A skeletal muscle spends approximately as much time lengthening as it does shortening. During shortening at other than zero load, a muscle does work; during lengthening, work is done on the muscle. The work done on a muscle to lengthen it is supplied by its antagonists or by the dissipation of potential or kinetic energy in structures to which the muscle is attached. For the functioning of a muscle, the periods of lengthening and work absorption (negative work) are not simply inconsequential interludes between the important periods during which there is shortening and positive work output. In many activities, the principal function of the muscles involved is to lengthen under load and to do negative work. For example, when a load is lowered, muscles lengthen in a controlled way and absorb the declining potential energy of the load. The net power output of a muscle during cyclic contraction and relaxation – which is the activity pattern characteristic of a muscle involved in running, swimming or flying – is determined in part by the work that the muscle absorbs during the re-lengthening following shortening (e.g. Josephson and Stokes, 1989; Rome et al., 1993). In swimming fish, the timing of trunk muscle activation changes with respect to the muscle length cycle as a swimming wave is propagated along the trunk, and at some trunk levels the muscle activation is sufficiently high during lengthening that the work absorbed during lengthening is similar in amplitude to the work produced during shortening (Wardle et al., 1995). The work absorbed during lengthening by some cockroach (Blaberus discoidalis) muscles during a normal running cycle exceeds that produced by the muscle during shortening, so the net work output over a full cycle by the muscle is negative (Full et al., 1998). The work output of a steering muscle of the blowfly (Calliphora vicina) flight system is similarly negative during locomotion (Tu and Dickinson, 1994). It seems likely that, as more systems are analyzed, many additional examples will be found of locomotory muscles whose principal function during...
normal movements is to absorb rather than to produce work. Muscle lengthening is a component of all motor activities, and the mechanics of lengthening is an important determinant of total muscle performance.

The work done by or on a muscle during shortening or lengthening is the integral, evaluated over the appropriate interval, of the product of muscle force and shortening distance (= $\int F \, dx$, where $F$ is muscle force and $dx$ is shortening distance). The principal factors that determine muscle force during a length change are the degree of muscle activation, the relationship between muscle force and muscle length, and the relationship between force and shortening or lengthening velocity. It is the latter, the force–velocity relationship of a lengthening muscle, that is of principal concern in this paper.

There is a wealth of information on the force–velocity properties of shortening muscles and muscle fibers, and a poverty of data on this relationship for lengthening muscle. In some skeletal muscles, for example frog fast limb muscles, there is a single-valued relationship between force and muscle shortening velocity. After some initial transient changes, the velocity is constant during shortening at constant force (isometric contraction), or the force is constant during shortening at constant velocity (isovelocity contraction), and the same relationship between force and velocity applies during either isovelocity or isometric shortening (e.g. Edman et al., 1976; Cecchi et al., 1978; Julian et al., 1986). The unique relationship between force and shortening velocity gives rise to the familiar force–velocity curves for striated muscle found in textbooks. It should be noted that force is not constant during isovelocity shortening and that velocity is not constant during isotonic shortening in all skeletal muscles. For example, in slow fibers of frogs, force declines continuously during isovelocity shortening (Floyd and Smith, 1971), and velocity may decline during isotonic shortening (Lännergren, 1978). These changes are attributed to shortening deactivation. Similarly, in some crustacean muscles, velocity declines continuously during isotonic shortening (Josephson and Stokes, 1987; Stokes and Josephson, 1994). If force in a muscle changes during isovelocity shortening or velocity changes during isotonic contraction, the force–velocity relationship cannot be represented by a single curve on a two-dimensional surface. Rather, the force–velocity relationship is a series of curves, or better still a surface, with time after shortening onset or total distance shortened as a third axis.

The force–velocity properties of lengthening muscle are seemingly more complex than those of shortening muscles. If, during an isometric tetanic contraction, a frog muscle or muscle fiber is suddenly loaded with a force, $F$, that is greater than the maximum tetanic force, $F_0$, there is an initial, very rapid lengthening followed by slower lengthening (Katz, 1939; Lännergren, 1978). In some studies, the velocity of the slower lengthening was reasonably constant, allowing the construction of a force–velocity curve for this part of the elongation (Katz, 1939; Lännergren, 1978, for frog slow fibers; Curtin and Edman, 1994); but in other studies, the velocity declined continuously during elongation so that there was no single-valued relationship between force and velocity (Mashima et al., 1972; Lännergren, 1978, for frog fast fibers). Most of the available studies on the force–velocity properties of lengthening muscle have used isovelocity stretch as the independent parameter. At low lengthening velocities, force rises through much or all of the stretch. At high lengthening velocity, force tends to rise continuously to a yield point, after which it continues to rise but at a lower slope than previously, or remains relatively constant, or sometimes declines momentarily or continuously (a representative but not exhaustive list of sources is: Gasser and Hill, 1924; Abbott and Aubert, 1952; Mashima et al., 1972; Sugi, 1972; Edman et al., 1978; Flitney and Hirst, 1978; Colomo et al., 1988; Månsson, 1994; Malamud et al., 1996; Constable et al., 1997). There are some published examples of force–velocity curves during isovelocity lengthening, but these have been obtained by measuring force at the yield point, during the more- or less steady force late in a stretch or at a selected time after the onset of stretch or a selected muscle length reached during stretch, and they do not apply to the whole of the lengthening (Joyce et al., 1969; Colomo et al., 1988; Lombardi and Piazzesi, 1990; Harry et al., 1990; Scott et al., 1996; Williams et al., 1998; for additional examples, see Fig. 2.21 in Woledge et al., 1985). After reviewing the force–velocity properties of lengthening muscles, Woledge et al. (1985) concluded ‘Muscles can clearly exert forces much larger than $P_0$ (=maximum isometric tension) while they are being stretched. It seems, however, that the force exerted may depend on more factors than the instantaneous value of the velocity of lengthening. What these other factors are and how their effects are exerted are questions requiring further investigation.’

This study is an examination of the force–velocity characteristics during lengthening of a crustacean muscle. The muscle used was muscle L2B (Young, 1975) of the green crab Carcinus maenas (L.). Muscle L2B is a respiratory muscle, one of a set that moves the scaphognathite (the gill bailer). It is a small muscle, approximately 10 mm long and 8 mg in mass, made up of parallel, apparently homogeneous muscle fibers (Stokes and Josephson, 1992). It is innervated by 3–4 excitatory axons and no inhibitors (Pasztor, 1968; Josephson and Stokes, 1987). Muscle L2B was selected largely because there is already a considerable body of information available about the organization and performance of the scaphognathite as a pump (see Mercier and Wilkens, 1984a,b, and references therein), on the basic contractile properties of the muscle (Josephson and Stokes, 1987) and on the mechanical power output of the muscle (Stokes and Josephson, 1988; Josephson and Stokes, 1989). An analysis of the mechanical properties of this crab muscle during lengthening should provide information that would be useful in analyzing and modeling the negative work portion of contractile cycles and the shape of the work loops previously obtained from this muscle.

Inhomogeneity of sarcomere properties within and between the fibers of a muscle can be expected to affect the force trajectory during muscle lengthening. During stretch, weaker sarcomeres will become extended faster and farther than stronger ones. To avoid sarcomere inhomogeneities, Julian and Morgan (1979) and Lombardi and Piazzesi (1990) examined
lengthening contractions in segments of single muscle fibers. If a segment is short, the sarcomere properties are likely to be rather homogeneous throughout that segment. Measurements from segments of muscle fibers are more instructive about the dynamics of contraction at the cross-bridge and sarcomere level than are those made from whole muscles. But animals control the movement of structures not by activating segments of single fibers, but rather by activating motor units made up of many muscle fibers and associated tendinous attachments. Sarcomere inhomogeneity is likely to be a normal contributor to the performance of motor units and muscles. Therefore, to understand muscle performance in intact organisms, the performance of whole muscle fibers and intact muscles is more relevant than that of homogeneous segments of muscle fibers.

Stimulating scaphognathite muscle L2B at 100 Hz produces a maximal or nearly maximal isometric contraction (15°C); stimulating at lower frequencies, down to approximately 20 Hz, produces responses that appear to be fully fused but in which the force achieved is less than that with 100 Hz stimulation (Josephson and Stokes, 1987). Thus, it is possible to adjust the level of muscle activation by changing the stimulation frequency. Being able to activate a muscle partially by using subsaturating stimulus frequencies allows an investigation of the force–velocity properties of the muscle under conditions that may be similar to those prevailing in the muscle during the rising activation early after the onset of stimulation and during the falling activation of relaxation. In the following, we consider first the response to lengthening of fully activated muscles, ones stimulated at 100 Hz, and then lengthening in partially activated muscles.

Materials and methods

Green crabs (Carcinus maenas) were obtained from the Supply Department, Marine Biological Laboratory, Woods Hole, MA, USA. Scaphognathitelevator muscle L2B and its nerve were isolated and mounted in a dish of crab saline (composition given in Govind and Lang, 1981, pH adjusted to 7.4 with NaOH before use). A cooling coil in the recording dish maintained the temperature of the saline at 15°C. The nerve to the muscle courses through the hepatopancreas, which must be dissected in order to free the nerve. The dissection of the nerve and muscle was carried out under continued perfusion with chilled sea water to reduce damage to the preparation from digestive enzymes released from the sectioned hepatopancreas. Additional information about the dissection is given in Josephson and Stokes (1987).

The proximal end of the muscle was fixed to the bottom of the recording dish, and the distal end was attached to an ergometer (design described in Malamud and Josephson, 1991). The ergometer was mounted on a lead screw which allowed adjustment of the length of the muscle. The nerve to the muscle was stimulated (1 ms shocks) with a suction electrode. Values of muscle length and force were collected with an analog-to-digital converter (12-bit resolution, sampling rate up to 10 kHz per data pair). Results were viewed and stored on a digital computer. During analysis, data were fitted to various functions using the CurveFit routine of SigmaPlot (SPSS Inc., Chicago, IL, USA).

The first task with each new preparation was to determine the optimal length of the muscle for producing tetanic tension. To do this, the muscle was set at a length judged to be shorter than the optimum and stimulated tetanically at 100 Hz for 0.5 s. The stimulation was repeated at 2 min intervals, lengthening the muscle between each trial in steps of 0.25 mm (approximately 2.5% of muscle length) until tetanic force began to decline with further increase in length.

The experiments to be described involved isovelocity lengthening of the muscle. The stretch was applied during the plateau of a tetanic contraction unless otherwise indicated. The length of the muscle before the stretch was set at or slightly shorter than the optimum. Trials with tetanic stimulation, with or without stretch, were paced regularly at 2 min intervals throughout an experiment. The stretch amplitude was approximately 0.44 mm (approximately 4.5% of muscle length). The lengthening velocities examined ranged from 0.3 to 16 mm s⁻¹, which is equivalent to muscle strain rates of approximately 0.03–1.6 L s⁻¹, where L is muscle length.

With isovelocity lengthening, as was used in this study, the distance of stretch is proportional to the elapsed time since the onset of stretch. The original recordings collected in this study were of force as a function of time and, because of our familiarity with this presentation, we have chosen to analyze force during stretch as a function of time. The force changes could have been analyzed equally well as a function of the distance of stretch, with basically similar conclusions (see Figs 2, 18).

Muscle performance tended to decline through a series of trials with stretch. Experiments were generally terminated if the tetanic force dropped to less than approximately two-thirds of its initial value. At the end of an experiment, the muscle was held at its optimum length and fixed at that length in 70% ethanol. After 20–60 min of fixation, the preparation was detached from the ergometer and recording dish, the muscle length was measured using an ocular micrometer, and the muscle was stored in 70% ethanol. After several days to several weeks of storage, the muscles were dissected free from adhering exoskeletal elements, rehydrated overnight in saline, dried on blotting paper and weighed with a torsion balance to the nearest 0.01 mg. The rehydrated mass was multiplied by 1.36 to compensate for mass loss during fixation and thus to obtain an estimate of the original wet mass (Josephson and Stokes, 1987). Muscle area was estimated as the ratio of muscle mass to muscle length. To enable comparison of results from muscles of differing size, muscle tension will generally be expressed as stress (=force/area) and velocity as strain rate (=velocity/initial muscle length).

The abbreviations used are defined at the end of the paper.

Results

Force during lengthening of a fully active muscle

Fig. 1 shows results from some of the trials of an experiment in which force was measured while the muscle
was lengthened at different velocities during the plateau of a tetanic contraction. The total distance of stretch in each case was approximately 4%. The length range over which the stretch occurred had been adjusted such that the maximum tetanic force ($F_0$) at the unstretched length was approximately equal to that in an isometric tetanus at the length reached at the end of stretch. Thus, changes in force during and after stretch were not due simply to changing position along the length–tension curve. Force rose monotonically but with a continuously declining slope during low-velocity stretch. With stretches at $0.6 L_s^{-1}$ and greater, there was an inflection on the rising force trace, and the slope beyond the inflection was substantially less steep than that before the inflection. Since the strain rate was constant in each of the trials, a decline in the slope of the stress versus time curve also indicates a decrease in the slope of the stress versus strain relationship, i.e. a decrease in the resistance to stretch or muscle stiffness. Thus, there was a continuous decline in stiffness at low lengthening velocities, and a discontinuous decline at high velocities. We will refer to the abrupt decrease in slope during high-velocity lengthening as yield. The minimal velocity for initiating yield was not determined precisely, but the average value was somewhere between 0.3 and $0.6 L_s^{-1}$. Clear yield occurred in only 10 of 35 trials from 21 preparations stretched at $3 \text{mm s}^{-1}$, which is approximately $0.3 L_s^{-1}$ for muscles of the sizes used. Only one out of 17 trials from 11 preparations with strain of $6 \text{mm s}^{-1}$ (approximately $0.6 L_s^{-1}$) failed to show pronounced yield.

Given the quite different natures of force–velocity relationships at low stretch rates, without yield, and at high rates, with yield, it is appropriate to consider separately force trajectories in the two velocity ranges.

**Characteristics of low-velocity stretch**

The force trajectory during low-velocity stretch at a constant

![Figure 1](image1.png)

**Fig. 1.** Stress during stretch at 0.3, 0.6 and $1 L_s^{-1}$ (where $L$ is muscle length). The time of stimulation is marked in A by a thickening of the time axis. The pre-stretch and post-stretch lengths were chosen such that the forces at the two lengths were nearly identical during isometric contraction (note, in A, the similarity between the force reached in the isometric contraction at the short length and that in the trace marked by the dotted line in which the passive muscle was stretched to the long length well before the onset of stimulation). Because the stretches were through the plateau of the length–tension curve, the changes in force reflect time-dependent consequences of stretch and not steady-state changes due to the shift along the static length–tension curve. As noted previously (Josephson and Stokes, 1989), the rate of relaxation is length-dependent and is slower following the isometric contraction at the long length (dotted trace) than after that at the short length. (B) An expansion of the portion of A in which stretch occurred. The arrow indicates the onset of yield for the fastest stretch.

![Figure 2](image2.png)

**Fig. 2.** Force increase during low-velocity stretch. (A) Increase in force as a function of time. The strain rate is indicated above each of the curves. The thin lines are exponential functions fitted to the force data (see equation 1). (B) The same force data as in A plotted as a function of muscle strain ($\Delta L/L_0$) plotted as a function of muscle strain ($\Delta L/L_0$) plotted as a function of muscle strain ($\Delta L/L_0$).
rate was reasonably well fitted by a single-exponential function of the form:

$$\Delta F = \Delta F_A [1 - \exp(-t/\tau)],$$  \hspace{1cm} (1)

where $\Delta F$ is the increase in stress above the pre-stretch level, $t$ is time after stretch onset, $\tau$ is a time constant and $\Delta F_A$ is the asymptotic value of stress at $t = \infty$ (Fig. 2). The constants of the exponential curves fitted to force trajectories – the asymptotic value and the time constant – varied with the velocity of lengthening (Fig. 3). The asymptotic value of the force was approximately an increasing exponential function of strain rate of the form:

$$\Delta F_A/F_0 = (\Delta F_A/F_0)_{AS} [1 - \exp[-(dS/dt)/\alpha]].$$  \hspace{1cm} (2)

Here $\Delta F_A/F_0$ is the asymptotic value of the original force curves as a fraction of the isometric force at the onset of shortening. Expressing the asymptotic force as a fraction of the isometric force reduces the preparation-to-preparation variability and makes it more appropriate to combine results from different experiments. $(\Delta F_A/F_0)_{AS}$ is the asymptotic value of the curve relating $\Delta F_A/F_0$ and strain rate, $dS/dt$ is the strain rate, and $\alpha$ is a rate constant with the same dimensions as strain rate. The change in the time constant for the force curves at differing strain rate was reasonably well fitted by a declining exponential function of the form:

$$\tau = \tau_A \exp[-(dS/dt)/\beta],$$  \hspace{1cm} (3)

where $\tau_A$ is the projected value of $\tau$ at zero strain rate and $\beta$ is a rate constant.

The values of $(\Delta F_A/F_0)_{AS}$, $\tau_A$, $\alpha$ and $\beta$ were estimated in two ways. In seven preparations, stretches at appropriate low velocities were obtained during an ascending series of strain rates followed by a descending series, as in Fig. 3. New exponential functions were fitted to the mean values for asymptote and time constant. The average values for the parameters (means ± s.d.) were: $(\Delta F_A/F_0)_{AS} = 0.767 ± 0.126; \alpha = 0.034 ± 0.013 s^{-1}; \tau_A = 0.698 ± 0.223 s; \beta = 0.120 ± 0.030 s^{-1}$. The second approach was to combine the constants obtained from exponential fits to data from all the trials at appropriate strain rates, and to fit new exponential functions to the full data sets (Fig. 4). The parameter values obtained in this way were: $(\Delta F_A/F_0)_{AS} = 0.764; \alpha = 0.038 s^{-1}; \tau_A = 0.650 s; \beta = 0.112 s^{-1}$. Averaging the values from the two approaches and rounding off gives the following relationships for predicting muscle force as a function of time ($t$) during lengthening at velocities below that which initiates yield:

$$\Delta F/F_0 = \Delta F_A/F_0 [1 - \exp(-t/\tau)].$$  \hspace{1cm} (4)

$$\Delta F_A/F_0 = 0.771 [1 - \exp[-(dS/dt)/0.36]]].$$  \hspace{1cm} (5)

$$\tau = 0.671 \{\exp[-(dS/dt)/0.12]\}.$$  \hspace{1cm} (6)

High-velocity stretch

There was considerable variation from preparation to preparation in the trajectories of force near the yield point, even for stretches at similar velocities (Fig. 5). In some preparations, yield appeared simply as a change in slope; in others, there were pauses or even brief reversals in the force increase at the yield point. In general, force increased linearly with stretch up to the yield point and again linearly, but at a reduced slope, above the yield point. The slope of the force increase with stretch before
yield was approximately four times greater than that after yield (ratio of pre-yield to post-yield slopes 3.99±1.11; mean ± S.D., N=49 values collected from 16 preparations).

For reasons that are unclear, there was a rather wide range in maximum isometric stress among the preparations of this study. The stress at yield (DFY) was strongly correlated with the isometric stress at the onset of stretch (F0); N=49 values from 16 preparations; strain rates range from 0.29 to 1.73 s⁻¹. The solid line is a least-squares regression line; the dotted lines indicate the 95% confidence interval. The equation for the regression line is: ΔFY=0.258F0+0.492 (r²=0.699, P<0.001), where ΔFY is in N cm⁻². (B) The increase in relative force at yield (ΔFY/F0) as a function of strain rate (dS/dt). The equation for the least-squares regression line (shown with 95% confidence intervals) is: ΔFY/F0=16.58dS/dt+24.27 (r²=0.465, P<0.001). L, muscle length.

increased with stretch was a linear function of strain rate (dS/dr) both before and after yield (Fig. 8).

Using the values of Fig. 8 relating the slope of the force increase to the strain rate, and those of Fig. 6B relating force at yield to strain rate gives the following force–velocity relationship for strain rate greater than that initiating yield:

pre-yield (dS<0.01)  
ΔF/F0 = [(51.4(dS/dr) − 7.3 s⁻¹)]τ ,  
and post-yield (dS>0.01)  
ΔF/F0 = [(12.37(dS/dr) − 1.3 s⁻¹)]τ + [0.169 s(dS/dr) + 0.242]  
(the second term in square brackets in equation 8 is the force at yield predicted by Fig. 6B).

**Stretch of partially activated muscles**

The effects of partial activation on the mechanical properties of lengthening muscle were investigated by stretching muscles that were being stimulated at suboptimal frequencies so that they developed submaximal tension. In most of these
experiments, the muscles were stimulated at 20, 50 and 100 Hz. At 15°C, 100 Hz stimulation gives a maximal or nearly maximal isometric contraction (Josephson and Stokes, 1987). Stimulation at 20 Hz and at 50 Hz produced maximal tetanic tension averaging 47% and 82%, respectively, of that with 100 Hz activation (Fig. 9A).

The force changes during lengthening of a partially activated muscle are qualitatively but not quantitatively similar to those in a fully activated muscle. In a partially activated muscle, as in a fully activated one, lengthening at low velocity gives an approximately exponential increase in force; stretching at high velocity produces force trajectories with an obvious yield (Figs 10, 12). The minimum strain rate required to evoke yield was similar in fully and partially activated muscles. In both, stretch at 3 mm s\(^{-1}\) (approximately 0.3 L s\(^{-1}\)) did not usually produce yield, but stretch at 6 mm s\(^{-1}\) (approximately 0.6 L s\(^{-1}\)) generally gave force traces with yield.

The force trajectory during stretch of a partially activated muscle is not simply a scaled down version of that in a fully activated muscle. If the force trajectory in a partially activated muscle were proportional to that in a fully activated one, reduced in amplitude by the same factor as is the maximum isometric tension, then the peak force reached during stretch should change with changes in stimulus frequency in the same way as does the maximum isometric force. This was generally not the case (compare Fig. 9A and 9B). At the lowest strain rate considered (0.03 L s\(^{-1}\)), the absolute value of the peak increase

Fig. 7. Strain at yield (\(=S_Y\)) appears to be independent of strain rate. Values are from the same trials and preparations as Fig. 6. The mean strain at yield was 1.05±0.25% (mean ± s.d.). \(L\), muscle length.

Fig. 8. Slopes of force increase during lengthening [d(\(\Delta F/F_0\))/dt] before (filled symbols) and after (open symbols) yield. \(N=48\) determinations from 16 preparations. The equations for the least-squares regression lines are: pre-yield, d(\(\Delta F/F_0\))/dt=51.4(dS/dt) –7.3 s\(^{-1}\) (\(r^2=0.866, P<0.001\)); post-yield, d(\(\Delta F/F_0\))/dt=12.37(dS/dt) –1.29 s\(^{-1}\) (\(r^2=0.850, P<0.001\)). dS/dt, strain rate; \(L\), muscle length.

Fig. 9. (A) Relative isometric force as a function of stimulation frequency; values are means ± s.e.m. The results are from sets of trials in which the muscle was stimulated successively at 20, 50 and 100 Hz. The tetanic force reached during 20 and 50 Hz stimulation was expressed as a fraction of that in the following 100 Hz trial. More than one set of trials at the different stimulation frequencies was obtained from most of the preparations. Multiple observations from the same preparation were averaged to give a single value for that preparation; \(N=11\) preparations. (B) Maximum increase in force during stretch (\(\Delta F_{\text{max}}\)) (\(\Delta L=0.44\) mm, approximately 4.5%). Values for the three stimulus frequencies tested were normalized to that at 100 Hz (\(\Delta F_{\text{max},100\text{Hz}}\)) for each preparation (means ± s.e.m., \(N=7\) preparations). \(L\), muscle length.
The relative force trajectory during stretches that were fast enough to initiate yield was, in general, less dependent on stimulus frequency than was the force trajectory during low-velocity stretch (compare Figs 10B and 12B). The relative force at yield \((ΔF_Y/F_0)\) declined by 13–26\%\, depending on strain rate, with an increase in stimulus frequency from 20 to 100 Hz (Fig. 13A). The muscle strain at yield \((S_Y)\) increased somewhat with stimulus frequency, by 10–35\%\, for the strain rates and frequencies considered (Fig. 13B). The slope of the relative increase in force during lengthening \(=[d(F/F_0)/dS]\) declined with increasing stimulus frequency for both the pre-yield and the post-yield segments; by 16–25\%\, with an increase in stimulus frequency from 20 to 100 Hz for the pre-yield portion, and by 41–50\%\, for the post-yield segment (Fig. 14).

**Lengthening during relaxation**

A few preparations were stretched at varied times during relaxation; the goal being to characterize further the stress–strain properties of lengthening muscle during

in force with stretch \((ΔF_{max})\) during 20 Hz or 50 Hz stimulation was actually greater than that with stretch during 100 Hz stimulation \((ΔF_{max,100Hz})\). The relatively large increases in force seen with slow stretch of partially activated muscles may be in part a consequence of stretch activation of previously quiescent cross-bridges. At lengthening velocities of \(0.1\text{ Ls}^{-1}\) and greater, \(ΔF_{max}\) was lower in partially activated than in fully activated muscles, but the reduction in \(ΔF_{max}\) at the lower stimulation frequencies, relative to that with 100 Hz stimulation, was less than the comparable reduction in the maximum isometric force. Not only the relative amplitude, but also the shape of the force change during stretch was different during saturating and sub saturating stimulation. Responses to equivalent stretch of a muscle when partially activated and when fully activated did not superimpose when each was normalized to its maximum isometric force (Fig. 10B, see also Fig. 12B). The asymptotic value of the exponential force rise during slow lengthening, normalized to the force at the onset of stretch (i.e. \(ΔF_{max}/F_0\)), declined by 50–60\%\, as the stimulus frequency was increased from 20 to 100 Hz (Fig. 11A). The time constant of the exponential force rise during slow lengthening \((τ)\) declined by approximately 40–50\%\, as the stimulus frequency was increased from 20 to 100 Hz (Fig. 11B). Thus, the relative increase in the asymptotic value of force during slow lengthening is greater, and the time taken for the force to reach the asymptote is longer, for a muscle stimulated at 20 Hz than for one stimulated at 100 Hz.

![Graph of stress vs. strain](image-url)
Mechanics of lengthening muscle

incomplete activation. These trials were all performed using a moderately high velocity of stretch (approximately \(0.6 \text{ L s}^{-1}\)), which produced force changes that were readily distinguishable from the normal decrease in tension at the end of a tetanus (Fig. 15). In the four preparations examined, the strain at yield was relatively constant, approximately 0.8 %, through at least the first 60–70 % of relaxation (Fig. 16A). The stress at yield, expressed as a fraction of the maximum isometric force (\(\Delta F_y/F_0\)), decreased throughout relaxation (Fig. 16B). However, the yield stress, when expressed as a fraction of the expected isometric force at a time equivalent to that at which yield occurred (\(\Delta F_y/F_2\); see Fig. 15B), was rather constant through the first 40 % of relaxation, after which it increased (Fig. 16B). The increase in relative yield force with increasing time of relaxation, and hence decreasing level of activation, is consistent with the increasing value of relative yield force with decreasing activation in preparations stimulated at subsaturating frequencies (Fig. 13A). The pre-yield slope, normalized to the force at stretch onset (\(F_1\)), was quite constant through 60 % of relaxation, but increased later (Fig. 17A). The post-yield slope, normalized to the force expected at the time of yield (\(F_2\)), generally increased throughout relaxation except in one preparation in which the normalized slope was negative (in this preparation, force tended to fall after yield rather than rise) and relatively constant throughout relaxation (Fig. 17B). Again, the results during the declining activation of relaxation are consistent with those in which activation was varied by varying stimulus frequency. In both approaches, the relative value of the pre-yield slope was not very dependent on the level of muscle activation (compare Figs 14A and 17A), while the relative value of the post-yield slope increased with decreasing activation (Figs 14B, 17B).

Discussion

The force–velocity–time surface for lengthening muscle

This study with a crustacean muscle confirms what has been apparent but generally not emphasized in many earlier studies of the mechanical properties of lengthening muscle, namely that force during lengthening is not a simple, instantaneous function of velocity. It appears to be generally the case that the force in an activated muscle rises continuously during low-velocity stretch. At high lengthening velocities, the force in a stretched muscle rises to a yield point, reached at approximately 1 % stretch, beyond which the force may

Fig. 12. (A) Force increase during stretch at \(1.06 \text{ L s}^{-1}\) in a muscle stimulated at different frequencies. For greater clarity, the traces have been offset from one another along the time axis by arbitrary distances. (B) The data in A, normalized for the isometric force existing just before the onset of stretch (\(F_0\)) and superimposed on an expanded time base. \(L\), muscle length.

Fig. 13. (A) Relative force at yield as a function of strain rate and stimulus frequency (values are means ± s.E.M., error bars are sometimes shown in one direction only for clarity). The yield force \(\Delta F_y\) is expressed relative to the isometric force preceding the onset of stretch \(F_0\). As in Fig. 11, the strain rates are approximate. Sample size: \(0.6 \text{ L s}^{-1}, N=5; 1 \text{ L s}^{-1}, N=11; 1.6 \text{ L s}^{-1}, N=3\). (B) Strain at yield (means ± s.E.M.). Sample sizes as in A. Figures in parentheses to the right of the curves indicate the percentage change in the mean value of the parameter as stimulus frequency was increased from 20 to 100 Hz. \(L\), muscle length.
actually decline, may remain reasonably constant or, as in the crab muscle, may continue to rise throughout lengthening (see Introduction for references). The important point is that, even when the lengthening velocity is constant, the force often is not. Thus, to predict the force in a lengthening muscle, even one in which activation is maintained constant by tetanic stimulation, may require information on the time of stretch or the distance of stretch as well as the stretch velocity. The force–velocity relationship is not a planar curve, but rather a surface with time or distance of stretch as a third axis (Fig. 18).

In a classic study of the mechanics of a cat limb muscle, Joyce et al. (1969) noted that the shape of the force increase during lengthening changed when the level of muscle activation was altered by changing the stimulation frequency. The shape of the force–velocity–time surface for muscle L2B also changes with the state of muscle activation. Stimulating the muscle at 20 or 50 Hz evokes tetanic contractions that are smoothly fused, but in which activation is partial and the maximum isometric force reached is smaller than that achieved with 100 Hz stimulation. The force trajectory during lengthening of a partially activated muscle has a different shape from that of a fully activated muscle, meaning that the trajectory of the partially activated muscle cannot be made to superimpose on that of a fully active muscle stretched at the same velocity by simply changing, in a linear manner, the scale of the force axis for the partially activated case. During slow stretch (0.03–0.3 $L_s^{-1}$), when the force rise is approximately exponential, the asymptote approached by the rising force is a greater fraction of the isometric force at the onset of stretch, and the time constant of the rise is longer, when the muscle is stimulated at 20 or 50 Hz and partially activated than when it is stimulated at 100 Hz and fully activated (Fig. 11). During more rapid stretch (0.6–1.6 $L_s^{-1}$), when there is yield in the force trace, the force at yield relative to the isometric force at the onset of stretch ($=\Delta F_Y/F_0$) and the relative slope of the force rise after yield are greater in the partially activated than in the fully activated muscle. Because the shape of the force trajectory at any given lengthening velocity is a function of the level of muscle activation, the shape of the force–velocity–time surface is also a function of muscle activation. The lengthening responses of partially activated muscles are not the same as those of fully activated muscles scaled down in proportion to the reduced isometric force at the onset of stretch.

**Implications for muscle models**

The finding that the shape of the force–velocity–time surface is a function of the level of muscle activation is somewhat unwelcome, for it greatly complicates the task of developing models of work output in active muscle. Recently three rather
similar treatments have been described in which work loops were reconstructed from measured or assumed properties of specific muscles; one by Curtin et al. (1998) based on dogfish muscle, one by Williams et al. (1998) for lamprey swimming muscle and one by Askew and Marsh (1998) using mouse soleus muscle. In each of these studies, the force–velocity properties for lengthening muscle were assumed to be describable by a single force–velocity curve, and changing muscle activation as a function of time following stimulation was simulated by introducing a time-dependent scaling factor. The expected force during shortening and lengthening was calculated as the expected force in a fully activated muscle at the relevant velocity and length multiplied by the scaling factor representing the instantaneous state of muscle activation. This approach assumes that the force–velocity curve for a partially activated muscle is adequately represented by a scaled-down version of that in a fully activated one. However, our results from crab muscles, which we think are likely to apply to other muscle as well, indicate that there are changes in the shape of the force–velocity relationship with changes in activation, and a simple linear scaling factor does not accurately predict the force–velocity properties of lengthening muscle during changing levels of activation.

In the modeling studies of Curtin et al. (1998) and Williams et al. (1998), there is impressive agreement between the predicted trajectory of force during sinusoidal strain and the force trajectory actually measured during sinusoidal length change in living muscles. This is so even though the force–velocity curves used in the models were time-invariant, other than for linear scaling of the force axis related to the degree of muscle activation. Our results with the crab muscle, and our reading of the literature on muscle lengthening in general, indicate that the force–velocity properties of lengthening muscle are a function of time and/or strain, even during tetanic stimulation when activation level is constant and, as emphasized above, the shape of the force–velocity function changes with changing levels of activation. It may be that changes in force–velocity curves with time of lengthening, and the changing shape of force–velocity–time surfaces with degree of activation, are minor subtleties that need not be included in models to produce reasonable results. However, it is possible that the models in question include factors that generate systematic errors whose effects offset errors introduced by the failure to allow for changes in the force–velocity relationships with time and with changing levels of activation. In their study, Askew and Marsh (1998) emphasize the differences between predicted and measured work loops, especially at higher operating frequencies. They attribute the discrepancy between the behavior of the model and that of real muscles to shortening deactivation and stretch activation in

![Fig. 16.](image-url) Fig. 16. (A) Muscle strain at yield during relaxation. Each symbol indicates the result from a different preparation. The stimulus frequency during the tetanic stimulation preceding relaxation was 100 Hz; the strain rate during stretch was 0.62–0.65 L s$^{-1}$ in the different preparations. The filled symbols are mean values from preparations in which trials were collected in a mirror series of increasing and then decreasing delays to the onset of stretch. The open symbols are from a preparation in which values were obtained only during a decreasing set of delays. (B) Force at yield in the preparations of Fig. 16 expressed relative to the isometric force at the plateau of the contraction ($F_y/F_0$) or relative to the expected isometric force at the time of yield ($F_y/F_2$). See Fig. 15 for a definition of these forces. $L$, muscle length.

![Fig. 17.](image-url) Fig. 17. Pre-yield slope (A) and post-yield slope (B) in the preparations of Fig. 16. See Fig. 15 for definitions of $F_1$ and $F_2$. 
the real muscles, features not included in the model. It seems likely that shortening deactivation and stretch activation are important factors determining the shapes of work loops (see, for example, Josephson and Stokes, 1989), but part of the difficulty in modeling real muscle behavior in the study of Askew and Marsh (1998) may be a consequence of using an overly simplistic force–velocity relationship for lengthening muscle, one that does not include changes in the force–velocity relationship associated with time and/or strain during stretch, and changes in the shape of the force–velocity–time relationship with differing levels of muscle activation.

The transition between force rise with and without yield

With low strain rates, force rises continuously along an approximately exponential course. At high strain rates, there is a pronounced yield point in the force record, and the pre-yield and post-yield segments are each approximately linear increases in force with distance of stretch. The transition between a force trajectory with yield and one without occurs somewhere between 0.3 and 0.6 \( L_s^{-1} \). Stretch at 0.3 \( L_s^{-1} \) usually produced force trajectories without obvious yield; stretch at 0.6 \( L_s^{-1} \) rarely failed to produce yield. The initial, approximately linear increase of force in stretches giving yield is not necessarily a discontinuous change from the exponential rise in force at low strain rates. The slope of the force rise, \( d(\Delta F)/dt \), for the exponential rise in force of equation 4 is:

\[
d(\Delta F)/dt = (\Delta F_A - \Delta F)/\tau.
\]  

At the beginning of the exponential force increase, \( \Delta F \) is small relative to the asymptotic value, \( \Delta F_A \), and the force rise is approximately linear with a slope of \( \Delta F_A/\tau \). The initial, approximately linear, portion of the force rise at higher lengthening velocities may be the approximately linear portion of an exponential increase, which becomes terminated by new processes brought in at yield. For a stretch velocity of 0.3 \( L_s^{-1} \), the predicted initial slope of the exponential force increase, using equations 5 and 6 to calculate \( \Delta F_A \) and \( \tau \), is 7.9 \( F_0 s^{-1} \), which is in good agreement with the value of 8.1 \( F_0 s^{-1} \) predicted for that strain rate from the regression line linking pre-yield slope and strain rate in preparations with yield (Fig. 8). At higher strain rates, however, the slopes predicted by equation 9 are far greater than those actually measured. At 1\( L_s^{-1} \), for example, the slope predicted by equation 9 is 4500 \( F_0 s^{-1} \), while that actually measured (Fig. 8) is approximately 44 \( F_0 s^{-1} \). To model the pre-yield force trajectory at higher lengthening velocities as an exponential function would require values for the time constant and/or the asymptote quite different from those predicted by the force changes during stretch at low velocities (Figs 3, 4).

Lengthening velocity and strain during normal muscle operation

At 15°C, and with sinusoidal strain, the maximum mechanical power output of muscle L2B occurs at a cycle

![Fig. 18. (A) The force–velocity–time surface for muscle L2B during isovelocity stretch predicted by equations 4–8 in the text. The trajectory along a velocity isogram, for example the path indicated by the larger arrow, is the change in force as a function of time during stretch. The trajectory along a time isogram, such as that indicated by the smaller arrow, is the force–velocity relationship at a given time after the onset of stretch. (B) The force–velocity–strain surface predicted when time in equations 4, 7 and 8 is replaced by the corresponding strain. Note that, since strain rate, \( dS/dt \), is constant, strain, \( S_= (dS/dt) t \), \( L \), muscle length.](image)

![Fig. 19. The strain experienced by a muscle under optimal conditions for power output (4Hz, strain 8% peak-to-peak). The trace is broadened for the lengthening portion of the cycle. The cross-hatched boxes show the portions of lengthening for which the strain rate \( dS/dt \) is less than 0.3 and therefore yield is not to be expected. The box with diagonal hatching is the portion of the cycle for which \( dS/dt \) is greater than 0.6 and during which yield might occur.](image)
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frequency of 4 Hz and a peak-to-peak strain of 8% (Stokes and Josephson, 1988; Josephson and Stokes, 1989). The maximum velocity experienced by the muscle in vivo, assuming that it operates under the conditions that maximize power output, is \( \pi \times (4 \text{ Hz}) \times (0.08 L) = 1.0 L \text{s}^{-1} \). The force–velocity–time properties of the crab respiratory muscle were determined for shortening velocities from 0.03 to 1.6 \( L \text{s}^{-1} \). Thus, the velocity range used to characterize the force–velocity properties of lengthening muscle covered the lengthening velocity range that the muscle experiences during operation at maximum power output. The total strain to which the muscle was subjected during experiments, approximately 4%, was approximately half that expected in vivo. Two considerations led us to choose a smaller strain than the muscle might normally experience. First, it has been widely noted that stretch is damaging to muscles (e.g. Wood et al., 1993; Lieber and Fridén, 1993). We assumed that using a smaller than normal strain would help to maintain the viability of the muscle preparations and allow us to collect information over a longer period than might otherwise be possible. Second, the length–tension curve for muscle L2B is rather narrow (Josephson and Stokes, 1987), much narrower, for example, than that of frog muscle. Using small strains made it easier to keep the muscle length during stretch near the peak of the length–tension curve, the region of the curve in which the static isometric force is maximal and relatively unaffected by length change. The strain amplitude used, 4%, does seem to be quite adequate for determining the trajectory of force changes during muscle lengthening.

The operating frequency of muscle L2B in intact crabs is 0.7 to 4–5 Hz (Hughes et al., 1969; Wilkens et al., 1984; Mercier and Wilkens, 1984a,b). The scaphognathite, the structure that is moved by muscle L2B, beats within a small chamber which limits its movement and, therefore, the strain range of the muscle. We assume that the strain experienced by muscle L2B in vivo is 8% peak-to-peak, i.e. the strain that is optimal for work output in isolated muscles, or less. With a strain of 8%, the strain rate of the muscle exceeds 0.6 \( L \text{s}^{-1} \) only if the frequency is 2.4 Hz or greater. Thus, over much of the normal operating range of the muscle, the cycle frequency is sufficiently low that the muscle does not reach the strain rate for yield. During sinusoidal length change at the optimal frequency (4 Hz) and strain (8% peak-to-peak) for power output, 59% of the lengthening half-cycle is at a velocity of 0.6 \( L \text{s}^{-1} \) or more, and therefore great enough to give yield (Fig. 19). At the optimal strain and frequency, the stretch velocity is 0.3 \( L \text{s}^{-1} \) or less and is thus within the range for which force rise is continuous and without yield only for brief periods, each lasting 9.7% of the lengthening half-cycle, at the beginning and at the end of the lengthening phase. However, even during high-frequency operation, the short portions of a cycle in which strain rate is below that required to produce yield may be functionally more significant than the longer period when the velocity is high enough to produce yield. Work output is maximal when muscle activation begins at approximately 90% of the way through the lengthening phase (Stokes and Josephson, 1988). The last 10% of lengthening, when the lengthening velocity is lower than that giving yield, is a time during which muscle activation is initially low but is rising rapidly. The time course of muscle activation in muscle L2B has not been adequately quantified, but it seems likely that, when the stimulation pattern and phase are appropriate for maximum power output, the muscle is at least partially activated at the end of the shortening phase and therefore at the onset of lengthening. Activation must fall rapidly early in lengthening since the resistance of the muscle to stretch during most of the lengthening phase is little different from that of an unstimulated muscle (see Fig. 9 of Josephson and Stokes, 1989). Under the conditions giving maximum power output, muscle activation is likely to be substantially higher at the beginning and the end of the lengthening phase, when the lengthening velocity is low, than during the middle of lengthening, when velocity is high. Muscle force during lengthening which, together with lengthening distance, determines the work absorbed during stretch, depends on both the velocity of lengthening and the degree of activation. Since the activation is low when the velocity is high, it is likely that it is the lower-velocity portions of the lengthening half-cycle, during which activation is falling (onset of lengthening) or rising (end of lengthening), that are most important in determining the work required to re-lengthen the muscle during cyclic contraction.

Crab muscles and frog muscles

Crustacean respiratory muscles are not common objects of research on muscle mechanics. It is appropriate, therefore, to consider how widely applicable might be the results obtained with the crab muscle. An appropriate study with which to compare the results from the crustacean muscle is that of Flitney and Hirst (1978) using the frog sartorius muscle, a muscle that everyone would agree is a standard, if not the standard, preparation for investigations of muscle mechanics. In the study of Flitney and Hirst (1978), as in ours, whole muscles were subjected to isovelocity stretch over a range of velocities. Flitney and Hirst (1978) distinguished two inflection points on the rising force during stretch: \( S_1 \) which was seen in some but not most trials; and \( S_2 \) which, at higher velocities, clearly corresponds with what we have called yield in the crab muscle. In the frog muscle, as in the crab muscle, the force trajectory during stretch was ‘rounded in form’ at low lengthening.

![Table 1](https://example.com/table1.png)

**Table 1. Some characteristics of isovelocity stretch in frog and crab muscles**

<table>
<thead>
<tr>
<th></th>
<th>Frog sartorius</th>
<th>Crab L2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum velocity for yield (( L \text{s}^{-1} ))</td>
<td>0.32</td>
<td>0.3–0.6</td>
</tr>
<tr>
<td>Strain at yield, 15 °C (%)</td>
<td>1.2–1.3*</td>
<td>0.5–1.5</td>
</tr>
<tr>
<td>Stress at yield, 15 °C, strain rate (= 0.87 L \text{s}^{-1} (F_0) )</td>
<td>1.35*</td>
<td>1.39‡</td>
</tr>
</tbody>
</table>

*Calculated as the mean of values at 12 and 18 °C; Table 1 of Flitney and Hirst (1978).
‡Calculated from regression line of Fig. 6B.

\( L \), muscle length; \( F_0 \), maximum isometric stress.

Frog values are from Flitney and Hirst (1978).
velocities and with obvious yield at high velocities. In both muscle types, the yield force increased with increasing strain rate. The minimal velocity required to achieve yield, the muscle strain at yield and the yield stress at a given high lengthening velocity are all remarkably similar in the crab muscle and in the frog muscle (Table 1). The responses of the crab respiratory muscle to stretch seem to be remarkably similar to those of stretched frog muscle, which leads us to anticipate that the results obtained with the crustacean muscle on the shape of the force–velocity–time surface, and the effects of variation in the degree of muscle activation on the mechanical responses to stretch, are likely to be features of most skeletal muscles.

List of abbreviations

\[
\begin{align*}
F & \quad \text{muscle stress (N cm}^{-2}\text{)} \\
F_0 & \quad \text{maximum isometric stress (N cm}^{-2}\text{, a function of stimulus frequency and muscle length)} \\
F_1, F_2 & \quad \text{measures used in characterizing responses to stretch during relaxation} \\
\Delta F & \quad \text{increase in stress above pre-stretch level during lengthening (N cm}^{-2}\text{)} \\
(\Delta F_\alpha/F_0)_{A_S} & \quad \text{asymptotic value of the ratio } \Delta F_\alpha/F_0 \text{ as a function of strain rate} \\
\Delta F_A & \quad \text{asymptotic value of } \Delta F \text{ during slow stretch (N cm}^{-2}\text{)} \\
\Delta F_{\text{max}} & \quad \text{maximum value of } \Delta F \text{ reached during a stretch value of } \Delta F_{\text{max}} \text{ during 100 Hz stimulation} \\
\Delta F_{Y,100} & \quad \text{muscle stress at yield during rapid lengthening (N cm}^{-2}\text{)} \\
L & \quad \text{muscle length (mm)} \\
L_0 & \quad \text{muscle length at the onset of stretch (mm)} \\
S & \quad \text{muscle strain } (\text{a} - L/L_0) \\
S_Y & \quad \text{strain at yield during rapid lengthening} \\
dS/dt & \quad \text{strain rate (s}^{-1}\text{, or } Ls^{-1}\text{)} \\
t & \quad \text{time after onset of stretch (s)} \\
\alpha & \quad \text{a rate constant used in describing the change in } \Delta F_\alpha/F_0 \text{ as a function of strain rate (s}^{-1}\text{)} \\
\beta & \quad \text{a rate constant used in describing the change in } \tau \text{ as a function of strain rate (s}^{-1}\text{)} \\
\tau & \quad \text{time constant for increase in stress during slow stretch (s)} \\
\tau_A & \quad \text{asymptotic value of } \tau \text{ as a function of strain rate (s)}
\end{align*}
\]

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References


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