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# MAXIMAL SHORTENING VELOCITIES, ISOMYOSINS AND FIBRE TYPES IN SOLEUS MUSCLE OF MICE, RATS AND GUINEA-PIGS

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## SUMMARY

1. Guinea-pig soleus contains only type I fibres and slow isomyosin, $SM_2$. Rat and mouse soleus contain about 70% of type I fibres and a mixture of isomyosins: slow, $SM_2$ and intermediate, IM. Many rat soleus muscles contain a third isomyosin of a slow type, $SM_1$.

2. The maximal velocity of unloaded shortening, $V_0$, is largest in mouse soleus (6·11 $L_f$ $s^{-1}$), slowest in guinea-pig soleus (1·67 $L_f$ $s^{-1}$) and intermediate in rat soleus (4·16 $L_f$ $s^{-1}$) ($L_f$ = fibre length).

3. In guinea-pig soleus, $V_0$ is equal to the maximal velocity ($V_{max}$) computed using the Hill force–velocity relationship; $V_0$ is approximately twice as large as $V_{max}$ in mouse and rat soleus.

4. $V_0$ measures the unloaded shortening velocity of the fastest fibres whereas $V_{max}$ is a function of the force–velocity characteristics of all the fibres contained in the muscle.

5. $V_0$ increases according to the isomyosin composition of the fibres in the sequence $SM_2 < SM_1 + IM < IM$.

## INTRODUCTION

Maximum shortening velocity is one of the most useful mechanical parameters for characterization of the various types of muscle fibres. It can be estimated by extrapolating the hyperbolic force–velocity relationship (Hill, 1938) to zero external load, $V_{max}$. It can also be measured from the time it takes a fully activated muscle fibre to take up a slack, $V_0$ (Edman, 1979). Julian, Rome, Stephenson & Stritz (1986) showed that $V_0$ equals $V_{max}$ in experiments on frogs where both velocities were carefully determined for the same single skeletal muscle fibre. Claflin & Faulkner (1985) measured both velocities in rat soleus muscle, a whole-muscle preparation with a heterogeneous fibre population, and observed that $V_0$ was 60% larger than $V_{max}$. They concluded that $V_0$ is a measure of the unloaded velocity of the fastest fibres whereas $V_{max}$ is a function of the force–velocity characteristics of all the fibres within a skeletal muscle preparation.

To test this interpretation we studied soleus from animals belonging to three species of the same taxonomic order (rodents). One preparation, from guinea-pig,

had a homogeneous population of fibres; the other two, from rat and mouse, were heterogeneous. $V_{max}$ and $V_0$ were measured sequentially on the same muscle. It is important to specify the conditions under which $V_{max}$ is measured since the discrepancy between $V_{max}$ and $V_0$ might be quite small if $V_{max}$ is determined by extrapolation to zero load of the velocities measured at very light loads (Julian *et al.* 1986). For this reason, we have estimated $V_{max}$ by the ratio of the velocity constant, $b$, to the force constant, $a/F_0$, of a force–velocity relationship in which the loads were varied between 95–98 and 10–20 % of the maximal isometric force $F_0$.

In isolated single fibres $V_0$ depends on the myosin heavy chain types and the content in myosin light-chain $LC_3f$ (Reiser, Moss, Giulian & Greaser, 1985; Sweeney, Kushmerick, Mabuchi, Stréter & Gergely, 1988). Since myosin heavy and light chains can combine in many ways to form an isomyosin (Staron & Pette, 1987), it is desirable to correlate $V_0$ with the complete myosin molecule. For this reason, the muscle was subjected to fibre typing and to electrophoretic analysis of isomyosins under non-denaturing conditions after the mechanical experiments. Besides confirming and extending the findings of Claflin & Faulkner (1985), our results enabled us to evaluate the relationships between $V_0$ and the isomyosin composition of the muscles.

## METHODS

### *Muscle preparation and stimulation*

Experiments were performed on whole soleus muscles isolated from fourteen rats (Wistar; body weight, 130–360 g), twenty-four mice (NMRI; body weight, 30–40 g) and eleven guinea-pigs (body weight, 110–150 g). The animals were anaesthetized with a subcutaneous injection of 2 ml $kg^{-1}$ body weight Thalamonal. The right soleus muscle was dissected free from the animal. The animals were killed by ether after the dissection. The distal tendon was tied to a fixed frame, which supported two parallel rows of eight platinum electrodes spaced 2 mm apart. The muscle was placed between the rows of electrodes. The proximal tendon was tied to a glass rod (250 mg in weight). The frame was placed in thermostated chamber containing a buffered physiological salt solution (in mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1; $KH_2PO_4$, 1; the pH of the solution was adjusted to 7·4 by equilibration with a gas mixture of 95 % $O_2$ and 5 % $CO_2$ and its temperature was kept at 20 ± 0·5 °C. The glass rod was attached to the force transducer of an electromagnetic ergometer. The muscle was slightly stretched at rest, until it resisted with a small resting force of approximately 5 mN. It was stimulated with capacitor discharges of alternating polarity to avoid electrolysis (time constant, 5 ms; output impedance, 100 Ω, 1 $H_z$). Its length was finely adjusted until the maximum isometric twitch force was obtained; the corresponding length defined the optimal length, $L_0$.

### *Measurement of $V_{max}$ and $V_0$*

The force–velocity relationship was obtained with the method of isovelocity releases (Cecchi, Colomo & Lombardi, 1978), using an ergometer described in an earlier work (Maréchal & Plaghki, 1979). The muscle was maximally tetanized at the optimal length. The isometric force attained a plateau, $F_0$, after an initial duration of stimulation of 0·6 s for mouse, 1·2 s for rat and 3 s for guinea-pig. The muscle was released at very high velocity (70 mm $s^{-1}$) in order to discharge the series elastic elements. The amplitude (25–100 μm) of the quick release was adjusted to the velocity of the following shortening at controlled speed in order to minimize the transients (see Fig. 1, insets). The force $F$ was measured at the beginning of the release at controlled velocity, just after the rapid fall caused by the quick release, and it was expressed relative to $F_0$. The tetanus with releases were repeated eighteen to twenty-two times, at different controlled velocities adjusted for each species, to cover the span of the force–velocity relationship (see Fig. 1) and arranged in a quasi-mirror order to minimize the influence of fatigue. The fall of force between the first and last tetanus averaged 5 % and was never higher than 10 %. The ratio $F/F_0$ was fitted to the velocities of release by a rectangular hyperbola (Hill, 1938; Fig. 1) using a non-linear least-squares regression based on the

Gauss–Newton method (Yamaoka, Tanigawara, Nakagawa & Uno, 1981). $V_{max}$ was computed as $b$ (velocity constant) divided by $a/F_0$ (force constant).

$V_0$ was determined by the slack test (Edman, 1979) as applied to whole muscle by Claflin & Faulkner (1985). Nine changes in length were applied during the plateau of an isometric tetanic contraction. The velocity of release was 70 mm $s^{-1}$. Step sizes, in order of application within each series, were 0·44, 0·88, 1·32, 1·78, 2·20, 1·98, 1·54, 1·10 and 0·66 mm. In all cases, the final length was $L_0$. The duration of unloaded shortening associated with each release, $t_L$, was measured from records obtained with a fast UV recorder (ME-100) as the interval between the instant of release and the point at which the force started to redevelop. This point was sometimes difficult to determine, due to the slow rise of the force and to some low-amplitude oscillations (Fig. 2, upper panels) at low frequency, approximately 500 Hz, originating in the electromagnetic device. In these cases, $t_L$ was estimated as the interval between the onset of the release and the intercept between a linear extrapolation of the rising force and the baseline. The unloaded shortening time intervals increased linearly with release amplitudes in the soleus muscles of all three species (Fig. 2, lower panels). The $V_0$ value was calculated as the inverse of the slope of the resulting line.

After the mechanical experiment, the muscle was blotted and weighed. It was placed on a plastic sheet and stretched to $L_0$; under a binocular microscope, the length of five to ten superficial fibres were averaged, giving the fibre length, $L_f$. We observed a mouse $L_f$ of $6·9 \pm 0·1$ mm, nearly equal to that reported by Luff (1981), $6·84 \pm 0·20$ mm. The ratio 'muscle fibre length, $L_f$/muscle length, $L_0$' was 0·68 for mouse soleus (in agreement with the published values of 0·69 for mouse soleus, Brooks & Faulkner, 1988) and 0·72 for rat and guinea-pig soleus. The mean cross-sectional area was estimated by dividing the muscle weight by its fibre length, assuming a muscle density equal to one (Close, 1972); the maximum tetanic stress, $S_0$, was calculated as the maximum tetanic force divided by the mean cross-sectional area. The muscle was rapidly frozen in isopentane at −150 °C and stored at −80 °C until further use.

*Fibre typing and isomysin analyses*

Eighteen of those frozen muscles, six of each species, were simultaneously analysed for their fibre types and native isomyosins. Cryostat sections (10 μm thickness) were obtained from three levels: proximal, central and distal. Three serial sections from each level were used for fibre typing by the classical myosin ATPase reaction at three pH values, 10·0, 4·45 and 4·28 (Brooke & Kaiser, 1970). Twenty sections from each level were homogenized at 4 °C and extracted with 100 μl of Guba–Straub solution (in mM): NaCl, 300; $NaH_2PO_4$, 100; $Na_2HPO_4$, 50; $MgCl_2$, 1; $Na_4P_2O_7$, 10; $NaN_3$ 0·1%; 2-mercaptoethanol, 0·1%; pH 6·5; centrifuged (10 min, 10000 r.p.m., 4 °C); then, 100 μl of glycerol were added to the supernatant and 50 μl of the mixture were diluted with 50 μl of buffer ($Na_4P_2O_7$, 200 mM; glycerol, 50%; bromophenol, 0·015%; 2-mercaptoethanol, 0·01%; pH 8·5; all percentages w/v) and 20 μl of an extract of taenia coli (added to provide an interval standard for the electrophoretic mobility). Finally, 10 and 20 μl of this mixture were put on top of a pyrophosphate polyacrylamide gel for electrophoretic analysis under native conditions (Hoh, McGrath & White, 1976). The mobility of the isomyosins and their relative proportions were estimated by computerized densitometry. Since no differences were observed between the sections from the three different levels, the data were pooled and averaged. In the remaining frozen muscles ($n = 29$), native isomyosins were separated and quantified as described in Maréchal, Schwartz, Beckers-Bleukx & Ghins (1984).

*Statistical analyses*

Results were expressed as mean ± S.E.M. Non-linear regressions were computed using a Truebasic program and statistical calculations were performed with the SPSS statisical package, both on a XT microcomputer.

## RESULTS

Examples of experiments to estimate $V_{max}$ by the force–velocity relationship and $V_0$ by the slack test method are presented in Figs 1 and 2. Isomyosin patterns and corresponding densitograms of the gels are shown in Fig. 3. Histochemical myosin

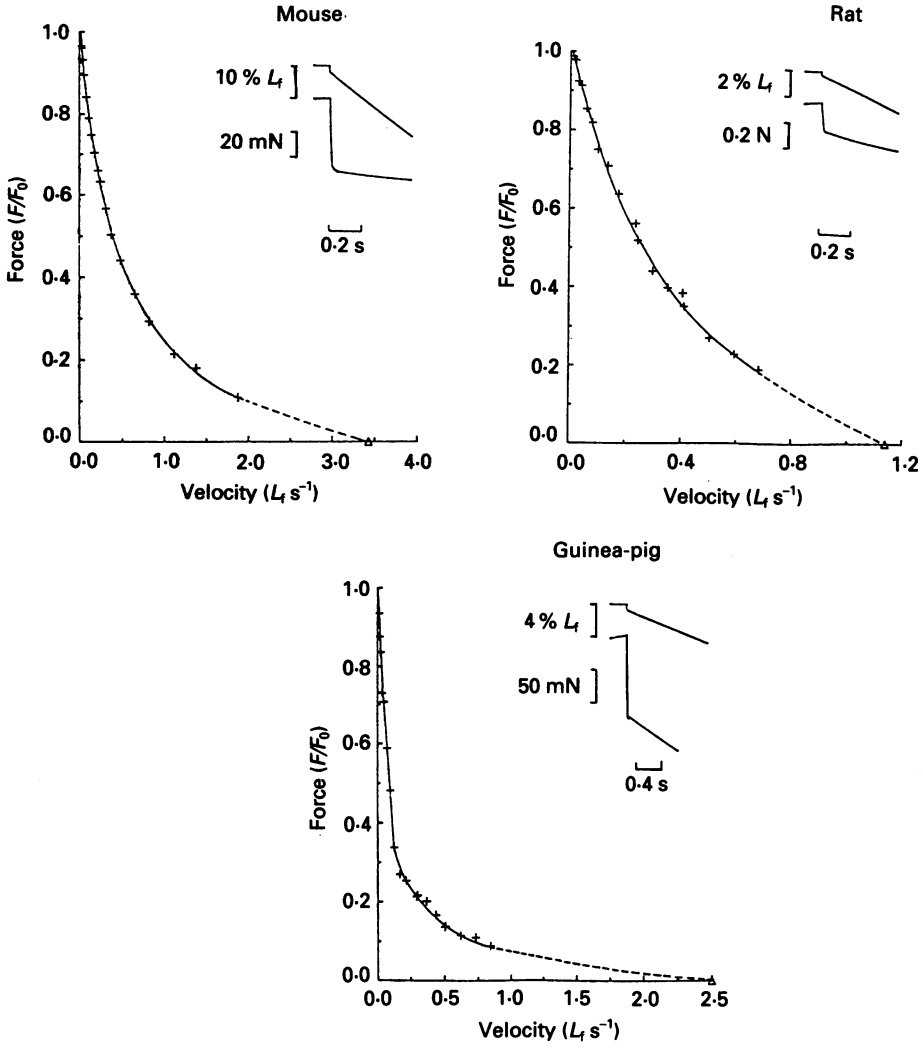


Fig. 1. Force–velocity relationships of soleus muscles of mouse (upper left), rat (upper right) and guinea-pig (lower). Insets: isometric tetanus with release at a constant velocity. The graph begins before the release when the tetanic isometric force, $F_0$, is maximal. Upper curves, muscle length; the velocity of release equals 0·17 fibre length s$^{-1}$ ($L_f$ s$^{-1}$) for the mouse soleus, 0·08 $Lf$ s$^{-1}$ for rat soleus and 0·02 $L_f$ s$^{-1}$ for guinea-pig soleus. Lower curves, force maintained during the release. Main graphs: force–velocity relationships. The ratio $F/F_0$ (+) is used as the estimate of the force maintained by the muscle when released at a constant velocity. The continuous lines show the Hill's hyperbolas computed by a least-squares method; the dashed lines point toward $V_{max}$(△), computed as the ratio of $b$ to $a/F_0$. *Mouse*: age, 120 days; body weight, 31 g; muscle weight, 9 mg; fibre length, 6·2 mm; maximum tetanic force, 194 mN; $b$ = 0·52 $L_f$ s$^{-1}$; $a/F_0$, 0·15; $V_{max}$, 3·4 $L_f$ s$^{-1}$ $V_0$, 7·2 $L_f$ s$^{-1}$; $SM_2$, 68 %; $SM_1$, 0 %; IM, 32 %. *Rat*: age, 97 days; body weight, 325 g; muscle weight, 186 mg; fibre length, 21 mm; maximum tetanic force, 1050 mN; $b$ = 0·46 $L_f$ s$^{-1}$; $a/F_0$, 0·43; $V_{max}$, 1·14 $L_f$ s$^{-1}$; $V_0$, 2·5 $L_f$ s$^{-1}$; $SM_2$, 73 %; $SM_1$ 18 %; IM, 9 %. *Guinea-pig*: age, 21 days; body weight, 120 g; muscle weight, 70 mg; fibre length, 14 mm; maximum tetanic force, 551 mN; $b$ = 0·10 $L_f$ s$^{-1}$; $a/F_0$, 0·04; $V_{max}$, 2·5 $L_f$ s$^{-1}$; $V_0$, 1·9 $L_f$ s$^{-1}$; $SM_2$, 100 % $SM_1$, 0 %; IM, 0 %.

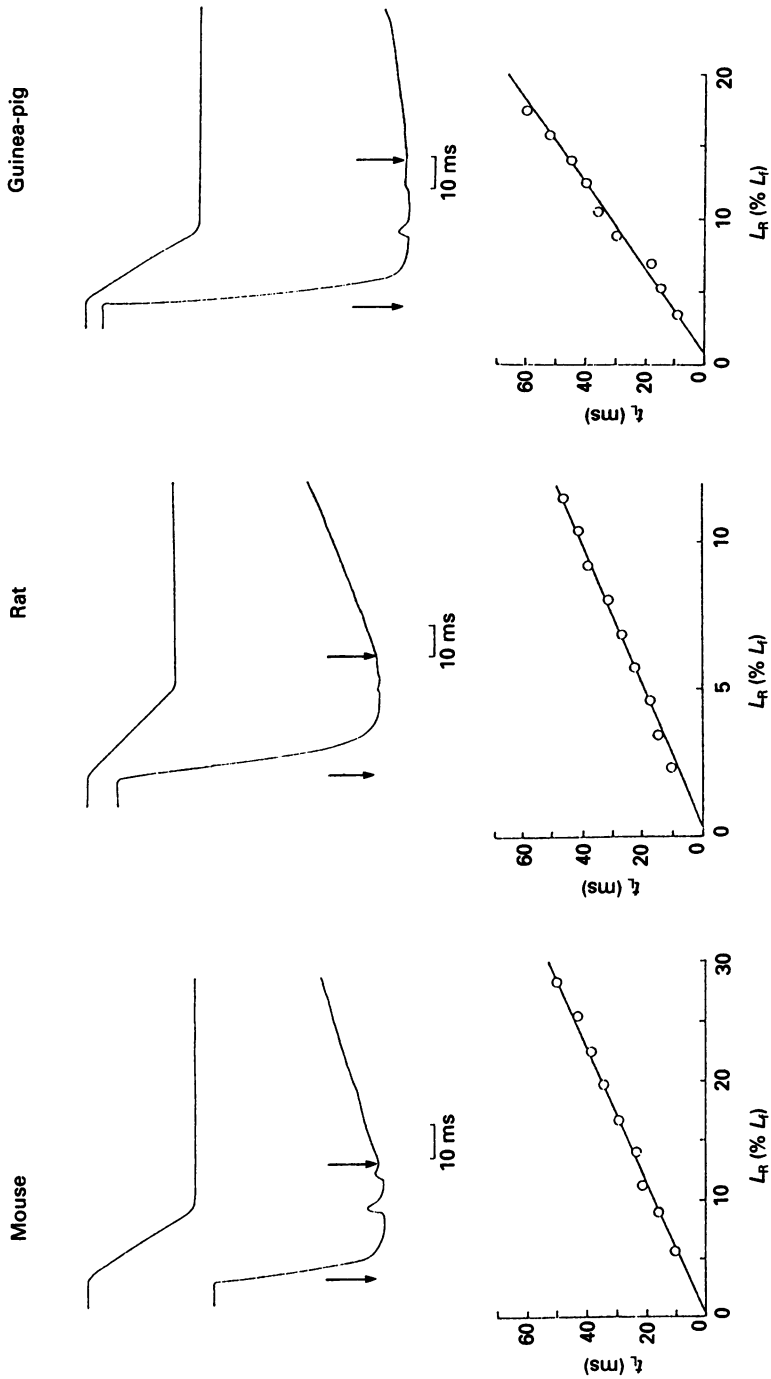


Fig. 2. Determination of unloaded shortening velocity ($V_0$) by the slack test of the muscles shown in Fig. 1. Upper panels, imposed length changes (upper curves) and resulting force response (lower curves). The release amplitude, $L_R$, equals 17 % $L_l$ for mouse soleus, 7 % $L_l$ for rat soleus and 10 % $L_l$ for guinea-pig soleus. The time $t_L$ required to take up the imposed slack is indicated by the arrows. Lower panels, $t_L$ for nine releases at various amplitudes. The line represents the least-squares regression of $t_L$ upon $L_R$. The unloaded shortening velocity ($V_0$) is computed from the slope of this line; it is equal to 5·4 $L_l$ s$^{-1}$ for the mouse soleus, 2·5 $L_l$ s$^{-1}$ for the rat soleus and 2·3 $L_l$ s$^{-1}$ for the guinea-pig soleus.

ATPase patterns are not shown, since they do not differ from numerous examples previously reported in the literature.

Table 1 summarizes the results obtained in all experiments. Soleus muscles from the three species exhibited the same maximum isometric stress, $S_0$; it appears to be

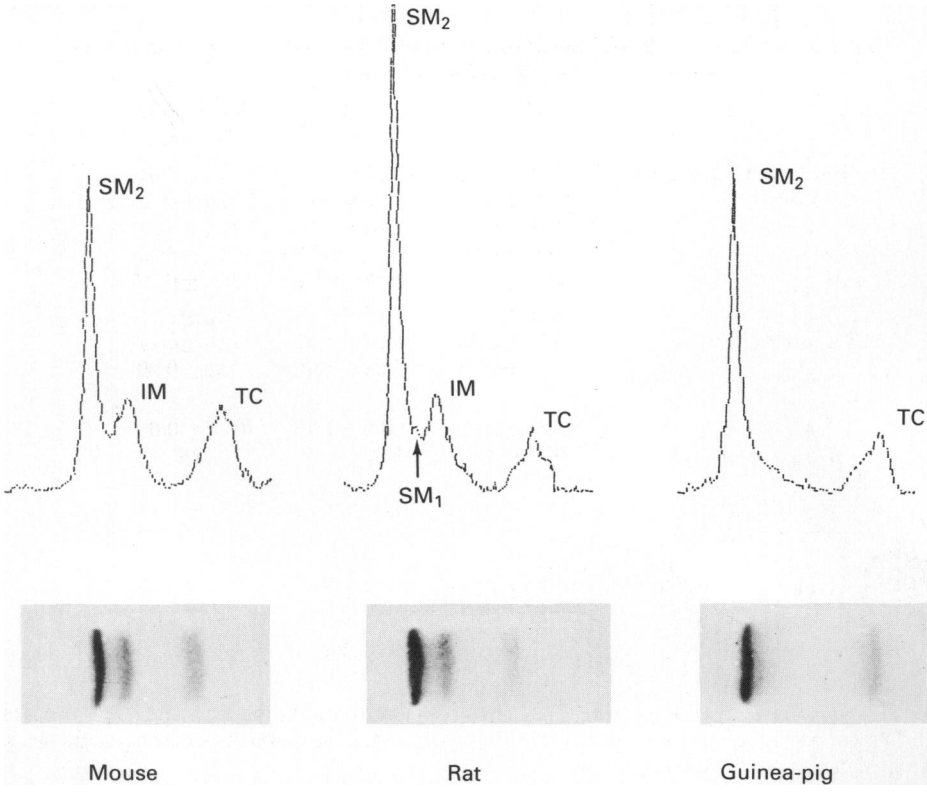


Fig. 3. Isomyosins of soleus muscles of mouse, rat and guinea-pig. Twenty $\mu$l of a muscle homogenate (final dilution 1:300 w/v) in a pyrophosphate buffer were put on top of the gel, underwent electrophoresis, were stained with Coomassie Blue and scanned with a densitometer. Photographs of the gels are shown in the lower panels and their absorbance at 535 nm, in arbitrary units, is shown in the upper panels. The mouse shows two zones, the rat three zones (the arrow points toward the small third zone) and the guinea-pig one zone. In every case, the zone to the right is taenia coli (TC) myosin, used as an internal standard to standardize the mobility of the other zones, its electrophoretic mobility being arbitrarily set at 147. The relative proportions of the soleus isomyosins were obtained by computer integration of the densitograms using the drop line method. *Mouse*: $SM_2$, 70%; IM, 30%; *rat*: $SM_2$, 79%; $SM_1$, 7%; IM, 14%; *guinea-pig*: $SM_2$, 100%.

rather low, but several workers have reported similar low values for soleus muscles (for instance, in mice: Rowe & Goldspink, 1969; Luff, 1981; Anderson, Bressler & Ovalle, 1988; in rats: Close, 1972; Witzmann, Kim & Fitts, 1983; and in guinea-pigs: Powell, Roy, Kanim, Bello & Edgerton, 1984). The Hill velocity constant $b$ of the guinea-pig soleus reaches only one-third that of the rat and the mouse. $V_0$ and $V_{max}$ are also lower in the guinea-pig. $V_0$ is approximately twice as large as $V_{max}$ in the rat and in the mouse whereas these two estimates of the maximum velocity are almost equal in the

guinea-pig. In accordance with previous reports, we find only one fibre type in the guinea-pig soleus muscles using the histochemical myosin ATPase assay, whereas the mouse and rat soleus are mixed muscles, containing type I and type IIA fibres (Close, 1967; Ariano, Armstrong & Edgerton, 1973; Lewis, Parry & Rowlerson, 1982).

TABLE 1. Mechanical, biochemical and histochemical characteristics of soleus muscles of various rodents

| | Mouse ($n = 24$) | Rat ($n = 14$) | Guinea-pig ($n = 11$) |
|---|---|---|---|
| Muscle weight (mg) | 11·0±0·16 | 145·0±9·0 | 72·9±2·8 |
| $L_f$ (mm) | 6·9±0·1 | 17·6±0·3 | 14·6±0·3 |
| $S_0$ (N cm$^{-2}$) | 14·8±0·8 | 16·8±1·1 | 14·7±2·2 |
| $b$ ($L_f$ s$^{-1}$) | 0·50±0·04 | 0·54±0·05 | 0·13±0·02 |
| $a/F_0$ | 0·15±0·01 | 0·33±0·05 | 0·10±0·02 |
| $V_{max}$ ($L_f$ s$^{-1}$) | 3·42±0·24 | 1·98±0·16 | 1·53±0·28 |
| $V_0$ ($L_f$ s$^{-1}$) | 6·11±0·30 | 4·16±0·26 | 1·67±0·17 |
| $V_0/V_{max}$ | 1·75±0·12 | 2·28±0·24 | 0·98±0·20 |
| $SM_2$ | | | |
| Mobility (%) | 99·8±0·5 | 98·2±0·4 | 99·8±0·6 |
| Proportion (%) | 69·9±3·0 | 76·2±5·2 | 100 |
| $SM_1$ | | | |
| Mobility (%) | — | 107·8±0·8 | — |
| Proportion (%) | 0 | 11·1±3·1 | 0 |
| IM | | | |
| Mobility (%) | 110·6±1·1 | 112·9±0·4 | — |
| Proportion (%) | 30·1±3·0 | 12·7±3·2 | 0 |
| Type I (%) | 67±5·3 | 73±1 | 100 |
| Type IIA (%) | 33 | 27 | 0 |

Results are reported as mean ±S.E.M. The electrophoretic mobility is expressed as percentage of the mobility of taenia coli myosin, arbitrarily set at 147; the proportions of isomyosins are as a percentage of the sum of all isomyosins.

The proportion of type I fibres in mouse soleus in markedly higher than that reported in other works, probably because the strain used here (NMRI) is different (C57BL/6J in Dribin & Simpson, 1977 and in Anderson *et al.* 1988). One isomyosin, $SM_2$, is present in the soleus muscles of all three species; this form migrates very slowly (electrophoretic mobility = 100). A second form, $SM_1$, which migrates faster (electrophoretic mobility = 108), is present only in rat soleus, in which it is not abundant (11% of total myosin) and not constant (observed in ten samples out of fourteen). A third form is observed in rat and mouse soleus; since its electrophoretic mobility (equal to 112) is intermediate, falling between that of the slow isoforms $SM_1$and $SM_2$and that of the fast muscle myosin isoforms($FM_1$, $FM_2$ and $FM_3$, with electrophoretic mobilities between 116 and 130; see Maréchal, Biral, Beckers-Bleukx & Colson-Van Schoor, 1988), it has been called 'intermediate isomyosin', IM. Thus, guinea-pig soleus contains only one isomyosin, $SM_2$ (in agreement with d'Albis, Pantaloni & Bechet, 1979), mouse soleus contains two isomyosins, $SM_2$ and IM (in agreement with Fitzsimons & Hoh, 1983), and rat soleus contains three isomyosins, $SM_1$, $SM_2$ and IM. The fast muscle myosin isoforms ($FM_1$, $FM_2$ and $FM_3$) are absent from the soleus muscles of the three rodents.

## DISCUSSION

### *Comparison of $V_{max}$ and $V_0$*

In this study, values of $V_{max}$ ($1{\cdot}98 \pm 0{\cdot}16\ L_f\ s^{-1}$) and $V_0$ ($4{\cdot}16 \pm 0{\cdot}26\ L_f\ s^{-1}$) obtained for rat soleus are in fair agreement with those reported by Claflin & Faulkner (1985), $3{\cdot}2 \pm 0{\cdot}1\ L_f\ s^{-1}$ and $5{\cdot}0 \pm 0{\cdot}1\ L_f\ s^{-1}$, respectively. The ratio $V_0/V_{max}$ is somewhat hig er in our experiments: $2{\cdot}28 \pm 0{\cdot}24$, to compare with $1{\cdot}6 \pm 0{\cdot}1$ in Claflin and Faulkner's experiments. This agreement is satisfactory since the methodology used in the two laboratories markedly differs. Claflin and Faulkner used controlled isotonic releases to evaluate $V_{max}$, whereas we used releases at controlled velocities. Additionally, they performed the slack tests at a higher velocity (700 mm $s^{-1}$ instead of 70 mm $s^{-1}$). Claflin and Faulkner concluded that $V_0$ measures the unloaded shortening velocity of the fastest fibres only, whereas $V_{max}$ represents a complex function of all the fibres within the muscle. They predicted that $V_{max}$ will only be equivalent to $V_0$ in muscles that are homogeneous with respect to the shortening velocities of their fibres. We tested this hypothesis by measuring $V_{max}$ and $V_0$ on guinea-pig soleus, which is homogeneous in its fibre type composition. For the sake of comparison, we also determined $V_{max}$ and $V_0$ on two heterogeneous muscles (rat and mouse soleus). In strong support of the hypothesis, we observed that $V_{max}$ equals $V_0$ in the homogeneous guinea-pig soleus, whilst $V_{max}$ is roughly twice as small as $V_0$ in heterogeneous muscles.

### *Fibre types, myosin isoenzymes and $V_0$*

All fibres of guinea-pig soleus are of type I and they contain only one isomyosin: $SM_2$ (Table 1). Therefore, $SM_2$ is associated with the slowest observed value of $V_0$ ($1{\cdot}67\ L_f\ s^{-1}$; 20 °C). Mouse soleus contains about 70 % type I fibres and 30 % type IIA fibres. Since the proportion of each fibre type is equal to that of the isomyosins $SM_2$ and IM respectively, it is reasonable to assume that a given fibre contains only one isomyosin: $SM_2$ being localized in type I fibres and IM in type IIA fibres. As isomyosin $SM_2$ of the mouse has the same subunit composition as that of the guinea-pig (Maréchal *et al.* 1988) it is very likely that type I fibres have the same value of $V_0$ in both animals. The high $V_0$ ($6{\cdot}11\ L_f\ s^{-1}$) observed in mouse soleus would thus characterize the isomyosin IM. The case of the rat soleus is more difficult to interpret since its *two* types of fibres (I and IIA) must accommodate *three* varieties of isomyosins ($SM_2$, $SM_1$ and IM). In the absence of direct determinations of the isomyosin content of individual fibres, we have to examine two hypotheses: (i) the fibres are homogeneous in the sense that they contain myosin heavy chain $MHC_I$, present in the isoforms either $SM_2$ or $SM_1$ (which differ by their light chain composition, Maréchal *et al.* 1988), whilst type IIA fibres contain myosin heavy chain $MHC_{IIA}$, present in the isoform IM. As there is no reason to think that isomyosin IM is different for rat and mouse, we expect $V_0$ to be equal in rat and mouse soleus. Since this is not the case, mouse soleus being 47 % faster, we have to postulate that rat IIA fibres cannot contract at the characteristic $V_0$ observed for mouse IIA fibres either because they are intrinsically slower or being few (no more than 11 % on the basis of IM proportion) they have to pull the slower fibres and thus contract under a sizeable internal load. (ii) Fibres are heterogeneous, containing a mixture of isomyosins with

two different heavy chains: type IIA fibres would contain isomyosins $SM_1$ and IM and perhaps some isomyosin $SM_2$. Their characteristic $V_0$ ($4{\cdot}16\ L_f\ s^{-1}$) would be intermediate between those of the slow $SM_2$ of guinea-pig ($1{\cdot}67\ L_f\ s^{-1}$) and the fast IM of mouse ($6{\cdot}11\ L_f\ s^{-1}$). The presence of $SM_1$ in IIA fibres would thus lower the characteristic high $V_0$ of isomyosin IM, a reasonable assumption since we expect $SM_1$ to be a slowly contracting isomyosin according to its subunit composition, which is identical to $SM_2$ except for the substitution of one slow alkaline light chain, $LC_1s$, by a fast one, $LC_1f$ (Maréchal *et al.* 1988). The second hypothesis seems more likely because several groups have reported that many single fibres contain a mixture of various myosin heavy and light chains (Billeter, Heizman, Reist & Jenny, 1981; Danielli-Betto, Zerbato & Betto, 1986; Staron & Pette, 1987; Biral, Betto, Danielli-Betto & Salviati, 1988).

In conclusion, the characteristic $V_0$ of isomyosins increases in the sequence: $SM_2 < SM_1 + IM < IM$. The subunit composition of these isomyosins is identical in mice, rats and guinea-pigs (d'Albis *et al.* 1979; Fitzsimons & Hoh, 1983; Maréchal *et al.* 1988). Using this information, we conclude that $V_0$ increases according to the following sequence: $MHC_I(LC_1s)_2 < MHC_I(LC_1s,LC_1f) + MHC_{IIA}(LC_1s,LC_1f) < MHC_{IIA}(LC_1s,LC_1f)$.

Taken together, all these results indicate that the shortening velocity of a fibre is controlled not only by the nature of its myosin heavy and light chains but also by the relative proportions of the isomyosins mixed in it.

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## REFERENCES

ANDERSON, J. E., BRESSLER, B. H. & OVALLE, W. K. (1988). Functional regeneration in the hind limb skeletal muscle of the mdx mouse. *Journal of Muscle Research and Cell Motility* **9**, 499–515.

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R. (1973). Hind limb muscle fibre populations of five mammals. *Journal of Histochemistry and Cytochemistry* **21**, 51–55.

BILLETER, R., HEIZMAN, C. W., REIST, U. & JENNY, E. (1981). Analysis of myosin light and heavy chain types in single human skeletal muscle fiber. *European Journal of Biochemistry* **116**, 389–395.

BIRAL, D., BETTO, R., DANIELLI-BETTO, D. & SALVIATI, G. (1988). Myosin heavy chain composition of single fibres from normal human muscle. *Biochemical Journal* **250**, 307–308.

BROOKE, M. H. & KAISER, K. K. (1970). Muscle fiber types: how many and what kind? *Archives of Neurology* **23**, 369–379.

BROOKS, S. V. & FAULKNER, J. A. (1988). Contractile properties of skeletal muscles from young, adult and aged mice. *Journal of Physiology* **404**, 71–82.

CECCHI, G., COLOMO, F. & LOMBARDI, V. (1978). Force–velocity relation in normal and nitrate-treated frog single fibres during rise of tension in an isometric tetanus. *Journal of Physiology* **285**, 257–273.

CLAFLIN, D. R. & FAULKNER, J. A. (1985). Shortening velocity extrapolated to zero load and unloaded shortening velocity of whole rat skeletal muscle. *Journal of Physiology* **359**, 357–363.

CLOSE, R. I. (1967). Properties of motor units in fast and slow skeletal muscle of the rat. *Journal of Physiology* **193**, 45–55.

CLOSE, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* **52**, 129–197.

D'ALBIS, A., PANTALONI, C. & BECHET, J. J. (1979). An electrophoretic study of native myosin isozymes and of their subunit content. *European Journal of Biochemistry* **99**, 261–272.

DANIELLI-BETTO, D., ZERBATO, E. & BETTO, R. (1986). Type 1, 2A and 2B myosin heavy chain electrophoretic analysis of rat muscle fibers. *Biochemical and Biophysical Research Communications* **138**, 981–987.

DRIBIN, L. B. & SIMPSON, S. B. (1977). Histochemical and morphological study of dystrophic (C57BL/6J $dy^2J/dy^2J$) and normal mice. *Experimental Neurology* **56**, 480–497.

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**, 143–159.

FITZSIMONS, R. B. & HOH, J. F. Y. (1983). Myosin isoenzymes in fast-twitch and slow-twitch muscles of normal and dystrophic mice. *Journal of Physiology* **343**, 539–550.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proceedings of the Royal Society* B **126**, 136–195.

HOH, J. F. Y., MCGRATH, P. A. & WHITE, R. L. (1976). Electrophoretic analysis of multiple forms of myosin in fast-twitch and slow-twitch muscles of the chick. *Biochemical Journal* **157**, 87–95.

JULIAN, F. J., ROME, L. C., STEPHENSON, D. G. & STRITZ, S. (1986). The maximum speed of shortening in living and skinned frog muscle fibres. *Journal of Physiology* **370**, 181–199.

LEWIS, D. M., PARRY, D. J. & ROWLERSON, A. (1982). Isometric contractions of motor units and immunohistochemistry of mouse soleus muscle. *Journal of Physiology* **325**, 393–401.

LUFF, A. R. (1981). Dynamic properties of the inferior rectus, extensor digitorum longus, diaphragm and soleus muscles of the mouse. *Journal of Physiology* **313**, 161–171.

MARÉCHAL, G., BIRAL, D., BECKERS-BLEUKX, G. & COLSON-VAN SCHOOR, M. (1988). Subunit composition of native myosin isoenzymes of some striated mammalian muscles. *Archives Internationales de Physiologie et de Biochimie* **97**, P14.

MARÉCHAL, G. & PLAGHKI, L. (1979). The deficit of the isometric tetanic tension redeveloped after a release of frog muscle at a constant velocity. *Journal of General Physiology* **73**, 453–467.

MARÉCHAL, G., SCHWARTZ, K., BECKERS-BLEUKX, G. & GHINS, E. (1984). Isozymes of myosin in growing and regenerating rat muscle. *European Journal of Biochemistry* **138**, 421–428.

POWELL, P. L., ROY, R. R., KANIM, P., BELLO, M. A. & EDGERTON, V. R. (1984). Predictability of skeletal muscle tension from architectural determinations in guinea-pigs' hind limbs. *Journal of Applied Physiology* **57**, 1715–1721.

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

ROWE, R. W. D. & GOLDSPINK, G. (1969). Surgically induced hypertrophy in skeletal muscles of the laboratory mouse. *Anatomical Records* **161**, 69–75.

STARON, R. S. & PETTE, D. (1987). The multiplicity of myosin light and heavy chain combinations in histochemically typed single fibres of the rabbit soleus muscle. *Biochemical Journal* **243**, 687–693.

SWEENEY, H. L., KUSHMERICK, M. J., MABUCHI, K., SRÉTER, F. & GERGELY, J. (1988). Myosin alkali light chain and heavy chain variations correlate with altered shortening velocity of isolated skeletal muscle fibers. *Journal of Biological Chemistry* **263**, 9034–9039.

WITZMANN, F. A., KIM, D. H. & FITTS, R. H. (1983). Effects of hind limb immobilization on the fatigability of skeletal muscles. *Journal of Applied Physiology* **54**, 1242–1248.

YAMAOKA, K., TANIGAWARA, Y., NAKAGAWA, T. & UNO, T. (1981). Pharmacokinetic analysis program (multi) for microcomputer. *Journal of Pharmacological Dynamics* **4**, 879–885.