EFFECTS OF pH ON CONTRACTION OF RABBIT FAST AND SLOW SKELETAL MUSCLE FIBERS

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ABSTRACT We have investigated (*a*) effects of varying proton concentration on force and shortening velocity of glycerinated muscle fibers, (*b*) differences between these effects on fibers from psoas (fast) and soleus (slow) muscles, possibly due to differences in the actomyosin ATPase kinetic cycles, and (*c*) whether changes in intracellular pH explain altered contractility typically associated with prolonged excitation of fast, glycolytic muscle. The pH range was chosen to cover the physiological pH range (6.0–7.5) as well as pH 8.0, which has often been used for in vitro measurements of myosin ATPase activity. Steady-state isometric force increased monotonically (by about threefold) as pH was increased from pH 6.0; force in soleus (slow) fibers was less affected by pH than in psoas (fast) fibers. For both fiber types, the velocity of unloaded shortening was maximum near resting intracellular pH in vivo and was decreased at acid pH (by about one-half). At pH 6.0, force increased when the pH buffer concentration was decreased from 100 mM, as predicted by inadequate pH buffering and pH heterogeneity in the fiber. This heterogeneity was modeled by net proton consumption within the fiber, due to production by the actomyosin ATPase coupled to consumption by the creatine kinase reaction, with replenishment by diffusion of protons in equilibrium with a mobile buffer. Lactate anion had little mechanical effect. Inorganic phosphate (15 mM total) had an additive effect of depressing force that was similar at pH 7.1 and 6.0. By directly affecting the actomyosin interaction, decreased pH is at least partly responsible for the observed decreases in force and velocity in stimulated muscle with sufficient glycolytic capacity to decrease pH.

INTRODUCTION

Fatigue, the decrease of force or power output which may accompany prolonged stimulation of glycolytic muscle, could result from changes at several points between the central nervous system (CNS) and the myosin cross-bridge. In cases where fatigue is not attributable to decreased CNS output (e.g., Bigland-Ritchie et al., 1986, and in isolated muscle preparations), decline of muscle function could be due to failure of excitation (Gardiner and Olha, 1987), impairment of excitation-contraction coupling (Caputo and Bolaños, 1987; Länngergren and Westerblad, 1986; Bigland-Ritchie et al., 1986; Fabiato and Fabiato, 1978; Nassar-Gentina et al., 1978), or metabolite inhibition of actomyosin (Nosek et al., 1987; Metzger and Moss, 1987*b*; Luney and Godt, 1987; Pate et al., 1987; Chase and Kushmerick, 1987, 1986; Kentish, 1986; Cooke and Pate, 1985; Edman and Mattiazzi, 1981; Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978). It has been suggested that multiple factors, protons and inorganic phosphate, may act additively (Cooke and Pate, 1985) or synergistically (Nosek et al., 1987) to cause fatigue and that even a single factor, protons, may have multiple actions (Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978). In contrast, it has also been suggested that there is no direct role of protons during fatigue; protons act indirectly on the contractile machinery by altering the relative proportion between ionized forms of inorganic phosphate (Dawson et al., 1986). To sort out mechanistically which factor(s) is (are) primarily responsible for fatigue, it is necessary to vary them in a controlled, independent manner, first examining their effects at the lowest common level of muscle function: the actomyosin interaction.

Intracellular acidification has long been known to correlate with muscle fatigue. To study the roles of protons in fatigue and in force production by actomyosin, demembranated muscle fibers have been used extensively. The pH dependence of skinned fiber force has been reported in several publications (Metzger and Moss, 1987*b*; Nosek, et al., 1987; Rees and Stephenson, 1987; Pate et al., 1987; Luney and Godt, 1987; Cooke and Pate, 1985; Johnston and Mutungi, 1985; Robertson and Kerrick, 1979; Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978), but quantitative differences exist, due in part to differences fiber type (Metzger and Moss, 1987*b*; Donaldson and Hermansen, 1978). In only two cases was pH varied over a wide range (Robertson and Kerrick, 1979; Johnston and Mutungi, 1985) and in no case was it clearly established that protons were adequately buffered such that no radial pH gradients existed throughout the fiber. Also, only a limited, and partially contradictory, body of evidence exists on the effect of pH on skinned fiber shortening velocity (Metzger and Moss, 1987*b*; Pate et al., 1987; Luney and

Godt, 1987; Cooke and Pate, 1985; Johnston and Mutungi, 1985).

Therefore we have systematically examined the pH-dependence of force and shortening velocity using fully Ca^{2+}-activated, demembranated muscle fibers from rabbit. Psoas (fast type IIb) muscle fibers were compared with soleus (predominantly slow type I) muscle fibers to look for qualitative differences between chemomechanical transduction by the two muscle types.

Portions of this work have been published in abstract form (Chase and Kushmerick, 1987).

METHODS

Bundles of rabbit psoas and soleus fibers were skinned in a solution that contained (mM) 25 EGTA, 77 imidazole, 6.5 $Mg(acetate)_2$, 6.5 Na_2ATP, 88 K^+, 85 acetate, 1 DTT, and 0.1 leupeptin, pH 7.1 at 0°C. The fiber bundles were soaked first in skinning solution plus 0.5% detergent (Brij 58 or Triton X-100, with no apparent differences in the microscopic appearance of, or in the results obtained from, fibers treated with either detergent) for 30 min at 4°C, then in skinning solution alone for 24 h at 4°C, and finally transferred to skinning solution plus glycerol (50% vol/vol) and stored at −20°C (for up to 3 mo). Permeabilized fiber segments were dissected in a cold bath (10°C) of glycerol/skinning solution plus 10 mg/ml bovine serum albumin (BSA). A new method of fiber attachment was developed. The ends of the fibers were chemically fixed, by applying glutaraldehyde (5% in H_2O, with 1 mg/ml Na-fluorescein added as a visual indicator) from a glass micropipette, and wrapped in aluminum foil clips (Fig. 1). BSA in the dissection solution served as an absorbent buffer for glutaraldehyde which diffused away from the site of application, and may also have been chemically fixed within the filament lattice at the ends of the fiber. The fixed and unfixed portions of a fiber could be distinguished at the end of an experiment by allowing the fiber to shorten actively until striations disappeared in the unfixed region; the striation pattern was maintained in the fixed regions, which did not shorten (Fig. 1).

The fiber segments were attached via the clips to wire hooks on a moving iron galvanometer motor (General Scanning, Watertown, MA) and a capacitance-type force transducer (Cambridge Technology, Cambridge, MA). Position was monitored by a capacitance-type transducer in the motor and was calibrated with respect to the motor arm. The galvanometer servo (Cambridge Technology) was switched between length- and force-clamp modes under computer control. Overall fiber length and relaxed sarcomere length (SL) were measured during the experiment with an optical micrometer. The reported values of SL for relaxing and activating conditions were determined from photographs. The length of the fiber's central, unfixed portion (FL) was determined by subtracting the length of the fixed regions (measured at the end of the experiment) from the total fiber length.

Experimental control, data collection, and analysis of raw data were carried out with a PDP-11/23 (Digital Equipment, Maynard, MA) with a slaved auxiliary Z-80 microprocessor (Sweeney et al., 1987). Data were sampled with 12-bit resolution at rates of up to 12.5 kHz per channel; length and force signals were first low pass filtered (1.5 kHz corner frequency) to avoid frequency aliasing.

Composition of experimental solutions was calculated by a previously described algorithm (Kushmerick et al., 1986) using the binding constants given in Table I. Total Ca and Mg concentrations in stock solutions were determined by atomic absorption spectroscopy. Standard conditions were (mM; abbreviations explained in Table I): 5 MgATP, 1 P_i, 4 EGTA or EDTA, pCa 4.75–4.0 (activating) or pCa > 8 (relaxing), 15 PCr, 1–1.5 mg/ml CK (creatine kinase, 160–435 U/ml; typically 290), 100 K^+ + Na^+, 3 Mg^{2+}, and 50 pH buffer (at pH 6.0 and 6.5, MES; at pH 7.1, MOPS or imidazole; at pH 7.3, MOPS; at pH 7.5, MOPS, Hepes, or TES; at pH 8.0, EPPS). The ionic strength (IS) was 160 mM in most experiments (adjusted with imidazole or Tris as the cation and acetate as

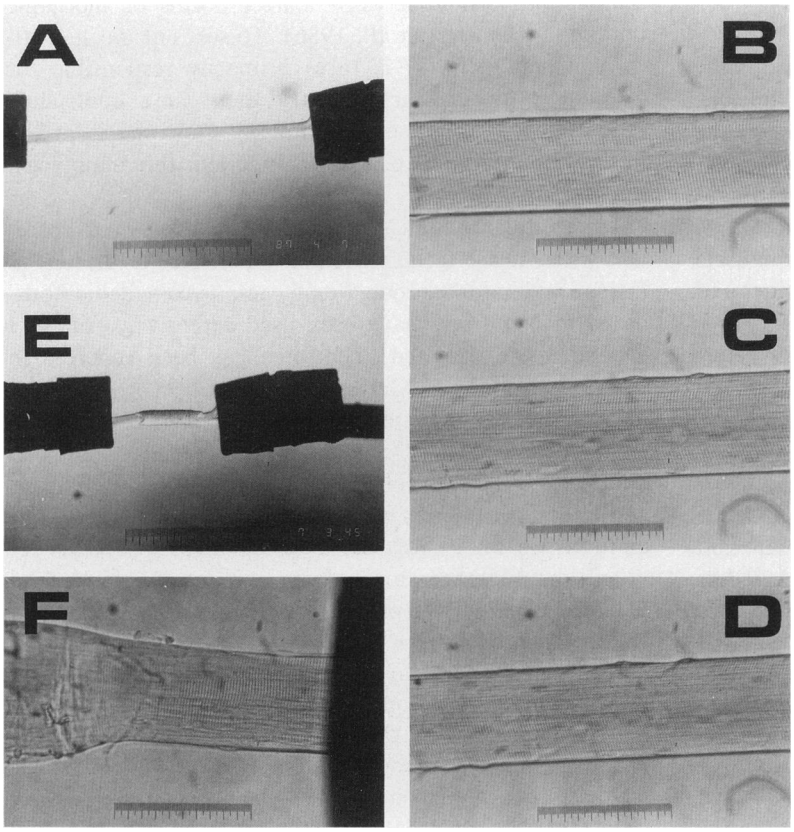

FIGURE 1 Photomicrographs of a rabbit psoas glycerinated fiber segment. (*A*) Fiber segment in relaxing solution: total length, 1.7 mm. (*B*) Relaxed fiber at mean SL, 2.57 μm. (*C*) Mean SL, 2.47 μm during the second activation at pH 7.1. The force was 220 mN/mm^2. (*D*) Mean SL, 2.28 μm during the eighth activation (fifth control after three experimental activations at pH 7.5). (*E*) Fiber segment after shortening in activation solution. Chemically fixed ends (0.3 mm) did not shorten. (*F*) High-power view of a fiber's fixed end. The fiber segment (not the same as that in other photos) had been shortened in activation solution, as was the fiber in *E*. Aluminum foil clip is at right of this photo. Entire calibration scale represents 830 μm at low power and 105 μm at high power.

TABLE I
EQUILIBRIUM BINDING CONSTANTS (K_Bs)

Compound/Equilibrium	K_B (12°C)	Reference
ATP, adenosine 5'-triphosphate		
$[HATP^{3-}]/[H^+][ATP^{4-}]$	2.96×10^6	4
$[H_2ATP^{2-}]/[H^+][HATP^{3-}]$	1.47×10^4	4
$[CaATP^{2-}]/[Ca^{2+}][ATP^{4-}]$	6.34×10^3	7
$[CaHATP^{1-}]/[Ca^{2+}][HATP^{3-}]$	1.05×10^2	7
$[MgATP^{2-}]/[Mg^{2+}][ATP^{4-}]$	9.04×10^3	4
$[MgHATP^{1-}]/[Mg^{2+}][HATP^{3-}]$	1.08×10^2	4
$[KATP^{3-}]/[K^+][ATP^{4-}]$	1.00×10^1	7
PO_4, inorganic phosphate (P_i)		
$[HPO_4^{2-}]/[H^+][PO_4^{3-}]$	7.11×10^{11}	4
$[H_2PO_4^{1-}]/[H^+][HPO_4^{2-}]$	5.87×10^6	4
$[H_3PO_4]/[H^+][H_2PO_4^{1-}]$	8.69×10^1	4
$[CaPO_4^{1-}]/Ca^{2+}][PO_4^{3-}]$	2.31×10^6	5
$[CaHPO_4]/[Ca^{2+}][HPO_4^{2-}]$	2.54×10^1	5
$[MgPO_4^{1-}]/[Mg^{2+}][PO_4^{3-}]$	2.51×10^3	5
$[MgHPO_4]/[Mg^{2+}][HPO_4^{2-}]$	4.02×10^1	5
$[KPO_4^{2-}]/[K^+][PO_4^{3-}]$	1.99×10^0	5
PCr, phosphocreatine		
$[HPCr^{1-}]/[H^+][PCr^{2-}]$	3.16×10^4	1
$[H_2PCr]/[H^+][HPCr^{1-}]$	5.01×10^2	1
$[H_3PCr^{1+}]/[H^+][H_2PCr]$	1.00×10^2	1
$[CaPCr]/[Ca^{2+}][PCr^{2-}]$	2.00×10^1	1
$[MgPCr]/[Mg^{2+}][PCr^{2-}]$	3.98×10^1	1
EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid		
$[HEGTA^{3-}]/[H^+][EGTA^{4-}]$	3.48×10^9	8
$[H_2EGTA^{2-}]/[H^+][HEGTA^{3-}]$	9.21×10^8	8
$[H_3EGTA^{1-}]/[H^+][H_2EGTA^{2-}]$	4.57×10^2	8
$[H_4EGTA]/[H^+][H_3EGTA^{1-}]$	1.00×10^2	8
$[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}]$	1.35×10^{11}	8
$[CaHEGTA^{1-}]/[Ca^{2+}][HEGTA^{3-}]$	2.16×10^5	8
$[MgEGTA^{2-}]/[Mg^{2+}][EGTA^{4-}]$	1.29×10^5	8
$[MgEGTA^{1-}]/[Mg^{2+}][HEGTA^{3-}]$	1.40×10^3	8
EDTA, ethylenediaminetetraacetic acid		
$[HEDTA^{3-}]/[H^+][EDTA^{4-}]$	2.25×10^{10}	8
$[H_2EDTA^{2-}]/[H^+][HEDTA^{3-}]$	1.76×10^6	8
$[H_3EDTA^{1-}]/[H^+][H_2EDTA^{2-}]$	4.29×10^2	8
$[H_4EDTA]/[H^+][H_3EDTA^{1-}]$	9.86×10^1	8
$[CaEDTA^{2-}]/[Ca^{2+}][EDTA^{4-}]$	6.61×10^{10}	8
$[CaHEDTA^{1-}]/[Ca^{2+}][HEDTA^{3-}]$	4.45×10^3	8
$[MgEDTA^{2-}]/[Mg^{2+}][EDTA^{4-}]$	5.26×10^8	8
$[MgHEDTA^{1-}]/[Mg^{2+}][HEDTA^{3-}]$	1.66×10^2	8
$[KEDTA^{3-}]/[K^+][EDTA^{4-}]$	6.31×10^0	8
Acetate, $[Hac]/[H^+][Ac^-]$	3.60×10^4	6
$[CaAc^+]/[Ca^{2+}][Ac^-]$	3.39×10^0	6
$[MgAc^+]/[Mg^{2+}][Ac^-]$	3.24×10^0	6
Lactate, $[HLa]/[H^+][La^-]$	4.54×10^3	4
$[CaLa^+]/[Ca^{2+}][La^-]$	1.17×10^1	6
$[MgLa^+]/[Mg^{2+}][La^-]$	8.51×10^0	6
MES, 2-[*N*-morpholino]ethanesulfonic acid		
$[HMES]/[H^+][MES^-]$	1.73×10^6	3
$[CaMES^+]/[Ca^{2+}][MES^-]$	5.01×10^0	3
$[MgMES^+]/[Mg^{2+}][MES^-]$	6.31×10^0	3
Imidazole		
$[HImid^+]/[H^+][Imid]$	1.96×10^7	4
MOPS, 3-[*N*-morpholino]propanesulfonic acid		
$[HMOPS]/[H^+][MOPS^-]$	1.79×10^7	2
Hepes, *N*-2 hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid		
$[HHepes]/(H^+][Hepes^-]$	4.59×10^7	3
TES, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid		
$[HTES]/[H^+][TES^-]$	4.57×10^7	3
EPPS, *N*-[2-hydroxyethyl]piperazine-*N'*-3-propanesulfonic acid		
$[HEPPS]/[H^+][EPPS^-]$	1.66×10^8	2
Tris, tris(hydroxymethyl)aminomethane		
$[HTris^+]/[H^+][Tris]$	3.53×10^8	3

References: (1) Dawson et al., 1974; (2) Good and Izawa, 1972, (3) Good et al., 1966; (4) Martell and Smith, 1982; (5) Smith and Martell, 1981; (6) Martell and Smith, 1979; (7) Smith and Martell, 1975; (8) Martell and Smith, 1974.

the anion). For calculating IS, protonated zwitterionic buffers were considered to have no charge (Rees and Stephenson, 1987). When the concentration of an anion was changed (e.g., P_i, MES, or lactate), an appropriate amount of acetate was also added or left out and the amounts of imidazole or Tris and total Ca and Mg were adjusted to maintain constant pH, pCa, pMg, and IS. Ca was added as $Ca(acetate)_2$ and Mg was added as $Mg(acetate)_2$; thus all solutions contained at least 20 mM acetate. To examine the effects of lactate at 50 mM, IS was increased to 190 mM (in both control and experimental solutions); 50 mM is on the upper end of the physiological intracellular lactate concentration range (e.g., Kushmerick and Meyer, 1985, and references therein). Determinations of pH, at 12°C, were accurate to ±0.01 pH unit and were made with a model PHM62 pH meter with a type GK2321C electrode (Radiometer America, Westlake, OH) which had been calibrated at 12°C with buffer standards with ±0.01 pH unit accuracy (Fisher Scientific, Springfield, NJ). pH buffers were chosen on the basis of proximity of log K_B (at 12°C; Table I) to the desired pH, where K_B is the equilibrium binding constant. In activating solutions, EDTA was used as a Ca^{2+} buffer because, over the pH range used, the log of its apparent binding constant for Ca^{2+} (calculated for the conditions described) is close to the desired pCa, whereas that of EGTA varies over several orders of magnitude. For the conditions used in these experiments, Mg^{2+} competes significantly with Ca^{2+} and H^+ for binding to EDTA (unlike EGTA). But we do not expect that there would be appreciable radial Mg^{2+} gradients within the fiber to alter the apparent affinity of the Ca^{2+} buffer for Ca^{2+}, whereas H^+ gradients are possible. In some control experiments, solution osmolarity was varied by dissolving solid sucrose in standard solutions; in these experiments, osmolarity was measured with a vapor pressure osmometer (Wescor, Inc., Logan, UT) and was within 6% of the computed value. Solutions were held in 300 μl slots in a temperature-controlled holder. Experiments were performed at 12°C because fiber degradation occurred more slowly than at 20°C. To change solutions, the fiber was raised out of one slot, a new slot was rotated into place, and the fiber was lowered into the new solution.

Before experimental measurements were made, all fibers were activated and relaxed once at pH 7.1 to insure that the striation pattern and force were stable under activating conditions. During activation, fibers were periodically (0.2 Hz, psoas; 0.067–0.1 Hz, soleus) shortened at a speed greater than their maximum velocity of shortening; experimental measurements were made during the steady-state period between shortenings (Sweeney et al., 1987). Force and velocity at an experimental pH were normalized to the average of two bracketing control measurements except at pH 8.0 where, because of rapid deterioration of psoas fibers, only the immediately preceding control value was used. (If the bracketing procedure had been used at pH 8.0, force would have been increased by 16 and 5% of the value given for pH 8.0, and velocity decreased by 19 and 11%, for psoas and soleus fibers, respectively.)

Shortening velocity was characterized in two ways. The velocity of unloaded shortening (V_{us}) was assessed using the slack test (Edman, 1979); four to six length steps (4–11% FL) were used (Fig. 2). To obtain a force-velocity curve, we made four to eight force-clamp steps to levels between 0.7 and 60% of the isometric force (Po) which immediately preceded the step (Fig. 3). The force baseline for each set of force steps was unambiguously determined by a separate slack release of the fiber; the force baseline determined in this manner is indicated in Fig. 3. For each force step, the force data were averaged and the velocity data were fitted to a line by linear least squares regression over a time period chosen individually for each force step. The initial time was chosen such that obvious force and velocity transients were avoided (15–20 ms after the step), and the final time was chosen such that the fiber had shortened by <12% of its initial length. The velocity (V) and relative force (P/Po) data thus obtained were fitted to Hill's (1938) equation.

$$V = V_{max}\,(a/Po)\,\frac{(1 - P/Po)}{(a/Po + P/Po)}, \qquad (1)$$

using a nonlinear least-squares regression program (RS/1, BBN Soft-

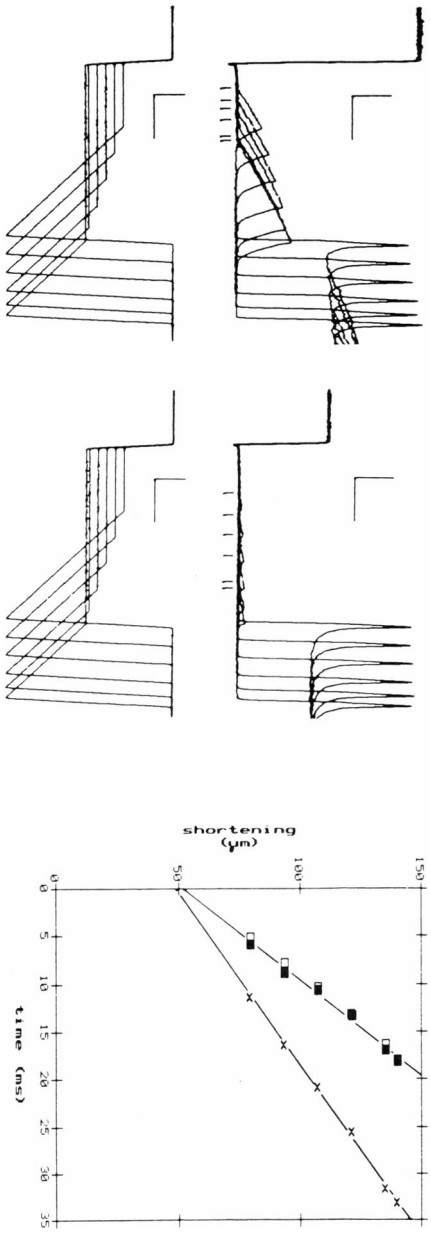

FIGURE 2 Measurement of unloaded shortening velocity. Plots of digitized data: pH 7.1 (*left*) and pH 6.0 (*center*). Calibrations: force (*upper*), 20 mg; length (*lower*), 50 μm; time, 10 ms. Small vertical lines below force baseline indicate times chosen for velocity determination. Before restretching, the fiber was shortened at $V > V_{us}$, dropping the force to zero. Psoas FL was 1.4 mm (mean SL, 2.46 μm in activating solution) and generated 233 mN/mm^2 force at pH 7.1. (*Right*) Plot of distance vs. time for data shown at left and center. The lines were drawn according to linear least squares regressions on the data. Solid squares correspond to data at left (pH 7.1); V_{us} was 5.0 mm/s. Crosses correspond to data at center (pH 6.0); V_{us} was 2.8 mm/s. Open squares correspond to a second control at pH 7.1.

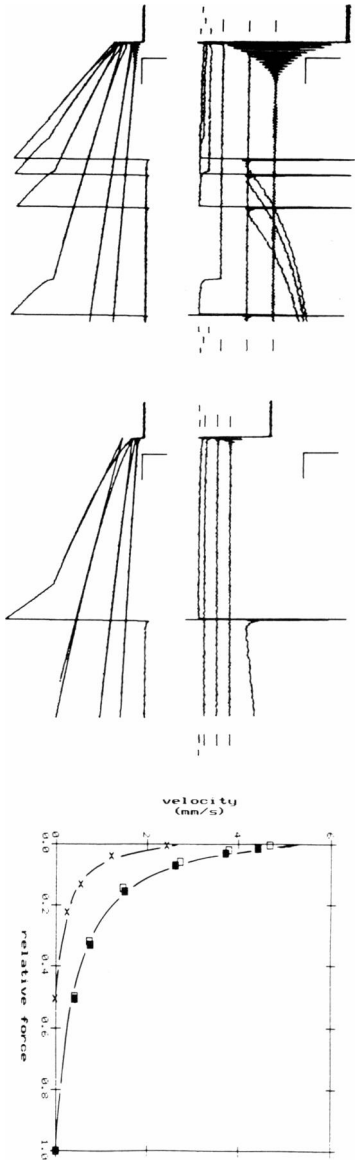

FIGURE 3 Measurement of force-velocity relation. The data are from the same fiber as in Fig. 2 and were obtained after completion of the slack tests. Plots of digitized data: pH 7.1 (*left*) and pH 6.0 (*center*). Calibrations: force (*upper*), 20 mg; length (*lower*), 50 μm; time, 10 ms. Small horizontal lines beside the force data indicate the average force levels obtained during analysis, and the baseline. Straight lines superimposed on length data are the linear least-square regressions of length on time. Note that before restretching the fiber was shortened at $V > V_{max}$ and force dropped briefly to the baseline. (*Right*) Plot of velocity vs. force for data shown at left and center. Solid squares correspond to data at left (pH 7.1); V_{max} was 5.4 mm/s. Crosses correspond to data at center (pH 6.0); V_{max} was 2.7 mm/s. Open squares correspond to a second control at pH 7.1. The lines were drawn according to the nonlinear least squares regressions on the data, fitted to Eq. 1 (Methods); the force axis for pH 6.0 data was compressed so that the maximum relative force was 0.51, the ratio of isometric force at pH 6.0 to that at pH 7.1 for this fiber.

ware Products Corp., Cambridge, MA). The fitted parameters were a/Po and V_{max} (the maximum velocity of shortening at $P = 0$).

Statistical analyses were performed using RS/1. Results are expressed as the mean ± SD. The t test was typically used; exceptions and significance levels are noted in the text.

RESULTS

Fibers with Chemically Fixed Ends

Chemical fixation of the ends of the skinned fibers was used to minimize end compliance associated with attachment of short fiber segments to the motor and force transducer. The total compliance we report below, obtained when FL was rapidly changed (e.g., during length or force steps), is less than that reported for some skinned fiber preparations (Cooke and Pate, 1985), but not others (Metzger and Moss, 1987*b*), and is greater than that reported for intact single muscle cells (Edman, 1979). Additionally, the fixation procedure allowed an accurate determination of FL because the fixed regions extended beyond the attachments; there was no uncertainty about what length of fiber within the attachment clips contributed to the measured mechanical properties. For the fibers used to construct Figs. 4 and 5, FL was 1.3–1.7 mm. SL in relaxing solution, determined after the first activation, was 2.56 ± 0.03 μm (n = 15; psoas) and 2.46 ± 0.14 μm (n = 13; soleus). SL at full activation (pH 7.1) was 2.41 ± 0.05 μm (n = 14; psoas) and 2.25 ± 0.15 μm (n = 12; soleus); photographs were not obtained for two fibers in activation solution. Length parameters were similar for fibers used in other experiments.

Effect of pH on Steady-State Isometric Force

Maximum isometric force at 12°C and pH 7.1 was, for psoas, 220 ± 20 mN/mm^2 (n = 15) and, for soleus, 153 ± 43 mN/mm^2 (n = 13). Maximum force increased monotonically with pH (Fig. 4). In relaxing solution, force was 0.9 ± 0.4% (n = 15) of isometric force at pH 7.1 for psoas fibers and 3.3 ± 1.3% (n = 13) for soleus fibers. Force in psoas fibers was more affected by pH than in soleus fibers

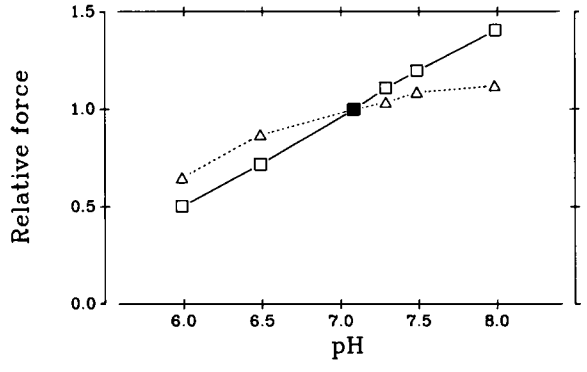

FIGURE 4 Effect of pH on steady-state isometric force. Force of psoas (*squares*) and soleus (*triangles*) fibers was normalized to the average of bracketing contractions at pH 7.1 (MOPS), except at pH 8.0 where peak force was normalized to the preceding control. Points are mean values (SDs were smaller than symbols); (n = psoas, soleus): pH 6.0 (3, 4); pH 6.5 (3, 5); pH 7.3 (7, 3); pH 7.5 (9, 11); pH 8.0 (3, 4). Lines were drawn to connect the points. Tris was the primary monovalent cation other than Na^+ and K^+; no imidazole was used.

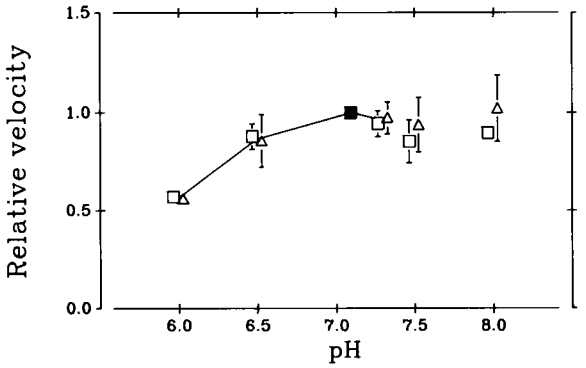

FIGURE 5 Effect of pH on velocity of unloaded shortening. V_{us} of psoas (*squares*) and soleus (*triangles*) fibers was normalized to the average of bracketing measurements at pH 7.1 (MOPS), except at pH 8.0, where velocity was normalized to the preceding control. Points are mean ± SD (in some cases, SD was smaller than symbol); number of points and conditions as in Fig. 4. Lines were drawn by eye. The psoas data were offset by −0.03 pH units, and the soleus data by +0.03, for clarity.

and this difference between fiber types was significant at all pH levels ($P < 0.01$). There was no significant effect of the pH buffer used (50 mM MOPS, Hepes, or TES, which have similar K_Bs) on force at pH 7.5 (data not shown; ANOVA; $P > 0.05$); therefore the data within each fiber type were pooled. When pCa was changed from 4.75 to 4.5 at pH 6.0, force changed by 1.9 ± 3.0% (n = 5 psoas + 3 soleus fibers), a number that was not different from 0 ($P > 0.05$). Similarly, when pCa was changed from 4.75 to 4.0 at pH 6.0, force changed by −1.0 ± 0.3% (n = 3 psoas fibers). No pCa-dependent changes in force (pCa 4.75–4.5) occurred at pH 6.5 (n = 3) or 7.1 (n = 4); other pH levels were not investigated. Thus, despite decreased Ca^{2+} sensitivity at acidic pH (Ashley and Moisescu, 1977; Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978; Robertson and Kerrick, 1979), these experiments were made under saturating Ca^{2+} conditions. There was no significant ($P > 0.05$) change in fiber diameter caused by pH changes (data not shown).

Rapid, irreversible deterioration of skinned fibers during activation at high pH was previously noted by Robertson and Kerrick (1979). Deterioration was not due to high pH alone, as a 10-min exposure to pH 8.0 relaxing solution did not alter force or velocity in pH 7.1 activating solution (data from two psoas fibers not shown).

Effect of pH on Shortening Velocity

Velocity of unloaded shortening at pH 7.1 was, for psoas, 3.4 ± 0.3 FL/s (n = 15) and, for soleus, 0.6 ± 0.2 FL/s (n = 13); these values represent the velocity (mm/s) normalized to the length of the unfixed portion of the fiber (mm), and adjusted to a standard sarcomere length of 2.60 μm by a multiplicative factor of SL/2.60, where SL is the mean sarcomere length (μm) measured at full activation (except in two fibers, where SL was only measured in relaxing solution). The corresponding slack test intercept

terms were 4.5 ± 0.7% FL (n = 15; psoas) and 4.8 ± 0.5% FL (n = 13; soleus). V_{us} increased with pH over the range of 6.0 to 7.1. It was maximum at about pH 7.1, the intracellular pH in vivo (Fig. 5). Above pH 7.1, V_{us} tended to be lower than at pH 7.1. This was statistically so for psoas, but not for soleus (at pH 7.5, 1 of 9 [psoas] and 2 of 11 [soleus] V_{us} measurements were greater than at pH 7.1, and the rest were lower). The slower rate of force redevelopment after a period of unloaded shortening at pH 6.0, in comparison with that at pH 7.1 (Fig. 2), was presumably related to the reduction in V_{us}. As with force, there was no significant effect of the pH buffer used (50 mM MOPS, Hepes, or TES; data not shown) on V_{us} at pH 7.5 (ANOVA; $P > 0.05$); therefore the data within each fiber type were pooled. The results for the two fiber types were statistically indistinguishable when compared with each other at each pH.

Force-velocity curves were obtained at pH 6.0 and 7.1 in four psoas fibers. Unloaded shortening velocities were also obtained with these fibers (three fibers were from those used to construct Figs. 4 and 5; the fourth fiber was from those used to construct Fig. 6), and therefore pairwise statistical tests were used. At pH 7.1, V_{us} and V_{max} were not significantly different (3.6 ± 0.6 vs. 3.3 ± 0.4 FL/s, respectively; $P > 0.05$). V_{us} at pH 6.0, normalized to V_{us} at pH 7.1, was not different from V_{max} at pH 6.0, normalized to V_{max} at pH 7.1 (0.59 ± 0.05 vs. 0.53 ± 0.1, respectively; $P > 0.05$). The Hill parameter, a/Po, was 0.09 ± 0.02 at pH 7.1 and was not significantly changed by pH (0.09 ± 0.03 at pH 6.0; $P > 0.05$). Similar values of a/Po, low in comparison to intact fibers, have also been obtained with skinned frog fibers (Julian et al., 1986).

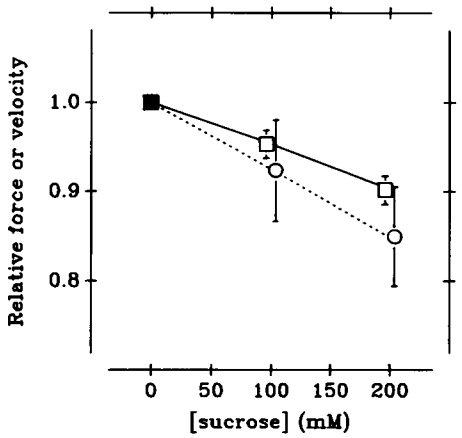

FIGURE 6 Effects of added sucrose on steady-state isometric force and velocity of unloaded shortening. Psoas force (*squares*) and V_{us} (*circles*) were normalized to the average of bracketing contractions with no added sucrose. Note that the minimum value on the ordinate is not 0. Osmolarity of the solutions was 250 mosmol/liter with no added sucrose. Points are mean ± SD; (n = force, velocity): 100 mM sucrose (8, 7); 200 mM (9, 8). Imidazole was the primary monovalent cation other than Na^+ and K^+; no Tris was used. The lines were drawn according to the linear least squares regressions constrained to go through 1 at no added sucrose. The force data were offset by −4 mM sucrose, and the velocity data by +4 mM, for clarity.

Experiments in the Presence of Lactate

Three psoas fibers were studied to assess the influence of lactate anion. At pH 7.1, the force in 50 mM lactate, relative to that in control solution without lactate, was 1.05 ± 0.02 (n = 3), a value that was not equal to 1 ($0.05 > P > 0.01$). At pH 7.1, V_{us} in 50 mM lactate, relative to that in control solution without lactate, was not different from 1 (0.96 ± 0.02; n = 3; $P > 0.05$). In the same fibers, force and V_{us} at pH 6.0 (0 lactate), relative to that obtained at pH 7.1 (0 lactate), were not significantly different from the values shown in Figs. 4 and 5 ($P > 0.05$). Thus the effect of 50 mM lactate on contractile function was minimal.

Control Experiments for Effects of Osmolarity on Force and Velocity

While maintaining constant IS, osmolarity can change significantly when varying the concentration of a proton-binding ligand that is not fully dissociated, i.e., at a pH near or above the pK_A of the ligand (e.g., MES in the pH 6 experiments to be described, or if imidazole was used as a cation for maintaining constant IS at high pH). Typically, solutions were 250 mosmol/l, but would be ~200 mosmol/l higher at pH 8.0 if imidazole (pK_A 7.3 at 12°C; note that $pK_A = \log K_B$) was used as the primary monovalent cation in addition to Na^+ and K^+.

Osmotic effects on fibers were studied in solutions with added sucrose. Both force and V_{us} of psoas fiber segments decreased by small but significant ($P < 0.01$) amounts with increased sucrose (Fig. 6). No differences were observed between results obtained at pH 7.1 or 6.0 ($P > 0.05$), normalized to data obtained at the same pH and with no added sucrose, so the data were combined for subsequent analyses. The force and V_{us} data at pH 6.0 (no added sucrose), relative to those at pH 7.1 (no added sucrose), were not different from those shown in Figs. 4 and 5 ($P > 0.05$). The effects of sucrose were not expected and presently remain unexplained; a similar effect on force has been obtained with skinned barnacle fibers (Ashley and Moisescu, 1977). One possible explanation is an increase in viscosity associated with added solute. Practically, these results were the reasons that we chose to use Tris rather than imidazole to adjust IS (while maintaining constant $K^+ + Na^+$) in experiments where pH was varied widely (particularly at alkaline pH). Unlike imidazole (pK_A 7.3 at 12°C), the major fraction of Tris (pK_A 8.6 at 12°C) was in the charged form at all pH levels used in these experiments. No significant change was observed in fiber diameter (data not shown), nor was a change expected as sucrose should readily permeate the filament lattice.

Effect of pH Buffer Concentration

At pH 6.0, psoas force increased as the pH buffer (MES) concentration was decreased (Fig. 7). The data shown in the figure were corrected for changes in solution osmolarity (see previous section); the largest correction was 3.5%, and the uncorrected data exaggerate the force increase at low buffer concentrations. MES concentrations >100 mM were not studied. Calculation of a model (see Discussion) indicates that if the pH buffer concentration could be increased from 0.1 to 1 M without increasing IS and osmolarity, force would be decreased by <1%. To make experiments at MES concentrations higher than 0.1 M necessitates an undesirable increase of ionic and osmotic strength, the latter due to adding uncharged pH buffer with the charged form.

There was no statistically significant effect of changing MES concentration on V_{us}, corrected for differences in osmolarity (data not shown; the maximum correction was 5.1%; if this correction had not been applied, the tendency would have been for V_{us} to increase as buffer concentration was decreased). The slope of the linear least squares regression of V_{us} on MES concentration, constrained to go through 1 at 100 mM MES, was not different from 0 ($P > 0.05$). The maximum standard deviation of velocity at any one pH buffer concentration was 6.5% (the numbers of observations were the same as for the force measurements). The force and velocity data at pH 6.0 (100 mM MES), relative to those at pH 7.1, were not different from those shown in Figs. 4 and 5, which were obtained with 50 mM MES ($P > 0.05$).

An assumption of the experimental design and model is that CK is "sufficiently" active (i.e., CK activity > ATPase activity) to maintain equilibrium conditions throughout the fiber. At pH 6.0, decreasing the amount of CK added by 2.7-fold had no effect on the results, suggesting that CK activity was sufficient in the conditions used (CK activity decreases with decreasing pH).

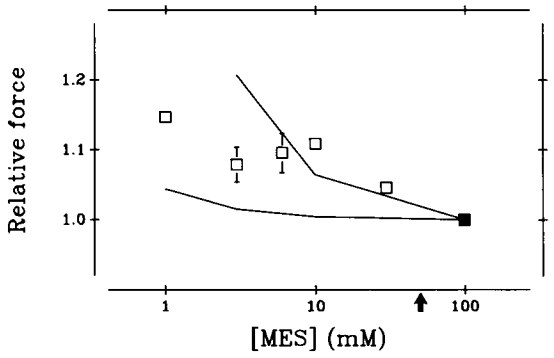

FIGURE 7 Effect of pH buffer concentration on steady-state isometric force. Psoas force, at pH 6.0 and various concentrations of pH buffer MES, was normalized to the average of bracketing contractions at pH 6.0 and 100 mM MES. Note that the minimum value of the ordinate and abscissa are not equal to 0. Points are mean ± SD (in some cases, SD was smaller than symbol); (*n*): 1 mM MES (4); 3 mM (4); 6 mM (2); 10 mM (5); 30 mM (2). The arrow indicates the buffer concentration used in other experiments (50 mM). Imidazole was the primary monovalent cation other than Na^+ and K^+; no Tris was used. The lines were drawn according to Eq. 5 (see Discussion). Model parameters were (lower, upper line): Q_H (0.1, 0.6 mM/s); fiber radius (25, 40 μm). Other parameters common to both curves were: D_H (5×10^{-5} cm^2/s); D_{MES} (2.5×10^{-6} cm^2/s); pK_A for MES (6.24).

Effects of P_i on Steady-State Isometric Force and Velocity of Unloaded Shortening at pH 7.1 and 6.0

In experiments with psoas fibers, force was decreased by 15 mM P_i at both pH 7.1 and pH 6.0 (Fig. 8). In Fig. 8, all of the data were expressed relative to control measurements at pH 7.1 and 1 mM P_i. If, instead, the force at 15 mM P_i was normalized to that at 1 mM P_i at the same pH, the relative force at 15 mM P_i was the same at pH 7.1 and 6.0 (0.70 ± 0.03 vs. 0.69 ± 0.03, respectively; $P > 0.05$). V_{us} was not affected by P_i at pH 7.1 ($P > 0.05$) but at pH 6.0, V_{us} was significantly lower in 15 mM Pi than in 1 mM Pi ($P < 0.01$; Fig. 8). The force and velocity data at pH 6.0 (1 mM P_i), relative to those at pH 7.1 (1 mM P_i), were not different from those shown in Figs. 4 and 5 ($P > 0.05$). Our calculations indicate that 35% of P_i was in the diprotonated, monovalent form ($H_2PO_4^{1-}$) at pH 7.1, and that 87% was in the diprotonated form at pH 6.0.

DISCUSSION

Validity of pH Effects

In our experiments, we have minimized variations of force and velocity due to variables other than pH: sub-maximal Ca^{2+} activation (fibers were maximally activated), proton gradients within the fiber, ionic strength, and osmolarity. The possibility of force changes due to submaximal activation, combined with pH-induced variation of Ca^{2+} sensitiv-

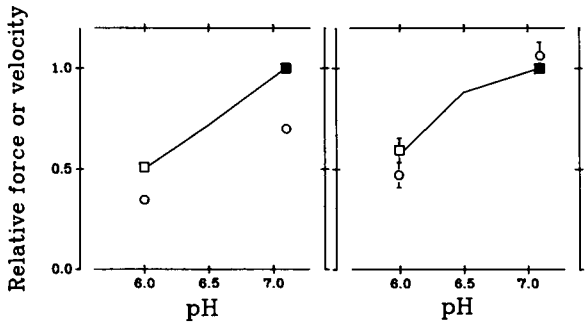

FIGURE 8 The effects of pH and P_i, alone and in combination, on steady-state isometric force and velocity of unloaded shortening. Psoas force (*left*) and V_{us} (*right*), at 1 mM P_i (*squares*) or 15 mM P_i (*circles*), were normalized to the average of bracketing contractions at pH 7.1, 1 mM P_i. Points are mean ± SD (in some cases, SD was smaller than symbol); (*n* = force, V_{us}): pH 7.1, 15 mM P_i (5, 5); pH 6.0, 1 mM P_i (7, 7); pH 6.0, 15 mM P_i (12, 8). Lines were copied from Fig. 4 (*left*) and Fig. 5 (*right*). Imidazole was the primary monovalent cation other than Na^+ and K^+; no Tris was used.

ity, was of particular concern at acid pH where this effect would be most likely to affect the results (Ashley and Moisescu, 1977; Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978; Robertson and Kerrick, 1979; Metzger and Moss, 1987*b*), but force was not increased by increasing Ca^{2+} concentration from pCa 4.75 to pCa 4.5 or 4.0 (see Results). Because force was maximal, there should not have been an influence of Ca^{2+} on the maximum speed of shortening either, as has been observed at submaximal levels of activation (Julian et al., 1986).

Increased acidity decreases the myofilament lattice spacing and could thus indirectly alter contractility. X-ray diffraction measurements of lattice spacing as a function of pH suggest that, at most, a 5–7% reduction occurs at pH 6 (Matsuda and Podolsky, 1986; Umazume et al., 1986), and our light microscopic measurements of fiber diameter did not show significant changes with pH. Equivalent changes (~5%) in fiber diameter induced by high-molecular weight polymers—which, unlike protons, decrease the filament lattice spacing by increasing osmotic pressure—do not decrease force (Godt and Maughan, 1981; Metzger and Moss, 1987*a*). Thus, small changes in interfilament spacing alone did not cause the large decrease in force at acidic pH. V_{us} is more affected by osmotic compression than force; when compressed by less than ~15% of the initial diameter, V_{us} is more affected in fast fibers than in slow fibers (Metzger and Moss, 1987*a*). Thus, part of the reduction of V_{us} observed in psoas fibers at acid pH may be due to decreased myofilament lattice spacing (according to Metzger and Moss, 1987*a*, a 5% reduction in cell diameter resulted in ~5% reduction in V_{us} of rat fast fibers, whereas there was no effect on slow fibers).

Proton concentration varies throughout an active fiber because protons are involved in both the ATPase and CK reactions. Net proton consumption occurs at all pH levels used in these experiments. The degree of alkalinization depends on two factors which vary with pH: (*a*) ATPase activity and (*b*) the stoichiometric coefficient of proton consumption per ATP hydrolyzed (coupled with rephosphorylation via the CK reaction; George and Rutman, 1960). For the conditions described in these experiments, the coefficient of the latter is about 0.85 at pH 6 and decreases to ~0.07 at pH 8. Because force increases with pH (Fig. 4), force would be higher with the fiber bathed in solutions with inadequate pH buffering. Force enhancement was observed at decreased buffer concentration (Fig. 7), which is consistent with alkalinization at the fiber's center. A second possibility, which our experiments cannot eliminate, is a specific inhibitory action of MES. However, no specific effects due to buffer species were observed at pH 7.5 (Results), where three buffers with appropriate K_Bs were available (MOPS, Hepes, and TES). Whereas alkalinization would also be expected to increase velocity (of the central portion of the fiber), the observed velocity did not increase within experimental error, presumably because the slower (more acidic), peripheral region acted in parallel to limit overall shortening velocity.

Protons are transported into the fiber, at steady state, by (*a*) diffusion from the bathing solution, and (*b*) release from mobile buffers, which must also diffuse from the bathing solution. The equation describing radial diffusion of protons, free or associated with buffers, into an infinitely long cylinder is

$$\frac{\partial([H^+] + \sum_j [HM_j] + \sum_k [HF_k])}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD_H \frac{\partial [H^+]}{\partial r}\right) + \frac{1}{r}\frac{\partial}{\partial r}\left(r \sum_j D_{HM_j} \frac{\partial [HM_j]}{\partial r}\right) - Q_H, \quad (2)$$

where M_j represents the jth mobile buffer, F_k represents the kth fixed buffer, and Q_H represents the proton consumption rate. This relation is similar to a facilitated diffusion model by Meyer et al. (1984), and is analogous to a less general description for diffusion in one dimension presented in Junge and McLaughlin (1987). Using the equilibrium relations

$$K_A = \frac{[H^+][A]}{[HA]} \quad (3)$$

$$[A]_{TOT} = [A] + [HA], \quad (4)$$

where A represents a buffer, fixed or mobile, Eq. 2 reduces to the steady-state relation

$$\frac{d^2[H^+]}{dr^2} = \frac{-1}{r}\frac{d[H^+]}{dr} + \frac{2\left(\sum_j \frac{D_{HM_j} K_{M_j} [M_j]_{TOT}}{([H^+] + K_{M_j})^3}\right)\left(\frac{d[H^+]}{dr}\right)^2 + Q_H}{D_H + \sum_j \frac{D_{HM_j} K_{M_j} [M_j]_{TOT}}{([H^+] + K_{M_j})^2}}, \quad (5)$$

with boundary conditions (*a*) $[H^+]$ at the fiber's outer edge is the same as that in the bath, and (*b*) the slope, $d[H^+]/dr$, is 0 at the fiber's center ($r = 0$).

To determine whether force enhancement at decreased pH buffer concentration could be explained by proton concentration heterogeneity, we evaluated Eq. 5 numerically. Using reasonable ranges of parameter estimates (stated in the legend of Fig. 7) and considering MES as the only proton ligand, the predictions of the model bounded the experimental results (lines of Fig. 7), and thus could easily account for the entire increase in force. A complete definition of the actual system in the fiber was not attempted, as this would require consideration of all proton-binding species (mobile proton-binding species other than MES), unstirred layers, and pH effects on the actomyosin ATPase, among other factors. A plateau of the dependence of force on MES concentration at low values of MES, observed in the data but not in the model-derived

curves, is expected due to the presence of other proton-binding species in the solutions.

The practical outcome from these results was that we chose 50 mM pH buffer to minimize pH gradients within the fiber, thus ensuring accurate force measurements. In studies which did not include appropriate pH buffering (e.g., Robertson and Kerrick, 1979, who used imidazole over a wide pH range), force may have been artifactually high, particularly at acid pH. Coincidently, 50 mM is approximately the total in vivo buffering capacity of cat soleus (Kushmerick, 1984) and frog muscle (Curtin, 1986).

pH Dependence of Skinned Fiber Contraction

Almost all studies, on several different fiber types from several species, qualitatively agree that force declines with acidification (Nosek et al., 1987; Rees and Stephenson, 1987; Metzger and Moss, 1987*b*; Pate et al., 1987; Luney and Godt, 1987; Cooke and Pate, 1985; Johnston and Mutungi, 1985; Robertson and Kerrick, 1979; Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978). In contrast, there was no effect of pH on barnacle muscle force (Ashley and Moisescu, 1977). We have observed a quantitatively greater effect of pH on force than has been previously reported for rabbit muscle. This we attribute primarily to minimization of pH heterogeneity within the fiber, but in some cases it may be related to differences in temperature. Because no study (including our own) used an absolute maximum force as a normalization reference, the conditions to which relative changes were referenced need to be considered in cross-comparisons. For example, as pointed out by Rees and Stephenson (1987), the choice of temperature to which data obtained at various temperatures were normalized can influence the apparent steepness of the force-pH relation. Rees and Stephenson showed that maximum Ca^{2+}-activated force at constant pH increased with temperature (1–35°C), and that the slopes of isothermal force-pH relations were similar when all of the data were normalized to the force obtained at pH 7.1 and 25°C. Thus, if their data had been renormalized to a single pH value at the various temperatures, the effect of pH would have appeared greater at low temperature.

Reported effects of pH on shortening velocity are varied. We have shown that acidification reduces shortening velocity and that the decrease in velocity at pH 6.0 was not an artifact of the slack method; velocity was reduced at all force levels. At alkaline pH, velocity was changed little from that at neutral pH. Cooke and Pate (1985), using rabbit psoas fibers, observed no effect of changing pH from 7.0 to 6.5 on V_{max} extrapolated from isotonic shortenings. It is possible that combining unbracketed data from several fibers masked any pH effect on the extrapolated V_{max}s. Metzger and Moss (1987*b*) found that V_{us} of rat fibers declines monotonically from pH 7.0 to 6.2, although they observed less of a decrease than we report for rabbit fibers, and they observed a greater decrease (at pH 6.2) for fast than for slow fibers. Our results are in qualitative agreement with the more recent results of Pate et al. (1987), who reported that psoas velocity declined as pH was decreased from 7.0 to 6.0, and our results are in partial agreement with Luney and Godt (1987), who reported a monotonically increasing relation between psoas velocity and pH between 6.5 and 7.5. Johnston and Mutungi (1985) have also reported depression of turtle muscle velocity at near zero load due to acidification (pH 6), and an almost equal depression of velocity at pH 8. It is not possible at this time to conclude what factors, other than fiber type, may have contributed to the differences in results.

Our results confirm Donaldson and Hermansen's (1978) finding that acidic pH decreases the force of fibers from fast muscles more than that of fibers from slow muscles, and also show that this relationship extends into the basic pH range. Metzger and Moss (1987*b*) have also confirmed this relationship in rat fibers over the pH range 7.0–6.2. Our comparison of fibers from psoas and soleus muscles showed only a quantitative difference in the effect of pH on force. Thus these two muscles, which are distinguished by their very different shortening velocities under the same conditions (e.g., Results) and each of which contains a predominant, distinct myosin isoform, probably have only quantitative differences in the kinetics of the cross-bridge cycle steps probed by altering pH. If this is so, the study of different fiber types can more rigorously test any generally applicable cross-bridge model.

Relevance to Muscle Fatigue

Decrease of intracellular pH due to lactic acid production is correlated with fatigue (Dawson et al., 1978; Renaud et al., 1986). We have shown that decreased pH, but not elevated lactate, caused large decreases in both force and velocity. Our results are in accord with Edman and Mattiazzi's (1981) evidence that intracellular pH change is largely responsible for changes in intact fiber force and velocity (although intracellular pH was not measured in their experiments, it is reasonable to expect that pH varied primarily over the acidic range where both force and velocity decrease with pH), and may at least partly explain the continuous decrease of mouse EDL shortening velocity during tetanic stimulation (Crow and Kushmerick, 1983). Furthermore, experiments on demembranated fibers activated by exogenous Ca^{2+} imply that the primary site at which protons act is the actomyosin interaction, rather than some step related to activation or excitation-contraction coupling. At Ca^{2+} concentrations below that which saturates thin filament regulation, proton-induced shifts of the force-pCa relation (Ashley and Moisescu, 1977; Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978; Robertson and Kerrick, 1979; Metzger and Moss,

1987*b*) provide an additional mechanism by which protons can affect the mechanics of a twitch in intact fibers.

Dawson et al. (1986) proposed that the diprotonated form of P_i is the physiological parameter which determines tetanic force during fatigue. Their hypothesis was based on a linear correlation between force and calculated $H_2PO_4^{1-}$ concentration during prolonged tetanic stimulation of frog muscle. In their experiments, $H_2PO_4^{1-}$ concentration increased for two reasons; (*a*) total P_i concentration increased, due to PCr hydrolysis, and (*b*) pH decreased, thus increasing the fraction of P_i which was in the diprotonated, monovalent form (pK_A, ~6.8). Using single, glycerinated fibers from rabbit psoas, Nosek et al. (1987) found that the relation between $H_2PO_4^{1-}$ concentration and force was very similar to that obtained by Dawson et al. (1986). The similarity between the skinned fiber and whole muscle results is somewhat surprising considering that the effect of pH on force was subtracted from the skinned fiber results (Fig. 1 of Nosek et al., 1987), but not from the intact muscle results (the effect of pH alone could not be subtracted because pH and P_i change in parallel in the protocol used for the intact muscle experiments). Our experiments with demembranated fibers, and other experiments by Kentish (1986) and Eisner et al. (1987) on cardiac muscle, show that pH and P_i independently affect force; it is not the diprotonated form of P_i alone that determines force, as suggested by Dawson et al. (1986). We therefore reject the hypothesis that $H_2PO_4^{1-}$ is the primary determinant of force during fatigue of intact muscle.

The suggestion that force is linearly dependent on $H_2PO_4^{1-}$ is inconsistent with the nonlinear decrease of force as total P_i is increased at constant pH (where $H_2PO_4^{1-}$ is a constant fraction of the total P_i; Kentish, 1986; Cooke and Pate, 1985). Our results are also inconsistent with the hypothesis that $H_2PO_4^{1-}$ is the only ionic form of P_i involved in the mechanism of force production. The main disparity between our results and those of Nosek et al. (1987) is on the effect of P_i at low pH. Nosek et al. (1987) reported a proportionally greater effect of P_i on force at low pH, which is consistent with the data of Cooke and Pate (1985). We observed that the effect of P_i on force is proportionally the same at neutral and low pH (Results and Fig. 8). One difference was that we used half as much total P_i (15 vs. 30 mM), although we would expect qualitatively similar results at all P_i concentrations. Clearly, the effects of P_i are not fully understood and deserve further attention.

As indicated above, fatigue is probably not caused by a single factor but is due to additive and possibly synergistic effects of several factors, with proton accumulation being a major contributor. pH is not the only factor that changes in intact fibers during prolonged stimulation (Dawson et al., 1978; Kushmerick et al., 1983; Kushmerick and Meyer, 1985). Other significant metabolite changes also occur, such as decreased PCr concentration and increased concentrations of ADP and P_i, which have separate effects (Cooke and Pate, 1985; Altringham and Johnston, 1985; Chase and Kushmerick, 1986 and 1988; Luney and Godt, 1987; Pate et al., 1987). The data in Fig. 8 show that although P_i did not affect V_{us} at pH 7.1 and had similar effects on force at pH 7.1 and 6.0, it reduced V_{us} at pH 6.0. In intact muscle, direct measurement of intracellular pH has shown that the decrease in force associated with fatiguing stimulation is larger than the decrease in force due to the pH change alone (Renaud et al., 1986), thus implying that at least one other factor (e.g., P_i or an inadequate amount of Ca^{2+} released to fully activate the contractile apparatus) is involved in causing fatigue. Although our results do not rule out other mechanisms of fatigue (see Introduction), protons clearly play a major role in inhibiting both force and velocity by directly inhibiting the actomyosin interaction during fatigue of fast muscle.

In contrast, aerobic slow muscle shows little or no change in force or velocity during a prolonged tetanus (e.g., Crow and Kushmerick, 1983). Under these conditions the pH changes very little, and transiently in a direction opposite to that of fast muscle (Kushmerick et al., 1983). Based on the results from skinned fibers, one would not expect any large changes in force and velocity during normal activity of a predominantly slow muscle because activity is accompanied by only small pH changes.

Mechanism(s) of pH Effects on Force and Velocity

Two steps in the actomyosin ATPase cycle which involve protons have been characterized: (*a*) proton release with P_i (product release from ATP hydrolysis), a step which is common to all ATPases, and (*b*) the release of a proton from myosin (presumably the result of a conformational change that alters the proton binding affinity of one or more ionizable groups) after ATP binding but before hydrolysis, and reciprocal proton absorption temporally proximal to the ADP release step (Bagshaw and Trentham, 1974; Koretz and Taylor, 1975). In addition, there may be other sites of proton action which, like step *b*, are not necessarily associated with the catalytic site of ATP hydrolysis.

According to the first mechanism, one would expect protons to act similarly to P_i, (*a*) decreasing force but (*b*) having little effect on velocity (Fig. 8; Cooke and Pate, 1985; Altringham and Johnston, 1985; Chase and Kushmerick, 1986; Luney and Godt, 1987). Although *a* is qualitatively correct, pH has a greater effect on force than does P_i with no apparent saturation over the range studied (6–8). Proposition *b* is clearly contradictory to what was observed. Thus, we conclude that protons do not exert their effect only through reversal of the P_i/proton release step in the ATP hydrolysis cycle.

The second mode of proton action described above does not satisfactorily explain the observed effects either. The

site(s) involved has (have) an apparent $pK_A > 8$ (Koretz and Taylor, 1975); thus, this mode of action would be important only at high pH and would not be involved at low pH, where protons have major effects on both force and velocity. Therefore the mechanism by which protons affect muscle contractility remains to be explained.

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