

# Correlated Reduction of Velocity of Shortening and the Rate of Energy Utilization in Mouse Fast-Twitch Muscle During a Continuous Tetanus

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**ABSTRACT** Isometric tetani of slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles of the mouse were studied at 20°C. The total energy cost for 3- and 9-s isometric tetani was measured as a function of length above  $L_0$  and partitioned into a filament overlap-dependent fraction and a smaller filament overlap-independent fraction. In both muscles, the rate of filament overlap-independent energy cost did not change with tetanic duration. In the EDL, but not in the soleus, the rate of filament overlap-dependent energy utilization was greater in a 3-s tetanus than in a 9-s tetanus. The force-velocity relationships were studied after 3 and 9 s of isometric tetanus. In the soleus,  $V_{max}$  was 2 fiber lengths/s and was not dependent on the duration of isometric tetanus. In contrast, in the EDL,  $V_{max}$  decreased from 5.9 fiber lengths/s at 3 s to 3.9 fiber lengths/s at 9 s. The velocity of unloaded shortening ( $V_{us}$ ) was examined by the slack test method as a function of the duration of isometric tetanus over the range of 1–15 s. In the soleus,  $V_{us}$  did not change, whereas in the EDL,  $V_{us}$  declined progressively from 6.4 to 3.2 fiber lengths/s after an isometric tetanus of increasing duration from 1 to 15 s. These results cannot exclude the hypothesis that in a maintained tetanus there is a decrease in the intrinsic cross-bridge turnover rate in the fast-twitch EDL, but not in the slow-twitch soleus muscle.

## INTRODUCTION

Stimulated fast-twitch mammalian skeletal muscles have a greater rate of energy utilization than slow-twitch muscles (Gibbs and Gibson, 1972; Goldspink et al., 1970; Wendt and Barclay, 1980; Wendt and Gibbs, 1973). These results are consistent with the more rapid mechanical properties of adult fast-twitch muscles (Close, 1972). However, the pattern of chemical energy

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utilization during a maintained isometric tetanus in the mouse muscles differed (Crow and Kushmerick, 1982*a*) in the following way: for short isometric tetani (up to 3 s duration), the rate of energy utilization in the fast-twitch extensor digitorum longus (EDL) was three times greater than that of the slow-twitch soleus; the rates were normalized to the average force per cross-sectional area. With continued stimulation, however, this rate in the fast-twitch muscle decreased so that from 12 to 15 s of isometric contraction, the rate was only one-half the initial value. We found no time-dependent change in the rate of energy consumption of the soleus during the 15-s tetanus, although in more prolonged stimulations of mouse soleus, a decreased rate of ATP usage and of relaxation of isometric tension was described (Edwards et al., 1975). In our work, quantitative comparison of the extents of recovery metabolism and of high-energy phosphate utilization during contraction verified that all the energy associated with contraction had been adequately measured in both muscles.

Several hypotheses could explain this change in the rate of high-energy phosphate splitting or apparent "economy" of the EDL with continued stimulation: (*a*) There may be selective fatigue or inactivation of one type of fiber; the EDL is composed of both fast-glycolytic and fast-oxidative-glycolytic fibers (Crow and Kushmerick, 1982*a*). (*b*) The fraction of total energy consumption that is independent of thick- and thin-filament interaction (and therefore of isometric force) may depend on stimulus duration. (*c*) The intrinsic kinetic properties of the actomyosin could be altered during the course of the contraction to result in a diminished rate of ATP splitting.

Time-dependent changes in the rate of filament overlap-independent or force-independent energy consumption can be estimated by measuring the total energy cost for an isometric contraction of constant duration as a function of muscle length. The experimental design allows an energy consumption independent of thick- and thin-filament overlap to be separated from the total energy consumption (Smith, 1972; Homsher et al., 1972). The experiment assumes that the isometric force is proportional to the degree of overlap of the thick and thin filaments (Gordon et al., 1966) and that sarcomere length increases with muscle length. We describe chemical energetic experiments examining this issue in mouse EDL and soleus muscles. The maximum speed at which a muscle shortens and the energy cost for isometric tension maintenance have been correlated with the rate of actomyosin ATP hydrolysis (Bárány, 1967; Goldspink et al., 1970; Gibbs and Gibson, 1972; Wendt and Gibbs, 1973). A modification of the kinetics of actomyosin interaction that results in a change in the isometric energy cost may also result in a reduction in the speed of shortening by the muscle. We made mechanical measurements to test this correlation.

Therefore, we designed experiments to test the hypothesis that the reduction in energy cost that occurs in an isometric tetanus of the fast-twitch EDL is due to a change in the rate of actomyosin energy consumption during the tetanus, and that this decrease is accompanied by a reduction in the velocity of shortening of the muscle.

## MATERIALS AND METHODS

The dissection, conditions of incubation, and characterization of the mouse EDL and soleus muscles have been described in detail elsewhere (Crow and Kushmerick, 1982a). Male CD-1 mice between the ages of 21 and 28 d were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and used in this study. All the experiments were performed at 20°C in a bicarbonate Ringers solution at pH 7.4 without added substrates.

*Measurement of Total Chemical Energy Utilization*

For measurement of the energy consumption of isometrically contracting muscle, the extent of recovery metabolism during and after the tetanus was derived from the recovery oxygen consumption ( $\xi O_2$ ) and lactate production ( $\xi \text{lac}$ ). These data were used to calculate the extent of energy utilization ( $\Delta \sim P_{\text{rec}}/g$ , in units of micromoles of  $\sim P$  hydrolysis per gram wet weight of muscle) with the in vitro stoichiometric coefficients relating the extent of substrate oxidation or product accumulation with ATP resynthesis. The values of these constants (Scopes, 1973; Kushmerick, 1977) for muscles metabolizing glycogen were:

$$\xi \sim P / \xi O_2 = 6.2;$$

$$\xi \sim P \xi \text{lac} = 1.5.$$

Thus,  $\Delta \sim P_{\text{rec}}/g = 1.5 \xi \text{lac} + 6.2 \xi O_2$ . The documentation of this approach, as well as the experimental details of these measurements, has been reported (Crow and Kushmerick, 1982a).

The total energy consumption in isometrically contracting muscles was partitioned into force-dependent and force-independent amounts by measuring  $\Delta \sim P_{\text{rec}}$  in muscles stretched to various degrees. The reference length was that which gave a maximum twitch force, and is designated as  $L_0$ . For the EDL, this length corresponded to the length of the muscle in the animal with the ankle fully extended. For the soleus,  $L_0$  corresponded to the in situ length with the ankle fully dorsiflexed. Each muscle was stimulated at  $L_0$  for 0.5 s at 66 Hz and allowed to recover for 30 min. Then the muscle was stretched by different amounts, but not to more than 1.3  $L_0$ , and allowed to achieve a steady metabolic state, defined as a steady rate of oxygen consumption while unstimulated. The muscle was then stimulated tetanically for 3 or 9 s. The extent of recovery metabolism was measured as the total oxygen consumption and lactate production minus the baseline of the stretched but unstimulated muscle. The isometric force during the tetanus was averaged by measuring the force-time integral as described (Crow and Kushmerick, 1982a) and by dividing that quantity by the stimulus duration. We define  $P_0$  as the average isometric force at  $L_0$ , and  $P_s$  as the average isometric force at longer lengths. The first tetanus at  $L_0$  was used as a reference to scale the data as the ratio  $P_s/P_0$ . The recovery metabolism from six to eight such tetani in the range of 1.0–1.3  $L_0$ , including regularly spaced contractions at  $L_0$ , was measured in single muscles.  $\Delta \sim P_{\text{rec}}$  was plotted as a function of  $P_s/P_0$  for 3- and 9-s tetani. The positive intercept on the ordinate at  $P_s/P_0 = 0$  gives the value of the force-independent energy consumption.

To establish quantitative relationships among sarcomere length, muscle length, and isometric force, measurements were made of average sarcomere length by optical diffraction, and of twitch and tetanic force as a function of muscle length in one soleus and five EDL muscles. The light source was a 5-mW He-Ne laser. Three diffraction orders were detected, but only the first and second were projected onto

a screen 144 mm from the muscle and recorded manually. The distance between each set of the first- and second-order patterns was measured and used to calculate the angle between the incident and diffracted beam. The formula  $d = m\lambda/\sin\theta$  was used, where  $d$  is taken to be the averaged sarcomere length,  $m$  is the diffraction order,  $\lambda = 0.623 \mu\text{m}$ , and  $\theta$  is the measured angle. Typically, the diffraction pattern consisted of (2–4 mm) broad bands, which indicates a dispersion of sarcomere lengths on the order of  $0.3 \mu\text{m}$ . In two EDL the lines were narrower,  $<1 \text{ mm}$ . For the muscles studied, the dispersion was independent of the location of the light beam. However, only the thinner portions of the muscles, near the lateral edges and near the tendons, gave useful patterns because of intense light scattering from the belly of the muscle. Once a stable diffraction pattern was obtained, the length of the muscle was set to  $L_0$ . The muscle length was increased or decreased by a micrometer screw adjustment. The diffraction pattern was recorded and a test twitch or tetanic contraction (0.3 s for EDL; 1.0 s for soleus) was obtained. Force-length observations were made at 2–3-min intervals over the range of 0.8–1.3  $L_0$ . The muscle rested for 30 min, the light beam was repositioned, and at least one additional set of measurements was made with each muscle.

#### *Mechanical Experiments*

**APPARATUS** Muscles were dissected, and 5-0 silk was tied to the tendons as close to the fibers as possible without damaging them, in order to reduce the tendon series compliance. The tendons were tied directly to cold-stretched stainless steel wire (0.38 mm diam) formed with a small hook for attachment to the force transducer and motor lever. During all measurements, the muscle was fully immersed in a continuously flowing solution of mammalian bicarbonate Ringers solution equilibrated with 95%  $\text{O}_2$ –5%  $\text{CO}_2$  (vol/vol) at  $20^\circ\text{C}$ . Muscles were stimulated in this solution via platinum plates placed on either side of the longitudinal axis of the muscle with bipolar pulses of 10–15 V amplitude and 66 Hz frequency.

The tension developed by the muscle was measured by a capacitor-type transducer (Harvard Apparatus, Millis, MA), which had a resonant frequency of 250 Hz.

Control and measurement of the position of the muscle was achieved with a galvanometer equipped with a differential-capacitor position detector (model 305; Cambridge Technology, Cambridge, MA). The compliance with a 4-cm lever was  $0.02 \mu\text{m/g}$ ; the inertia was  $4.7 \text{ g}\cdot\text{cm}$ . The position detector had a typical displacement resolution of  $1 \mu\text{m}$ . The total system compliance with the muscle attached to the force transducer and servomotor was  $2.3 \mu\text{m/g}$ .

**ISOTONIC RELEASES** Quick releases of the muscle to different loads were used to obtain the force-velocity properties of the muscles. To obtain isotonic releases, the output of the tension transducer was used to generate an error signal which, when applied to the positional input of the servomotor, allowed the muscle to shorten against a constant force. A rapid change in length caused by shortening of the series elastic elements, and a slower length change as the muscle shortened at constant velocity and load, were analyzed as described by Jewell and Wilkie (1958).

The force-velocity constants,  $b/L_0$  and  $a/P_0$ , were obtained from the data by a linearized form of the Hill (A. V. Hill, 1938) force-velocity equation (Julian, 1971; Rall and Schottelius, 1973). A plot of the fractional load,  $P/P_0$ , as a function of  $[L_0(1 - P/P_0)/V]$  yields a linear relationship with a slope =  $b/L_0$  and a  $y$  intercept =  $-a/P_0$ . The  $V_{\text{max}}$  was determined by extrapolation of this curve to  $P/P_0 = 0$ . Here  $P_0$  is the isometric force just before the release.

**VELOCITY OF UNLOADED SHORTENING** The velocity of unloaded shortening

( $V_{us}$ ) was determined by a slack-test method from the time course of events following a rapid release of the muscle during the plateau of isometric tension to zero force (Edman, 1979). Length changes at a velocity greater than the maximum velocity of shortening of the muscle, typically 200–500 mm/s, were used. The intervals between the time when the tension trace hit the zero-force baseline to the onset of force redevelopment were plotted against the length change. The slope of this relationship for a family of such measurements performed at different length excursions is the velocity of unloaded shortening. The zero-time intercept of this relationship provided an estimate of the series compliance of the system. The length excursions used were chosen so that release occurred symmetrically about  $L_0$ . The length changes were always limited to a maximum of 15% of the muscle fiber length ( $\sim 1.0$ – $1.5$  mm). An example of records obtained in one EDL muscle is given in Fig. 5C.

After each tetanus and release, both in the isotonic experiments and in the slack test experiments, the muscle was allowed to recover for at least 30 min; this recovery period is more than five time constants for aerobic recovery (Crow and Kushmerick, 1982a).

When required, muscle lengths were converted into fiber lengths using the factors previously measured (Crow and Kushmerick, 1982a): fiber length  $\div$  muscle length = 0.83 in the soleus and 0.68 in the EDL. No adjustment was made for the 4–6° angle between fiber orientation and long axis of the whole muscle.

#### *Statistical Analysis of Data*

Unless otherwise stated, all measurements are given as the mean  $\pm$  1 SD. Regression analyses were performed using a Gauss-Newton method (Duggleby, 1981). The statistical significance was inferred from the unpaired  $t$  test (Armitage, 1971). When  $n_1 \neq n_2$ , the degrees of freedom were adjusted as described by Beyer (1966).

## RESULTS

### *Isometric Properties of Mouse Soleus and EDL Muscles*

The time courses of a twitch and a 12-s isometric tetanus at  $L_0$  and 20°C for representative soleus and EDL muscles are shown in Fig. 1. The peak twitch force was substantially greater in the fast-twitch EDL muscle, but the maximum tetanic force was similar (Table I). The EDL was faster than the soleus in its time-dependent properties: time-to-peak twitch tension ( $T_c$ ) and twitch relaxation time ( $T_{1/2r}$ ). As expected from its greater aerobic capacity, the soleus was able to withstand prolonged stimulation with little decline of force. In the EDL, after 15 s of stimulation, the mean tetanic force had declined by  $\sim 20\%$ . These characteristics indicate that the muscles used, from 3–4-wk-old mice, have the mechanical characteristics of muscles from fully mature mice (Close, 1972).

### *Sarcomere Length*

The data shown in Fig. 2 establish relationships between average sarcomere length and isometric tetanic and twitch force ( $A$ ), and overall muscle fiber length ( $B$ ) for one EDL muscle. The measurements are based on optical diffraction patterns obtained in unstimulated muscle just before the test contraction. The average sarcomere length that gave maximum tetanic force

was  $2.7 \pm 0.02 \mu\text{m}$  (SD) in 10 experiments from five EDL muscles. The average sarcomere length that gave maximum twitch force was  $2.9 \pm 0.1 \mu\text{m}$  (SD). The light scattering from soleus muscles was usually sufficiently intense to obscure the diffraction pattern, but one muscle gave useful data: the sarcomere length that gave maximum tetanic force was  $2.9 \mu\text{m}$ . Extrapolation of the sarcomere length vs. tetanic force curves to zero force for 13 sets of measurements (including 2 sets from the soleus) gave an average sarcomere length of  $3.9 \pm 0.1 \mu\text{m}$  (SD). The definition of  $L_0$  used in these experiments is based on maximum twitch force, so  $L_0$  corresponded to a sarcomere length of  $\sim 2.9 \mu\text{m}$ . The sarcomere length increased in a nearly linear manner with the muscle stretch in good agreement with predictions of filament sliding,

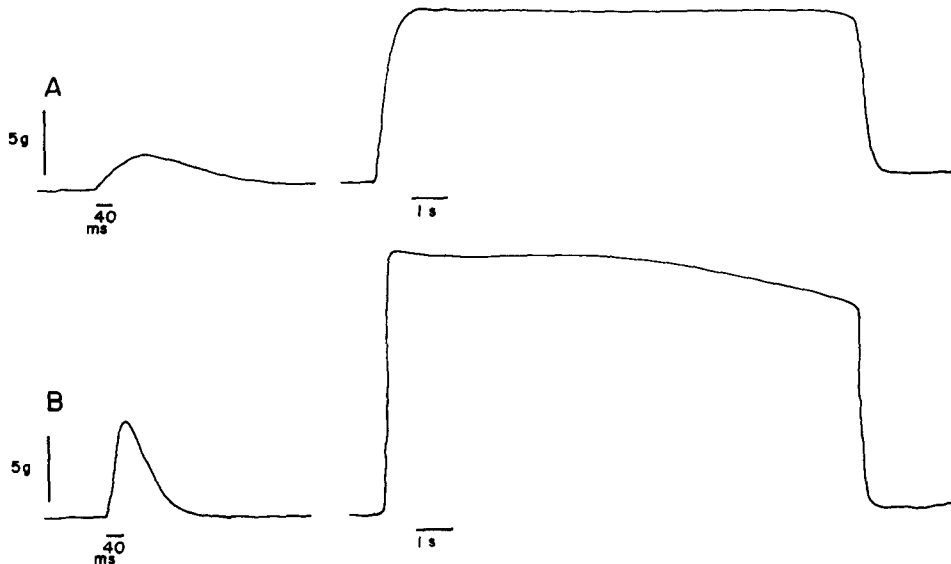


FIGURE 1. The time course of the isometric mechanical responses in mouse soleus (A) and EDL muscles (B) from tracings of actual experimental records at  $20^{\circ}\text{C}$ . In each part, an isometric twitch (left) and a 12-s tetanus (right) are shown. The horizontal bars represent 40 ms for the twitch and 1 s for the tetanus. The vertical bars represent 5 g of force for both the twitch and tetanus.

with a thick filament length of  $1.6 \mu\text{m}$  and a thin filament length + Z line of  $1.15 \mu\text{m}$  (Page and Huxley, 1963; Close, 1972). Because of series compliance, the sarcomere length during the twitch and tetanus was less than that in resting muscles. In three tetani of the muscle studied in Fig. 2, the sarcomere length decreased by  $0.3 \mu\text{m}$  from a pre-tetanus sarcomere length of  $2.9 \mu\text{m}$ , and the width of the diffraction pattern increased to  $\sim 5 \text{ mm}$ . Thus, by taking series compliance into account, the maximum tetanic force occurred at  $\sim 2.4 \mu\text{m}$ .

#### *The Energy Cost in Muscles Stretched to Vary Filament Overlap*

The decrease in energy cost observed in the mouse EDL might arise from a time-dependent reduction in the rate of filament overlap-independent ATP

hydrolysis. To test this hypothesis, we estimated the fractional contribution of force-independent energy cost to the total energy cost by determining the total energy consumption in progressively stretched muscles. In Fig. 3, the energy utilization values estimated from recovery metabolism ( $\Delta \sim P_{\text{rec}}$ ) in the soleus (A) and EDL (B) muscles stimulated for 3 and 9 s were plotted as a function of the average force in the stretched muscle ( $P_s$ ) relative to the force ( $P_0$ ) obtained at  $L_0$ . Fatigue of force or creep of force in stretched muscles was insignificant with these stimulus durations. In all cases, total energy consumption was a linear function of  $P_s/P_0$ . Extrapolation of the regression line to  $P_s/P_0 = 0$  provided an estimate of the force-independent (filament overlap-independent) energy cost. The basis for this interpretation is the data described in the previous section; those data indicate that the abscissa scale in Fig. 3 corresponds to an average sarcomere length of  $2.4 \mu\text{m}$  at  $P_s/P_0 = 1$  and of  $3.9 \mu\text{m}$  at  $P_s/P_0 = 0$ . In the soleus, the total energy cost for a 9-s tetanus at  $L_0$  (i.e.,  $P_s/P_0 = 1$ ) was three times that for a 3-s tetanus; the result

TABLE I  
*Isometric Properties of Mouse Soleus and EDL at 20°C*

	Soleus	EDL
$P$ (N/mm <sup>2</sup> )*	0.25±0.05	0.25±0.05
$P_t$ (N/mm <sup>2</sup> )	0.06±0.01	0.10±0.02
$P_t/P_0^\dagger$	0.25±0.02	0.42±0.04
$T_c$ (ms)	100±5	40±3
$T_{1/2}$ (ms)	130±23	46±8

\* The abbreviations used are:  $P$ , maximum isometric tetanic tension per cross-sectional area in newtons per square millimeter;  $P_t$ , maximum twitch force developed per cross-sectional area (units as above);  $T_c$ , time-to-peak twitch tension;  $T_{1/2}$ , time to half-relaxation in the twitch.

† The standard deviation of this ratio was obtained using the method of analysis described by Armitage (1971).

of this experiment was similar to what was previously reported (Crow and Kushmerick, 1982a). There was no change in the averaged filament overlap-dependent energy consumption rate in a 3- or 9-s tetanus (Table II).

In the EDL (Fig. 3B and Table II), the total energy cost for a 9-s tetanus at  $L_0$  (i.e.,  $P_s/P_0 = 1$ ) was only twice the energy for a 3-s tetanus, despite the fact that the force-time integral for a 9-s tetanus was approximately three times that of a 3-s tetanus. These data confirm the previously reported reduction in rate of energy utilization in the EDL during a tetanus (Crow and Kushmerick, 1982a). There was no change in the rate of filament overlap-independent energy consumption in a 3- or 9-s tetanus (Table II). The size of this energy consumption is greater in the EDL than in the soleus; compare  $0.44 \mu\text{mol} \sim P/\text{g}\cdot\text{s}$  for a 3-s tetanus of the soleus with the corresponding value of  $1.21 \mu\text{mol} \sim P/\text{g}\cdot\text{s}$  in the EDL. On the other hand, the averaged rate of filament overlap-dependent energy consumption during the 9-s tetanus ( $1.74 \mu\text{mol} \sim P/\text{g}\cdot\text{s}$ ) was 60% of that observed in the 3-s tetanus ( $2.91 \mu\text{mol} \sim P/\text{g}\cdot\text{s}$ ). These data show that the decrease in total isometric energy consumption by the EDL was due primarily to a reduction in the rate

of filament overlap-dependent energy consumption. Therefore, there is a reduction in the extent of ATP hydrolysis by actomyosin ATPase per unit of force-time integral during a maintained isometric tetanus of EDL, but not of soleus muscles. There was no evidence for a decrease in the rate of filament overlap-independent energy cost in either muscle.

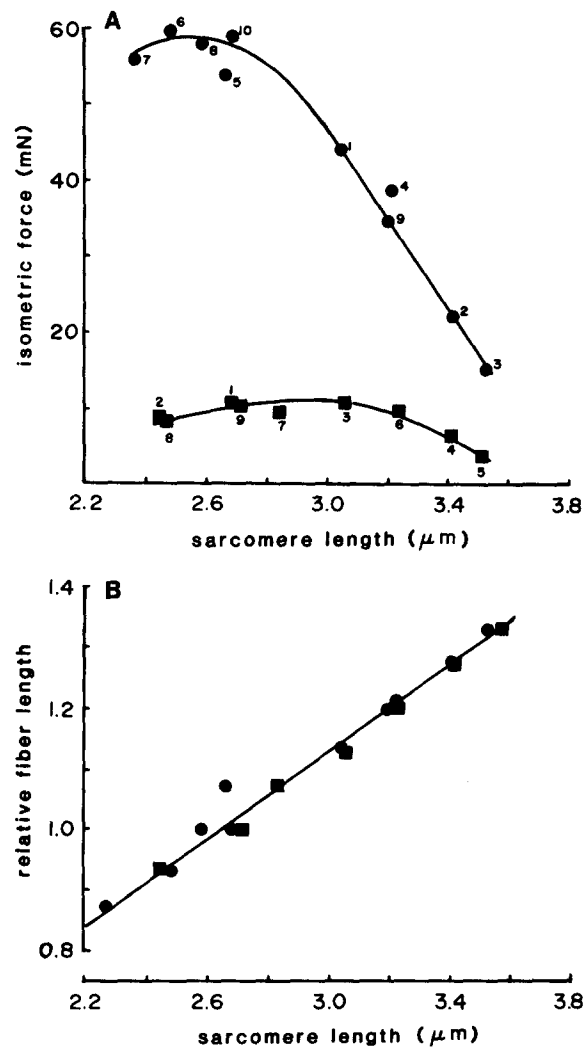


FIGURE 2. Isometric force-length curve of an EDL muscle and the relation between its relative fiber length and sarcomere length at 20°C. Panel A shows 0.5 s isometric tetanic force (●) and twitch force (■) as a function of sarcomere length measured by optical diffraction before the test contraction). The numbers adjacent to the data indicate the order of measurements. Panel B shows the linear relationship between fiber length and sarcomere length in the same muscle. Circles indicate the tetanic series and squares represent the twitch series. The muscle fiber length at  $L_0$  was 7.5 mm. The line was drawn from the regression equation  $y = 0.36x + 0.05$ .



*Force-Velocity Relationships*

The force-velocity curves generated from a series of isotonic releases in three soleus and three EDL muscles are presented in Fig. 4. Figure 4A gives the relationship for the EDL measured after 3 (closed circles) and 9 s (closed squares) of isometric tetanic stimulation. The points at  $P/P_0 = 0$  marked by the arrows represent the values obtained from the velocity of unloaded shortening,  $V_{us}$  (see next section), and agree well with the value determined by extrapolation of the curve to zero load. In the inset to Fig. 4, the fractional load ( $P/P_0$ ) was plotted as a function of  $[L_0(1 - P/P_0)/V]$  in order to obtain the characteristic constants,  $a/P_0$  and  $b/L_0$ , which are given in Table III.

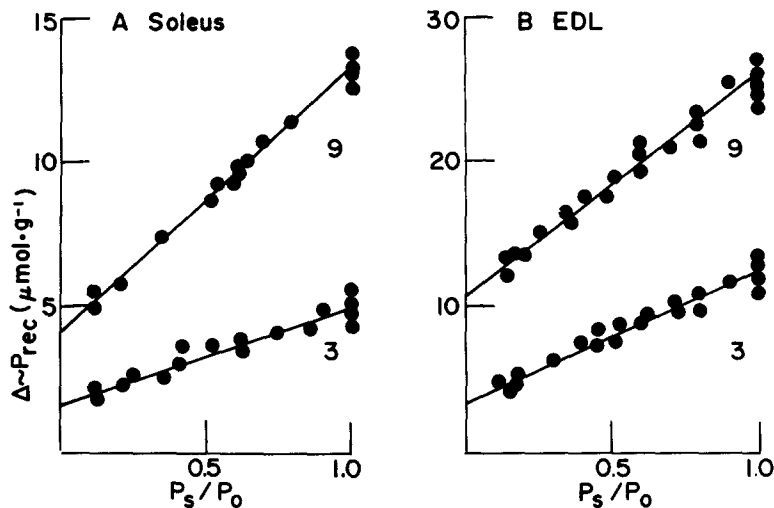


FIGURE 3. The energy cost in stretched muscles stimulated for 3 or 9 s at 20°C. A: soleus; B: EDL. The ordinate is the energy cost estimated from the extent of recovery metabolism ( $\Delta \sim P_{rec}$ ) and is expressed in  $\mu\text{mol} \sim P/g$ .  $P_0$  is the average isometric force in a 3- or 9-s isometric tetanus at  $L_0$ .  $P_s$  is the same parameter for muscles stretched to various lengths greater than  $L_0$ . The ratio,  $P_s/P_0$ , plotted on the abscissa, corresponds to the relative isometric force at the stretched length compared with force at  $L_0$ . The lines through the points are linear regression functions. Each set of data is from four or five muscles as indicated by the number of data at  $P_s/P_0 = 1$ .

Stimulation of the EDL isometrically for 9 s prior to release caused a reduction in the velocity of shortening for all loads and a reduction in the  $V_{max}$  by 24% from that observed after a 3-s tetanus. The tetanus durations and load were selected randomly, and after each tetanus the muscles were left unstimulated for at least 20 min to recover aerobically. The reductions in isotonic velocity of shortening were fully reversible after this recovery. Fig. 4B presents curves showing no change in the force-velocity relationship for the soleus after either 3 (open circles) or 9 s (open squares) of isometric tetanic stimulation.

*The Velocity of Unloaded Shortening after Isometric Tetani of Various Durations*

To ascertain whether the reduction of  $V_{\max}$  in the EDL correlates with the reduction in the rate of energy utilization observed during the course of an isometric tetanus, the extent and time course of the reduction in shortening velocity after preceding isometric tetani of various durations were measured by the slack-test method.

In Fig. 5, the family of curves generated from this procedure for releases after tetani of 1, 3, 6, 9, 12, and 15 s are shown. Examples of records obtained in one EDL are given in panel C. For the soleus (Fig. 5B), all the data were superimposable upon a common line, which indicates that both  $V_{us}$  and the total series compliance were independent of the duration of the preceding tetanic activity. For the EDL, however, separate curves that shared a common intercept were generated. As the duration of stimulation preceding

TABLE II  
*Fractionation of the Total Energy Cost Associated with Contraction*

Muscle	Tetanus duration	Total energy consumption*	Filament overlap-independent energy consumption	Filament overlap-dependent energy consumption	Filament overlap-independent cost/total cost
	<i>s</i>	$\mu\text{mol/g}\cdot\text{s}$	$\mu\text{mol/g}\cdot\text{s}$	$\mu\text{mol/g}\cdot\text{s}$	
Soleus	3	1.46 ( $\pm 0.10$ )	0.44 ( $\pm 0.04$ )	1.00 ( $\pm 0.07$ )	0.31 ( $\pm 0.06$ )
	9	1.46 ( $\pm 0.16$ )	0.45 ( $\pm 0.02$ )	1.01 ( $\pm 0.09$ )	0.31 ( $\pm 0.04$ )
EDL	3	4.08 ( $\pm 0.38$ )	1.21 ( $\pm 0.11$ )	2.91 ( $\pm 0.05$ )	0.29 ( $\pm 0.04$ )
	9	2.82 ( $\pm 0.15$ )	1.12 ( $\pm 0.15$ )	1.74 ( $\pm 0.06$ )	0.39 ( $\pm 0.02$ )

Mean values  $\pm$  standard deviation.

\* Data were obtained from linear regression analyses of data in Fig. 3 and with the assumption of a linear decrease in tetanic force with increasing sarcomere length (Fig. 2).

the release was increased, the slope of the line, and hence the  $V_{us}$ , decreased. Between 12 and 15 s of pre-release tetanic stimulation, no further reductions in the velocity of shortening occurred. The data from all isotonic force-velocity and  $V_{us}$  experiments are summarized in Table III. Both estimates of maximal shortening velocity ( $V_{\max}$  and  $V_{us}$ ) agree. The reduction in velocity of shortening in the EDL as a function of preceding isometric tetanic duration clearly stands in contrast with the results obtained in the soleus. The curvature of the force-velocity curves, estimated by the parameter  $a/P_0$ , is similar in EDL and soleus, and appears to be independent of tetanic duration.

## DISCUSSION

The hypothesis tested by these experiments is that the reduction in chemical energy cost per unit of developed force that occurred during a maintained isometric tetanus in mouse EDL muscles (Crow and Kushmerick, 1982a) was

due to a decrease of the intrinsic actomyosin ATPase rate. Two types of experiments were used. The total isometric energy cost was divided into a filament overlap-dependent portion and a filament overlap-independent,

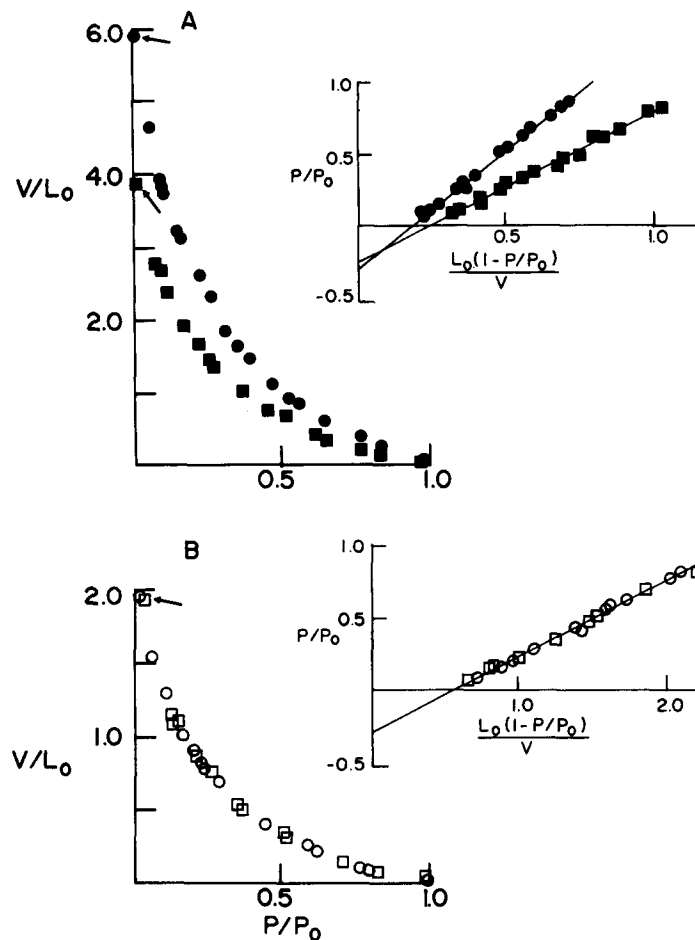


FIGURE 4. Force-velocity curves for mouse soleus and EDL at 20°C. In each curve the shortening velocity in fiber lengths per second ( $V/L_0$ ) is plotted as a function of the fractional load ( $P/P_0$ ) for that velocity. The arrows in A and B at  $P/P_0 = 0$  point to the values obtained for the velocity of unloaded shortening, as described in Fig. 5. The insets in A and B are plots of the fractional load ( $P/P_0$ ) as a function of  $L_0(1 - P/P_0)/V$ . The intercept of such a plot yields the characteristic constant,  $-a/P_0$ , and the slope is equal to  $b/L_0$ ; these values are given in Table III. (A) Force-velocity relationship for the EDL obtained from isotonic releases after 3 (●) or 9 s (■) of isometric tetanic stimulation. Results represent determinations from at least four muscles with at least six releases performed on each muscle; closely overlapping data are not plotted. (B) Force-velocity relationship for the soleus obtained from isotonic releases after 3 (○) or 9 s (□) of tetanic stimulation. Results represent the determination on three muscles with at least eight releases per muscle; closely overlapping data are not plotted.

presumably non-actomyosin, portion. For these experiments, energy cost was measured in terms of total recovery metabolism ( $\Delta \sim P_{\text{rec}}$ ), a measure of the total chemical energy cost, in muscles progressively stretched in the range  $L_0$  to  $1.3 L_0$ . Sarcomere length increased and isometric force decreased with muscle length, so that extrapolation of energy cost to zero isometric force (Fig. 3), which occurred at an average sarcomere length of  $3.9 \mu\text{m}$  (Fig. 2), provided an estimate of the non-actomyosin energy cost associated with contractile activity. Only the force-dependent (filament overlap-dependent) portion of the energy cost decreased during a maintained tetanus, and this decline occurred in the fast-twitch EDL, not in the slow-twitch soleus muscle.

TABLE III  
*Force-Velocity Properties of the Mouse Soleus and EDL*

Isometric tetanus	EDL				Soleus				
	Duration (s)	1	3	9	15	1	3	9	15
$V_{\text{max}}^*$	— <sup>‡</sup>	5.88 (±0.21)	3.91 (±0.51)	—	—	2.01 (±0.21)	1.95 (±0.31)	—	—
$V_{\text{un}}^{\S}$	6.38 (±0.15)	5.75 (±0.13)	4.07 (±0.06)	3.21 (±0.10)	1.88 (±0.02)	1.88 (±0.05)	1.89 (±0.04)	1.88 (±0.03)	—
SC <sup>¶</sup>	4.5 (±0.2)	4.6 (±0.6)	4.5 (±0.8)	4.2 (±0.9)	3.7 (±0.5)	3.8 (±0.4)	3.8 (±0.9)	3.7 (±0.8)	—
$a/P_0^*$	—	0.26 (±0.03)	0.26 (±0.02)	—	—	0.25 (±0.01)	0.26 (±0.02)	—	—
$b/L_0^*$	—	1.53 (±0.06)	1.02 (±0.02)	—	—	0.50 (±0.01)	0.51 (±0.02)	—	—

\* Determined from a linearized transform of the data in Fig. 4, A and B; units are fiber lengths/s.

<sup>‡</sup> Not measured.

<sup>§</sup> Determined by direct measurement (Fig. 5); units are fiber lengths/s.

<sup>¶</sup> Series compliance expressed in percent fiber length.

The second indication of actomyosin ATPase rate was based on the established correlations between maximal velocity of shortening and actomyosin ATPase activity (Bárány, 1967). If the results just summarized are due to a diminished actomyosin ATPase rate, the velocity of unloaded shortening should also decrease as the duration of the preceding isometric tetanus increased. The EDL muscle showed this behavior, but, as expected, the soleus did not. The relative reduction in force-dependent energy cost and in velocity of unloaded shortening can be obtained from the data summarized in Tables II and III. The total energy cost in the EDL declined from  $12.2 \mu\text{mol/g} \sim P$  ( $4.08 \mu\text{mol/g} \cdot \text{s}$  at 3 s) of an isometric tetanus to a value of  $25.4 \mu\text{mol/g}$  ( $2.82 \mu\text{mol/g} \cdot \text{s}$ ) at 9 s of continued isometric tetanus; these data are comparable to the values found in our earlier experiments

(Crow and Kushmerick, 1982a). The filament overlap-independent (force-dependent) energy cost fell from 2.91 to 1.74  $\mu\text{mol/g}\cdot\text{s}$ , or to a value 0.59 times the initial one. The velocity of unloaded shortening averaged 5.75 fiber lengths/s at 3 s of a preceding isometric tetanus, and 4.07 fiber lengths/s at 9 s of a preceding isometric tetanus (Table II). Thus, the velocity of shortening declined to a value 0.71 times the initial one over the stated intervals. We therefore infer, from both mechanical and chemical energetic data, that a decreased rate of actomyosin ATPase occurs during a maintained isometric tetanus of the fast-twitch EDL. Neither the energy cost nor the velocity of shortening changed during a maintained tetanus of the soleus.

The relative decrease in force-dependent energy cost may be greater than that of the decrease in velocity of shortening, as the data just summarized suggest. Our experiments did not specifically address this quantitative issue, and another set of experiments of a different design is needed. The predicted relationship for actomyosin ATPase rate under unloaded isotonic vs. isometric conditions depends on the details of the molecular model chosen (Huxley, 1957; Eisenberg et al., 1980). Furthermore, to explain the decreased velocity of shortening, more than one kinetic constant of the cross-bridge cycle must be changed because the shape of the force-velocity curves as determined by the parameter  $a/P_0$  (Table III) is independent of the maximal velocity of shortening.

It is possible that some aspect of fiber fatigue contributes to the observed reduction in both energy cost and intrinsic shortening velocity. Since the chemical measurements have taken into account the average force developed per cross-sectional area (Crow and Kushmerick, 1982a), it would be necessary to postulate the existence of at least two classes of muscle fibers in the EDL that differ in their mechanical and energetic properties such that only the fibers with lower rates of actomyosin ATP splitting remain active in the prolonged tetani studied. The EDL is composed of fast-twitch glycolytic (FG) and fast-twitch oxidative glycolytic (FOG) fibers (Crow and Kushmerick, 1982a). Therefore, for selective fiber inactivation or fatigue to operate as a mechanism to explain our results, these two fiber types must differ in their mechanical and energetic properties. Such experiments require studies of identified single FG and FOG fibers and have not been reported. However, such an interpretation is possible. A recent study by Luff (1981) on the mechanical properties of the mouse EDL, soleus, and diaphragm muscles indicates that the mouse diaphragm was intermediate in its mechanical properties between the slow-twitch soleus and fast-twitch EDL, despite the fact that the diaphragm is predominantly composed of histochemically identified FOG or fast-twitch "red" fibers (Gauthier and Padykula, 1966; Gauthier, 1969). In addition, there is immunocytochemical evidence for the presence in mammalian muscles of a small population of fast-twitch fibers containing slow-twitch myosin (Lutz et al., 1979; Gauthier and Lowey, 1979). Whether or not these fast-slow myosin-type fibers occur in mouse EDL, or are of the FOG type, is not known.

Another possibility is that the formation of rigor complexes capable of

maintaining tension in the absence of ATP splitting in some fibers obscures the true time course of isometric force, provides an internal load, and therefore reduces the measured velocity of shortening at zero external load. It was not possible to make optical diffraction measurements, which might have detected more than one population of sarcomere lengths, during rapid shortening. Such a hypothesis, however, is inconsistent with the observation that the effects on velocity of shortening are readily reversible during metabolic recovery. The reason for this is that resynthesis of the high-energy phosphates is necessary to reverse the postulated rigor condition, and the

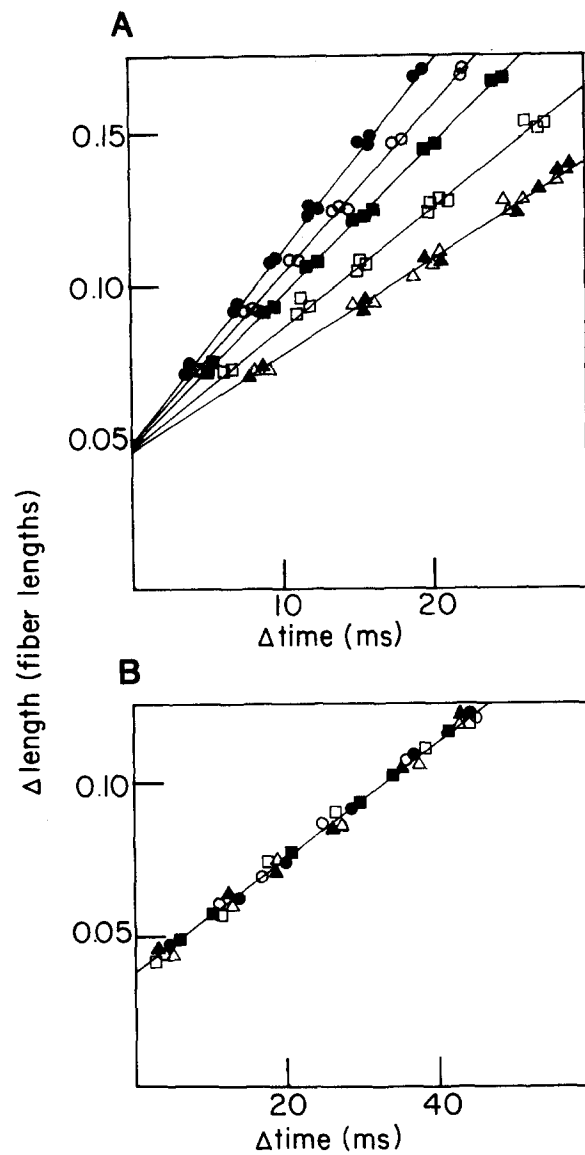


FIGURE 5.

rate of resynthesis during aerobic recovery is not much influenced by different stimulation durations in frog or mouse muscles (D. K. Hill, 1940; Mahler, 1978; Crow and Kushmerick, 1982a).

Furthermore, the data obtained on stretched muscles indicate that the energetic and mechanical changes seen in the EDL are independent of the depletion or accumulation of metabolic intermediates. The reduction in rate of force-dependent energy utilization in a 9-s tetanus in the EDL is maintained with stretch even though the total energy consumption is decreased as a result of the reduced filament overlap (Fig. 3 and Table II). Had the depletion or accumulation of metabolic intermediates per se been the cause for the observed reduction in energy cost, the relationship between energy utilization and  $P_s/P_0$  would have been concave in Fig. 3, because depletion or accumulation of these intermediates occurred to a lesser extent in the stretched muscles.

The filament overlap-independent or force-independent energy costs are defined as those that remain in muscles stretched an average sarcomere length of 3.8  $\mu\text{m}$ . One important mechanism of this energy cost is the

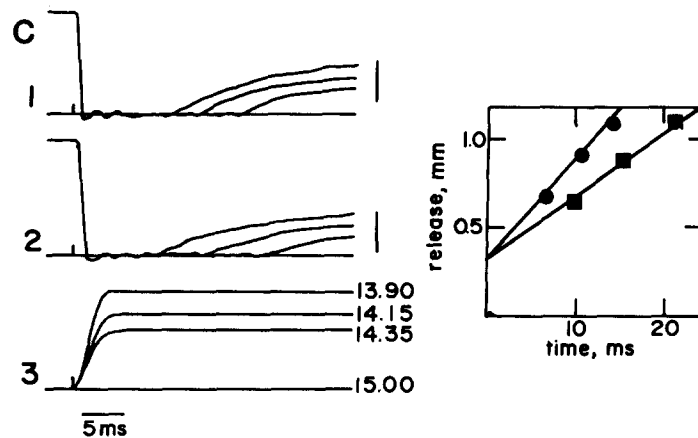


FIGURE 5. Measurements of the velocity of unloaded shortening ( $V_{us}$ ) by the slack test method. The slope of the line gives  $V_{us}$ . The various symbols indicate the duration of isometric tetanus before the quick release. A: EDL; B: soleus. ● = 1 s; ○ = 3 s; ■ = 6 s; □ = 9 s; ▲ = 12 s; △ = 15 s. (C) Examples of measurements of the velocity of unloaded shortening ( $V_{us}$ ) for a mouse EDL. Traces labeled 1 and 2 are the output of the force transducer for muscles stimulated isometrically for 1 and 9 s, respectively, before release. The vertical bars to the right of these curves are tension calibration bars. These represent 5 g for trace 1 and 4 g for trace 2. Traces labeled 3 are the length of the muscle obtained from the position detector; the numbers to the right are the length of the muscle in millimeters for the examples shown. The horizontal bar represents the time calibrations. At the right, the time at which the force remained at zero is plotted against the length of the release as described in Materials and Methods.

sarcoplasmic reticulum ATPase and  $\text{Ca}^{2+}$  cycling during a tetanus. Measurements of aequorin luminescence indicate that  $\text{Ca}^{2+}$  release in response to depolarization may not be independent of muscle stretch (Blinks et al., 1978). Nonetheless, Homsher et al. (1972) and Smith (1972) noted that the energy output of the frog semitendinosus muscle decreased in a linear fashion as the force declined with stretch of the muscle. Similarly, in the stretched mouse soleus and EDL, both isometric force and total energy consumption fell linearly (Figs. 2 and 3) as sarcomere length increased. The filament overlap-independent energy cost is almost three times larger in the EDL than in the soleus. Its value agrees with our earlier but less precise estimate, which was based on regression analysis of various tetanic durations (compare Fig. 1 of Crow and Kushmerick, 1982a, with Table II). The relative magnitude of the filament overlap-independent energy cost compared with the total energy cost for a brief tetanus was similar in mouse EDL and soleus,  $\sim 0.3$ , a finding that is consistent with myothermal measurements of rat (Wendt and Gibbs, 1973; Gibbs and Gibson, 1972) and mouse (Wendt and Barclay, 1980) muscles. The filament overlap-independent energy cost rose proportionally with the duration of the stimulus (3 and 9 s; Fig. 3) in both soleus and EDL muscles. This observation alone disproved the hypothesis that the change in total energy cost in the EDL is due to changes in filament overlap-independent energy consumption. The data therefore indicate that there is a constant rate of the filament overlap-independent energy consumption during tetanization of mouse muscles, with the rate in EDL almost three times larger than in soleus muscles.

Changes in the "economy" of force maintenance and velocity of shortening with continued stimulation have also been observed in smooth muscle (Bozler, 1930; Butler et al., 1982; Dillon et al., 1981). We have shown (Crow and Kushmerick, 1982b) that the reduction in isometric energy cost in the EDL is correlated with phosphorylation of its myosin light chains. Thiophosphorylation of myosin light chains in glycerinated rabbit psoas fibers was recently reported to be associated with a 50% decrease in fiber (Cooke et al., 1982). Phosphorylation of vertebrate skeletal muscle myosin may therefore represent a new kind of control mechanism in striated muscle by which the kinetic properties of the myosin molecule are modified to reduce the intrinsic turnover rate. Definitive tests of such a mechanism await further investigations of mechanics and energetics with single-fiber segments in which the degree of phosphorylation, content of ATP and phosphorylcreatine and their hydrolysis products, pH and ionic strength, are well controlled.

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