-1}$) under each load (N mm$^{-2}$) is less than under control conditions (Fig. 2). The results also show that the hyperbolic relation between force and velocity is less curved in moderate fatigue than it is in the rested state. This means that the fibre's ability to produce power is reduced less than in proportion to the reduction in $P_0$, $P_0$* and $V_{max}$. The fibre's ability to do work at a high rate is thus spared from the deleterious effects of the factor(s) that reduce isometric force and high velocity shortening.

#### Stiffness

During shortening of rested fibres, force and stiffness decrease as velocity increases (Ford, Huxley & Simmons, 1985). We found that this also occurs in the moderately fatigued state (Fig. 8*B*). In terms of Huxley's (1957) two-state model decreased stiffness with increasing velocity reflects the reduction in the proportion of attached bridges as the time available for attachment is reduced. The decreased force is due to (1) reduction in the proportion of bridges producing positive force, (2) on average each of these attached bridges being less extended (has smaller positive $x$ value) and producing less positive force, (3) an increase in the proportion of attached bridges at negative $x$ values where negative force is produced. The fact that force and stiffness decreased with increasing velocity in fatigue provides evidence that at least in general terms these principles also apply in moderately fatigued intact fibres during shortening.

The results for stiffness during shortening in the moderately fatigued state agree with those for isometric conditions (Edman & Lou, 1990) in showing that fatigue reduces force more than it reduces stiffness. In terms of cross-bridge operation, this means that both the number of attached bridges and the average force per attached bridge is reduced by fatigue. The change in the average force per bridge may be due to a shift in the distribution of bridges among those in the attached state so that the proportion in a low-force state is increased.

### $V_{\max}$, $V_0$ and $a/P_0^*$

The results for fatigue show that both $V_0$ and $V_{\max}$ are reduced in moderate fatigue. As stated in Results the reduction in $V_0$ is not significantly different from that predicted from the results of Edman & Mattiazzi (1985). We observed a smaller reduction in $V_{\max}$ than $V_0$. This reflects the fact that the rested state $V_0$, measured by the slack test method, is larger than $V_{\max}$, found by extrapolation of the force–velocity relation, in agreement with the findings of Edman (1979) and Josephson & Edman (1988). They concluded that velocity changes more per unit change in force in the region of very low force than expected from the shape of the rest of the force–velocity relation.

Our results show that there is less discrepancy between $V_0$ and $V_{\max}$ in moderate fatigue than in the rested state. The increase in $a/P_0^*$ in fatigue indicates that the force–velocity relation is less curved in fatigue than in rested muscle, and this effect may extend into the very low force region. If this is the case, it would explain why extrapolation of the force–velocity relation for fatigue gives a value for $V_{\max}$ which is closer to $V_0$, the unloaded shortening velocity, than in the rested state.

### Stretch

The results show that moderate fatigue has less effect on the fibre's performance during stretch than during isometric contraction or shortening (Fig. 1). There are a number of ways this could be explained.

First, it could be that fatigue reduces force during shortening and isometric contraction because the increase in $P_i$ shifts attached bridges from a high-force to a low-force attached state (Pate & Cooke, 1989). Stretch allows rapid reattachment into the low-force attached state and thus shifts the bridges into this state (Lombardi & Piazzesi, 1990). Thus during stretch most attached bridges may move into and remain in the low-force state, independent of the level of $P_i$. In other words, the bridges may be quite similar during stretch in the fatigued and unfatigued fibre, and consequently, the force during stretch would be similar in the fatigued and unfatigued fibre. There will, of course, be a higher force during stretch than during shortening or isometric contraction because the stretched bridges will be higher up their stress–strain curves.

Second, the reduction in the maximum velocity of shortening during fatigue (Edman & Mattiazzi, 1981; and results presented here) indicates that the rate of bridge detachment during rapid shortening is reduced in fatigue. It could be that bridge detachment in fatigue is slower during stretch, as well as during shortening, so that the bridges which are attached remain so over a longer distance of movement than in the unfatigued state. This would affect both the proportion of bridges that are attached and the force produced by each bridge. These two factors would both tend to enhance force during stretch in fatigue, and could result in the force during stretch being the same in the fatigued and unfatigued state. If the bridges remained attached for longer during stretch in fatigued than in the unfatigued state, then during stretch in the fatigued state there would be a greater increase in attached bridges (as a proportion of those attached under isometric conditions) than during stretch in the unfatigued state. This would require that the increase in stiffness during stretch (above that under isometric conditions) would be greater than the 10% increase above isometric that has been found in the unfatigued state by Lombardi & Piazzesi (1990). Whether this actually occurs could be tested by comparing the instantaneous stiffness during stretch and isometric conditions in the fatigued and unfatigued state.

### Implications of the stretch results for *in vivo* function

It should be noted that the effect of fatigue on stretch that we have observed may act as a 'protective' mechanism *in vivo* to prevent overstretch of the fatigued muscle. Studies of human subjects have shown that eccentric exercise, that is exercise involving stretch of active muscle, is particularly effective at causing muscle damage and delayed soreness (Jones, Newham, Round & Tolfree, 1986; Newham, Jones & Clarkson, 1987). Our results indicate that muscle's ability to resist stretch is relatively well maintained during fatigue. It appears that the muscle's resistance to damage by stretch is preserved during fatigue, despite the deterioration of other aspects of performance.

## Acidification, $P_i$ and fatigue

The results presented here show that the effects of intracellular acidification in the rested state are in some respects similar to the effects of moderate fatigue, but there are some significant quantitative differences. Fatigue had a larger effect on isometric force than acidification did, whereas the effects on $V_{\max}$ were not different. Changes in the concentrations of metabolites, $P_i$ and $H^+$, and their known effects on the mechanical properties of skinned fibres, offer a simple explanation of these effects.

### Effects on isometric force, $P_0$

Experiments on skinned fibres have shown that isometric force is reduced by both increased $P_i$ and increased $H^+$. It is very likely that $P_i$ concentration increases in moderate fatigue studied here; Kawano, Tanokura & Yamada (1988) have measured the time constant for recovery of $P_i$ to its initial value to be approximately 30 min after a tetanus of frog muscle at 4 °C. The time constant was independent of tetanus duration in the range tested, 0·2–10 s. $P_i$ recovery would therefore not be complete under the moderate fatigue conditions used here, so $P_i$ concentration would increase. From skinned fibre experiments we know this will change cross-bridge behaviour to reduce isometric force (for example, Altringham & Johnston, 1985; Chase & Kushmerick, 1988; Cooke *et al.* 1988; Godt & Nosek, 1989; Stienen *et al.* 1990; Dantzig, Goldman, Millar, Lacktis & Homsher, 1992; Stienen *et al.* 1992 and references therein).

It is likely that lactic acid is produced during moderate fatigue studied here. The size of intracelluar pH changes would depend on the effectiveness of intracellular buffering and the fluxes of lactic acid and of $H^+$ out of the fibre. To the extent that intracellular pH is acidified during fatigue, we expect that the effects of acidification will add to those of $P_i$ accumulation in reducing isometric force, as they have been shown to do in skinned fibres (Chase & Kushmerick 1988; Cooke *et al.* 1988; Godt & Nosek, 1989).

The fact that we found a significantly larger change in isometric force than in $V_{max}$ could easily be due to an increase in the concentration of both $P_i$ and $H^+$ in fatigue, but an increase in $H^+$ and no change in $P_i$, during intracellular acidification.

### Effect on $V_{max}$

The evidence from skinned fibre experiments about whether $P_i$ affects $V_{max}$ is more complicated. At pH 7·4 (Altringham & Johnston, 1985) and at pH 7·0 (Chase & Kushmerick, 1988; Cooke *et al.*, 1988) $P_i$ does not have a significant effect on $V_{max}$. At pH 6·0, there is disagreement. Chase & Kushmerick (1988) found that maximum shortening velocity was significantly depressed by $P_i$, whereas Cooke *et al.* (1988) found that it was not.

There is, however, general agreement that $V_{max}$ is strongly dependent on pH, $V_{max}$ being reduced by acidification (Chase & Kushmerick, 1988; Cooke *et al.* 1988).

The fact that we find the same size reduction in $V_{max}$ in moderate fatigue as with intracellular acidification, suggests that the change in $V_{max}$ is due to increased $H^+$ in both cases and that $V_{max}$ is unaffected by increased $P_i$ in fatigue.

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