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# THE FORCE–VELOCITY RELATIONSHIP IN VERTEBRATE MUSCLE FIBRES AT VARIED TONICITY OF THE EXTRACELLULAR MEDIUM

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SUMMARY

1. The relationship between active force and velocity of shortening was studied during tetanic contraction of isolated semitendinosus muscle fibres of the frog (0·5–2·0° C). Measurements were carried out with the fibre immersed in isotonic (1·00$R$) Ringer solution and in solutions that were made hypotonic by reduction of NaCl (osmolality 0·62 and 0·81 of normal Ringer) and hypertonic by addition of sucrose (osmolality 1·22 and 1·44 of normal Ringer).

2. The force–velocity relation was hyperbolic at loads lower than 80 % of measured isometric force ($P_0$) but exhibited a reversed curvature between 0·8$P_0$ and $P_0$. The maximum velocity of shortening was determined in two different ways: (i) by extrapolation to zero load from force–velocity data truncated at 0·8$P_0$ (computer fitting of hyperbola, least-squares method) and (ii) by recording the time required to take up the slack of the fibre after a quick release during tetanus.

3. Isometric force and maximum speed of shortening both changed inversely with the tonicity of the extracellular medium. Immersion of the fibre in 0·81$R$ hypotonic solution caused active tension and shortening velocity to increase by 10 ± 1 % (mean ± S.E. of mean, $n$ = 14) and 12 ± 1 %, respectively. Conversely, force and shortening velocity decreased by 12 ± 1 % ($n$ = 13) and 22 ± 2 % when normal Ringer was replaced by 1·22$R$ hypertonic solution. These changes doubled when the tonicity was altered from normal Ringer to 0·62$R$ and 1·44$R$, respectively.

4. Changes in fibre cross-sectional area equivalent to those obtained in the 0·81$R$ and 1·22$R$ solutions (+11 % and −13 %, respectively) were produced by varying the sarcomere length within the range 2·0–2·5 $\mu$m in the normal Ringer solution. Maximum velocity of shortening remained

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very nearly constant under these conditions, indicating that the shortening velocity, like the isometric force, is not critically dependent on changes in myofilament lattice width over the range considered.

5. The results support the view that *both* shortening velocity and active force are modulated by changes of the intracellular ionic strength above and below the level that normally exists in the intact muscle fibre.

## INTRODUCTION

Active tension of striated muscle has been shown to vary as an inverse function of the tonicity of the extracellular medium (Howarth, 1958; Edman & Andersson, 1968; April & Brandt, 1973). Evidence obtained in studies on isolated muscle fibres (Edman & Andersson, 1968; April, Brandt, Reuben & Grundfest, 1968; April & Brandt, 1973) supports the idea that this effect of tonicity is attributable to altered intracellular ionic strength rather than to a change in myofilament lattice width. Further support for this view is provided by the finding (Gordon & Godt, 1970; Thames, Teichholz & Podolsky, 1974) that ionic strength affects force production of skinned muscle fibres (which maintain a virtually constant myofilament spacing; Matsubara & Elliott, 1972) in much the same way as can be deduced from experiments on intact fibres.

The influence of tonicity on the *shortening velocity* is still incompletely understood. Howarth (1958) found in experiments on frog whole sartorius muscle that the shortening velocity at small loads, like maximum isometric force, decreased when the tonicity of the Ringer solution was raised. No increase in velocity was produced, however, by reducing the tonicity of the extracellular fluid. Results in apparent disagreement with these findings have been obtained in studies on skinned muscle fibres. Thus Thames *et al.* (1974) found that the maximum speed of shortening of frog skinned fibres was independent of ionic strength as the KCl concentration was increased above 140 mM. Reduction of the KCl concentration below 140 mM, on the other hand, was found to decrease the shortening velocity. These differences in results between intact muscle and skinned fibres could mean that tonicity affects the shortening velocity of the intact muscle through a more complex mechanism than by merely changing the intracellular ionic strength.

The present investigation is an attempt to further elucidate the nature of the contractile change that is produced by altering the tonicity of the extracellular medium. For this purpose the effects of hypertonic and hypotonic solutions have been studied on the relationship between active force and shortening velocity in isolated muscle fibres of the frog. Experiments have been designed to test whether the change in myofilament

spacing that associates a change in tonicity of the extracellular fluid may affect the velocity of shortening of the fibre. The results support the view that the maximum speed of shortening, similar to the active force, is inversely related to the intracellular ionic strength. A brief account of the results has been published previously (Edman & Hwang, 1975).

## METHODS

*Preparation.* Single fibres dissected from the ventral head of the semitendinosus muscle of *R. temporaria* were used. The frogs had been stored at a temperature of +4° C for more than 7 days before use. The techniques used for dissection and mounting of the fibre have been described (Edman & Kiessling, 1971). The experimental arrangement was essentially the same as described previously (Edman, 1975). The fibre was mounted horizontally in a thermostatically controlled Plexiglas chamber between a tension transducer and a lever attached to the moving coil of an electromagnetic puller (see below). The position of the tension transducer and/or the vibrator-lever system could be adjusted to give the desired rest length of the fibre.

*Bathing solutions and temperature.* The following solutions were used (mM). The osmotic strength (given within parentheses) of these solutions is expressed as a multiple of the value for the normal Ringer (I) which was measured to be 225 m-osmole/kg water (Andersson, 1973). For calculation of the relative strength of the modified Ringer solutions (II–IV) the osmotic coefficient of NaCl was taken to be 0·93 (Conway, 1952). The osmotic coefficient of sucrose was calculated according to the formulae provided by Dydyńska & Wilkie (1963). It was furthermore assumed that 1 l. Ringer solution contains 997 g water (Dydyńska & Wilkie, 1963).

I. Normal Ringer solution (1·00*R*): NaCl 115·5, KCl 2·0, $CaCl_2$ 1·8, Na phosphate buffer 2·0, pH 7·0.

II. Hypotonic Ringer (0·62*R* and 0·81*R*): The same composition as I except for NaCl which was reduced to 69·3 and 92·4 mM, respectively.

III. Hypertonic Ringer (1·22*R* and 1·44*R*): Solution I + 50 and 98 mM sucrose, respectively.

IV. Isotonic low-sodium Ringer (1·01*R*): The same composition as solution I except for addition of 46·2 mM sucrose and reduction of NaCl to 92·4 mM.

The solutions were freshly made up on the day of the experiment. The fibre was immersed in one of the above solutions for 45 min before a series of force–velocity recordings was carried out. Such a series of recordings usually spanned over a time period of 45–60 min. The temperature varied from 0·5 to 2·0° C between the different experiments and was maintained constant to ± 0·1° C throughout an experiment. The water used for washing of glassware and for preparation of solutions was deionized and double distilled in a borosilicate glass distiller. All chemicals used were of analytical grade.

*Stimulation.* The fibre was stimulated by passing current through a multi-electrode assembly (Edman, 1975) or between two platinum plate electrodes placed on either side of the preparation. Fused tetanic contractions were produced by passing a 1 sec train of 1 msec rectangular pulses with a frequency of 18–22/sec. Before a series of force–velocity recordings was started the fibre was stimulated to produce six or more isometric tetani at 2 min intervals. Care was taken to stimulate the fibre regularly at 2 min intervals throughout a series of force–velocity recordings. Using this procedure the tetanic tension varied insignificantly (less than 1 %) over many

hours of experimentation. In one series of experiments, to be described separately, tetanic contractions were produced at 30 min intervals.

*Tension recording.* An RCA 5734 mechano-electric transducer fitted with a tubular glass extension (compliance 1·5 $\mu$m/mN) was used. The frequency response of the tension transducer was approximately 750 Hz when the glass lever was submerged in the bath.

*Electromagnetic puller.* A description of the puller and its servo control system, including the displacement transducer, has been given previously (Edman, 1975). The puller was used in the present study to produce either position clamp (for recording of isometric force) or tension clamp (for recording of isotonic shortening of the fibre). An appropriate position clamp was achieved by using the signal from the displacement transducer (both in its original form and after differentiation) for feed-back control. Under the conditions used the tip of the lever could be held constant to within 2·5 $\mu$m. Tension clamp was produced by exchanging the signal from the displacement transducer in the feed-back loop for the signal from the tension transducer. A rapid switch from position feed-back to force feed-back was achieved by means of a relay. An appropriate degree of damping of the lever movements was achieved by adjusting the high-frequency response of the feed-back loop. Constant clamp tension (variation $<$ 0·01 mN) could be produced at any selected level from about 0·1 mN and higher. The triggering of the tension clamp was synchronized with the first pulse of the stimulus train. By means of a delay unit the onset and duration of the tension clamp could be set (with an accuracy of 2 msec) to the desired values during the plateau of the tetanus.

*Determination of fibre length and cross-sectional area.* The distance between the insertions of the fibre to the tendons was measured to the nearest 0·05 mm using a Zeiss Stereo II microscope at 6 × magnification. For determination of the cross-sectional area the fibre was mounted in a separate trough at approximately 2·2 $\mu$m sarcomere length. Two perpendicular diameters were measured (at 500 × magnification) at a given site along the fibre by turning the preparation 90° around its longitudinal axis. The cross-sectional area was calculated from the two diameters by assuming that the cross-section had an elliptical shape. The cross-section was determined in this way at ten equally spaced sites along the fibre and a mean value was calculated for each fibre.

*Determination of sarcomere length.* The sarcomere length at rest and during activity was determined from the laser diffraction pattern using the approach described by Cleworth & Edman (1972).

*Experimental procedure.* Typical recordings from an experiment are illustrated in Fig. 1. The fibre immersed in one of solutions I–IV (see above) was stimulated to produce a fused isometric tetanus at 2 min intervals. At a given time during the isometric plateau phase the lever was released to allow the fibre to shorten at a predetermined force. This was achieved by switching from position- to tension-feed-back control of the electromagnetic lever. After 150–200 msec the system was again changed to position feed-back and the lever brought back to its original position. The resting sarcomere length was adjusted appropriately (2·25–2·30 $\mu$m) so as to obtain a sarcomere spacing of 2·18–2·22 $\mu$m at the outset of the isotonic phase. It can be seen in Fig. 1 that following an initial period of oscillations (covering approximately 15–30 msec after the release) the tension was maintained stable throughout the shortening phase. Straight shortening ramps were obtained at different loads provided that the shortening did not go below approximately 1·95 $\mu$m sarcomere length. In some experiments to be described separately, the resting fibre length was varied during the experiment to allow force–velocity measurements to be made at different selected sarcomere lengths.

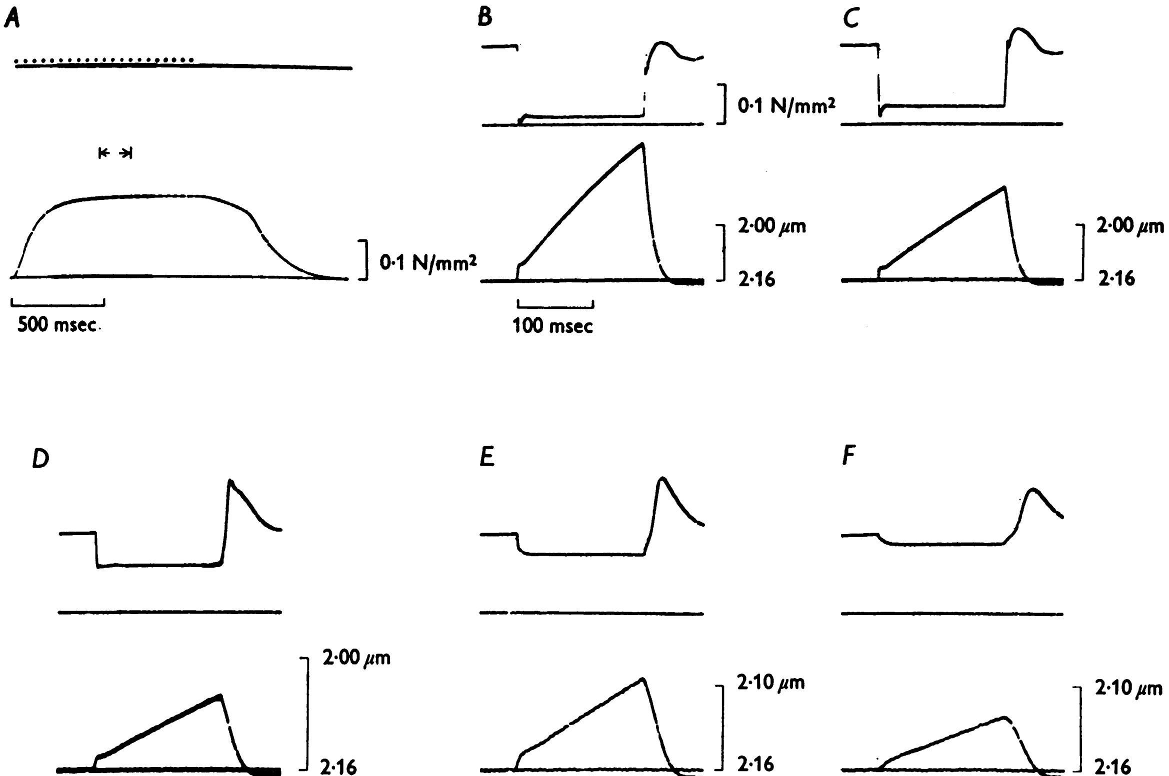


Fig. 1. Oscilloscope records illustrating: *A*, fused isometric tetanus and *B–F*, active shortening against various loads (tension clamp) in single muscle fibre. The tension clamp steps were performed during time interval of tetanus indicated by horizontal arrows in *A*. The shortening records indicate position of electromagnetic lever calibrated to indicate actual sarcomere length. Tension calibration the same in *A–F*. Cross-sectional area: $110 \times 10^{-4}$ mm$^2$. Temp. 1·1° C.

Experiments were performed to determine the shortening velocity of an unloaded fibre. For this purpose a quick release was carried out during the plateau of a fused tetanus to allow the fibre to shorten between approximately 2·20 and 2·00 μm sarcomere lengths, the release movement being fast enough to slacken the fibre. Knowing the total length change the velocity of unloaded shortening could be determined from the time interval between release and onset of tension redevelopment. Fig. 4 illustrates typical recordings from such an experiment. The velocity of shortening determined in this way will be referred to as $V_0$ in the following.

*Recording and measurement of responses.* The signals from the tension and displacement transducer were displayed on a Tektronix 5103N storage oscilloscope and photographed on 35 mm film. The film records were magnified 20× in a Nikon comparator (Profile projector model 6C). The over-all magnification of CRO traces to projector screen was 3·4×. Isometric and isotonic tensions were measured to 0·5 μN. Duplicate readings by two observers agreed within 5·4 ± 4·1 (S.D.) μN. Measurements of the shortening velocity were made within the straight portion of the shortening record (not earlier than 20 msec after the release) as the fibre passed between approximately 2·15 and 1·95 μm sarcomere lengths. The slopes of the shortening records were measured to the nearest minute of arc on the Nikon vernier scale and were converted to muscle lengths per sec (l./sec). Duplicate readings of the slope by two observers agreed within 0·005 ± 0·006 (S.D.) l./sec.

No account was made for the resting tension at the sarcomere lengths considered. The resting tension was < 0·002 N/mm² for sarcomere lengths up to 2·5 μm.

*Computer analysis.* The force–velocity data were fitted with Hill's (1938) equation by means of the least-squares method using a computer programme (Edman & Nilsson, 1972; Edman, Mulieri & Scubon-Mulieri, 1976). The Hill equation (1938) is given by

$$(P+a)(V+b) = b(P_0+a),$$

in which $P$ denotes force, $V$ velocity of shortening and $a$ and $b$ are constants with dimensions of force and velocity, respectively.

Student's $t$ test on paired observations was used in the statistical analysis.

## RESULTS

### *1. The force–velocity relation as a function of the tonicity of the extracellular medium*

*Normal Ringer solution.* The relationship between force and velocity of shortening was studied in single muscle fibres during tetanic activity using the approach illustrated in Fig. 1. Force–velocity data obtained from a fibre in normal Ringer solution are shown in Fig. 2. Hill's equation (1938) has been fitted to all data points (dashed line) and, in addition, to velocity values obtained at loads ⩽ 80 % of the *measured isometric force* ($P_0$) (continuous line). The characteristics of the force–velocity relation as depicted in Fig. 2 accords well with previous results (Edman *et al.* 1976). It can be seen that whereas Hill's equation provides an excellent fit to data truncated at $0·8P_0$, velocities recorded at higher loads are clearly lower than predicted from values obtained below $0·8P_0$. Accordingly, the curve derived from truncation at $0·8P_0$ intersects with the abscissa at a force ($P_0^*$) that is considerably larger than the measured

isometric force, $P_0$. The ratio $P^*_0/P_0$ was $1{\cdot}34 \pm 0{\cdot}04$ (mean ± S.E. of mean, $n = 10$).

The hyperbolas derived from all force–velocity data and from truncated data differ only insignificantly from each other at intermediate and low loads (Fig. 2). $V_{max}$, the predicted velocity of shortening at zero load, was $1{\cdot}95 \pm 0{\cdot}08$ l./sec ($n = 10$) when determined from truncated data. A

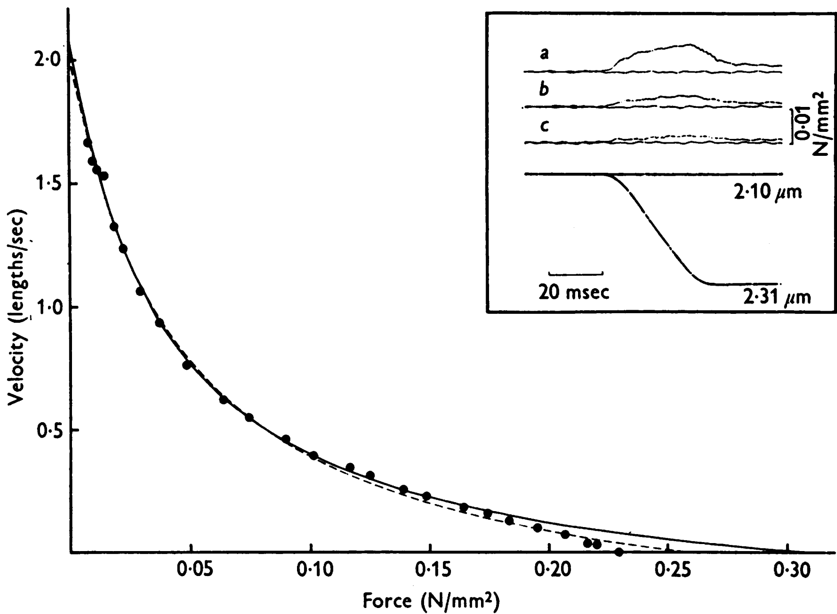


Fig. 2. Force–velocity relation in single muscle fibre. Hill's (1938) equation fitted by computer programme to all data (dashed line) and to data truncated at $0{\cdot}8P_0$ (continuous line). Isotonic Ringer solution. Note that the two curves nearly coincide above $0{\cdot}5P_0$. Cross-sectional area: $132 \times 10^{-4}$ mm². Temperature: 1·2° C. Inset: tension increase during stretch (0·21 μm/sarcomere) of resting fibre immersed in *a*, hypertonic (1·22*R*); *b*, isotonic, and *c*, hypotonic (0·81*R*) solutions. Cross-sectional area: $135 \times 10^{-4}$ mm². Temperature: 1·91° C.

4% lower value, $1{\cdot}88 \pm 0{\cdot}07$ l./sec, was obtained when the analysis was based on all force–velocity values. The values of $V_{max}$ and $P^*_0$ presented in the following sections have been determined from force–velocity data truncated at $0{\cdot}8P_0$.

*Hypotonic and hypertonic Ringer solutions.* A series of force–velocity recordings was first carried out in the normal Ringer solution. The fibre was thereafter equilibrated for about 45 min in the 0·81*R* hypotonic or 1·22*R* hypertonic solution and a new series of release recordings was performed. In some experiments the fibre was again immersed in normal

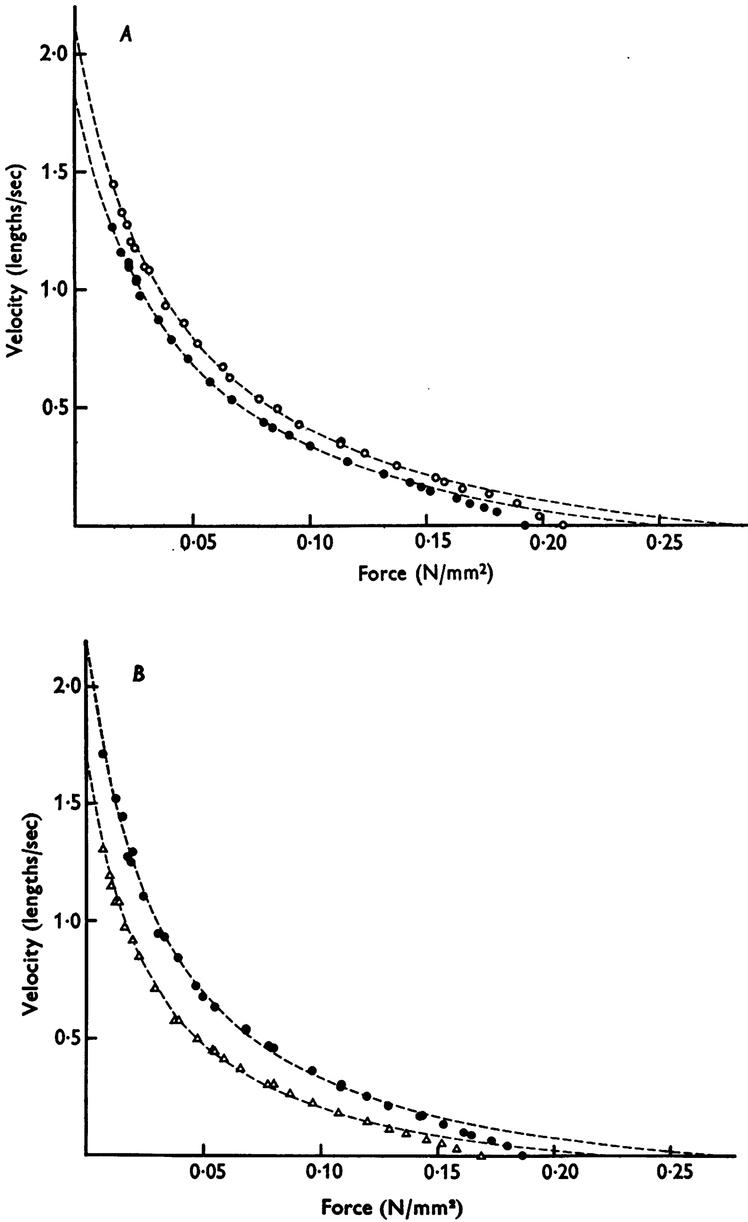


Fig. 3. Force–velocity relations in single muscle fibres: $A$, in $0{\cdot}81R$ hypotonic medium (open circles) and $B$, in $1{\cdot}22R$ hypertonic medium (open triangles). Filled circles in $A$ and $B$: recordings in isotonic Ringer solution. Hyperbolas fitted to data truncated at $0{\cdot}8P_0$. Cross-sectional area: $A$, 155 $\times 10^{-4}$ mm$^2$; $B$, $161 \times 10^{-4}$ mm$^2$. Temp. $A$, 1·1° C, $B$, 0·5° C.

Ringer for 45 min and another series of force–velocity measurements were made. In each of the solutions studied the fibre was able to produce a fused tetanic contraction at 2 min intervals over several hours.

As is illustrated in Fig. 3*A*, $V_{max}$ and $P_0$ both increased by immersing the fibre in the hypotonic solution. In six experiments (Table 1) $V_{max}$ increased by an average of 11 % and $P_0$ by 9 % of the value recorded in normal Ringer solution. Opposite effects were obtained by changing to hypertonic solution (Fig. 3*B*). In five experiments (Table 1) $V_{max}$ and $P_0$ decreased by 26 and 13 %, respectively. The changes of $P_0$ and $V_{max}$ showed a high statistical significance.

TABLE 1. Maximum force ($P_0$) and velocity of shortening ($V_{max}$) in hypotonic (0·81*R*) and hypertonic (1·22*R*) solutions. Data obtained in ordinary Ringer solution (1·00*R*) have been taken as unity (mean ± S.E. of mean, paired observations). Student's *t* test: *** significance at the 0·1 % level; ** significance at the 1 % level

| | 0·81*R* (*n* = 6) | 1·22*R* (*n* = 5) |
|---|---|---|
| $V_{max}$ | 1·11 ± 0·02** | 0·74 ± 0·02*** |
| $P_0$ | 1·09 ± 0·01*** | 0·87 ± 0·01*** |
| $P_0^*/P_0$ | 1·00 ± 0·06 | 0·95 ± 0·03 |
| $a/P_0^*$ | 1·08 ± 0·17 | 1·10 ± 0·05 |

The general shape of the force–velocity curve was not markedly changed by the osmotic interventions used. Thus as can be deduced from Table 1, $a/P_0^*$ was not significantly different between normal Ringer and the hypotonic and hypertonic solutions. The degree to which the force–velocity curve deviated from a true hyperbola in the high-force region was also found to be nearly the same in the different solutions. This is indicated by the fact that the ratio $P_0^*/P_0$ was not significantly affected by the changes in tonicity of the extracellular fluid.

A series of experiments was performed in which only $P_0$ and the velocity of unloaded shortening after quick release, $V_0$ (see Methods) were measured. The ordinary stimulation schedule with 2 min intervals between the tetani was used. As is evident from Fig. 4 and Table 2 (series I) the effects on $V_0$ and $P_0$ produced by the 0·81*R* and 1·22*R* solutions were quite similar to the effects on $V_{max}$ and $P_0$ presented in Table 1 and exhibited a high degree of statistical significance.

The possibility was considered (also see Okada & Gordon, 1972) that the effects produced by hypotonicity might be due to the lowering of the Na concentration *per se*. In order to test this point an experiment was performed in which a complete force–velocity analysis was carried out first in normal Ringer solution (I), and thereafter in *isotonic* low-sodium Ringer (IV). The experimental data obtained in solutions I and IV

yielded virtually identical force–velocity curves. The calculated value for $V_{max}$ was 2·13 l./sec in both solutions. The measured isometric force was 0·230 and 0·229 N/mm² in solutions I and IV, respectively. These results support the conclusion reached by Okada & Gordon (1972) that reduction of the Na concentration (at least to 0·8 of the normal concentration) does not by itself affect the contractile behaviour of the fibre.

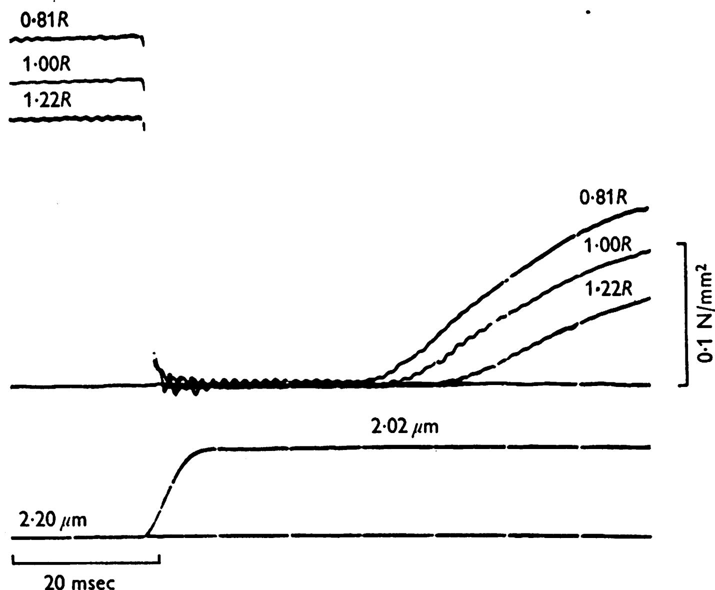


Fig. 4. Redevelopment of tension after quick release of single muscle fibre during plateau of isometric tetanus at different tonicities of the extracellular medium. Photographically superimposed records of tension (upper) and lever movement (lower). Sarcomere length at the onset of release, 2·20 μm and at the beginning of tension rise, 2·02 μm. Cross-sectional area: 95 × $10^{-4}$ mm². Temp. 2·0° C.

Attempts were made to investigate the force–velocity relation in solutions of lower tonicity than 0·8*R*. However, reducing the NaCl to 0·7 of the normal concentration rendered the fibre unable to produce a fused tetanic contraction at 2 min intervals and thus made it impracticable to perform a complete analysis of the force–velocity curve. It was possible, on the other hand, to produce a 1 sec fused tetanus after the fibre had been kept resting for half an hour in a low-Na medium containing ⩾ 0·6 of the normal NaCl concentration. Experiments were therefore performed in which the fibre was stimulated to produce a 1 sec tetanus after immersion for 30 min in 0·62, 0·81, 1·22 and 1·44*R* solutions. Before each of these solutions were administered, the fibre was immersed in ordinary

(1·00$R$) Ringer solution for 30–60 min and stimulated at 30 min intervals during this time. Isometric force ($P_0$) and velocity of unloaded shortening after quick release ($V_0$) were determined during each contraction. The results are presented in Table 2, series II.

TABLE 2. Maximum force ($P_0$) and velocity of shortening ($V_0$) in hypotonic (0·62$R$ and 0·81$R$) and hypertonic (1·22$R$ and 1·44$R$) solutions. Data obtained in ordinary Ringer solution (1·00$R$) have been taken as unity (mean ± S.E. of mean, paired observations). Series I: contraction intervals, 2 min. Series II: contraction intervals, 30 min. The effects presented in series I are statistically significant at the 1 % level

| | | 0·62$R$ | 0·81$R$ | 1·22$R$ | 1·44$R$ |
|---|---|---|---|---|---|
| I | $V_0$ | — | 1·15 ± 0·02 ($n$ = 5) | 0·78 ± 0·03 ($n$ = 5) | — |
| | $P_0$ | — | 1·11 ± 0·02 ($n$ = 5) | 0·88 ± 0·02 ($n$ = 5) | — |
| II | $V_0$ | 1·21 ± 0·02 ($n$ = 4) | 1·12 ± 0·02 ($n$ = 3) | 0·85 ± 0·01 ($n$ = 3) | 0·60 ± 0·01 ($n$ = 4) |
| | $P_0$ | 1·26 ± 0·07 ($n$ = 4) | 1·08 ± 0·02 ($n$ = 3) | 0·89 ± 0·01 ($n$ = 3) | 0·74 ± 0·03 ($n$ = 4) |

The effects on $V_0$ and $P_0$ produced by changing from normal tonicity to 0·81$R$ and 1·22$R$ were similar to those recorded at 2 min stimulation intervals (cf. Table 2, series I). Changing from normal Ringer to 0·62$R$ and 1·44$R$ solutions approximately doubled the effects on $V_0$ and $P_0$.

## 2. *Changes in fibre diameter*

For the further analysis of the contractile effects of altered tonicity (section 3) it was essential to quantify the changes in fibre diameter that occurred as the normal Ringer solution was exchanged for the hypotonic and hypertonic solutions. Measurements of the cross-sectional area (see Methods) were first carried out in the normal Ringer solution and, thereafter, at 30–60 min intervals, with the fibre immersed in 0·81$R$ and 1·22$R$ solutions and finally after immersion in normal Ringer. The cross-sectional area attained a steady-state value within 30 min after the fibre had been transferred into a new medium. The mean cross-sectional area was 1·11 ± 0·02 (S.E. of mean, five fibres) and 0·87 ± 0·01 (six fibres) in 0·81$R$ and 1·22$R$ solutions, respectively, relative to the values derived in normal Ringer. These changes remained constant over 2 hr observation time. Reversal of the cross-sectional area occurred (within 2 %) when the fibre was re-immersed in normal Ringer after exposure to the hypotonic and hypertonic solutions.

*3. The force–velocity curve in relation to sarcomere length and fibre diameter*

A change in tonicity of the bathing fluid will affect both the intracellular ionic strength and the diameter of the fibre (see section 2). The aim of the present experiments was to find out whether the observed changes in $V_{max}$ (section 1) could be attributed to the alteration in fibre diameter. For this purpose the force–velocity relation was determined at

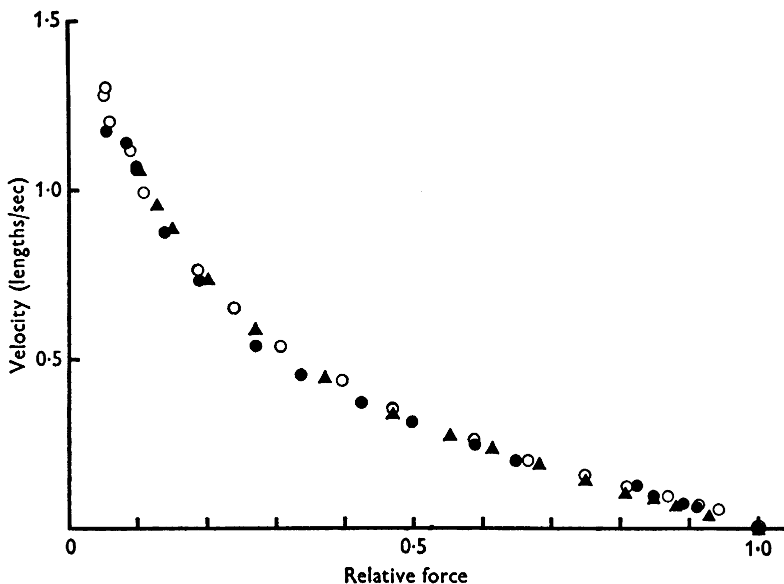


Fig. 5. Force–velocity relations in single muscle fibre recorded at three different sarcomere lengths in isotonic Ringer solution. Data normalized with respect to the $P_0$ value recorded at each length. Sarcomere length and computed values of $V_{max}$: ○, 2·03 μm, 1·59 l./sec. ●, 2·23 μm, 1·56 l./sec. ▲, 2·43 μm, 1·57 l./sec. Cross-sectional area: $133 \times 10^{-4}$ mm². Temp. 1·2° C.

sarcomere lengths ranging between 2·0 and 2·5 μm in the normal Ringer solution. As a fibre maintains a nearly constant volume, a change in sarcomere length from 2·20 μm to 2·00 and 2·45 μm, respectively, represents ± 10 % changes in cross-sectional area, i.e. approximately the same alterations in diameter as produced by the 0·81*R* and 1·22*R* solutions. The results of one experiment are illustrated in Fig. 5. It can be seen that the velocity of shortening at different relative loads was very similar at the three sarcomere lengths studied yielding almost identical values of $V_{max}$. Table 3 summarizes the results of force–velocity determinations at different sarcomere lengths in four fibres, also including measurements close to

2·5 μm. In each individual fibre the value of $V_{max}$ was found to vary by less than 4 % for sarcomere lengths between 2·0 and 2·5 μm. The results would thus seem to make clear that a 20 % change in cross-sectional area of the fibre does not affect $V_{max}$ substantially.

Force–velocity measurements were not pursued above 2·5 μm sarcomere length for the following reasons. The tension creep during a tetanus is appreciable at sarcomere lengths greater than 2·5 μm making the determination of $P_0$ uncertain. Secondly, the occurrence of passive tension at more stretched lengths makes it difficult to determine the actual load of the contractile system during the shortening phase.

TABLE 3. Maximum velocity of shortening ($V_{max}$) determined at different sarcomere lengths in four fibres

| Expt. | Temp. (° C) | Sarcomere length (μm) | Tetanic force $P_0$ (N/mm²) | $V_{max}$ (l./sec) |
|---|---|---|---|---|
| 22.i.1975 | 1·2 | 2·14 | 0·252 | 1·80 |
| | | 2·51 | 0·210 | 1·79 |
| 24.i.1975 | 1·2 | 2·23 | 0·208 | 1·56 |
| | | 2·03 | 0·202 | 1·59 |
| | | 2·43 | 0·190 | 1·57 |
| 04.ii.1975 | 0·8 | 2·22 | 0·218 | 1·87 |
| | | 2·02 | 0·215 | 1·84 |
| | | 2·42 | 0·196 | 1·80 |
| 05.iii.1975 | 1·2 | 2·20 | 0·230 | 2·05 |
| | | 2·48 | 0·197 | 2·01 |

*4. Changes in passive resistance to stretch*

Experiments were designed to find out whether a change in tonicity might affect a resting visco-elastic force within the fibre which could alter the effective load on the contractile system during shortening. The fibre was stretched in the relaxed state by 0·20 μm/sarcomere from a sarcomere length of 2·10–2·20 μm. The speed of stretch, 2·5 lengths/sec, was chosen to roughly correspond to the maximum *shortening* velocity of the fibre under the conditions used. The tension response to stretching was recorded after the fibre had been immersed in the respective solution for 30 min. The inset of Fig. 2 illustrates oscilloscope records from such an experiment. In four experiments the mean tension response to stretching was 0·00190 ± 0·00045 N/mm² in the ordinary (1·00*R*) Ringer solution. It decreased to 0·00116 ± 0·00029 N/mm² in the 0·81*R* solution and increased to 0·00533 ± 0·00108 N/mm² in the 1·22*R* solution. The implications of these results are discussed in the following section.

## DISCUSSION

The present study on isolated skeletal muscle fibres has shown that a decrease in tonicity below that existing in the normal Ringer solution increases both the maximum isometric force (also see Edman & Andersson 1968; Okada & Gordon, 1972; April & Brandt, 1973) and the maximum speed of shortening. An increase in tonicity was found, in an analogous way, to decrease both these parameters. Similar effects of hypertonicity have previously been observed by Howarth (1958) in experiments on whole sartorius muscle of the frog. There is an apparent difference, however, between the present results and those obtained on whole muscle as concerns hypotonicity. A decrease in tonicity to 60 % of the normal value was found by Howarth (1958) to have no clear effect on either the tetanic force or the velocity of shortening of the whole sartorius muscle. However, we have shown in this study that there is a failure of activation if single fibres are stimulated more frequently than once every 30 min at low ($\leq 70$ %) Na concentration. Howarth's observation that the tetanic force is unchanged (interval between tetani is not stated in his study) might therefore be explained if a fraction of the fibres in the muscle was unable to produce a fused tetanic contraction on repeated stimulation. This would counteract the increase in contractile force that is produced by the fully excitable fibres. On the other hand, the failure of the whole muscle to exhibit an increase in shortening velocity in the hypotonic medium remains unexplained. It is clear, however, that with a single fibre preparation both tension and shortening velocity at zero load are steadily increased as the tonicity is reduced to 0·6 of the normal.

A change in shortening velocity would occur if tonicity alters the effective load on the contractile system by affecting a passive resistive force in the fibre. This possibility was investigated by measuring the tension increase during stretch of resting fibres at different tonicities of the extracellular fluid. If an equally large resistive force is produced during *shortening* the present data would mean that the contractile system experiences an 'intrinsic' load that is less than 1 % of $P_0$ during shortening at zero external load. This intrinsic load would decrease by about one third in the $0{\cdot}81R$ hypotonic solution and would more than double in the $1{\cdot}22R$ hypertonic medium. The apparent shift of the force–velocity curve that may result from such changes of the intrinsic load would, however, only account for 1/30–1/10 of the shift that actually occurs in response to the osmotic intervention.

An essential step in the analysis of the tonicity effects was to find out whether the observed changes in mechanical performance could be related to the alterations in fibre diameter *per se* or to altered intracellular ionic

strength. The approach used was based on the fact that the fibre maintains a virtually constant volume as its length is changed (e.g. Huxley, 1953; Elliott, Lowy & Worthington, 1963; Elliott, Lowy & Millman, 1967; Huxley, 1969). Thus by altering the sarcomere length appropriately (at a given tonicity of the extracellular fluid) it was possible to vary the fibre diameter without affecting the volume, and hence the intracellular ionic strength. The finding that $V_{max}$ remains very nearly constant under these conditions almost certainly means that the shortening velocity is not critically dependent on either the myofilament lattice width or the area of overlap between the A and I filaments. The alternative would be that changes in overlap area and fibre diameter both affect $V_{max}$ but that these effects just cancel out one another at each length. Such a mechanism would seem highly unlikely in view of the fact that overlap area and fibre diameter vary as quite different functions of sarcomere length (a linear function and an inverse square root function, respectively) over the range studied. On the basis of these results it is therefore reasonable to conclude that the effect on $V_{max}$ that is produced by a change in tonicity of the bathing fluid is not attributable to the change in myofilament spacing but rather to the alteration of the intracellular ionic strength. The same conclusion was previously reached concerning the effects of tonicity on the isometric tetanic force of frog (Edman & Andersson, 1968) and crayfish (April *et al.* 1968; April & Brandt, 1973) single muscle fibres.

Results obtained in studies of skinned muscle fibres (Gordon & Godt, 1970; Thames *et al.* 1974) support the view that $P_0$ varies inversely with the intracellular ionic strength. However, there is a notable difference between the intact fibre and the skinned preparation as concerns the effect of ionic strength on the shortening velocity. Thus, according to Thames *et al.* (1974), $V_{max}$ of the skinned fibre is not markedly affected by raising the ionic strength above the control value, 140 mM-KCl, whereas $V_{max}$ *declines* as the ionic strength is reduced below this point. Evidence was presented in the same study, however, that the skinned fibre develops a partial rigor state during contraction at the reduced ionic strength which probably accounts for the decline of $V_{max}$. This partial rigor state was found to persist even after reimmersion of the fibre in the relaxing (Ca-free, ATP containing) medium. No effects of this kind appear in the intact fibre as indicated by the fact that the fibre relaxes completely after contraction in 0·62 and 0·81$R$ hypotonic solutions (also see Fig. 2 in Edman & Andersson, 1968). There is thus reason to believe that the changes in $V_{max}$ that have been observed in the skinned preparation (Thames *et al.* 1974) are not representative of the intact muscle fibre.

The possibility should be considered as to whether the tonicity induced contractile changes may also be attributed to altered kinetics of activator

Ca in the excitation–contraction coupling. Evidence obtained in studies of barnacle muscle fibres has shown (Ashley & Ridgway, 1970) that hypertonicity suppresses the release of Ca into the myoplasm in response to a single electrical stimulus (also cf. Homsher & Briggs, 1968). However, the rate of sequestration of Ca by the sarcoplasmic reticulum has also been shown to decrease as the KCl concentration of the medium is raised (Martonosi & Feretos, 1964; De Meis, 1968) and it is therefore uncertain whether in fact the steady-state Ca concentration in the myofibrillar space does undergo any marked change during tetanic stimulation in the hypertonic medium. Experiments on crayfish muscle fibres (April *et al.* 1968) would seem to indicate that altered metabolism of activator Ca is not the prime mechanism behind the contractile effects of altered tonicity. It was demonstrated that tonicity affects the tension response to a *given* amount of calcium injected into the fibre in much the same way as occurs during tetanic activation of frog muscle fibres.

The above conclusion that $V_{max}$ and $P_0$ are inversely related to the intracellular ionic strength is in good agreement with biochemical data which show that a similar inverse relationship to the ionic strength also exists for the rate of splitting of ATP by isolated myofibrils (Bendall, 1964) and acto-heavy meromyosin (Rizzino, Barouch, Eisenberg & Moos, 1970; Burke, Reisler, Himmelfarb & Harrington, 1974). This similarity in results is of interest in view of the observation (Bárány, 1967) that the maximal shortening velocity is quantitatively related to the myosin ATPase activity (determined *in vitro*) in skeletal muscle of a great variety of animal species. This finding supports the idea that the velocity of shortening at zero load reflects the speed at which the myosin bridges are able to interact with the actin components of the thin filament. The present results obtained on the intact muscle fibre are fully consistent with this view. They suggest that the intracellular ionic strength modulates the rate of interaction between actin and myosin and, by virtue of this action, serves as a regulator of the speed of shortening of the muscle. Contrary to previous observations on whole muscle (see above) the results described here suggest that changes in ionic strength both above and below the normal level in the fibre are effective in altering the mechanical performance of the contractile system.

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