pH-induced effects on median frequency and conduction velocity of the myoelectric signal

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BRODY, LEE R., MARK T. POLLOCK, SERGE H. ROY, CARLOS J. DE LUCA, AND BARTOLOMÉ CELLI. *pH-induced effects on median frequency and conduction velocity of the myoelectric signal.* J. Appl. Physiol. 71(5): 1878–1885, 1991.—H^+ accumulation at the sarcolemma is believed to play a key role in determining the electrophysiological correlates of fatigue. This paper describes an in vitro method to externally manipulate muscle pH while measuring the resultant effect on surface-detected median frequency (MDF) and conduction velocity (CV) parameters. Hamster muscle diaphragm strips (n = 8) were isolated with the phrenic nerve intact and placed in an oxygenated Krebs bath (26°C). The muscle was clamped to a noncompliant load cell to measure isometric contractile tension. Tetanic contraction was developed via 40-Hz supermaximal stimulation of the phrenic nerve. Differential signals were recorded from three electromyogram (EMG) detection surfaces for computation of CV (via the phase shift in the EMG signals) and MDF. Repeated trials were conducted at bath pHs of 7.4, 7.0, and 6.6. Bath pH was altered by aerating predetermined concentrations of O_2 and CO_2 into the bath. Decreases in bath pH resulted in decreases in both initial MDF and initial CV. The differences in initial MDF and initial CV were significant ($P < 0.001$) for each of the bath pH conditions. In general, the change in bath pH resulted in an equal percent change in initial MDF and initial CV. This suggests that the change in bath pH caused a decrease in CV without significantly altering the fundamental shape of the M wave. In contrast, the EMG was altered differently during stimulated contractions. During stimulation, the rate of decay of CV was 65% of the rate of decay of MDF. Because the rate of decay was unequal during stimulation, it is concluded that the fundamental M-wave shape was significantly altered in addition to changes in the CV. These results indicate that *1*) the MDF-CV relationship is situational and dependent on the specific biochemical changes in effect and *2*) during a sustained contraction, the change in MDF is caused by more than a change in pH and CV.

electromyogram; fatigue; electrical stimulation; diaphragm muscle; muscle pH

SURFACE ELECTROMYOGRAPHY is increasingly being used to provide objective measures of localized muscle fatigue in both the clinical and research environments. For nearly 80 years, the frequency components of the surface myoelectric (ME) signal have been known to change in a predictable way during forceful isometric contractions (20). This spectral shift can be easily exploited by measuring a characteristic component of the ME signal power density spectrum. The spectral parameter that most accurately reflects spectral shifts has been shown to be the median frequency (MDF) (18, 25). Despite widespread use of spectral analysis of the ME signal, the mechanisms that cause the frequency shift have not been clearly identified.

A mathematical model described by Lindstrom et al. (14) and supported by Stulen and De Luca (25) argues that a decrease in the average muscle fiber conduction velocity (CV) will cause a spectral shift. From a theoretical standpoint, a decrease in the velocity at which action potentials travel past a stationary detection electrode will result in a decrease in the spectral content of the recorded waveform. This has been supported by many empirical studies that describe a linear relationship between spectral parameters and CV during sustained isometric contractions (2, 8, 24, 26).

The physiological factors that affect MDF and CV are not well established. Typically, studies that have attempted to describe this relationship have included in vivo human investigations that have measured a particular physiological parameter (i.e., pH) and associated its change with characteristic changes of the ME signal during fatigue (5, 16). This approach is limited by the inability to distinguish between physiological changes that cause the observed ME signal changes and those that do not affect the ME signal. Factors such as substrate depletion (e.g., ATP and phosphocreatine), accumulation of metabolites (e.g., P_i and ADP), and motor unit recruitment have not been controlled during these indirect attempts at establishing a causal relationship between the ME signal and specific biochemical components. Therefore, previous approaches were limited to studying associative relationships, which merely described cooccurrence of biochemical and electrical changes. Causal relationships can be more directly established using an in vitro model. The advantage of this approach is that the muscle environment can be altered in a controlled manner so that a specific intramuscular change is induced without introducing confounding fatigue processes.

The purpose of this study was to selectively alter the pH of a nerve-muscle preparation and record the resultant alterations in the ME signals. Specifically, we determined the effects of bath pH on the MDF and CV recorded from an isolated diaphragm. We also addressed the question of whether the relationship between MDF and CV during induced changes in bath pH is different

from that during stimulated contractions that fatigue the muscle preparation. This analysis was conducted to determine whether the changes in MDF that occur during fatigue are the result of more than just changes in muscle pH.

MATERIALS AND METHODS

Animal preparation. Eight adult LVG Syrian Golden hamsters (Charles River Laboratories, Wilmington, MA) were used in this study. After an injection of pentobarbital sodium (Nembutal, 6 mg/100 g body wt ip) a tracheostomy was performed via a cervical incision and the animal was connected to a small rodent ventilator. The left phrenic nerve was isolated from its entry into the thorax to the diaphragm. The diaphragm, phrenic nerve, and attached ribs were rapidly removed after exsanguination and placed in oxygenated cold Krebs solution (4°C) containing (mM) 124 NaCl, 4 KCl, 1 $MgCl_2$, 1 KH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, and 6.5 glucose. A 5-mm-wide costal diaphragm strip with intact phrenic nerve was isolated using a template, and its tendons were placed between two Plexiglas clamps. The strip was then placed in a jacketed bath containing Krebs solution maintained at 26°C by a circulating pump. The solution was continuously aerated with 95% O_2-5% CO_2, which maintained the extracellular pH (pH_e) at 7.4. The clamps were mounted on a stand that allowed for the adjustment of muscle length and for the measurement of isometric force. The unattached end of the nerve was hooked to a bipolar stimulating electrode. After a 30-min equilibration period at pH_e 7.4, supermaximal stimulating voltage was determined by progressively increasing voltage until peak twitch tension was maximal; the voltage was set at 1.5 times this value. This voltage setting produced a maximal twitch tension at all three pH values during preliminary experiments, indicating that the voltage was supermaximal in all the experimental conditions. The muscle was then adjusted to its optimal length, defined as the length at which peak twitch amplitude could be generated. The supermaximal electrical impulses were delivered at 40 Hz (0.2-ms pulse duration) for 3 s through a stimulus isolation unit. The complete setup of the experiment may be seen in Fig. 1.

Experimental procedures. Experiments were designed to evaluate the effects of bath pH changes on the ME signal MDF and CV. Each strip was exposed to normal bath conditions (pH_e 7.4) and two levels of respiratory acidosis (pH_e 7.0 and 6.6). Decreases in bath pH were achieved by increasing the CO_2 concentration of the aerating gas mixture. Solutions at these pH levels were previously equilibrated, and pH changes were achieved by a drain-and-replace technique. The sequence of pH exposure was randomized to either 7.4, 7.0, and 6.6 or 6.6, 7.0, and 7.4. Three repeated contractions were performed at each bath pH level, with a 5-min rest period allowed between successive contractions. During preliminary experiments, a 5-min recovery period ensured complete recovery as determined by the return of tension, MDF, and CV to baseline. A 15-min equilibration period between bath pH changes was allowed to ensure a static relationship between pH_e and resting intracellular pH (pH_i). This equilibration period was shown to be sufficient by us in preliminary experiments by noting stable values of tension, MDF, and CV.

Bath pH was continuously monitored with a pH meter. The bath solution was analyzed for P_{CO_2} and P_{O_2} by a System 1304 blood gas analyzer calibrated between samples. The pH, P_{CO_2}, P_{O_2}, and temperature were recorded twice for each bath condition.

ME signal detection technique. A special electrode probe with three detection bars was developed for differential detection of ME signal activity (Fig. 2). The detection bars were made of compliant gold-plated springs so that their presence minimally affected the contractile properties of the muscle preparation. The interelectrode spacing was 2.38 mm. The detection bars were mounted on a pivoting arm, so that the position of the bars with respect to the muscle could be easily adjusted by a micromanipulator. Care was taken to place the bars perpendicular to the direction of fiber orientation. The detection array was placed so that all three bars were located on the distal side of the motor end-plate region.

Because of the size constraints and impedance properties of the bath, signal-conditioning circuitry was located both inside and outside of the bath. High-impedance buffer amplifiers (gain = 1) were connected directly to each detection surface in the bath. Insulated cable connected the buffers to a circuitry box located near the bath. Functionally, the circuitry box consists of an instrumentation amplifier (differential gain = 10), a bandpass filter (20–2,000 Hz), and an adjustable gain (range 10–500).

The conditioning circuitry provided three differential ME signals. The differential output from the outermost two bars (*bar 1 − bar 3*) was used to compute MDF. A comparison of the other two differential signals [(*bar 1 − bar 2*) and (*bar 2 − bar 3*)] was used to provide an estimate of average muscle fiber CV (see *ME signal processing*).

ME signal processing. The ME signals and stimulus synchronization pulse were recorded on analog tape. The ME signals were differentially amplified with a band pass of 1–1,000 Hz and sampled at a rate of 4,096 Hz. The samples were digitized by a twelve-bit analog-to-digital converter and stored on the disk of a IBM PC. To reduce signal noise, both time-windowing and signal-averaging techniques were implemented. Only a 16-ms window after each stimulation pulse was actually digitized; the 1st ms was later discarded, eliminating the stimulus artifact in the response. Twenty successive responses were then averaged, yielding one average 15-ms M wave representing each 0.5-s epoch of the ME signal.

MDF and CV were computed with numerical algorithms. MDF was calculated by zero padding each epoch response, resulting in a frequency resolution of 2 Hz. CV was computed as d/τ, where d = 2.38 mm and τ was the time delay between the two ME signals. The delay was calculated by identifying the time shift required to minimize the mean squared error between the two Fourier transforms by use of a method described previously (15).

Results are presented as means ± SD. A one-way analysis of variance (repeated-measure design) was con-

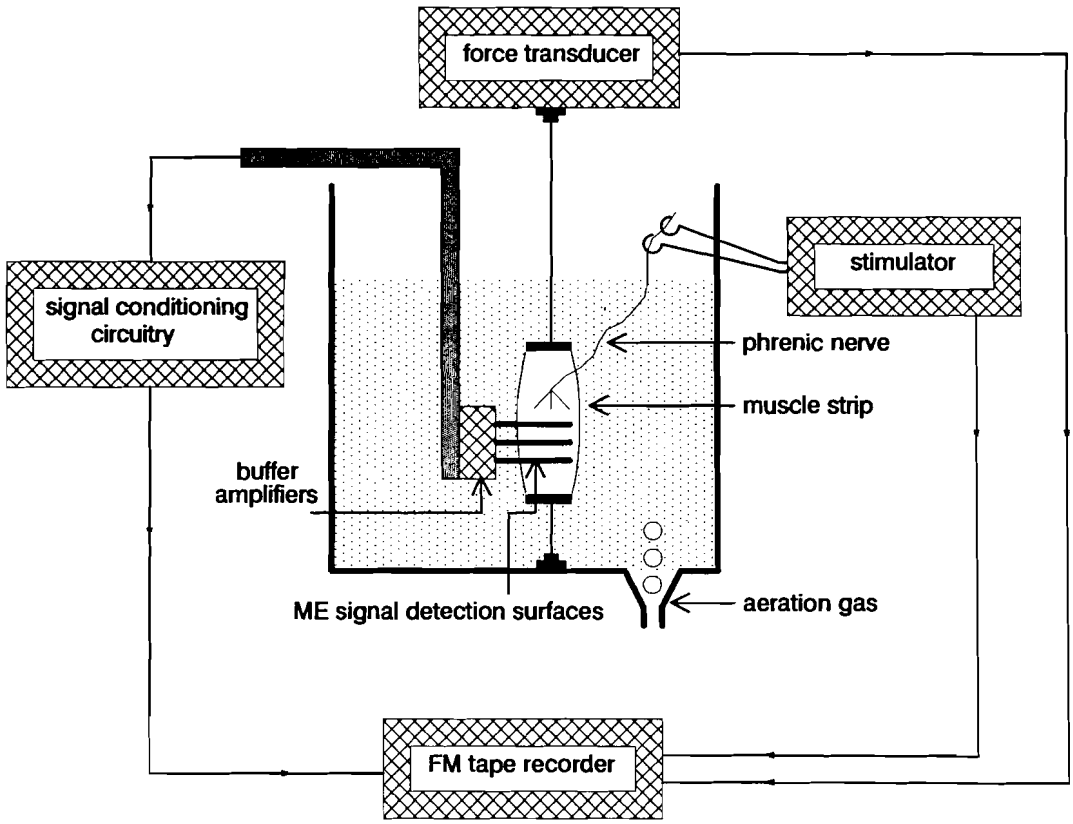

FIG. 1. Schematic representation of nerve-muscle preparation located in experimental chamber. ME, myoelectric.

ducted to determine whether bath pH resulted in a significant change in initial MDF and initial CV. Pairwise comparisons for normalized initial MDF and normalized initial CV were conducted for data at pH 7.0 and 6.6 to determine whether changes in bath pH resulted in equal percent changes in initial MDF and initial CV. The percent change in MDF and CV during elicited contractions was also analyzed using a pairwise comparison for the three bath pH conditions. Statistical significance was determined as $P < 0.01$.

RESULTS

Typical MDF vs. time and CV vs. time plots for one preparation are presented in Fig. 3. In general, the initial values of both parameters were highly repeatable for the three consecutive contractions at each bath pH. There were no cases in which the three initial values of MDF or CV varied around their mean by >3%. Initial values of MDF and CV are defined as values calculated at $t = 0.25$ s, and final values of MDF and CV are defined at $t = 2.75$ s. In two preparations, a total of four contractions were eliminated from the analysis. In both of these preparations, the first contraction after an increase in bath pH (from 6.6 to 7.0 and from 7.0 to 7.4) was excluded for the same reason. In these four instances, the initial values of MDF and CV of the second contraction increased further change after the bath pH, indicating that the nerve-muscle preparation had not come to metabolic equilibrium with the bathing solution.

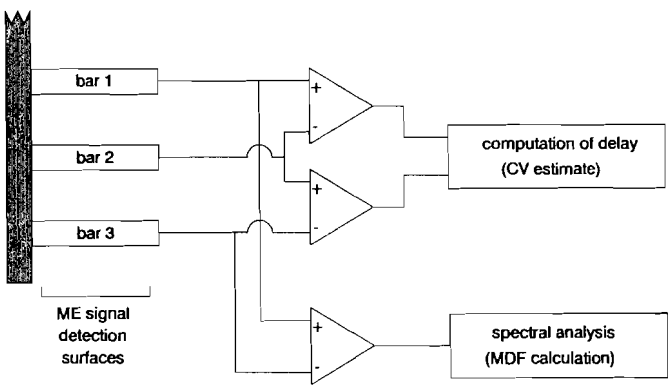

FIG. 2. Schematic representation of ME signal detection configuration. MDF, median frequency; CV, conduction velocity.

Effects of bath pH on the initial values of MDF and CV. The mean initial MDF vs. bath pH and mean initial CV vs. bath pH are shown in Fig. 4. The results were similar regardless of the ordering of pH changes. In each preparation, the initial value was normalized with respect to the average of the three initial values at pH 7.4. The mean initial MDF was 1.000 ± 0.008, and the mean initial CV was 1.000 ± 0.012. The relatively low standard deviation is representative of the high repeatability of the ME signal recordings at this pH level. Decreasing the bath pH resulted in decreases in both initial MDF and initial CV in each of the eight preparations. The mean normalized initial MDF at bath pH 7.0 was 0.948 ± 0.027 and 0.854 ± 0.029 at bath pH 6.6. The mean normalized initial CV at bath pH 7.0 was 0.947 ± 0.033 and 0.863 ± 0.035 at bath pH 6.6. The differences in the initial values (both initial MDF and initial CV) at different pHs were significant ($P < 0.01$) in all cases. In general, the bath pH

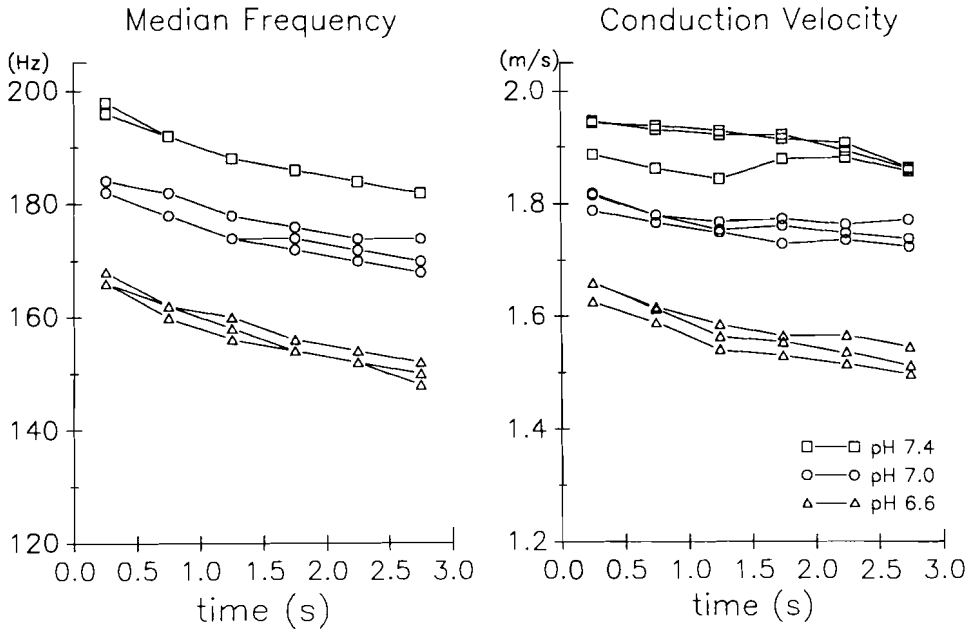

FIG. 3. Typical set of ME data for 1 preparation. Time course of MDF and CV are shown for different bath pH conditions. □, Contractions at bath pH 7.4; ○, contractions at bath pH 7.0; △, contractions at bath pH 6.6.

change resulted in an equal percent change in initial MDF and initial CV. There was no statistically significant difference between normalized initial MDF and initial CV values at pH 7.0 or 6.6.

Changes in MDF and CV during contractions. Mean percent changes of MDF and CV during contraction are presented vs. bath pH in Fig. 5. Percent change is defined as

$$\text{percent change} = \frac{(\text{initial value} - \text{final value}) \times 100\%}{\text{initial value}}$$

At bath pH 7.4, MDF decreased by 11.25 ± 1.95% and CV decreased by 7.20 ± 2.77%. At bath pH 7.0, MDF decreased by 10.86 ± 2.35% and CV decreased by 6.43 ± 2.44%. At bath pH 6.6, MDF decreased by 12.80 ± 2.56% and CV decreased by 8.54 ± 2.09%. The mean percent decrease in MDF was significantly greater than the mean percent decrease in CV at all three bath pHs ($P < 0.01$). On the average, the rate of decay of CV was only 65% that of MDF.

Relationship between MDF and CV. A plot of MDF vs. CV is presented in Fig. 6. A linear MDF-CV relationship during a contraction is evident for each of the three bath pHs. Least squared regression lines ($r > 0.99$) were fit to the data points for each bath pH. The slopes of the regression lines are similar, indicating that the MDF-CV relationship during stimulated contractions is similar at each bath pH. The mean value of the three slopes is 1.56 ± 0.11. There is also a linear relationship between initial MDF and initial CV across bath pHs ($r > 0.99$). However, the slope of this regression line ($m = 1.07$) is quite different from the slopes of the regression lines corresponding to data from stimulated contractions at a particular bath pH. This difference in slope reflects the different relationship between initial MDF and initial CV after changes in bath pH and the relationship between MDF and CV during stimulated contractions.

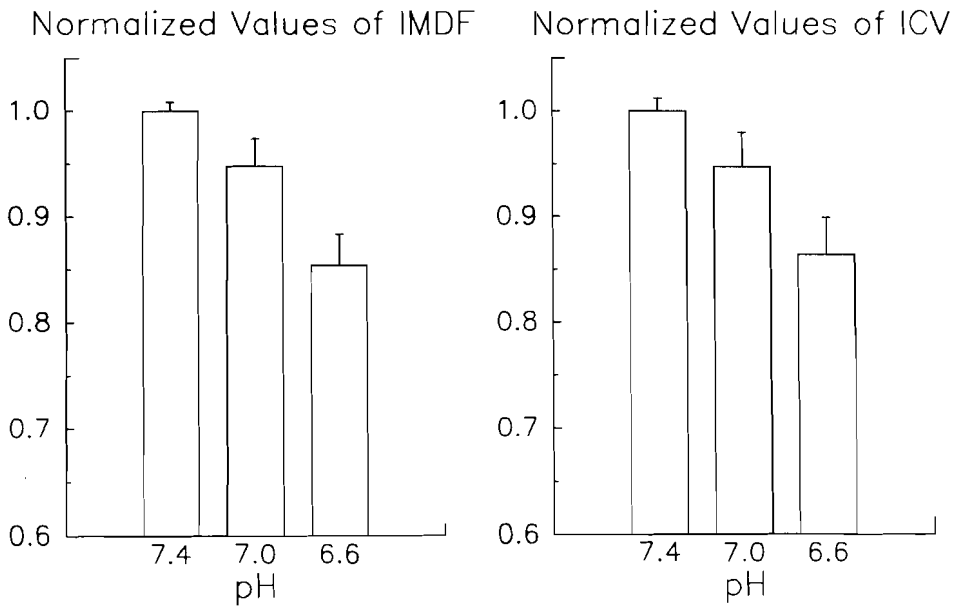

FIG. 4. Initial MDF (IMDF) and initial CV (ICV) vs. bath pH for all 8 preparations (means ± SD, $n = 68$). Each parameter is normalized with respect to data at bath pH 7.4. At bath pH 7.0 and 6.6, normalized values of initial MDF and initial CV are not significantly different, indicating that changes in bath pH resulted in an equal percent change of MDF and CV.

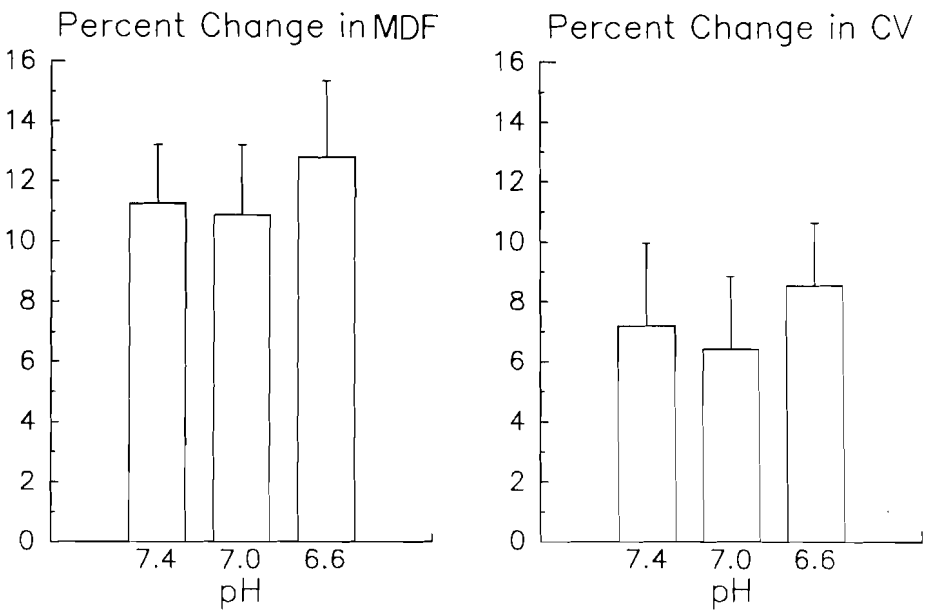

FIG. 5. Percent changes in MDF and CV as a function of bath pH during 3-s elicited contractions (means ± SD, n = 68). In all 3 bath pH conditions, percent change in MDF was greater than percent change in CV.

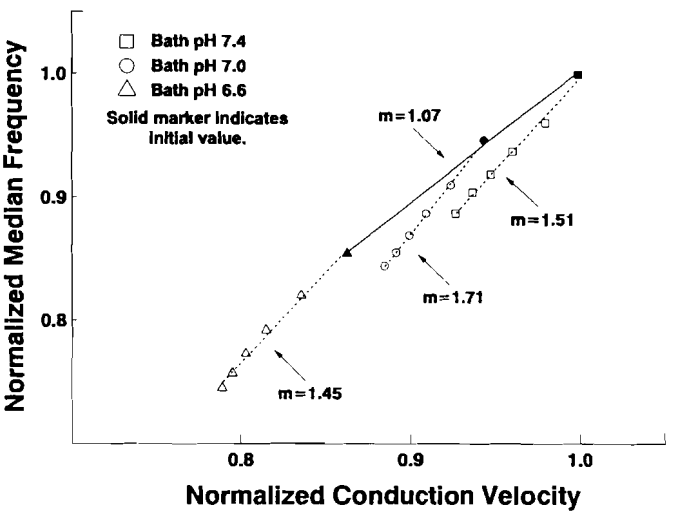

FIG. 6. Mean normalized MDF vs. CV for all 8 preparations for all 3 bath pH conditions. Results are normalized with respect to initial MDF and initial CV at bath pH 7.4. Regression lines are drawn through data points representing elicited contractions at bath pH 7.4 [squares: slope (m) = 1.51], bath pH 7.0 (circles: m = 1.71), and bath pH 6.6 (triangles: m = 1.45). Also a regression line is drawn through initial values of MDF and CV at the 3 bath conditions (filled symbols: m = 1.07). Different slope of this last regression line reflects different relationship between MDF and CV during stimulated contractions compared with relationship between initial MDF and initial CV after changes in bath pH.

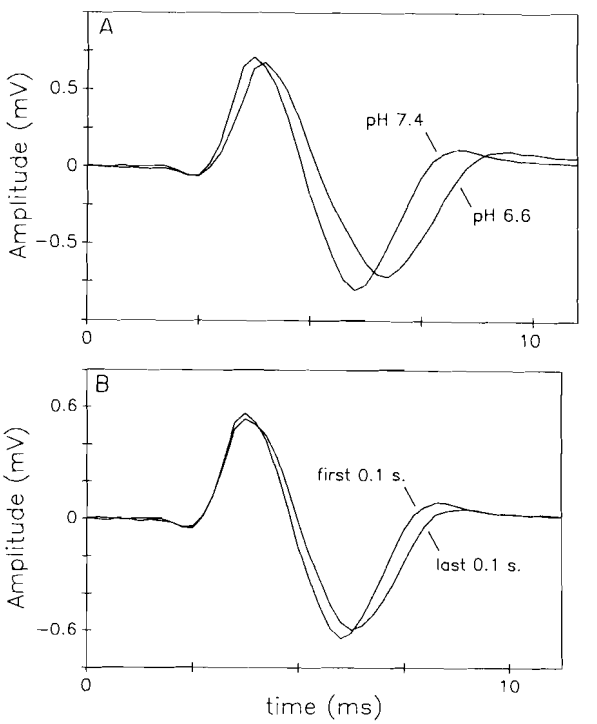

FIG. 7. M-wave widening as a function of bath pH and as a function of time during elicited contractions. *A*: 2 M waves from beginning of 2 separate contractions (same preparation) at bath pH 7.4 and pH 6.6. *B*: 2 M waves from beginning and end of 3-s contraction.

DISCUSSION

The spectral changes reported in this paper were solely due to changes in the M wave, because firing rate was kept constant by the stimulator. Therefore a decrease in the ME signal's MDF corresponds to a widening of the individual M wave (7). This widening of the M wave can be the result of decreases in the CV of the action potentials, changes in the fundamental shape of the M wave independent of CV (several possible physiological causes will be discussed), or a combination of both CV-induced changes and fundamental shape changes. M-wave widening occurred both as a result of a change in the bath pH and during sustained contractions. Both cases are depicted in Fig. 7. Figure 7*A* presents an M wave from the first 0.1 s of a contraction at pH 7.4 and an M wave from the first 0.1 s of a contraction at pH 6.6. Figure 7*B* presents an M wave from the first 0.1 s of a contraction and one from the last 0.1 s of the same contraction at pH 7.4. In both cases the widening of the M-wave shape resulted in the observed decrease in MDF.

CV acts as a linear operator on the action potential spectrum (7). When the CV of an action potential is scaled, its spectrum is scaled by the same factor. This relationship holds true for the spectrum of the M wave, although noise is effectively added to the relationship because the spectrum of the M wave is formed by a non-

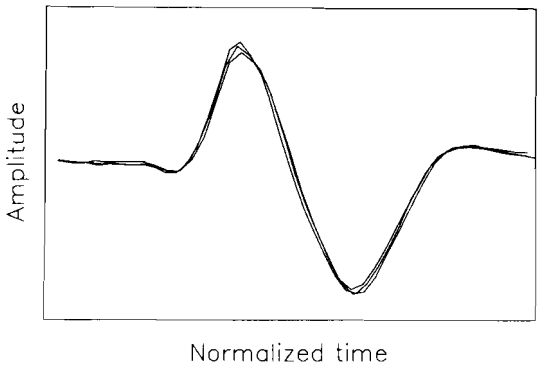

FIG. 8. Three time-scaled M waves from contractions at 3 different bath pHs are superimposed. Each M wave is from first 0.1 s of contraction at bath pH 7.4, 7.0, and 6.6. M waves are time scaled by normalizing their CVs to that obtained at pH 7.4. Time scaling resulted in identical waveforms, indicating that waveshape changes that occurred when bath pH was altered were due to CV changes.

linear unpredictable summation of the spectra of the individual action potentials. Therefore, when the CV of the action potentials changes with no concurrent change in the fundamental shape of the potentials, equal percent changes in CV and MDF occur.

MDF changes may also occur with no concurrent CV change. An example of a mechanism that would produce spectral changes without CV changes is the alteration of the fundamental shapes of the muscle fiber action potentials that comprise the M wave. Changes in the individual action potential waveshapes would cause their summation (the M wave) to change shape without a change in the average CV.

The present study has shown that a decrease in bath pH results in a significant decrease in both MDF and CV. This is in agreement with the concept that the accumulation of metabolic byproducts causes a decrease in both MDF and CV (7, 18). It was observed that the percent changes in initial MDF and initial CV were nearly equal, thereby indicating that changes in bath pH caused changes in the MDF simply through a change in CV with no accompanying fundamental shape to the M wave. This concept is illustrated in Fig. 8. An M wave from the first 0.1 s of a contraction at bath pH 7.4, 7.0, and 6.6 is superimposed. The CVs at pH 7.0 and 6.6 are normalized to that of pH 7.4 by time scaling the two waveforms. The resulting waveforms illustrate that there is no significant change in the fundamental M-wave shape at different bath pHs.

This result may be contrasted to our observations of changes in the ME signal during the 3-s sustained contractions. In this case, CV changed by much less than MDF. Depicted in Fig. 9 are three M waves from a stimulated contraction at bath pH 7.4. One of the M waves is from the first 0.1 s of the contraction, one from the middle of the contraction, and one from the last 0.1 s of the contraction. When the CVs were again normalized to that of the beginning of the contraction, we observed a systematic widening of the repolarization phase of the M wave. Therefore the spectral changes revealed by MDF decay during contraction were due both to changes in CV and changes in the fundamental shape of the M wave.

Theoretically, several possible mechanisms can produce a change in the fundamental shape of the M wave during stimulated contractions. These possibilities can be aggregated into three categories.

A stimulation pulse delivered at the costal end of the phrenic nerve produces an M wave at the ME signal detection electrode. A delay between the stimulus and the response arises from the propagation times along the nerve (presynaptic), across the neuromuscular junction (synaptic), and along the muscle fibers proximal to the detecting electrode (postsynaptic). One possible mechanism for the fundamental shape change observed during stimulation is that nonuniform changes in the delay between stimulation pulse and individual muscle fiber action potential caused the action potentials to sum, forming an altered shape. Theoretically, any one of the three components of the delay could contribute to this mechanism. However, this mechanism has been eliminated as a possible cause of the M-wave shape changes because changes in all phases of the M-wave shape would be expected. This prediction is in contrast with the observed shape changes that were limited to the repolarization phase (Fig. 9).

Another possible mechanism that could have caused the observed M-wave shape changes is the derecruitment of fibers during the stimulation. The shape change would be produced by gradually eliminated components of the M wave. This mechanism was also eliminated as a possibility because it would also produce a decrease in the total energy of the M wave. This is in contrast with our observations as depicted in Fig. 9.

The third (and most likely) possible mechanism that would produce the observed M-wave shape changes is a change in the fundamental shape of the individual muscle fiber action potentials. Changes in membrane ion gradients are believed to play a key role in action potential waveform changes, because the shape of the waveform is determined by rates of depolarization and repolarization of the sarcolemma. It is known that changes in both Na^+ and K^+ gradients (across the sarcolemma) occur during sustained contractions, producing muscle fatigue (12). This explanation comfortably explains how some phases of the M wave remain intact, while only the repolarization phase undergoes distortion during fatigue.

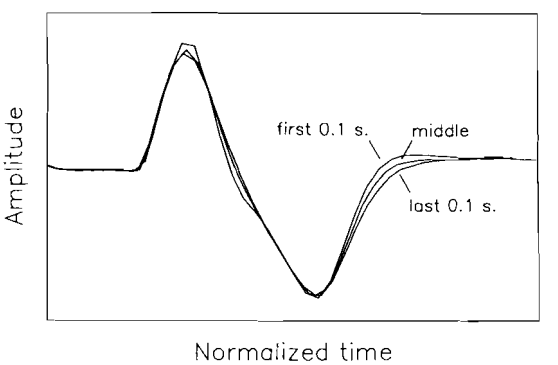

FIG. 9. Three time-scaled M waves from 1 contraction are superimposed. M waves are taken from beginning, middle, and end of contraction at bath pH 7.4. M waves are time scaled by normalizing CV to its initial value. This normalization procedure results in waveforms that have a systematic widening of repolarization phase, indicating that waveshape changes that occurred during stimulation cannot be solely attributed to CV changes.

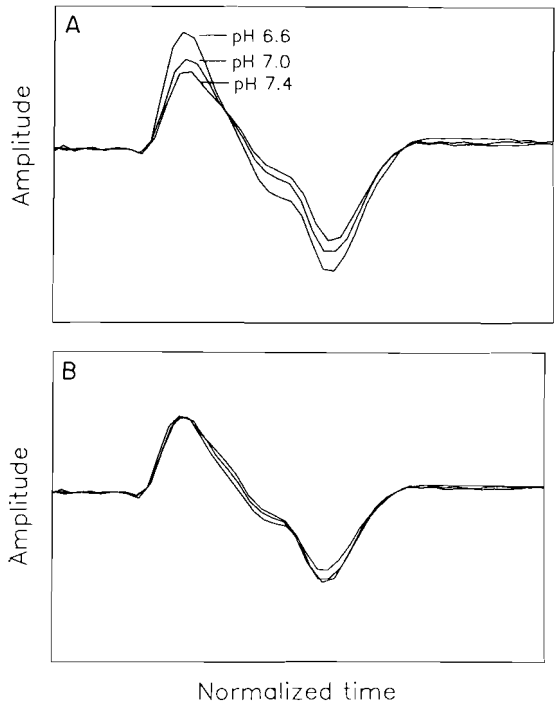

FIG. 10. Three time-scaled M waves from contractions at 3 different bath pHs are superimposed. Each M wave is from the first 0.1 s of contractions at bath pH 6.6, 7.0, and 7.4. M waves are time scaled by normalizing their CVs to that obtained at pH 7.4. Data were collected by starting at bath pH 6.6 and ending at pH 7.4. *A*: progressive loss of total energy in M waves as bath pH is increased from 6.6 to 7.4. To better observe M-wave shape changes due to this bath pH sequence, time-scaled waveforms are amplitude scaled to normalize total energy in each M wave to that at bath pH 7.4 (*B*). When these M waves are both time and amplitude scaled, shapes are nearly identical, indicating that this bath pH sequence caused amplitude scaling but no systematic waveshape changes (other than time scaling due to CV changes).

Therefore it appears that the changes in MDF that occurred during stimulation were due to both CV changes and changes in the fundamental shape of the individual muscle fiber action potentials.

It must be noted that the sequence of bath pH levels (7.4, 7.0, and 6.6 vs. 6.6, 7.0, and 7.4), while having no statistical effect on the MDF-CV relationship, did have an effect on the changes observed in the M-wave shape. Figures 7–9 present typical changes in the M-wave shape observed during preparations that began at pH 7.4 and ended at pH 6.6. When the pH order was reversed, an additional change in the M-wave shape was noted. During this reversed pH sequence, we observed a systematic decrease in the energy of the M wave across bath pH. This behavior is presented in Fig. 10*A*, where the M waves from the first 0.1 s of a stimulated contraction at pH 6.6, 7.0, and 7.4 are superimposed. The CV values at pH 6.6 and 7.0 are normalized to that of pH 7.4. As the bath pH is increased, the total energy in the M wave systematically decreased.

In Fig. 10*B*, the energy in each of the M waves is normalized to that obtained at pH 7.4. It is apparent that the M-wave shape is consistent in all cases. The changes observed in the shape of the M wave for decreases in bath pH were more consistent than those observed for the reversed pH sequence. In all cases tested during the reversed pH sequence, the amplitude and waveshape observed at pH 7.4 did not completely recover to the initial values of tests that started at pH 7.4. This incomplete recovery could be the result of failure of the ion pumps to restore polarization after being kept at acid pH, development of unbuffered intracellular acidosis, or selective failure of neuromuscular transmission.

We did not directly address the issue of whether the pH_e or pH_i modification affects the CV of the ME signal. Although we did not measure pH_i, we note that Adler et al. (1) documented the relationship between bath pH and pH_i under experimental conditions nearly identical to ours. His results indicate that pH_i is significantly lowered in both of our acidosis conditions (bath pH 7.0 and 6.6). We strongly suspect that the ME signal CV is related specifically to pH_i. This is supported by theoretical models of mechanisms of CV changes, our preliminary studies, and work performed by Juel (11), who has shown that the single-fiber action potential CV decreased with decreases in pH_i and was independent of pH_e in mouse skeletal muscles.

In this study we have shown that a change in pH caused changes in both the MDF and CV. In apparent contrast, in a recent study of myophosphorylase-deficient patients, Mills and Edwards (17) observed a considerable decrease in the spectral parameters with no accompanying change in muscle pH. Although it is certainly possible that factors other than the pH may cause the spectral parameters to change, the work of Mills and Edwards is ambiguous in this regard, because they measured the spectral changes during a force-decreasing isometric contraction. It has been well established (6, 23) that the value of the spectral parameters of the ME signal decreases as the force output of the muscle decreases. Thus, their work leaves uncertainties about the relationship of pH and the value of the spectral parameters. Furthermore the fact that we observed divergent behavior between MDF and CV during stimulated contractions indicates that spectral changes can be independent of pH. It is therefore likely that several factors beside pH affect the ME signal spectrum.

Although the majority of studies indicate a correlative relationship between pH and ME signal spectral parameters, there are some notable exceptions. These exceptions, which describe divergent behaviors of pH and ME signal spectral parameters, occur during the recovery phases of ME signal and pH after fatiguing contractions. In particular, using ^{31}P nuclear magnetic resonance spectroscopy, Miller et al. (16) showed that, after a fatiguing sequence of the adductor pollicis muscle, changes in the M-wave shape recovered within 4 min, while pH took >20 min to recover to its prefatigue state. We have also observed these trends in our ^{31}P nuclear magnetic resonance spectroscopy studies, in which we noted a faster recovery of MDF than pH in the tibialis anterior (22). This disparity indicates the possibility that, at least under certain experimental conditions, the causal relationship between pH and the ME signal during fatigue is different from that during recovery.

There is considerable debate in the literature concerning the relationship between ME signal spectral parameters and CV. Although some studies have described a relationship of direct proportionality between spectral

parameters and CV (2, 8, 24), others have noted exceptions where spectral parameters decreased with no or limited concurrent decrease in CV (4, 19, 26). Our results help clarify these seemingly contradictory reports. We have observed that the changes in MDF during sustained contractions are due to both CV-induced changes and changes produced by mechanisms independent of CV. Therefore, spectral changes are not completely reflective of CV changes, and occasional deviations from a direct proportionality between the two should be expected. Furthermore the MDF-CV relationship can be thought of as situational, in which the specific biochemical changes that occur during a muscle task dictate the relationship.

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