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THE FORCE–VELOCITY RELATION OF ISOLATED TWITCH AND SLOW MUSCLE FIBRES OF *XENOPUS LAEVIS*

BY J. LÄNNERGREN

From the Nobel Institute for Neurophysiology and the Department of Physiology II, Karolinska Institutet, S-104 01 Stockholm, Sweden*

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SUMMARY

1. A study has been made of the relation between force and speed of shortening, or lengthening, in isolated twitch and slow muscle fibres, dissected from the iliofibularis muscle of *Xenopus laevis*. Both after-loaded and quick-release contractions were studied. Twitch fibres were stimulated electrically to give tetanic contractions (5–20 °C); slow fibres were activated by a rapid change to solutions with high K concentration (30–75 mM; experiments at 21–24 °C).

2. The velocity of slow fibres was constant during shortening over 10% length change in after-loaded contractions, except at forces exceeding about 0·8 of isometric tension, P_0. In quick-release experiments, shortening velocity was found to depend not only on the relative load, P/P_0, but also on the instant when the release was made. With increasing time after onset of contraction the initial rate of shortening decreased; also, a progressive fall in speed during shortening became more marked.

3. The fall in initial shortening speed with time of release from the onset of a contracture was more pronounced at high $[K]_o$ than at low.

4. The relation between the relative force, P/P_0, and shortening velocity, V, in after-loaded contractions (75 mM-K) and quick-release contractions (45 mM-K, early releases) in slow fibres could be represented by a hyperbola with the constants $a = 0{\cdot}10P_0$, $b = 0{\cdot}11$ lengths/sec; extrapolated $V_{max.}$ was 1·10 lengths/sec.

5. Isometric tension and maximum shortening velocity in slow fibres were very nearly constant between 32 and 75 mM-K. a/P_0, however, was clearly reduced at 32 mM-K, representing a more curved P-V relation.

6. Force–velocity data for twitch fibres (quick-release contractions, 20 °C) were reasonably well fitted by a hyperbola ($a = 0{\cdot}38P_0$, $b = 1{\cdot}97$ lengths/sec, $V_{max.} = 5{\cdot}20$ lengths/sec), but a systematic deviation was observed for forces exceeding $0{\cdot}6P_0$.

7. a/P_0 for twitch fibres was found to be independent of temperature in the range 5–20 °C. Q_{10} for b was 2·24 (10–20 °C), and 2·86 (5–10 °C).

8. $V_{max.}$ for twitch fibres was calculated to be 6·34 lengths/sec at 22·5 °C, the average temperature in the slow fibre experiments. The maximum shortening velocity in twitch fibres is thus 6 times higher than in slow fibres.

9. When loads in the range $1{\cdot}1$–$1{\cdot}4P_0$ were quickly applied to an actively contract-

* Present address.

ing slow fibre, lengthening of the fibre occurred in two phases, an initial rapid phase, followed by a phase of extremely slow lengthening. In corresponding experiments on twitch fibres lengthening was rapid at first and then gradually became slower.

10. Factors affecting the shape of the force–velocity curve are discussed. Calculations based on A. F. Huxley's (1957) model for muscle contraction indicated that cross-bridge turnover rate is about 15 times lower in slow than in twitch fibres.

INTRODUCTION

Amphibian skeletal muscle contains two major fibre types, twitch fibres and slow fibres. The two types differ with respect to innervation, fine structure, histochemical properties, and mechanical performance (for recent reviews, see Peachey, 1961, 1968; Hess, 1970; Lännergren, 1975*a*).

The isotonic shortening properties of frog twitch fibres have been extensively studied, especially at low temperature, 0–5 °C. Amphibian slow fibres, however, have mainly been studied under isometric conditions and there are few studies of their shortening speed under isotonic conditions. Aidley (1965) recorded shortening speed of whole rectus abdominis muscles during the late, maintained phase of a potassium contracture, which probably represents slow fibre activity. Floyd & Smith (1971), also using frog muscle, measured tension during constant-velocity releases in the whole iliofibularis muscle during selective, indirect activation of slow fibres. In both instances a form of 'inactivation' was observed with a progressive decrease in mechanical activity with shortening, which made it difficult to obtain reliable force–velocity curves. The only study of the shortening speed of isolated slow fibres is that by Costantin, Podolsky & Tice (1967), in which they found that the shortening induced by local application of Ca to a skinned fibre was about 10 times slower in slow than in twitch fibres.

In the present study another Anuran species has been used (*Xenopus laevis*), partly because the animals are much easier to keep in good condition in captivity, partly because slow fibres can be readily identified in the dissecting microscope. The morphological characteristics of various fibre types in *Xenopus* have been described previously (Lännergren & Smith, 1966; Smith & Ovalle, 1973). The major aim of the study was to obtain force–velocity data for slow fibres and compare those with corresponding data for twitch fibres. For technical reasons the slow fibre experiments were performed at room temperature. Since no force–velocity data for *Xenopus* twitch fibres are available, and since most frog fibre data refer to low temperature, it was considered necessary to perform parallel experiments on *Xenopus* twitch fibres. 'Inactivation' was observed during isotonic releases also in *Xenopus* slow fibres, but its effect could be minimized by timing the isotonic release appropriately. The results show that in *Xenopus* the maximum shortening velocity is about 6 times lower in slow than in twitch fibres; at intermediate loads the difference in shortening speed is much greater because the force-velocity curve is much more curved for the slow fibres. The lengthening response to the application of loads, exceeding isometric force, is quite different in the two fibre types.

A preliminary report of some of the results has been given (Lännergren, 1976).

METHODS

Fibre preparation, measurement of cross-sectional area, and mounting. Medium-sized (7–10 cm nose-tail length) specimens of *Xenopus laevis* were used. They were transported by air from South Africa and kept in tap-water filled plastic tanks at room temperature. The animals were fed minced meat once a week and showed no signs of deterioration for periods of several years. Experiments were performed at all times of the year without indications of seasonal variations in the results.

Dark-field illumination was used during the dissection. Slow fibres were identified in the tonus bundle of the iliofibularis muscle on the basis of their extreme transparency (Lännergren & Smith, 1966; Nasledov, Zachar & Zacharová, 1966) and isolated with aid of forceps and iris scissors with carefully thinned and sharpened tips. Their response to stimulation was tested at the beginning of the experiment, the criterion for correct identification being the absence of twitches on electrical stimulation and a large, maintained contracture in 30 mM-K solution. Twitch fibres were dissected in the same way, but from the area just outside the tonus bundle; they were of the large, granular type (the second type in the classification of Lännergren & Smith, 1966, see also Smith & Ovalle, 1973). Fibres selected as twitch fibres were tested for their ability to give twitches and tetani before the experiment was begun.

After a fibre had been isolated, its cross-sectional area was measured. The fibre was held just taut, and by turning it, the largest (a) and smallest (b) diameter was measured at three places. Cross-sectional area was calculated as $\pi/4.ab$ for each place and the mean was taken.

Fine stainless-steel hooks were then tied to the trimmed-down tendons with 20 μm nylon thread and the fibre was transferred to the experimental trough. This was made of acrylic plastic (Perspex^R, I.C.I.) with a glass bottom and was provided with a perfusion channel, 2·5 mm deep, 2·5 mm wide, and 40 mm long, in which the fibre was suspended between an isotonic lever and a force transducer. The position of the force transducer could be altered, thus allowing adjustment of fibre length.

Measurement of sarcomere spacing and fibre length. A cover-slip was placed on top of the channel and sarcomere length was measured with a high power microscope fitted with a Leitz UMK 50 objective (× 32) and a × 25 micrometer eye-piece. The length of the fibre was adjusted to give a sarcomere spacing of 2·3 μm as determined from at least three separate measurements; this value was chosen since it has been shown that the isometric tension-length curve of slow fibres has a flat maximum in the range 2·0–2·4 μm (Nasledov & Lebedinskaya, 1971; confirmed here in a separate series of experiments on four slow fibres, activated by applying 30 mM-K solution). The high-power microscope was then removed and a Leitz stereomicroscope, carried on a screw-operated arm, was positioned above the fibre in order to measure fibre length. The position of the microscope, in the longitudinal direction of the fibre, was indicated to the nearest 0·01 mm by a dial-type distance gauge, fitted to the microscope arm. Using an eye-piece with a hair cross and 50 × magnification the microscope was positioned above one tendinous insertion of the fibre, then moved to the other end. By reading off the difference in position from the distance gauge the length of the fibre could then be calculated.

Force transducer. A piezo-resistive transducer was used (AE 801, Akers Electronics, Horten, Norway) with a thin glass tube cemented to the silicon beam. The tube protruded 4 mm and was provided with a small steel hook at the end. The resonance frequency in air was 2 kHz and the compliance 12 μm/mN.

Isotonic levers. A horizontal lever arrangement was used, similar to that described previously (Lännergren, 1971), but in the present experiments the lever was made of balsa wood and it was pivoted on jewelled bearings. The fibre shortened against a constant force, provided by a spiral spring, the tension of which could be set by changing the position of a clamp, holding the peripheral end of the spring (Fig. 1). In after-loaded contractions the lever was prevented from stretching the unstimulated fibre by a stop; in quick-release contractions (against forces smaller or greater than the isometric force of the fibre) the movement of the lever was initially checked by a claw with diamond tips, lightly gripping a thin steel tab cemented in a vertical position to the lever. The lever could be released by actuating a solenoid, causing the claw to swing away from the tab. Inertial oscillations of the lever were damped by silicone oil, contained in a well under the lever, into which dipped a thin, T-shaped metal strip, cemented to the under-side of the lever. Two different levers were used: in the slow fibre experiments (which were performed first) the

length of the lever was 75 mm and the equivalent mass 29 mg; for the twitch fibre experiments the lever was shortened to 45 mm to reduce inertia, the equivalent mass was then 17 mg. The compliance of the free part of the lever, i.e. the part beyond the claw, was 3·9 μm/mN in both cases. With the shorter lever the force at the tip increased less than 2 % for 1 mm fibre shortening.

The position of the lever was recorded photo-electrically. A small vane (0·7 × 2·5 mm), attached to the lever at one third of the distance from the fulcrum, cast a shadow on the central part of a differential photo-diode (BPX 48, Siemens), arranged in 'reverse' mode, i.e. the common cathode was connected to a constant voltage source (+ 5·6 V); the anodes were connected via 10 kΩ resistors and a common 2 kΩ balance resistor to ground.

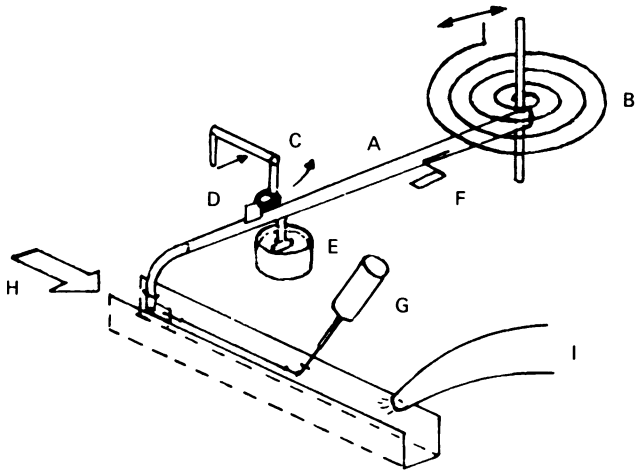

Fig. 1. Schematic drawing of arrangement used to record isotonic contractions of isolated muscle fibres. A, horizontal balsa wood lever with L-shaped tip of glass-tubing dipping into perfusion channel; B, adjustable spiral spring attached to lever axis; C, movable claw, lightly gripping steel tab (D) attached to lever; pull by solenoid (straight arrow) causes claw to swing away, releasing lever; E, dash-pot with silicone oil; F, vane, partially obstructing light falling on photo-diodes (not shown); G, force transducer; H, flow of solution from two-way stop-cock into perfusion channel; I, suction.

The potential difference between the anodes, which was related to the position of the vane, was sensed by a differential operational amplifier. Sensitivity was 178 mV/mm lever tip movement; a linear response was obtained over 1·6 mm movement of the lever tip.

Measurements of unloaded shortening velocity (V_0). A few experiments were performed on slow fibres to determine the shortening velocity of the unloaded fibre using the approach of Edman & Hwang (1977). The isotonic lever was substituted by a servo-controlled galvanometer (model G-100 PD, General Scanning Inc., Watertown, Mass.) with a 47 mm long, rigid tripod arm mode of bamboo attached to the axis. The fibre was connected to the tip of the arm and by using a suitable command pulse a quick (2 msec) length change could be imposed on the fibre. The fibre was first made to contract isometrically, a shortening step somewhat larger than required to slacken the fibre (about 0·2 mm; s_1) was then applied and the time between the release and the onset of tension redevelopment (t_1) was measured. Part of the shortening after release is due to instantaneous recoil of series elastic elements; to take this into account the procedure was repeated after the usual period of rest (10 min) using a larger step (about 0·5 mm; s_2) resulting in a longer time (t_2) before the onset of tension redevelopment. The shortening velocity was then calculated as $(s_2 - s_1)/(t_2 - t_1)$ and expressed as fibre lengths/sec.

Stimulation and recording. Twitch fibres were activated by passing 1 msec current pulses of alternating polarity between electrodes made of 2·5 mm wide strips of Pt foil, cemented to the walls of the perfusion channel and extending well beyond the length of the fibre. The fibre was

positioned halfway between the electrodes, which were 2·5 mm apart. Pulse amplitude was about 1·5 times threshold; stimulus frequency was 100 Hz at 20 °C, 50 Hz at 10 °C, and 30 Hz at 5 °C. The stimulator also triggered an adjustable delay unit; this actuated the solenoid which served to release the isotonic lever. Releases were made at about 200 ms from the start of tension rise at 20 °C, at about 300 msec at 10 °C, and at about 400 msec at 5 °C.

Slow fibres were activated by a rapid change from Ringer solution to a K-rich solution (see below). Releases were made by means of a hand-operated switch and were performed at various times (see Results).

The signals from the force and length transducers were amplified by solid-state, DC amplifiers and displayed on strip-chart recorders. In the twitch fibre experiments a U.V. galvanometer recorder (S.E. Laboratories, model 3006) was used. The natural frequency of the galvanometers used was 2·5 kHz. Load, expressed as per cent of maximum isometric tension, and shortening speed, calculated from the slope of the length trace during shortening, were measured directly from these records. In most of the slow fibre experiments a Sanborn 320 heated-pen recorder was used. With the pen-deflexion used the frequency response was flat within 3 dB up to 90 Hz. For slow fibres all values for shortening speed refer to the initial slope of the length trace, immediately after series elasticity recoil. Load is expressed as per cent of the succeeding isometric tension level (after-load experiments) or the preceding level (quick-release experiments). Isometric tension varied less than 10 % from the first to the last contraction in a series; in the majority of the experiments the difference was less than 5 %.

Temperature control. The slow fibre experiments were performed at room temperature, 21–24 °C. The experiments on twitch fibres were made at 20 °C, and in some cases also at 10 and 5 °C. Temperature control was obtained by maintaining a steady, slow flow of cooled Ringer solution through the perfusion channel. Cooling was achieved by letting the Ringer solution pass through a heat exchanger made from a 12 cm long piece of thin-walled steel tubing, surrounded by a water-jacket, which was perfused with an ethanol-water mixture from a Hetofrig Ultra Cryostat (Heto, Birkeröd, Denmark). The efficiency of the heat exchanger was increased by inserting a spiral of stainless-steel wire into the tubing, as well as surrounding it with another spiral, thus causing turbulence in the fluids. The heat exchanger was placed immediately before the stop-cock at the inlet to the perfusion channel. The temperature in the channel was monitored continuously at a point 3 mm downstream of the fibre. It varied with the rate of flow but could be maintained within ± 0·2 °C during an experiment. The temperature gradient in the channel was about 0·08 °C/mm at 5 °C, and 0·04 °C/mm at 10 °C. The temperatures given are those measured near the end of the fibre.

Solutions and solution changes. The composition of the Ringer solution used was (mM): NaCl 115, KCl 2·5, $CaCl_2$ 1·8, Na phosphate buffer (pH 7·2) 3·0. Solutions with increased K-concentration (range 30–75 mM-K) were made up according to Hodgkin & Horowicz (1959) and had a [K].[Cl] product of 300 mM^2. Double-distilled water and chemicals of analytical grade were used throughout.

A continuous slow flow of Ringer solution was maintained through the perfusion channel also in the slow fibre experiments. A change to a test solution could be quickly made with the aid of a stop-cock system. In most cases gravity-feed was used, giving a flow rate of 3–4 cm/sec; in some of the after-loaded contraction experiments high-K solution was introduced by means of a hand-operated syringe, giving a flow rate of 10–20 cm/sec. With gravity-feed the solution change was 90 % complete within 0·4 sec, as determined by switching between fluids of different conductances and measuring the change in resistance between electrodes placed at the normal position of the fibre.

Experimental procedure. Twitch fibres. After mounting and testing the response to single and repetitive stimulation, sarcomere length was adjusted to 2·3 μm and fibre length measured. Isotonic releases were first made at 20 °C, starting at about $0{\cdot}3P_0$, then ascending to the highest load, followed by a descending series to the lowest load; sometimes additional intermediate values were taken. Another, similar series was taken at 10 °C; if maximum isometric tension at the end of this was within 95 % of the first contraction at 10 °C a third series was made at 5 °C. The interval between tetani in each series was 3–4 min.

Slow fibres. Slow fibres were set up in the same way except that 30 mM-K solution was used to induce the first contraction. Recordings were then made at 10 min intervals using K-concentrations in the range 30–75 mM. Since the number of reproducible contractions which could be obtained was limited (usually twenty to twenty-five) and since it was desirable to perform other

experiments than $P-V$ measurements on a fibre, only those loads which were most critical for the shape of the force–velocity curve were used. Intermediate values were taken first, followed by lower and higher loads in alternation. In all but two of the fibres depicted in Fig. 6*A* a control was taken at the initial load; the maximum difference in shortening speed was 7 %. The quick-loading experiments were usually performed after a number of shortening runs. The experiment was terminated either when isometric tension had fallen to less than 90 % of the initial value or, more often, when relaxation after return to Ringer solution was beginning to be incomplete.

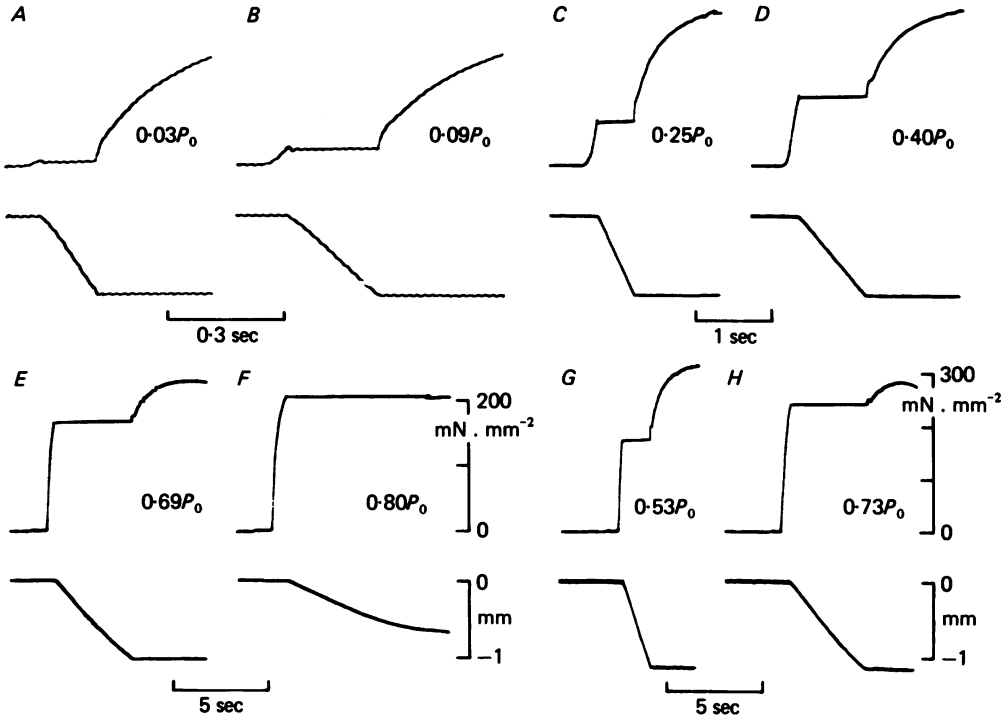

Fig. 2. After-loaded contractions of slow muscle fibres in 75 mM-K solution. Upper traces, tension; lower traces, length (shortening downwards). Records *A–F* from fibre 760311, cross-sectional area (*A*) $4{\cdot}45 \times 10^{-3}$ mm^2, length at 2·3 μm sarcomere spacing ($L_{2{\cdot}3}$) 9·3 mm; records *G*, *H* from fibre 760325, $A = 6{\cdot}58 \times 10^{-3}$ mm^2, $L_{2{\cdot}3} = 9{\cdot}0$ mm.

RESULTS

Isotonic shortening experiments on slow fibres

Quick-release vs. after-loaded contractions; 'inactivation'

It has been demonstrated for frog twitch fibres at low temperature that the rate of isotonic shortening against a very light load is constant over a wide range of sarcomere length (Gordon, Huxley & Julian, 1966); the velocity appears to be determined solely by the load, and values obtained with after-loaded and quick-release contractions are the same (Jewell & Wilkie, 1958; Edman, Mulieri & Scubon-Mulieri, 1976).

As with frog slow fibres (see Introduction) the behaviour of *Xenopus* slow fibres was found to be more complex. After-loaded contractions were studied first, using a K concentration of 75 mM for activation, which is well beyond the value required to

produce maximum isometric tension (about 30 mM; see also Lännergren, 1975*b*). In these contractions the rate of shortening was nearly constant over 1 mm except in the highest force range (Fig. 2) where a gradual slowing down occurred; also, with heavy loads the redevelopment of tension was slower after shortening. Fibre lengths ranged between 7·3 and 9·3 mm; 1 mm shortening thus corresponded to about 12 % change in sarcomere length.

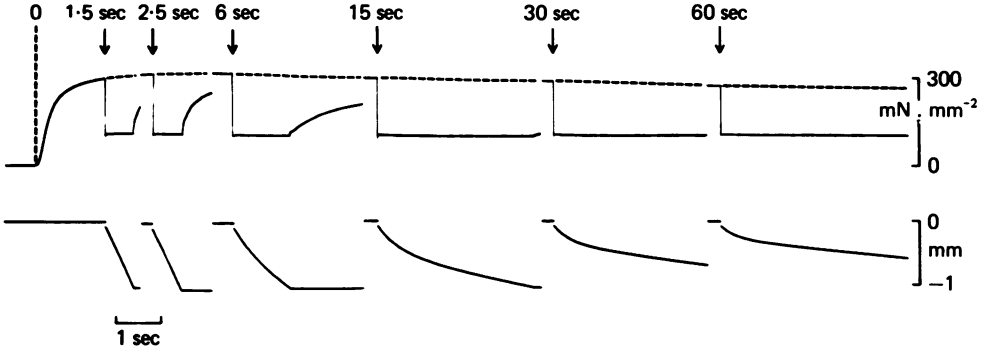

Fig. 3. Composite picture showing the influence of contraction time before release on shortening speed (load = $0{\cdot}33P_0$) in 45 mM-K solution. Releases were performed at progressively later times in separate runs at times indicated above tension record. Interrupted line indicates time course of isometric contraction. Shortening speed in a control release at 2·5 sec made at the end of the series (not shown) was 7 % higher than in the first 2·5 sec release. Fibre 760525, $A = 4{\cdot}53 \times 10^{-3}$ mm², $L_{2{\cdot}3} = 8{\cdot}6$ mm.

Because the level of activation at which the shortening occurs is different for different loads in after-loaded contractions, it was decided to study quick-release contractions as well, starting when isometric tension had reached a high value. The rise of isometric tension is rapid in 75 mM-K, but tension is not well maintained and starts to decay after about 5 sec (upper traces in Fig. 4*C*). The rate of rise is only slightly lower in 45 mM-K and tension is much better maintained (upper traces in Fig. 4*B*). Accordingly, many of the quick-release experiments were performed at this concentration. To begin with, the release was performed at the time of maximum tension, i.e. 5–10 sec from the start of tension rise. In nearly all cases the length trace was then curved, that is, shortening speed decreased continuously and was reduced by 15–35 % after 0·5 mm shortening.

Fig. 3 illustrates how shortening capability varies with the time of release during a long, maintained contracture (45 mM-K). Early in the contracture shortening speed is relatively high and constant; when the release is made progressively later a deceleration during shortening becomes increasingly pronounced; also, the initial speed of shortening clearly decreases. At the end of the 60 sec contracture in Fig. 3 initial shortening velocity was reduced by nearly 60 % and the fibre was able to shorten by only about 7 %; still, isometric tension was within 85 % of the maximum value. The decrease in shortening capability will be referred to as 'inactivation'. The underlying mechanism is not clear at present, and will require further investigation.

The effect of $[K]_o$ *on inactivation*

Releases were made against a load of about one third of P_0 at different times at three different K concentrations: one which was just sufficient to induce full isometric tension (30–32 mM-K), 45, and 75 mM-K. The results from five different fibres are summarized in Fig. 4. It can be seen that inactivation, measured as a fall in initial speed of shortening, developed more quickly the higher the $[K]_o$. At 30–32 M-K the decrease in initial shortening speed with time was moderate; however, even in early releases there was often a slight slowing down during shortening. This was observed also in after-loaded contractions.

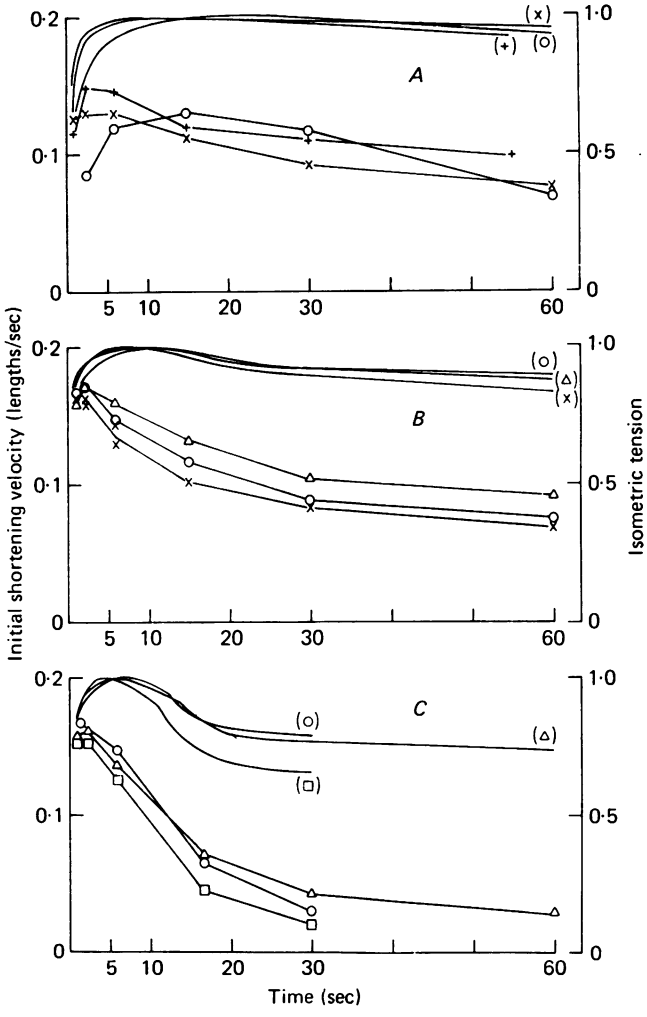

Fig. 4. Data collected from five different slow fibres, each denoted by a different symbol, showing decrease in shortening velocity for a load of $0{\cdot}33$–$0{\cdot}35P_0$ with time after start of tension rise (data points connected by straight lines). K concentration used to induce contraction was 30–32 mM in *A*, 45 mM in *B*, and 75 mM in *C*. Continuous lines at the top of each panel are tracings of isometric contraction records of the same fibres (ordinate on right-hand side in each panel). Cross-sectional area of fibres ranged between $4{\cdot}53 \times 10^{-3}$ and $10{\cdot}36 \times 10^{-3}$ mm², max. isometric tension was 266–338 mN/mm².

The effect of inactivation on maximum shortening velocity

The experiments above demonstrate that the ability of a fibre to shorten against a moderate load was progressively reduced during a maintained contracture and that the reduction was accelerated at high $[K]_o$. In order to find out whether the maximum shortening velocity was affected in the same way separate experiments were performed on two fibres in which the unloaded shortening speed was determined at various times by measuring the time required for the onset of redevelopment tension after a shortening step, large enough to slacken the fibre, had been applied (see

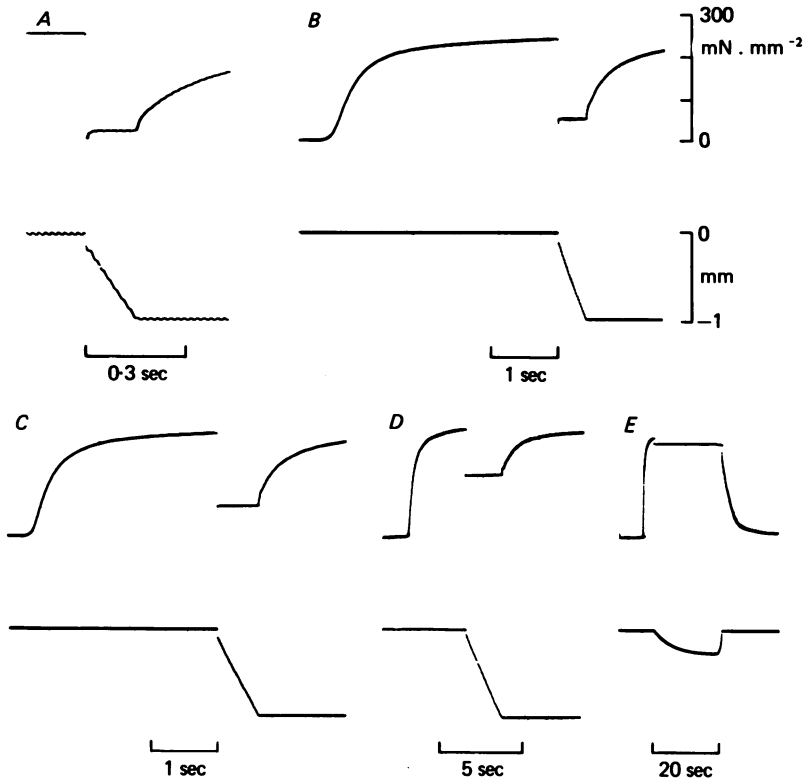

Fig. 5. Original records from a single slow fibre showing isotonic shortening after quick-release against various loads in 45 mM-K solution. Upper traces, tension; lower traces, length (shortening downwards). Records taken in order *C*, *D*, *B*, *E*, *A*. Fibre 770330, cross-sectional area $5{\cdot}50 \times 10^{-3}$ mm^2, $L_{2{\cdot}3} = 9{\cdot}1$ mm.

Methods). Maximum shortening velocity determined in this way (V_0) was found to be 1·08 and 1·22 lengths/sec, respectively, when measured at 2·5 sec from the start of tension rise in 45 mM-K solution and 1·00 and 1·12 lengths/sec, respectively, at 30 sec. Thus, V_0 was much less affected by inactivation than shortening speed against a load. The rate of tension redevelopment, however, was markedly reduced, the initial rate at 30 sec being only 50 % of that at 2·5 sec.

Force–velocity relation in the range $0 < P < P_0$

Force–velocity data from seven fibres are collected in Fig. 6*A*. For three of the fibres after-loaded contractions were used (75 mM-K, filled symbols), in four of the fibres quick-releases were used (45 mM-K, open symbols), performed at a time when shortening speed was found to be highest at this K concentration (Fig. 4*B*). Original records from one fibre are shown in Fig. 5. There is no clear-cut difference between

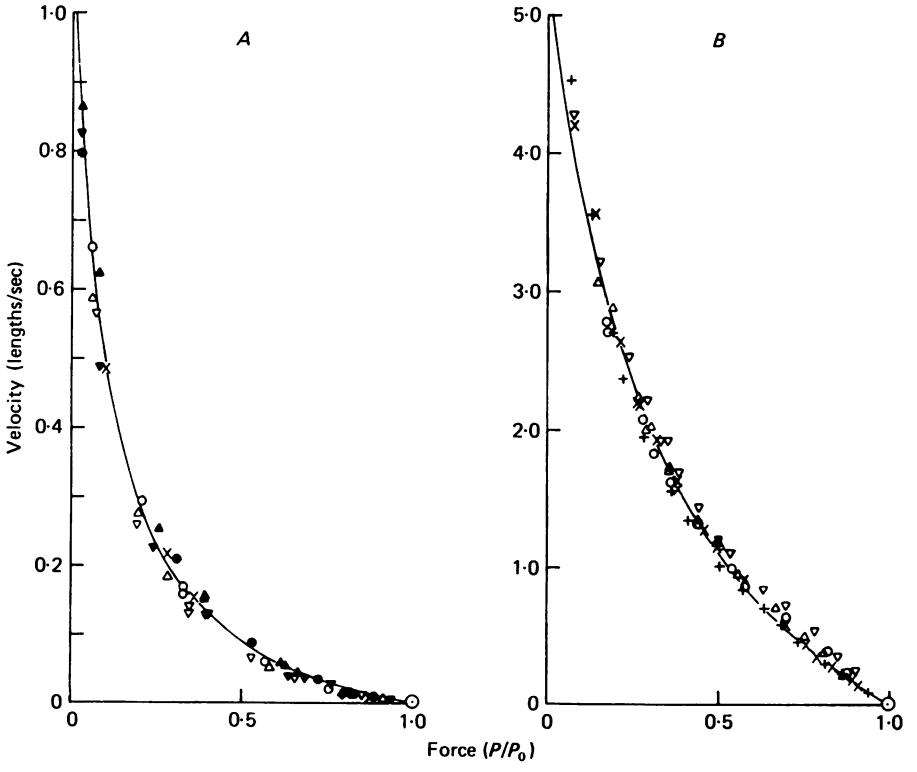

Fig. 6. Force–velocity data from seven slow fibres (*A*), and five twitch fibres (*B*). In *A* filled symbols represent values obtained in 75 mM-K solution (after-loaded contractions), other symbols values recorded in 45 mM-K solution (quick-release, 2–3 sec after start of tension rise), temperature 21–24 °C. Hyperbola drawn with $a = 0{\cdot}1P_0$, $b = 0{\cdot}11$ lengths/sec. Data points in *B* obtained in quick-release contractions starting about 0·2 sec from start of tension rise. Electrical stimulation, 100 Hz, 20 °C. Hyperbola drawn with $a = 0{\cdot}38P_0$, $b = 1{\cdot}97$ lengths/sec. Data points from one fibre of those studied at 20 °C omitted for clarity; they would fall between those marked by + and ×.

the two sets of data, hence both probably represent the maximum shortening velocity the fibres are capable of at the particular loads. This is also indicated by the results in Fig. 4*B* and *C*, which show that the maximum shortening velocity during quick-release against about $0{\cdot}33\,P_0$ was not increased by going from 45 to 75 mM-K solution. In two fibres (not included) after-loaded and quick-release contractions (at 0·44 and $0{\cdot}55\,P_0$, respectively) were performed on the same fibre at 45 mM-K; shortening speed differed less than 10% in each case.

The curve fitted to the values in Fig. 6*A* is drawn according to Hill's 'characteristic equation' (Hill, 1938); $V(P+a) = b(P_0-P)$. Values for the constants a and b were found by re-plotting the data as $(1-P/P_0)/V$ *vs.* P/P_0 and fitting a straight line to the values (Fig. 7*B*). The value for a thus found was $0{\cdot}10\,P_0$, and for b 0·11 lengths/sec; the ensuing value for $V_{max.}$, maximum shortening velocity, is 1·10 lengths/sec.

Apart from providing a means for obtaining the constants a and b, the linear plot of the data brought out one experimental finding more clearly, namely a marked deviation of the values from a hyperbola at higher loads. As can be seen from Fig. 7*A* all values above $0{\cdot}6P_0$ lie above a straight line fitted to the low-load values, i.e. the observed shortening velocity in this range is lower than predicted by Hill's equation.

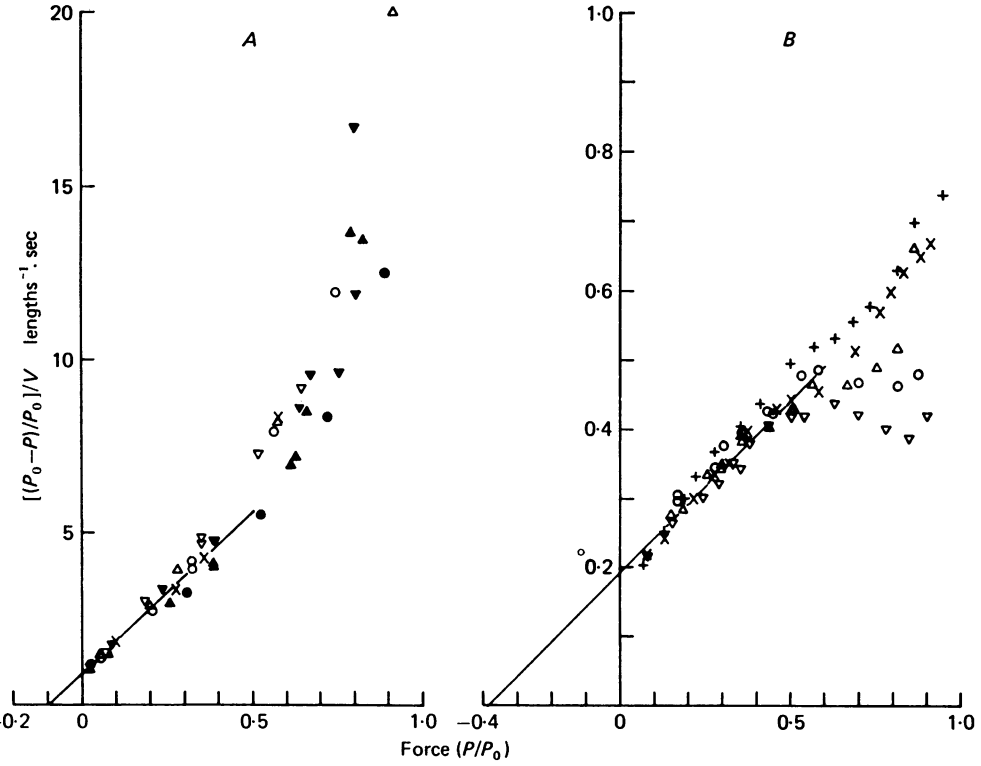

Fig. 7. *A* and *B*. Same data as in Fig. 6*A* and *B*, respectively, but plotted according to the linear form of Hill's equation. Regression analysis was applied to data for individual fibres below 0·5 and $0{\cdot}6P_0$, respectively; the mean values for slope and intercept for each fibre group were used for drawing the straight lines in *A* and *B*.

Effect of $[K]_0$ *on the force-velocity relation*

These experiments were carried out with the intention of finding out whether $[K]_0$, in the range where isometric tension is maximal, has any effect on V_{max}. Force-velocity measurements were performed alternating between 32 and 45 mM-K solutions; releases were made at about 6 and 2·5 sec, respectively, i.e. at times when shortening velocity should be optimal in each solution. The results from three fibres are shown in Fig. 8. Isometric force in 32 mM-K was 94, 97, and 100 %, respectively,

of P_0 in 45 mM-K. It is seen that V at very low loads was also very nearly the same; at intermediate loads however, the 32 mM-K values were clearly below the 45 mM-K values. The best-fitting hyperbola for the 32 mM-K values had $a = 0{\cdot}06\ P_0$ (45 K = $0{\cdot}09\ P_0$), $b = 0{\cdot}07$ lengths/sec (45 K = 0·10 lengths/sec); that is, the P–V curve was more curved at the lower $[K_0]$. Possible interpretations of this finding are considered in the Discussion.

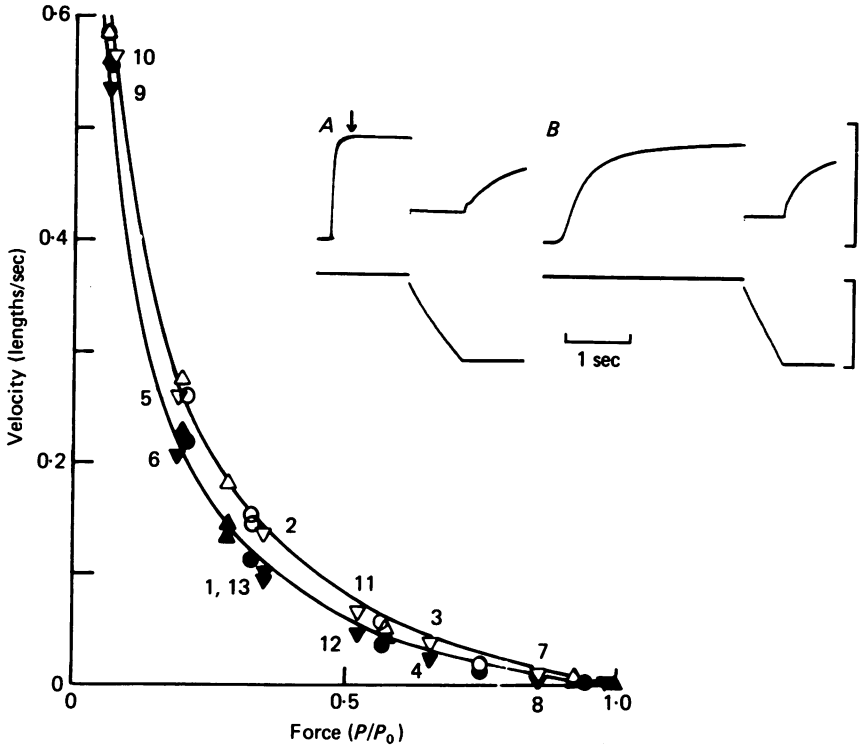

Fig. 8. Force–velocity data obtained from quick-release contractions in three slow fibres, each represented by a different symbol. Filled symbols denote values obtained in 32 mM-K solution, open symbols values recorded in 45 mM-K solution. The numbers indicate, for one fibre (▽, ▼), the order in which the measurements were made; the order was similar for the other two fibres. The velocity values for one fibre (○, ●) have been scaled down by 11·4% to facilitate comparison. Cross-sectional area was 4·53–5·50 × 10^{-3} mm², max. isometric tension 260–317 mN/mm², $L_{2\cdot3}$ 8·6–9·9 mm. Constants used for construction of hyperbolas were $a = 0{\cdot}09P_0$, $b = 0{\cdot}10$ lengths/sec (45 mM-K) and $a = 0{\cdot}06P_0$, $b = 0{\cdot}07$ lengths/sec (32 mM-K). *Inset*, sample records from fibre 770330 (△, ▲), *A*, quick-release in 32 mM-K, *B*, in 45 mM-K solution. Arrow in *A* marks instant when paper speed was changed from 1 to 20 mm/sec, in *B*, chart speed was 20 mm/sec throughout. Scale bars represents 300 mN/mm² for force traces and 1 mm shortening for length traces.

Isotonic shortening experiments on twitch fibres

The main reason for performing these experiments was to obtain twitch fibre data from the same animal species and at the same temperature so that a direct comparison with the slow fibre results could be made. Initial experiments were done at room temperature, but it was soon realized that the much higher shortening speed

of the twitch fibres made the recording system inadequate. The situation was improved by making the lever lighter and by working at lower temperatures.

Quick-release contractions (at the peak of isometric tension) were carried out on altogether six fibres, electrically stimulated (100 Hz); four of these were studied at both 20, 10, and 5 °C. Fig. 6*B* shows force–velocity data collected at 20 °C from five of the fibres. The shortening velocity was strictly uniform over the shortening distance permitted (1 mm), except at forces above $0{\cdot}85\,P_0$, where a slight slowing down occurred after about 0·8 mm shortening; for forces in the range $0{\cdot}90$–$0{\cdot}95\,P_0$ a marked bend of the length trace was seen after about 0·5 mm shortening, so that no fibre shortened more than about 0·6 mm.

The data in Fig. 6*B* are re-plotted in Fig. 7*B*, using the linear form of Hill's equation. By fitting a straight line to the values for each fibre in the range 0·07–$0{\cdot}6\,P_0$ the following mean values for Hill's constants were found: $a = 0{\cdot}38\,P_0$ (range 0·27–0·45) $b = 1{\cdot}97$ lengths/sec (range 1·53–2·24); $V_{max.} = 5{\cdot}20$ lengths/sec (range 4·64–5·89). Inspection of Figs. 6*B* and 7*B* shows that the experimental values of velocity deviate from a hyperbola both at the low ($< 0{\cdot}15\,P_0$) and the high ($> 0{\cdot}55\,P_0$) force end, being too high in both instances.

The effect of temperature

Figure 9 shows force-velocity values for one fibre at the three different temperatures used (filled symbols). When the values of velocity obtained at the lower temperatures were multiplied by a constant factor the resulting values (open symbols) were seen to fit very well to those obtained at 20 °C. The same result was obtained in the three other fibres. This shows that the shape of the force–velocity curve is not affected by temperature in this range. The good agreement after scaling also argues against the possibility that, for instance, the inertia of the lever seriously affected the measurements at 20 °C. The values for various force–velocity parameters at different temperatures are summarized in Table 1.

TABLE 1. The effect of temperature on force–velocity constants for *Xenopus* twitch fibres

Temp. (°C)	a/P_0	b (lengths/sec)	$V_{max.}$ (lengths/sec)
20 ($n = 6$)	0·38 (0·27–0·47)	1·97 (1·53–2·24)	5·20 (4·64–5·89)
10 ($n = 4$)	0·35 (0·31–0·42)	0·88 (0·79–1·03)	2·55 (2·38–2·81)
5 ($n = 4$)	0·35 (0·30–0·45)	0·52 (0·42–0·63)	1·49 (1·31–1·66)

Values are mean and range. $V_{max.}$ values have been calculated as $b/(a/P_0)$.

Assuming that a/P_0 is 0·38 and the temperature coefficient for b is 2·24 also at temperatures above 20 °C, $V_{max.}$ for twitch fibres would be 6·34 lengths/sec at 22·5 °C, which is the average temperature at which the slow fibre experiments were performed.

Isotonic lengthening with loads greater than P_0

Slow fibres. The fibre was first made to contract isometrically by applying 30–45 mM-K solution, releases were then made after 5–6 sec against forces which were 1·05–1·3 times P_0. The resulting lengthening response (Fig. 10*A*) had two distinct phases (1) an initial rapid give, followed by (2) an extremely slow lengthening, hardly

perceptible for forces up to $1{\cdot}2\,P_0$. The amplitude of the initial give was almost linearly related to the size of the force step (Fig. 11*B*) and amounted to about 10 % of the fibre's length at $1{\cdot}25\,P_0$.

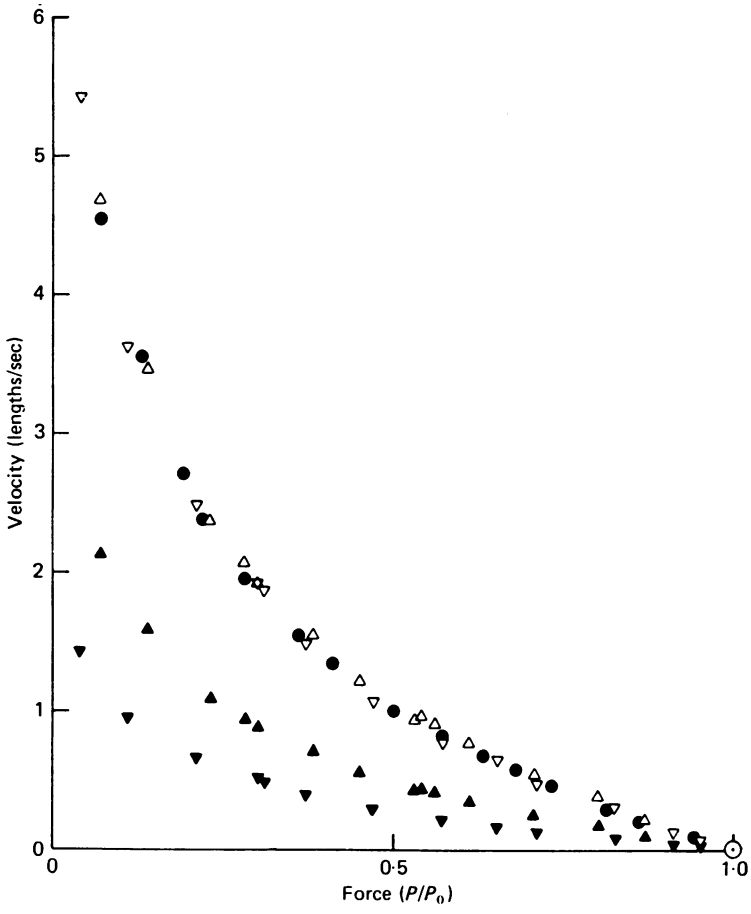

Fig. 9. Force–velocity relation in a single twitch fibre recorded at three different temperatures. Values obtained at 20·0 °C are given by ●, at 10·0 °C by ▲, and at 4·9 °C by ▼. Force is given as P/P_0, actual values were 2·55 mN at 20·0 °C, 2·04 mN at 10·0 °C, and 1·63 mN at 4·9 °C. Open symbols denote ordinate values at 10·0 and 4·9 °C multiplied by 2·19 and 3·78, respectively. Fibre 771103, cross-sectional area $9{\cdot}95 \times 10^{-3}$ mm^2, $L_{2\cdot3}$ 14·7 mm.

The fibre was observed in the dissecting microscope while the load was applied, and the lengthening was uniform as far as could be discerned. It is unlikely that the rapid elongation during phase 1 represents damage to the fibre, since perfectly normal contractions could be obtained after the usual period of rest, 10 min, and identical responses could be obtained several times in a fibre. Fig. 11*A* shows the relation between the load and the speed of lengthening during phase 1 and 2, respectively, and the load; shortening speeds for forces smaller than P_0 are also given. As is evident from the Figure, the force–velocity curve shows a very marked change in slope at P_0, even more marked than for frog twitch fibres (Katz, 1939).

Twitch fibres. A few experiments of this kind were performed on twitch fibres. Fig. 10*B* shows records from one experiment. The lengthening response was clearly different in that no distinct phases could be seen, instead there was a gradual decrease in lengthening speed. Except for at very low forces, the speed did not settle down to a steady value within the working range of the length transducer (about 1·5 mm), hence it was not possible in these experiments to obtain P–V data in the range $P > P_0$ for the twitch fibres.

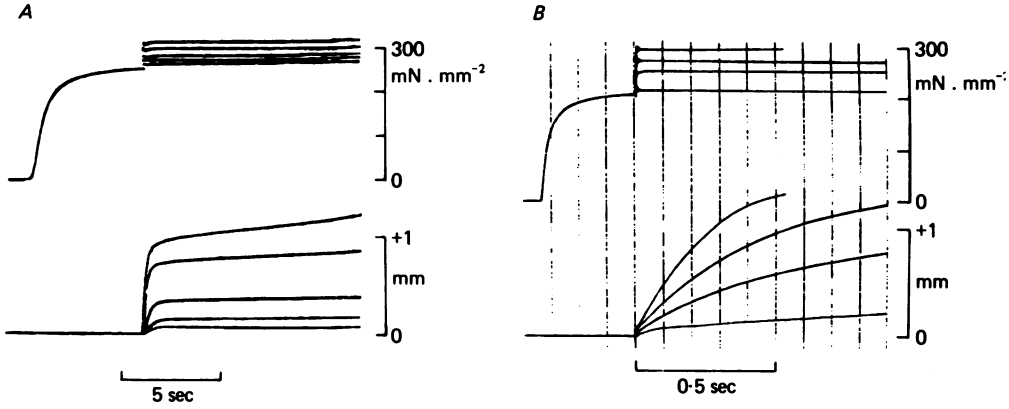

Fig. 10. Records of isotonic lengthening in single fibres. *A*, slow fibre, photographically superimposed records taken at 10 min intervals, activation by application of 30 mM-K solution, temperature 23·8 °C. Fibre 770422, cross-sectional area $4{\cdot}65 \times 10^{-3}$ mm², $L_{2\cdot3}$ 11·1 mm. *B*, corresponding records from a twitch fibre, electrical stimulation, 50 Hz, temperature 10·0 °C. Fibre 771103 (same as in Fig. 9).

DISCUSSION

The main result of the present study is that the speed of isotonic shortening in *Xenopus* slow fibres is related to the load in a manner analogous to that earlier observed in frog twitch fibres and in many other muscles, but the value for maximum shortening velocity, $V_{max.}$, is unusually low, 1·10 lengths/sec at room temperature. The form of the curve describing the relation between force and shortening velocity is also unusual, being more curved than for most other preparations studied.

Inactivation

A conspicious feature in many of the isotonic shortening experiments was a decrease in shortening speed with distance shortened. This has been noted also in studies on frog slow fibres (see Introduction). It was also observed here, using releases against a load of about $\frac{1}{3}$ of P_0, that the deceleration was more marked when shortening was preceded by a long period of isometric contraction; under this condition there was also a progressive fall in initial shortening speed. Experiments with imposed shortening steps showed that $V_{max.}$ was little affected by a preceding contraction; however, the rate of tension redevelopment when free shortening ended was markedly reduced at late times. Judging from these data, this would mean that inactivation mainly manifests itself as an increased curvature of the force–velocity relation. A possible underlying mechanism for increased curvature is discussed below in connexion with the effects of altered $[K]_o$ on the P–V relation.

The decrease in shortening capability against a load was observed although isometric tension was well maintained; thus the ability to bear tension and perform work could be partially dissociated. A similar phenomenon has been reported for crayfish fibres and frog semitendinosus muscles, activated by K-depolarization (Kawai, Brandt & Orentlicher, 1977). In their experiments shortening capability

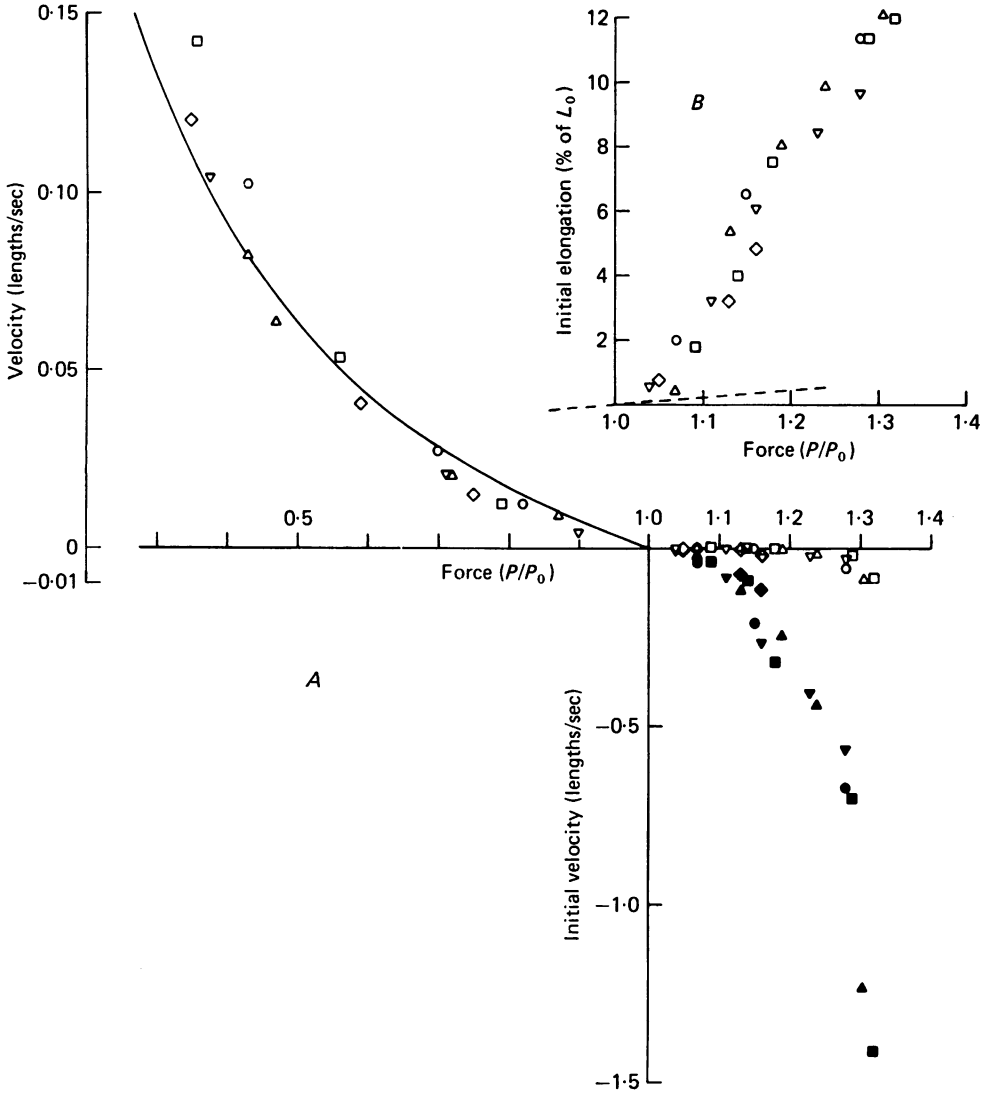

Fig. 11. *A*, open symbols, relation between force and speed of shortening or slow lengthening (phase 2, see text) in slow fibres. Filled symbols, speed of initial, rapid lengthening (phase 1). Five different fibres, activated by application of 30–32 mM-K solution, quick-release or quick-loading performed 5–6 sec after start of tension rise. Hyperbola drawn with $a = 0{\cdot}06P_0$, $b = 0{\cdot}07$ lengths/sec. *B*, relation between loading force and extent of initial give in per cent of $L_{2\cdot3}$. Interrupted line indicates length–tension relation for the undamped series elasticity calculated from quick-release experiments in the range $0{\cdot}1$–$0{\cdot}8P_0$. Same fibres as in *A*. Cross-sectional area range $3{\cdot}32$–$4{\cdot}93 \times 10^{-3}$ mm^2, max. isometric tension 266–344 mN/mm^2, $L_{2\cdot3}$ 7·3–11·1 mm.

was not tested; instead the ability to perform oscillatory work was determined and this decreased markedly within 1 min, although tension remained high. Kawai *et al.* interpreted their data as being due to a gradual decrease in actomyosin ATP-hydrolysis rate, possibly caused by a depletion of a myosin-hydrolysis product complex. Whether this is the correct explanation and whether it applies also to amphibian slow fibres cannot be decided at present.

Comparison with earlier studies on frog slow fibres

The only published force-velocity data for amphibian slow fibres are those of Floyd & Smith (1971) for which isovelocity releases of whole frog iliofibularis muscles were used, with selective nerve stimulation of slow fibres. $V_{max.}$ was estimated to be 0·11 lengths/sec, i.e. ten times lower than the value found here. Their low value is probably partly explained by inactivation, which was quite prominent in their experiments; the release was performed 10 sec from the start of stimulation and the tension was measured after 1 mm shortening. Another factor which might contribute to an underestimate are visco-elastic and frictional effects from the large number of passive twitch fibres present, although Floyd & Smith tried to correct for this. Also, there were, for technical reasons, very few observations in the high-speed range.

A probably more accurate determination of shortening speed of frog slow fibres against low loads is that by Costantin *et al.* (1967) in which the rate of sarcomere shortening in single 'skinned' slow fibres was measured in response to direct application of calcium with a pipette. The mean value was 1 μm/sec sarcomere, range 0·8–1·5 μm/sec sarcomere, which is less than half the value reported here ($1{\cdot}10 \times 2{\cdot}3 = 2{\cdot}52$ μm/sec sarcomere). It must be noted, however, that the latter is an extrapolated value to zero tension; in the skinned fibre experiments actively shortening sarcomeres were in series with passively stretched ones, which must have presented some load. Because of the steepness of the $P–V$ relation for slow fibres (Fig. 6*A*) a load of $0{\cdot}05P_0$ brings down V by nearly 40% from $V_{max.}$. It is also possible that *Xenopus* slow fibres are in fact faster than frog slow fibres; the time required to reach 90% of full isometric tension was about 1 sec in the present experiments, whereas the time for frog slow fibres appears to be about 2·5 sec (Nasledov *et al.* 1966; their Fig. 6*A*).

The shape of the force-velocity curve of slow fibres

A notable property of the slow fibre $P–V$ relation is the strong curvature as demonstrated in Fig. 6*A* with a a/P_0 value of 0·10 against 0·38 for the twitch fibres. A similar feature has been observed by Katz (1939) and by Woledge (1968) when comparing the properties of tortoise muscles with those of frog twitch muscles and by Rall & Schottelius (1973) comparing anterior (slow) and posterior (fast) latissimus dorsi muscles of the chicken. A more curved $P–V$ relation thus seems to be a common feature of slowly contracting muscles (see Close, 1972, for a related discussion of mammalian muscle). Woledge also showed by thermal measurements that tortoise muscle is more efficient in converting free energy into work and he has discussed possible causal connexions between the dynamic and energetic properties. It is interesting to note here that Floyd & Smith found that the maintenance heat rate

was about 30 times lower in slow than in twitch frog fibres. There is thus an obvious possibility that the turnover rate of cross-bridges is lower in slow than in twitch fibres.

Turnover rates cannot be determined directly from mechanical experiments. However, calculations can be made, based on A. F. Huxley's (1957) model for muscle contraction. Cross-bridge turnover rate of twitch fibres (r_{tw}) relative to the turnover rate of slow fibres (r_{sl}) can be expressed as the ratio of the sums of bridge attachment and detachment rates, i.e. $(f_1+g_1)_{tw}/(f_1+g_1)_{sl}$. Following Huxley's theory, (f_1+g_1) can be found by obtaining a fit of a constructed P–V curve (Simmons & Jewell, 1974, eqn. (1), p. 121) to the experimental data. When this was done $(f_1+g_1)_{tw}$ was found to be 520 sec^{-1} and $(f_1+g_1)_{sl}$ 35^{-1} (with $(g_2)_{tw} = 1460\ sec^{-1}$ and $(g_2)_{sl} = 250\ sec^{-1}$, obtained from the $V_{max.}$ values). Thus, turnover rate would be 15 times lower in slow than in twitch fibres.

It might be argued that the large difference in the form of the P–V curve for slow and twitch fibres observed here is partly due to the different modes of activation used for the two fibre types. While this possibility exists, it is made less likely by the results obtained from one particular fibre. This was a very slow twitch fibre (contraction time 135 msec), obtained unintentionally during the dissection, which gave long-lasting contractions in 30–75 mM-K solution. P–V data were obtained from this fibre, both during electrical stimulation (50 Hz) and during K-depolarization (45 mM-K); the maximum difference between the two sets of data was 8·3 %.

The effect of $[K]_o$ *on the force–velocity relation*

The present experiments have shown that in the range where peak isometric tension is reached (32–75 mM-K), $V_{max.}$ is also very nearly constant as demonstrated in Fig. 6*A* (comparison of 45 and 75 mM-K values) and Fig. 8 (32 and 45 mM-K values from the same fibres). However, as shown by the results in Fig. 8 the shortening speed at intermediate loads was lower in 32 than in 45 mM-K solution; thus the best-fitting hyperbola was more curved at 32 mM-K ($a/P_0 = 0{\cdot}06$) than at 45 mM-K ($a/P_0 = 0{\cdot}09$).

One possible explanation for this finding would be, following the reasoning above, and using the framework of Huxley's model (1957), that the rate constants for cross-bridge attachment and detachment, f_1+g_1, are influenced by $[K]_o$, possibly via an effect on internal free calcium concentration, $[Ca]_i$. The results might then be taken as support for the view (Julian & Sollins, 1973) that $[Ca]_i$ regulates not only the number but also the kinetics of attached cross-bridges, as opposed to the opinion (Podolsky & Teichholz, 1970; Thames, Teichholz & Podolsky, 1974) that at normal internal ionic strength $[Ca]_i$ only influences the number of attached bridges, i.e. P_0.

The force–velocity relation of Xenopus twitch fibres

Deviations from a hyperbola. As can be seen from Fig. 6*B* the force–velocity data from twitch fibres were reasonably well fitted by a hyperbola. A closer inspection, however, reveals that the fit is more satisfactory in some regions than in others. This is demonstrated more clearly when the data are plotted according to the linear form of Hill's equation (Fig. 7*B*), from which it is seen that the velocity values in the range $0{\cdot}6$–$0{\cdot}9P_0$ are too high; there is a tendency in the same direction for values

below $0{\cdot}2P_0$. A very similar result was obtained by Edman *et al.* 1976 (e.g. their Fig. 5) and Edman & Hwang (1977, e.g. their Fig. 5) in experiments on frog twitch fibres. Edman *et al.* found that a better fit could be obtained if values above $0{\cdot}8P_0$ were omitted and the hyperbola was extrapolated to about $1{\cdot}3P_0$. This approach has not been used here, first, because there is no *a priori* reason that velocity should be related to force in a hyperbolic fashion; a hyperbola just happens to be a convenient way of describing the P–V relation (and useful for comparing data from different fibre types); secondly, because it was found that the slow fibre values deviated from a hyperbola in the opposite direction for loads above about $0{\cdot}5P_0$ (Figs. 6*A* and 7*A*).

The effect of temperature: a/P_0. No clear change in a/P_0 was found in the range 5–20 °C (Table 1). Previous studies of amphibian muscles have given somewhat conflicting results. Thus, Katz (1939) found a higher value at 10·9 °C (0·38) than at 0 °C (0·26), whereas Hill (1938) obtained quite divergent values between 8·9–13·5 °C (0·19–0·29) and a lower value at 19·8 °C (0·18).

The effect on b. The value for b was found to be reduced at lower temperatures (Table 1). The mean Q_{10} was 2·24 in the range 10–20 °C, and 2·86 in the range 5–10 °C, values which are within the range observed by Hill and Katz.

Comparison with frog twitch fibres. The value of a/P_0 observed here for twitch fibres is higher than 0·25, the 'standard' value for frog fibres, derived mainly from measurements on sartorius muscles at 0 °C. There are, however, examples of a/P_0 values similar to those found here. Thus, Julian & Sollins (1973) found an average a/P_0 of 0·39 for six frog fibres studied at 0 °C; Edman *et al.* (1976) obtained a mean a/P_0 of 0·29 ($n = 16$), but two of their fibres (their Figs. 4 and 5) gave values of a/P_0 of 0·41 and 0·58, respectively (calculated over the range $0{\cdot}05$–$0{\cdot}65\,P_0$).

Extrapolated $V_{max.}$ at 5 °C for the *Xenopus* fibres was found to be 1·49 lengths/sec (Table 1). Edman *et al.* and Edman & Hwang obtained values of 1·7–1·8 lengths/sec at 1–2 °C; Julian & Sollins' values were slightly higher, 1·85 lengths/sec (0 °C). Assuming a Q_{10} of 2·86 also in the range 0–5 °C, the present value extrapolates to 1·21 lengths/sec at 1 °C. The maximum shortening velocity of the *Xenopus* twitch fibres studied here would thus be 30–40 % lower than that of frog twitch fibres.

The lengthening response of twitch and slow fibres

As was shown in Fig. 10 the response to a sudden application of a load greater than the isometric tension was different in twitch and slow fibres. The twitch fibre response could not be studied in its full extent and will not be discussed here; it appears to be similar to that of frog twitch fibres (Katz, 1939). In slow fibres the lengthening response had two clear-cut phases, an initial give, followed by very slow lengthening. A qualitatively similar response was obtained by Katz from tortoise retractor penis muscles. There are quantitative differences between the tortoise and the slow fibre responses, however. First, the initial lengthening of the tortoise muscle to a load of, for example, $1{\cdot}3\,P_0$ was only about 0·01 muscle lengths whereas the corresponding figure for *Xenopus* slow fibres was 0·1 muscle lengths. Another difference relates to the speed of lengthening during the second phase, after the initial give. From Katz's paper (his Figs. 1 and 4) it appears that the lengthening speed for a given increment of load above the isometric level (e.g. $P_0 + 0{\cdot}3P_0$) was at least as high as

the shortening speed for the same decrement below the isometric level ($P_0 - 0{\cdot}3P_0$), that is, in tortoise muscle there appears to be very little change in slope of the P–V curve at P_0 (against a five to sixfold decrease in twitch fibres; his Fig. 5); in the *Xenopus* slow fibres there was always a striking change in slope at P_0, so that the P–V curve was practically horizontal in the range $1{\cdot}0$–$1{\cdot}2\,P_0$.

The differences are surprising in view of the similarity of the force–velocity parameters for the tortoise and the *Xenopus* slow fibre preparations and cannot be explained at present. A hypothesis, which might be tested by applying loads at various times during a contracture, would be that the differences are in some way related to the inactivation process in amphibian slow fibres.

Slow fibres are generally assigned a 'tonic' or postural role in the animal. The results of the present investigation are consistent with such a view and indicate that a demand for long-term tension production at low metabolic cost is met by a specialization of the contractile machinery, in terms of low cross-bridge cycling rate.

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