Journal of Physiology (1988), **395**, *pp.* 679–694
With 8 text-figures
Printed in Great Britain

MAXIMUM VELOCITY OF SHORTENING RELATED TO MYOSIN ISOFORM COMPOSITION IN FROG SKELETAL MUSCLE FIBRES

BY K. A. P. EDMAN, C. REGGIANI,* S. SCHIAFFINO† AND G. TE KRONNIE
From the Department of Pharmacology, University of Lund, S-223 62 Lund, Sweden and Institute of General Pathology, University of Padova, Italy†

(*Received 21 May 1987*)

SUMMARY

1. The velocity of unloaded shortening (V_0), the myofibrillar ATPase activity and the immunoreactivity to two monoclonal antibodies (A1 and A2) that were raised against the myosin heavy chains were studied in single fibres of the anterior tibialis muscle of *Rana temporaria*. V_0 was recorded for the fibre as a whole using the slack-test method. Myofibrillar ATPase activity was determined by means of a quantitative histochemical technique.

2. A highly significant, direct relationship was found to exist between V_0 and the myofibrillar ATPase activity recorded in the same single fibres. Both V_0 and the myofibrillar ATPase activity changed in proportion to the cross-sectional area of the fibres.

3. Muscle fibres that had first been characterized with respect to V_0 and myofibrillar ATPase activity were exposed to monoclonal antibodies A1 and A2. Thin fibres, having relatively low V_0 and low myofibrillar ATPase activity, reacted preferentially with A1. Thick fibres, on the other hand, exhibiting relatively high V_0 and high myofibrillar ATPase activity, were preferentially stained by A2. A third category of fibres reacted with both A1 and A2. The results support the view that the variability in shortening velocity and myofibrillar ATPase activity that exists among twitch fibres in frog skeletal muscle is based on differences in myosin heavy-chain composition.

4. Attempts were made to elucidate further the previous observation (Edman, Reggiani & te Kronnie, 1985) that the velocity of unloaded shortening (V_0) differs along the length of individual muscle fibres. To this end discrete segments (0·5–0·7 mm in length) of intact fibres were delineated by opaque markers of hair that were placed on the fibre surface. The change in length between two adjacent markers (one segment) was recorded photo-electrically while the fibre was released to shorten against a very small load between 2·2 and 2·0 μm sarcomere lengths. In the majority of fibres (eight out of eleven preparations), V_0 and myofibrillar ATPase activity exhibited similar patterns of variation along the fibre. Pooled data from thirty-three segments of twelve fibres showed a positive correlation between V_0 and myofibrillar ATPase activity ($P < 0·05$).

* On study leave from the Institute of Human Physiology, University of Pavia, Italy.

5. The possibility was explored that the myosin isoform composition might vary along the length of an individual muscle fibre. For this purpose bundles of fibres were cross-sectioned at 0·5–1 mm intervals along their entire length and the reactivity to monoclonal antibody A2 was tested at each location. Clear differences in immunoreactivity tc A2 were demonstrable along the length of ten out of sixty-six fibres examined. These results provide evidence that the myosin heavy-chain composition may differ from one region to another within a single muscle fibre. Such a variation in myosin isoform composition may, at least partly, account for the segmental differences in contractile properties that are found to exist along the length of single muscle fibres.

INTRODUCTION

The velocity of shortening at zero load is generally thought to reflect the maximum speed at which the myosin cross-bridges are able to interact with the thin filaments during muscle activity. In line with this view the velocity of shortening against a small load has been found to correlate with the myosin ATPase activity (measured *in vitro*) in different muscles of a variety of animal species (Bárány, 1967). There is reason to believe that the differences in mechanical performance and in myosin ATPase activity that have been observed among muscles and individual fibres are based on structural heterogeneity of the contractile proteins. In accordance with this view several isoforms of both heavy and light chains of myosin have been identified in skeletal muscle fibres, but the evidence suggests that only the heavy chains are of relevance as a determinant of the myosin ATPase activity (Wagner, 1981).

Functional and morphological differences among individual fibres of amphibian skeletal muscles have been described. Twitch fibres of the tibialis anterior muscle of *Rana temporaria* exhibit considerable variability in maximum velocity of shortening (Edman, 1979). Based on their microscopic appearance five fibre types have been classified in the iliofibularis muscle of *Xenopus laevis* (Lännergren & Smith, 1966; Lännergren, 1979). These fibre types exhibit distinct force–velocity curves and have recently been shown to have a characteristic isomyosin distribution (Lännergren & Hoh, 1984). Using a qualitative histochemical ATPase measurement and immunohistochemical techniques, Rowlerson & Spurway (1985) have distinguished four different fibre types in frog skeletal muscle. Recently it has been demonstrated (Edman *et al.* 1985) that the velocity of unloaded shortening, V_0, varies to a substantial degree even within individual fibres of frog skeletal muscle. This opens the possibility that the kinetic properties of the contractile system may actually differ from one region to another along the same fibre.

It has been the aim of the present investigation to correlate the contractile properties (measured as V_0) of whole muscle fibres and of individual fibre segments with the myofibrillar ATPase activity and the myosin isoform composition. For this purpose a quantitative histochemical method was used to study the myofibrillar ATPase activity in whole single fibres and in discrete segments of single fibres. Monoclonal antibodies directed against the myosin heavy chains were employed to characterize differences in isomyosin composition. Evidence will be presented to

show that a relationship exists between V_0 on the one hand and the myofibrillar ATPase activity and the myosin isoform composition on the other, both in whole fibres and at segment level. Some of the results have been reported previously in a preliminary form (te Kronnie, Reggiani & Edman, 1985; te Kronnie, Reggiani, Schiaffino & Edman, 1986).

METHODS

Mechanical measurements

Preparation and mounting. Single fibres were isolated from the anterior tibialis muscle of *Rana temporaria*. Before use the frogs had been cold-adapted at +4 °C for at least 1 week. The dissection was carried out by means of fine-tipped scissors and care was taken to avoid any appreciable stretching of the preparation during this procedure. In experiments where surface markers were to be attached to the fibre (see below) connective tissue was carefully cleaned from the fibre along its entire length. The fibres were mounted horizontally in a thermostatically controlled Perspex chamber between a force transducer (AE 801, Aksjeselskapet Mikroelektronikk, Horten, Norway) and an electromagnetic puller. Details of the methods used for attachment of the fibre between the force transducer and the puller, and for alignment of the fibre in the chamber, have been given earlier (Edman & Reggiani, 1984).

The bathing solution had the following composition (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; Na_2HPO_4–NaH_2PO_4, 2·0; pH 7·0. Pre-cooled solution was continuously circulated through the chamber (volume *ca.* 2·5 ml) at a speed of approximately 5 ml/min. A thin glass slide covered the upper surface of the chamber along the whole length of the fibre. The temperature of the bathing solution was maintained constant to ±0·1 °C throughout an experiment. There was no temperature gradient greater than ±0·1 °C along the length of the fibre. The range of temperature between different experiments was 1·1–2·5 °C.

Stimulation. Rectangular pulses of 0·2 ms duration were delivered between two platinum plate electrodes that were placed on either side of the fibre approximately 2 mm from it. The stimulus strength was *ca.* 25% above threshold. A train of pulses (frequency 14–22 Hz) was given to produce a fused tetanus of 0·5 s duration. The pulse frequency was just sufficient to produce mechanical fusion in the individual fibre. The fibres were tetanized at regular 2 min intervals throughout the experiment.

Segment length recording. The technique used to record length changes of consecutive segments along a single muscle fibre has previously been described (Edman & Reggiani, 1984). Opaque markers of black dog's hair (*ca.* 50 μm wide) were placed on the upper surface of the fibre, approximately 0·5 mm apart, along the entire length of the preparation. The outermost markers were located *ca.* 0·2 mm from the edge of the tendon. The markers could be firmly attached to the fibre, and no detectable change in their position was generally recorded during a whole experiment. The distance between two adjacent markers (one segment) was recorded by means of a photodiode array, as described in detail previously (Edman & Reggiani, 1984). The segment length measurement could resolve length changes of 0·1% and had a time resolution of 0·25 ms. During recording of large length changes (as during V_0 measurements, see below) the error in the mesurement increased due to slight misalignment of the fibre and skew of the markers. The total error that might be encountered in a segment V_0 measurement under such conditions would be at most 3–4% and generally only a fraction of this value (Edman *et al.* 1985).

Recording and analysis of responses. Signals from the force and displacement transducers and from the photodiode array (used for segment length recording) were displayed on a Tektronix 5113 storage oscilloscope and photographed on 35 mm film. Measurements from the film records were made on a Nikon model 6C profile projector using the stage micrometer readings. Measurements of segment shortening velocity were made within the straight portion of the shortening records (not earlier than 20 ms after the release) to the nearest minute of arc on the Nikon vernier scale and were converted to muscle lengths per second (L/s).

The unloaded velocity of shortening, V_0, was determined for the whole fibre using the slack-test method (Edman, 1979).

Measurement of ATPase activity

After the unloaded velocity of shortening had been measured, the fibre was transferred to a separate trough where it was mounted together with a bundle of eight to twelve fibres at room temperature (21 °C). The rationale of mounting the single fibre together with a bundle of fibres was to provide control values for the density of staining in the individual sections of the fibre that were used for histochemical and immunocytochemical assays as described below. The fibre bundle was dissected from the tibialis anterior muscle, generally from the same animal as was used for dissection of the single fibre. The fibre and the bundle were held just above slack length (approximately 2·1 μm sarcomere spacing). The preparations were depolarized by replacing the ordinary Ringer solution by a solution containing 117·5 mM-KCl. The latter medium was thereafter exchanged for a 117·5 mM-KCl solution containing 15% gelatine. After allowing the gelatine to set for *ca.* 30 min at +4 °C, a block of gelatine containing the single fibre and the bundle was cut out, wrapped in aluminium foil and frozen by submersion in liquid nitrogen. The preparation was thereafter stored at −30 °C.

The ATPase activity was measured in individual sections of the muscle fibre using the quantitative histochemical method described by Van der Laarse, Diegenbach & Maslam (1984). The preparations were sectioned transversally in a cryostat within 48 h after freezing. Sections were collected on coated slides and stored at +4 °C before incubation in the ATP-containing medium. The incubation medium for the ATPase measurement had the following composition: 80 mM-Tris maleate, 2·7 mM-$Pb(NO_3)_2$, 6·8 mM-$CaCl_2$, 2·5 mM-Na_2H_2ATP, 2 g gelatine/100 ml, pH 7·2. The sections were incubated for 15 min at +18 °C without stirring the medium. This temperature was chosen, rather than 1–2 °C which was used for the mechanical measurements, in order to avoid an unduly long incubation time for the histochemical reaction. After incubation the sections were first rinsed three times (for 10 s) in distilled water. The slides were thereafter immersed in an aqueous solution containing 1% sodium disulphide (pH 7·5) for 60 s and were finally rinsed in distilled water. Within 75 h after the reaction, the light absorbance of the brownish lead sulphide precipitate on the sections was determined in a Zeiss cytospectrophotometer at 550 nm using a 20 × immersion objective lens. The absorbance (A) was calculated in arbitrary units according to the following expression: $A = -\ln(I_1/I_2)$, where I_1 and I_2 denote the light intensity measured within the fibre domain and immediately outside the specimen, respectively. To determine the mean absorbance (A_F) of a fibre, five sections were investigated at each of five different locations spaced at approximately 0·8 mm intervals along the fibre. The mean absorbance derived in this way provided a measure of the mean ATPase activity of the fibre.

In experiments where individual fibre segments were studied, the preparation was embedded in gelatine with the hair markers (delineating the segments, see above) still attached to the fibre surface. The embedding procedure, including the initial depolarization of the fibre, was the same as described above. Before freezing the gelatine block the inter-marker distances were remeasured at 40 × magnification to ascertain that no change in the location of the markers had occurred during the embedding. The markers were used for identification of the individual segments during the subsequent cryosectioning of the fibre. For ATPase studies of individual segments, those segments were selected which showed a clear difference in shortening velocity. Usually two or three segments were studied in each fibre. Serial sections (16 μm thick) were collected and assayed from the whole length of the relevant segments so that the total of the section thicknesses corresponded to the segment length. The sum of absorbances of all sections from a given segment was normalized with respect to the segment's length. The value so derived, A_S, provided a measure of the ATPase activity *per unit length* of that particular segment.

Immunocytochemistry

Two monoclonal antibodies, BF-34 (A 1) and BF-23 (A 2), were obtained by fusing NS-0 mouse myeloma cells with spleen cells of mice that were immunized against bovine fetal skeletal myosin. Both antibodies were specific to myosin heavy chains as determined by immunoblotting. Indirect immunofluorescence procedures were used to determine the pattern of reactivity to the two antibodies in different fibre types within the anterior tibialis muscle of *Rana temporaria*. The antibody reactivity was studied in the following preparations of the anterior tibialis muscle: (1) whole muscle, (2) bundles of fibres and (3) single fibres that had previously been characterized with respect to their maximum shortening velocity (V_0) and ATPase activity. Transverse cryosections

of these preparations were incubated with the undiluted culture supernatants of clones BF-34 and BF-23 and thereafter incubated with appropriate dilutions of fluorescein-conjugated anti-mouse immunoglobulin G. The sections were viewed and photographed in a Leitz fluorescence microscope.

In order to characterize the reactivity of a whole isolated single fibre or of individual fibres within a bundle, five serial sections were collected for immunostaining in each of five different regions along the preparation. The antibody reactivity was consistently found to be the same in all five sections examined within any given region studied. Attempts were made to investigate whether the immunocytochemical properties varied from one region to another along an individual muscle fibre. In these experiments five serial sections were collected in fibre regions that were separated by approximately 1 mm. A change in staining of a given fibre between different regions was judged by comparing its antibody reactivity with that exhibited by other fibres in the same section. Such a comparison could be achieved also in the case of an isolated fibre by mounting the fibre in parallel with a fibre bundle before the gelatine embedding and freezing (see above).

Determination of cross-sectional area and location of motor end-plate

The cross-sectional area of individual muscle fibres was determined from projected images of transverse cryosections. Traces of the fibre contour were produced at five different locations along the length of the fibre, using a projection microscope. The area was determined by cutting out and weighing the traces.

In order to determine the location of the motor end-plate, single fibres or bundles of two to three fibres were dissected from the anterior tibialis muscle and stained for acetylcholinesterase activity according to Karnovsky & Roots (1964). The length of the end-plate and the distance from the edge of the end-plate to the proximal and distal tendons were measured in a Zeiss stereo-microscope (40× magnification) to the nearest 10 μm.

RESULTS

Relationship between maximum velocity of shortening, ATPase activity and cross-sectional area in single muscle fibres

The velocity of unloaded shortening (V_0) was determined in eight single muscle fibres using the slack-test method (Edman, 1979). The measured values of V_0 ranged between 1·81 and 2·67 L/s. The ATPase activity (A_F) was determined in the same eight fibres (see Methods). The values of A_F varied between 0·179 and 0·247 units. As illustrated in Fig. 1, there was a positive correlation between V_0 and A_F. A linear regression of V_0 (L/s) upon A_F (units) yields the following relationship: $V_0 = 11{\cdot}57\ A_F - 0{\cdot}29$ (correlation coefficient, 0·90: $P < 0{\cdot}01$; $n = 8$).

The possibility was considered that maximum velocity of shortening and ATPase activity are related to fibre width. Figure 2 shows a histogram of the fibre cross-sectional area based on measurements from 304 fibres of one anterior tibialis muscle (dashed line) and 125 fibres in twelve bundles of different anterior tibialis muscles (continuous line). As can be seen, there is a wide distribution of fibre width, the cross-sectional area varying between approximately $1{\cdot}0 \times 10^{-3}$ and $33{\cdot}0 \times 10^{-3}$ mm^2. The highest frequencies of fibres (15–25%) are found within the range $1{\cdot}0 \times 10^{-3}$–$7{\cdot}0 \times 10^{-3}$ mm^2.

Figure 3*A* illustrates the relationship between V_0 and cross-sectional area (A_{cs}) in eighteen single fibres. In these experiments V_0 varied between 1·71 and 2·68 (mean 2·20) L/s and the cross-sectional area between 4·9 and 28·2 (mean $14{\cdot}125) \times 10^{-3}$ mm^2. A regression analysis provides the following relation between maximum speed of shortening (L/s) and cross-sectional area (mm^2): $V_0 = 29{\cdot}05\ A_{cs} + 1{\cdot}797$ (correlation coefficient, 0·74; $P < 0{\cdot}001$; $n = 18$).

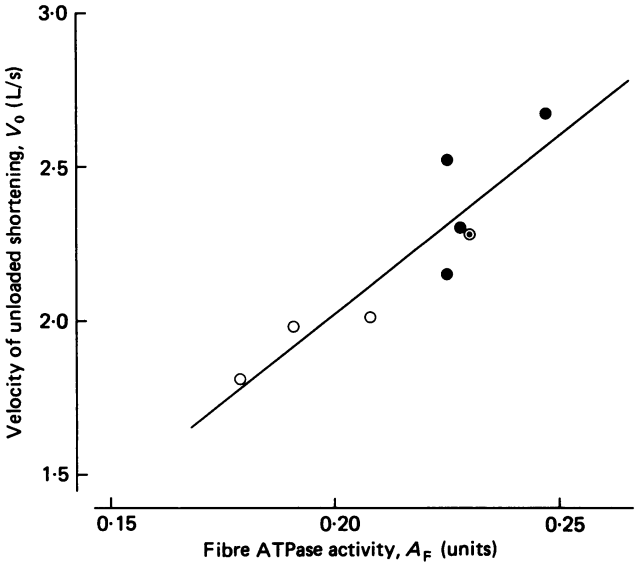

Fig. 1. Relationship between velocity of unloaded shortening (V_0, L/s) and myofibrillar ATPase activity (A_F, units) in eight anterior tibialis muscle fibres of the frog. Line: least-squares regression of V_0 upon A_F. ○, fibres reacting with the antibody A1. ●, fibres reacting with the antibody A2. ⊙, fibre reacting with both A1 and A2.

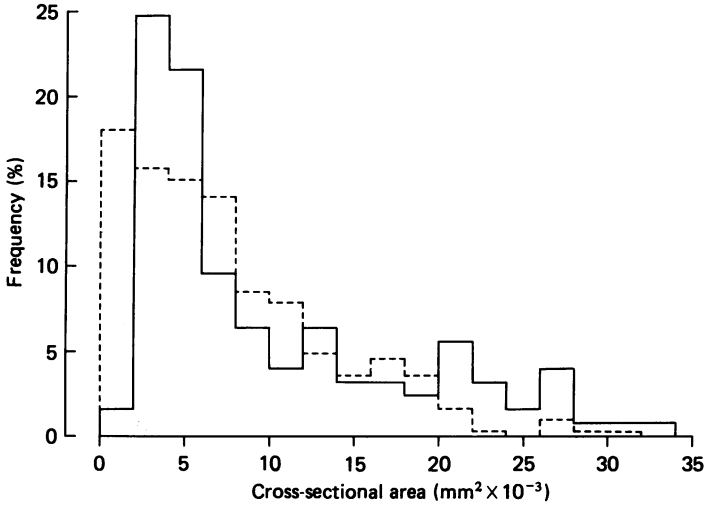

Fig. 2. Histogram illustrating distribution of fibre cross-sectional area in anterior tibialis muscle of the frog. Continuous line: measurements from 125 fibres in twelve isolated bundles of different muscles. Dashed line: measurements from 304 fibres of one muscle. Frequency is expressed as percentage of the total fibre number in respective series.

The relation between ATPase activity and fibre cross-sectional area was studied in twenty-one single fibres (Fig. 3*B*). In this series of experiments the cross-sectional area (A_{cs}) ranged between 1·4 and 25·2 (mean 12·050) $\times 10^{-3}$ mm^2. The ATPase

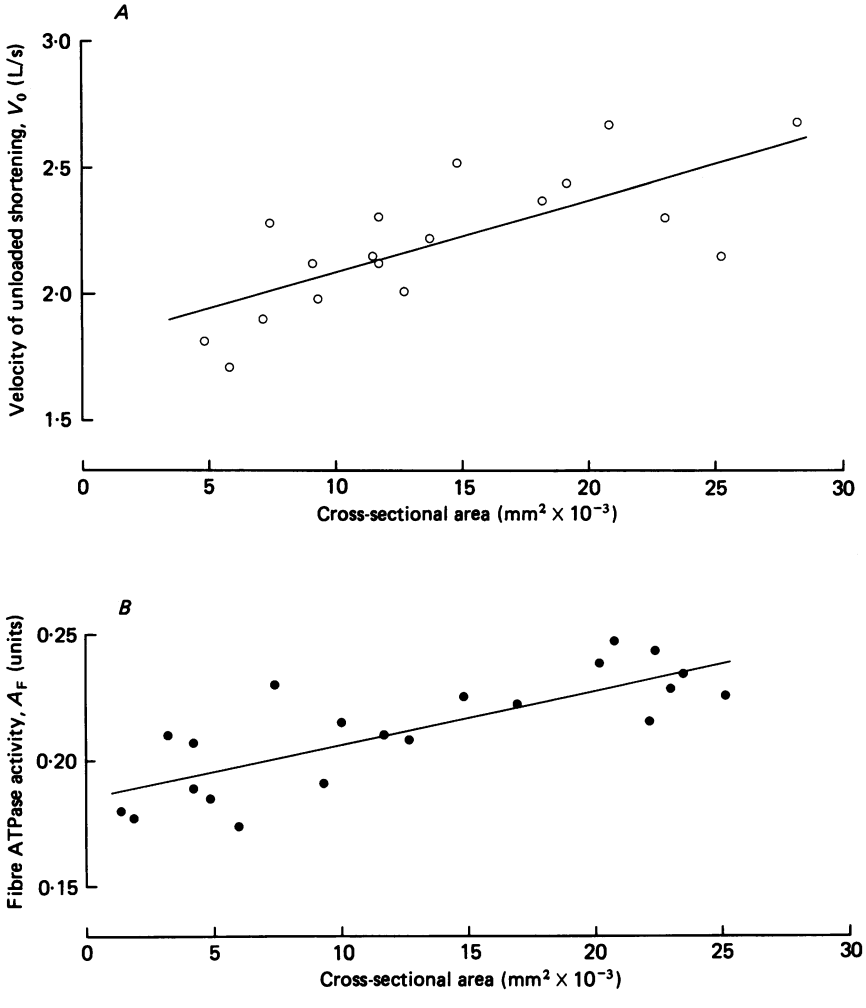

Fig. 3. *A*, relationship between velocity of unloaded shortening (V_0) and cross-sectional area in eighteen single fibres of frog's anterior tibialis muscle. Line: least-squares regression of V_0 upon cross-sectional area. *B*, relationship between ATPase activity (A_F) and cross-sectional area in twenty-one fibres of frog's anterior tibialis muscle. Line: least-squares regression of A_F upon cross-sectional area.

activity (A_F) varied between 0·175 and 0·249 (mean 0·212) units. The following relationship between fibre ATPase activity (units) and cross-sectional area (mm^2) was obtained: $A_F = 2{\cdot}143\,A_{cs} + 0{\cdot}185$ (correlation coefficient, 0·79; $P < 0{\cdot}001$; $n = 21$).

Maximum velocity of shortening and ATPase activity studied in individual segments along single muscle fibres

It has previously been demonstrated (Edman *et al.* 1985) that maximum velocity of shortening may vary substantially from one region to another along the length of an individual frog muscle fibre. In view of the relationship between shortening velocity and ATPase activity that has been found to exist in whole fibres (see previous Results section), it was of interest to find out if a similar relationship exists at segment level. In twelve fibres the maximum velocity of shortening, V_0, was determined in individual segments (0·5–0·8 mm in length) along the fibre. In each of these fibres one to four segments were selected for a quantitative determination of the myofibrillar ATPase activity, A_S (see Methods). In accordance with our previous experiments (Edman *et al.* 1985) the difference between the highest and the lowest values of V_0 in a given fibre ranged between 10 and 35% of the fibre mean. Figure 4 summarizes the results of parallel measurements of ATP splitting rate and V_0 in eleven fibres in which more than one segment were investigated. It can be seen that in the majority of the fibres (eight experiments) the ATPase and V_0 measurements showed similar trends. In three experiments (Fig. 4*C*, *G* and *H*), however, the segmental variations of the two measurements did not agree with one another. Figure 5 presents a diagram where V_0 is plotted against the ATPase activity for the segments studied, including one experiment where only one segment was investigated. A regression analysis yields the following relationship between segment shortening velocity (V_0, L/s) and segment ATPase activity (A_S, units): $V_0 = 0{\cdot}013\,A_S + 1{\cdot}66$ (correlation coefficient, 0·38; $P < 0{\cdot}05$; $n = 33$). Although no definite conclusion can be drawn at the present time, the results do suggest that the measured differences in V_0 along single muscle fibres are related to variations in myofibrillar ATPase activity.

Immunocytochemical characterization of fibres of anterior tibialis muscle

Two monoclonal antibodies, designated A1 and A2, directed against the heavy chains of myosin were used to characterize individual fibres of the anterior tibialis muscle of the frog. As is illustrated in Fig. 6, A1 reacted preferentially with fibres of small diameter, whereas the opposite was true for A2 which preferentially stained thick fibres. A group of fibres of small or intermediate size reacted with both A1 and A2 (cf. central fibre in Fig. 6*C* and *D*).

In eight single fibres the reactivity to A1 and A2 was correlated with the maximum velocity of shortening (V_0), ATPase activity (A_F) and cross-sectional area. Figure 7 illustrates results obtained with two single fibres tested for their immunoreactivity, together with a small fibre bundle which was used as a reference. A1 can be seen to react with the relatively thin fibres in the section, including single fibre 1. The latter exhibited comparatively low V_0 (1·81 L/s) and also relatively low ATPase activity (0·179 units). A2, on the other hand, stained the larger fibres, including single fibre 2, which had a relatively high V_0 (2·30 L/s) and also a fairly high ATPase activity (0·228 units). The reactivity of the two antibodies with fibres of different kinetic properties is summarized in Fig. 1. Here ○ denotes reactivity with A1 and ●

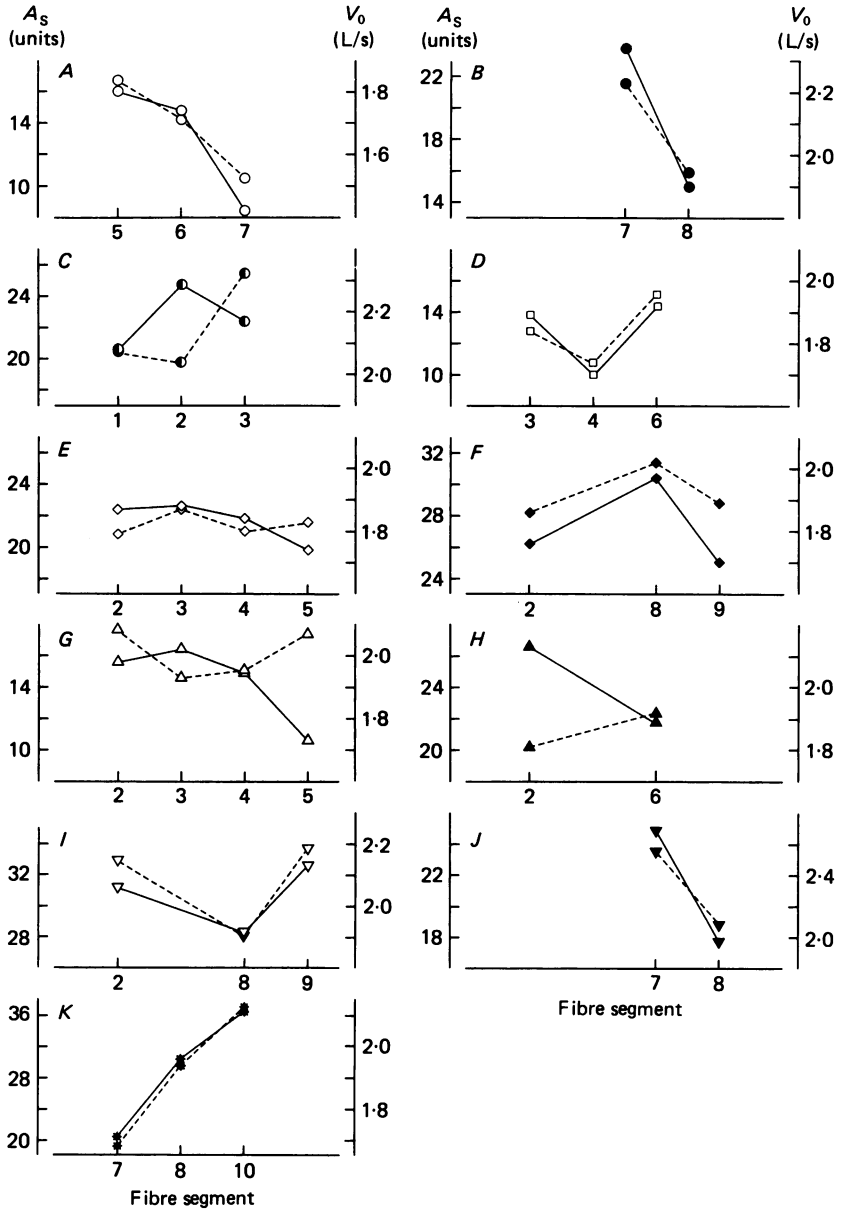

Fig. 4. Velocity of unloaded shortening (V_0, right-hand ordinates) and myofibrillar ATPase activity (A_S, left-hand ordinates) measured in the same segments of eleven muscle fibres (diagrams *A–K*). V_0 data connected by continuous line, A_S data by dashed line. Abscissa: number of consecutive segments along fibre. Units of ATPase activity normalized with respect to segment lengths (see Methods).

reactivity with A2. The results confirm that A1 reacts with relatively slow-twitch fibres having low ATPase activity, whereas A2 reacts with fibres characterized by higher V_0 and higher ATPase activity.

A few exploratory experiments were performed to investigate if the reactivity to one of the two antibodies, A2, differed along the length of an individual fibre. To this end five transverse serial sections were produced in different regions spaced along the length of a fibre bundle. No difference in antibody staining was detectable among the sections within a given region. However, in certain fibres (ten out of sixty-six fibres in six muscles) there were clear differences in the reactivity to A2 from one region to

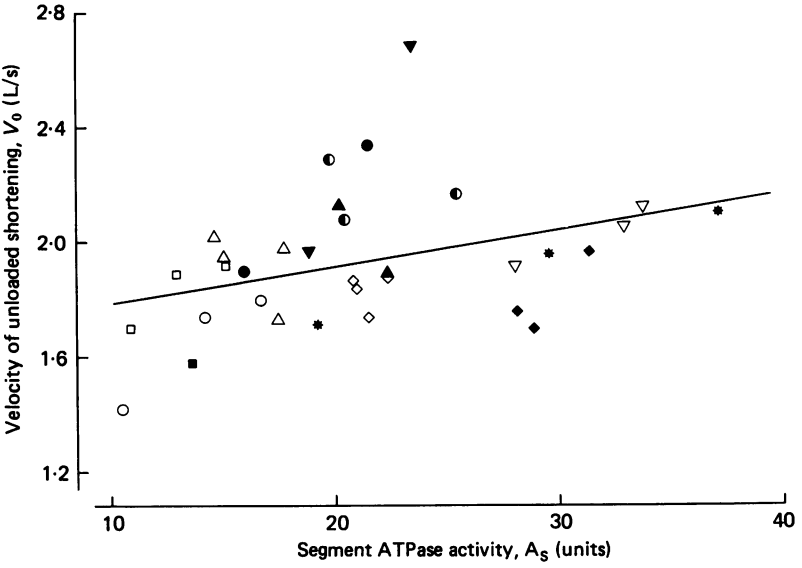

Fig. 5. Relationship between myofibrillar ATPase activity (A_S) and velocity of unloaded shortening (V_0) determined in thirty-three segments of twelve single fibres. Data from the individual fibres shown in Fig. 4 denoted by the same symbols. Line: least-squares regression of V_0 upon A_S.

another. This change in antibody staining was ascertained by comparing the reaction with that of adjacent fibres in the same cross-section.

Figure 8 shows results from one experiment. Illustrated are three sections (*A*, *B* and *C*) located approximately 0·8 mm apart along the fibre bundle and incubated with the same antibody A2. Fibres 1 and 2 serve as a control in order to follow the variations in reactivity of fibres 3, 4 and 5. In the section shown in Fig. 8*A*, fibres 1–5 can all be seen to react with A2. By contrast in the section shown in Fig. 8*B*, only fibres 1 and 2 react with A2, whereas fibres 3, 4 and 5 here show no detectable reactivity. As demonstrated in Fig. 8*C*, however, illustrating a section that is further away from that shown in Fig. 8*A*, fibres 3 and 4 again show reactivity to A2, as do control fibres 1 and 2. Fibre 5, however, is unreactive in section *C*.

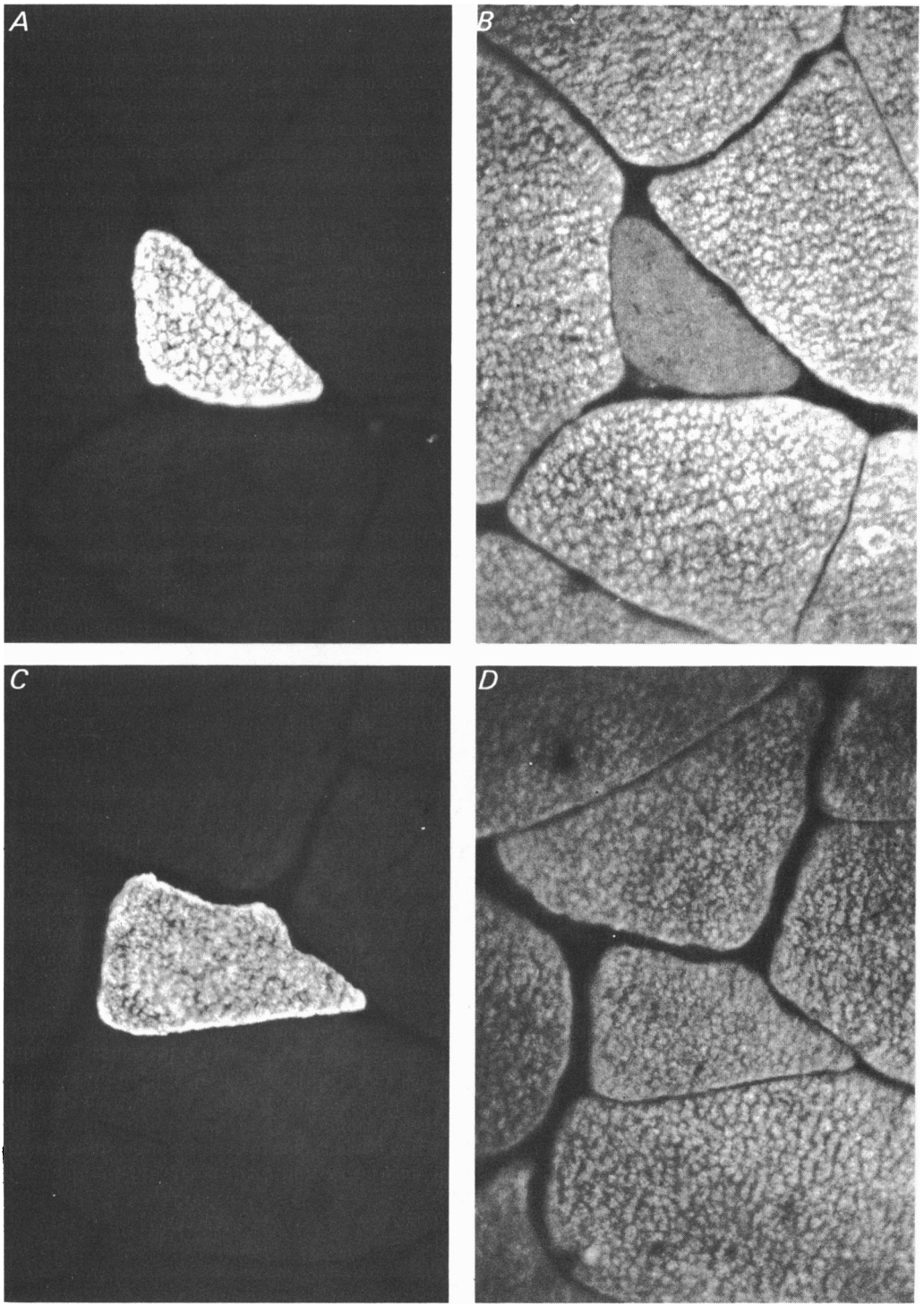

Fig. 6. Immunofluorescence staining of anterior tibialis muscle using two monoclonal antibodies directed against myosin heavy chains. *A*, *B* and *C*, *D* are two serial sections from two different muscles, respectively. *A* and *C*: reactivity to antibody A1. *B* and *D*: reactivity to antibody A2. Thin fibres were preferentially stained by A1 whereas thick fibres were preferentially stained by A2. A category of thin and intermediate fibres reacted with both A1 and A2 as exemplified by the central fibre in *C* and *D*.

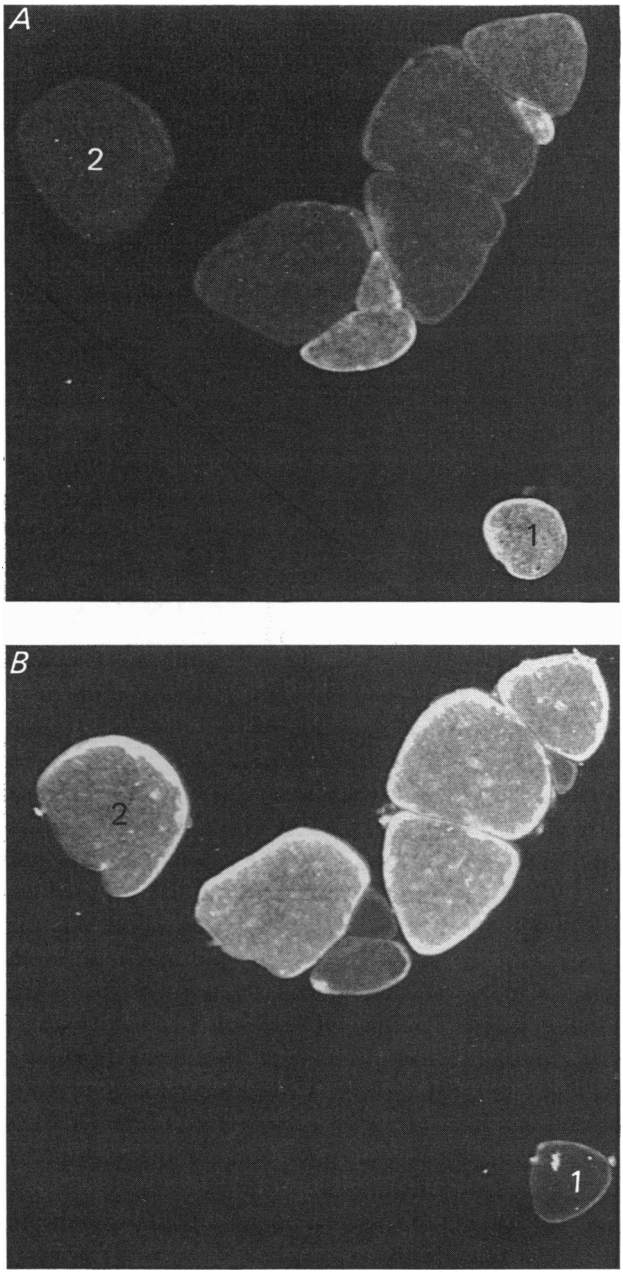

Fig. 7. Immunoreactivity of fibre bundle and two isolated fibres (numbered 1 and 2) that had been characterized with respect to maximum speed of shortening (V_0) and myofibrillar ATPase activity (A_F). *A* and *B*: immunofluorescence staining of two serial sections exposed to monoclonal antibodies A1 and A2, respectively. Note that A1 reacted with thin fibres including fibre 1 (having relatively low V_0 and low A_F) whereas A2 reacted with thicker fibres including fibre 2 (having relatively high V_0 and high A_F). For further information, see text.

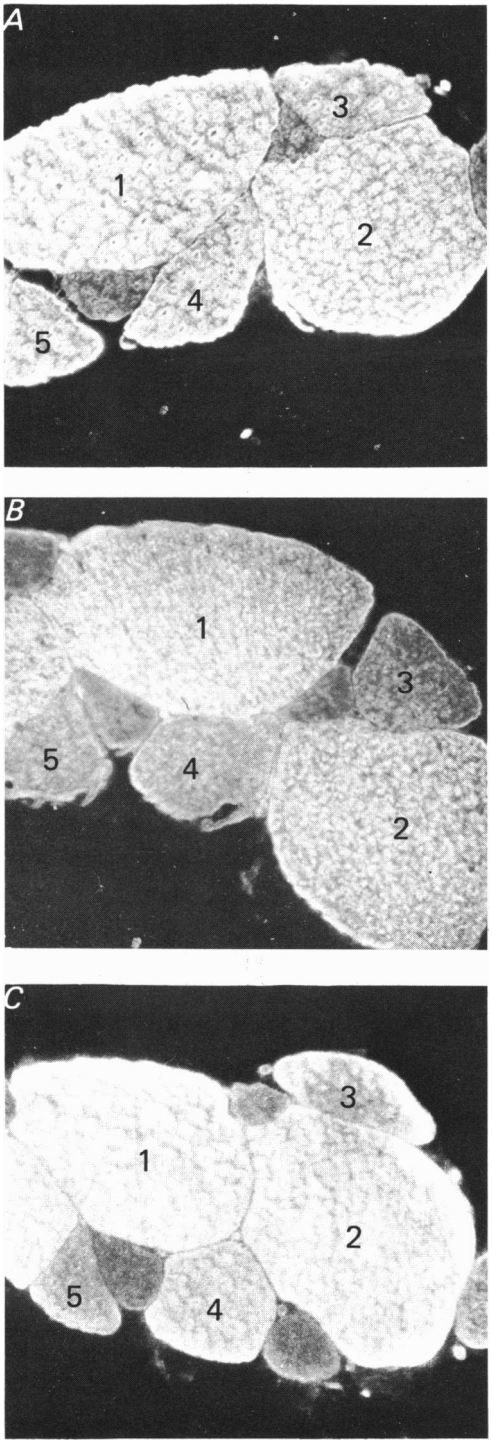

Fig. 8. Differential reactivity to antibody A2 along individual fibres (numbered 1–5) of the anterior tibialis muscle of the frog. *A–C*: immunofluorescence staining of three sections located approximately 0·8 mm apart along the fibre bundle. Thick fibres 1 and 2 reacted with antibody A2 at all three locations. Thin fibres 3, 4 and 5 reacted with A2 in section *A*. Fibres 3 and 4 were unreactive in section *B* but again showed reactivity to A2 in section *C*. Fibre 5 was unreactive in both sections *B* and *C*.

Location of motor end-plate

The results obtained in the present study and in earlier experiments (Edman *et al.* 1985) indicate that V_0 and ATPase activity vary along the length of a muscle fibre. It was therefore of interest to find out if these differences in kinetic properties along the fibre might bear some relation to the location of the motor end-plate. To this end the distance from the centre of the end-plate to each fibre–tendon junction was measured in fifteen isolated fibres (see Methods). The end-plate was found to be located close to the mid-point of each fibre. In the fifteen fibres investigated the spacing between the proximal fibre–tendon junction and the centre of the end-plate was $52{\cdot}0 \pm 2{\cdot}8\,\%$ of the total fibre length. This relative invariability of the location of the end-plate makes it seem unlikely that the motor end-plate is a major determinant of the irregular pattern of V_0 along the fibre.

DISCUSSION

The present experiments, performed on single muscle fibres, confirm that maximum velocity of shortening is proportional to the myofibrillar ATPase activity, as previously suggested by studies on whole muscles (Bárány, 1967). A sampling method was used that provided a mean value of the ATPase activity of the fibre (A_F), in this way accounting for the local differences in ATP splitting rate that were found to exist along single muscle fibres. The value so obtained can thus be presumed to form a valid counterpart of the shortening velocity that was measured from the fibre as a whole.

Previous results suggest that maximum speed of shortening (Reiser, Moss, Giulian & Greaser, 1985) and myosin ATPase activity (Wagner, 1981) are both related to the myosin heavy-chain composition. The present results support this view by demonstrating that fibres of different functional properties react differentially with monoclonal antibodies directed against myosin heavy chains. One antibody, A1, was thus found to react with fibres of relatively small diameter exhibiting low maximum velocity of shortening and low myofibrillar ATPase activity. A second antibody, A2, reacted with comparatively large fibres that showed high maximum velocity of shortening and high ATPase activity. A third class of fibres could be identified which reacted with both A1 and A2. This third category of fibres may therefore contain a mixture of the two isomyosins or, alternatively, be composed of a third isoform of myosin that reacts with both A1 and A2. It is reasonable to conclude from these findings that the large variability in force–velocity characteristics that exists among fibres in frog striated muscle (Edman, 1979; Edman *et al.* 1985) is based on differences in the heavy-chain composition of the myosin in the individual fibres.

Attempts to classify amphibian twitch fibres according to their myosin isoform composition have previously been made by Lännergren & Hoh (1984) and by Rowlerson & Spurway (1985). These studies, however, were not specifically designed to identify differences in the heavy-chain composition of the myosin. Rowlerson & Spurway (1985), using histochemical and immunohistochemical methods, found evidence for the existence of three subgroups of twitch fibres in frog skeletal muscle.

Lännergren & Hoh (1984), studying *Xenopus* iliofibularis muscle, identified four types of twitch fibres according to the force–velocity characteristics and the gel electrophoretic mobilities of myosin. Our own results do not, however, give any clear indication that there actually exist distinct subgroups of fibres in a muscle. It would appear more likely that functional properties, such as force–velocity characteristics and myofibrillar ATPase activity, vary in a smooth continuous way within the fibre population. Such a graded variation in fibre properties may be achieved by the co-existence of different isomyosins in various proportions within the individual muscle fibres. In support of this view, the maximum shortening velocity and the curvature of the force–velocity relation may be found to vary within a given fibre to the same extent as among fibres in a muscle (Edman *et al.* 1985).

Earlier studies (Wilson & Woledge, 1985; te Kronnie *et al.* 1986) have shown that maximum velocity of shortening is related to fibre width. The present experiments confirm and extend this observation by demonstrating that the fibre diameter is positively correlated with both maximum speed of shortening and myofibrillar ATPase activity. The rationale of this relationship between contractile properties and fibre width is unclear at the present time.

Our previous finding that force–velocity characteristics vary along the length of a muscle fibre (Edman *et al.* 1985) was further explored in the present investigation. A most interesting outcome of this study is the finding that the reactivity to one specific antibody directed against the myosin heavy chains may differ along individual fibres. This observation was made using antibody A2 which reacts with relatively large and fast muscle fibres. Later experiments have confirmed this finding, and furthermore, demonstrated that similar non-uniform reactivity along individual fibres also holds true for A1, the antibody preferentially staining thin (and relatively slow) fibres (K. A. P. Edman & C. Reggiani, unpublished data). These observations would seem to provide clear evidence that in certain fibres more than one myosin isoform exists and, furthermore, that the myosin isoforms are non-uniformly distributed, resulting in a predominance of one or the other isoform in different segments. It is of interest to note in this connection that the co-existence of myosins of different heavy-chain composition has been previously demonstrated within single myocardial cells (Samuel, Rappaport, Mercadier, Lompre, Sartore, Triban, Schiaffino & Schwartz, 1983) and extraocular muscle fibres (Wieczorek, Periasamy, Butler-Browne, Whalen & Nadal-Ginard, 1985). Regional differences in the myosin isoform composition would be expected to give rise to segmental differences in myofibrillar ATPase activity along the fibre. The results obtained in this study, showing non-uniform ATP splitting rates along the fibres, are in general agreement with this view. In the majority of the experiments there was indeed a fair agreement between the ATPase activity and the maximum speed of shortening measured in various segments of the fibre. However, further experiments are required to establish the quantitative relationship between shortening velocity and ATP splitting rate at segment level.

In conclusion, the present results provide evidence in support of the view that differences in kinetic properties of the contractile system are based on differences in myosin isoform composition both at whole-fibre level and at segment level in the individual fibre. A quantitative evaluation of the myosin isoform composition

related to the mechanical performance of the contractile system would be of great interest in the light of the present results.

This work was supported by a grant from the Swedish Medical Research Council (project 14X-184). G. te Kronnie was the recipient of a Visiting Scientist Fellowship (K83-14V-6654) from the Swedish Medical Research Council. C. R. was supported by a Visiting Scientist Fellowship from the Swedish Institute.

REFERENCES

BÁRÁNY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *Journal of General Physiology* **50**, 197–216.

EDMAN, K. A. P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *Journal of Physiology* **291**, 143–160.

EDMAN, K. A. P. & REGGIANI, C. (1984). Redistribution of sarcomere length during isometric contraction of frog muscle fibres and its relation to tension creep. *Journal of Physiology* **351**, 169–198.

EDMAN, K. A. P., REGGIANI, C. & TE KRONNIE, G. (1985). Differences in maximum velocity of shortening along single muscle fibres of the frog. *Journal of Physiology* **365**, 147–163.

KARNOVSKY, M. J. & ROOTS, L. (1964). A direct colouring thiocholine method for cholinesterases. *Journal of Histochemistry and Cytochemistry* **12**, 219–224.

LÄNNERGREN, J. (1979). An intermediate type of muscle fibre in *Xenopus laevis*. *Nature* **279**, 254–256.

LÄNNERGREN, J. & HOH, J. F. Y. (1984). Myosin isoenzymes in single muscle fibres of *Xenopus laevis*: analysis of five different functional types. *Proceedings of the Royal Society* B **222**, 401–408.

LÄNNERGREN, J. & SMITH, R. S. (1966). Types of muscle fibres in toad skeletal muscle. *Acta physiologica scandinavica* **68**, 263–274.

REISER, P. J., MOSS, R. L., GIULIAN, G. G. & GREASER, M. L. (1985). Shortening velocity in single fibers from adult rabbit soleus muscle is correlated with myosin heavy chain composition. *Journal of Biological Chemistry* **260**, 9077–9080.

ROWLERSON, A. & SPURWAY, N. C. (1985). How many fibre types in amphibian muscles? A comparison of *Rana* and *Xenopus*. *Journal of Physiology* **358**, 78*P*.

SAMUEL, J. L., RAPPAPORT, L., MERCADIER, J. J., LOMPRE, A. M., SARTORE, S., TRIBAN, C., SCHIAFFINO, S. & SCHWARTZ, K. (1983). Distribution of myosin isoenzymes within single cardiac cells. An immunohistochemical study. *Circulation Research* **52**, 200–213.

TE KRONNIE, G., REGGIANI, C. & EDMAN, K. A. P. (1985). Segmental differences in maximum speed of shortening and myofibrillar ATPase activity in frog skeletal muscle fibres. *Journal of Muscle Research and Cell Motility* **6**, 108.

TE KRONNIE, G., REGGIANI, C., SCHIAFFINO, S. & EDMAN, K. A. P. (1986). Shortening velocity correlated with myosin isoform composition and myofibrillar ATPase activity in frog single muscle fibres. *Journal of Muscle Research and Cell Motility* **7**, 77.

VAN DER LAARSE, W. J., DIEGENBACH, P. C. & MASLAM, S. (1984). Quantitative histochemistry of three mouse hindlimb muscles: the relationship between calcium stimulated myofibrillar ATPase and succinate dehydrogenase activities. *Histochemical Journal* **16**, 529–541.

WAGNER, P. D. (1981). Formation and characterization of myosin hybrids containing essential light chains and heavy chains from different muscle myosin. *Journal of Biological Chemistry* **256**, 2493–2498.

WIECZOREK, D. F., PERIASAMY, M., BUTLER-BROWNE, G. S., WHALEN, R. G. & NADAL-GINARD, B. (1985). Co-expression of multiple myosin heavy chain genes, in addition to a tissue-specific one, in extraocular musculature. *Journal of Cellular Biology* **101**, 618–629.

WILSON, M. G. A. & WOLEDGE, R. C. (1985). Lack of correlation between twitch contraction time and velocity of unloaded shortening in fibres of frog anterior tibialis muscle. *Journal of Physiology* **358**, 81*P*.