Muscle buffer capacity estimated from pH changes during rest-to-work transitions

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ADAMS, GREGORY R., JEANNE M. FOLEY, AND RONALD A. MEYER. *Muscle buffer capacity estimated from pH changes during rest-to-work transitions.* J. Appl. Physiol. 69(3): 968–972, 1990.—Gated phosphorus nuclear magnetic resonance (^{31}P-NMR) spectra were acquired after 5 or 9 s of 5-Hz stimulation in rat and cat skeletal muscles, respectively. Net phosphocreatine (PCr) hydrolysis was associated with an intracellular alkalinization of 0.08 ± 0.01 and 0.05 ± 0.003 pH units in isolated perfused cat biceps and soleus, respectively, and 0.12 ± 0.02 in the superficial predominantly fast-twitch white portion of gastrocnemius of anesthetized rats. The net change in $[H^+]$ expected from PCr hydrolysis was calculated, and apparent buffer capacity (β) in intact muscles was calculated from $\beta = \Delta[H^+]/\Delta pH$. The β of the same muscle types was also estimated from titration of muscle homogenates between pH 6.0 and 8.0. The contribution of P_i to total β of the homogenates was subtracted to ascertain the non-P_i β for each muscle. The non-P_i β values were added to the actual amount of P_i present in the stimulated muscles to calculate a predicted β at pH 7. The apparent β calculated from PCr and pH changes in intact muscles and the predicted β from homogenate titrations were in good agreement (38 ± 9 vs. 38 slykes in cat biceps, 21 ± 7 vs. 30 in cat soleus, and 30 ± 6 vs. 27 in rat gastrocnemius). The results indicate that changes in pH during the first few seconds of contraction can be entirely accounted for by proton consumption via net PCr hydrolysis.

alkalinization; phosphorus nuclear magnetic resonance spectroscopy; rat; cat

THE INTRACELLULAR pH of skeletal muscle becomes transiently alkaline within seconds after the onset of a series of twitch contractions (12, 17, 18). In many recent studies using phosphorus nuclear magnetic resonance (^{31}P-NMR) for pH measurements (4, 8, 12, 17, 18), both the magnitude and time course of the initial alkalinization suggest that it is at least partly due to proton consumption associated with net phosphocreatine (PCr) hydrolysis

$$\begin{array}{l} ATP + H_2O \longrightarrow ADP + P_i + \alpha_a\ H^+ \\ PCr + ADP + H^+ \longleftrightarrow creatine + ATP \\ \hline PCr + H_2O + \alpha_p\ H^+ \longrightarrow creatine + P_i \end{array}$$

where the stoichiometric coefficient $\alpha_p = (1 - \alpha_a)$ is 0.4 at pH 7 and increases at lower pH (6, 8). For example, if an intracellular buffer capacity of ~40 slykes is assumed for mammalian skeletal muscle (2, 3, 15), net hydrolysis of 10 μmol/g PCr at pH 7 should produce a maximum alkalinization of 0.10 pH units. This small alkalinization is consistent with what is typically observed in NMR studies, suggesting that PCr hydrolysis might be entirely responsible for the transient alkalinization. In contrast, based on multiequilibrium calculations from metabolic data, Connett (5) recently reported a much larger transient alkalinization (0.75 pH units) after 5 s of twitch contraction in dog gracilis muscle. This peak alkalinization is much too large to account for by net PCr hydrolysis alone. The calculated alkalinization was reversed after 15 s, and thus the early peak alkalinization would have been missed in NMR studies in which data accumulation was averaged over the first 15 s or more of a contraction series (9, 11).

The purpose of this study was to determine whether the transient alkalinization observed during the first few seconds of a series of contractions can be quantitatively accounted for by net PCr hydrolysis. This was accomplished by comparing the buffer capacities (β, slykes) of three different mammalian muscles calculated from titrations of muscle homogenates in vitro with their buffer capacities estimated from the observed alkalinization during brief series of contractions. The latter was calculated assuming that net PCr hydrolysis was the only significant metabolic reaction affecting pH, i.e., $\Delta[H^+] = \alpha_p \times \Delta[PCr]$, and hence

$$\beta = \alpha_p \times \Delta[PCr]/\Delta pH \qquad (1)$$

PCr and pH were measured at discrete times by gating acquisition of ^{31}P-NMR scans to specific times during and after repeated bursts of 5-Hz contractions.

METHODS

^{31}P-NMR studies. These studies were performed on three animal muscle preparations described previously: the superficial 2- to 3-mm (predominantly fast-twitch glycolytic) portion of the rat gastrocnemius muscle in situ (8, 11) and the isolated arterially perfused cat biceps brachii (fast-twitch) and soleus (slow-twitch) muscles (9, 12).

Male Sprague-Dawley rats (300–350 g) were anesthetized with pentobarbital sodium (50 mg/kg ip), and an intraperitoneal catheter was inserted to allow delivery of further anesthetic as needed. The sciatic nerve was dissected free, cut, and placed within a bipolar platinum electrode. Rats were mounted in a specially designed NMR probe with the Achilles tendon attached to a force transducer as described previously (11). Stimulation voltage and muscle length were adjusted to produce maximum twitch force.

Cats of either sex weighing 3–4 kg were sedated with ketamine (11 mg/kg im) and anesthetized with pento-

barbital sodium (30 mg/kg iv). The biceps [7.5 ± 0.5 (SE) g, n = 3] or soleus muscles (4.3 ± 0.5 g) were vascularly isolated, excised, and perfused at constant flow (0.2–0.4 ml·min^{-1}·g^{-1}) via the arteries with a 20% suspension of sheep erythrocytes in Krebs-Henseleit solution containing 3.5% bovine serum albumin, 5 mmol glucose, 0.15 mM sodium pyruvate, and 30 mg/l papaverine hydrochloride (12). Perfusion pressure was 90–110 Torr. After muscle dissection, the cats were killed with pentobarbital. Perfused muscles were attached to a force transducer and platinum stimulation electrodes in a specially designed 7.4-cm-diam NMR probe that is otherwise similar to the 5-cm probe described previously (12). Muscle length and stimulation voltage were adjusted to produce maximum twitch force, and temperature was maintained at 37 ± 2°C during the experiments.

Before muscle stimulation, fully relaxed ^{31}P-NMR spectra (162 MHz, 7,000-Hz sweep width, 2K complex data, 90° nominal pulse width, 15-s interpulse interval) were acquired from each muscle. Muscles were then stimulated with 6- (rat) or 10- (cat) s trains at 5 Hz, with 4.5 min between each train. Acquisitions of ^{31}P-NMR spectra were gated to specific times during and after the trains of stimulation by triggering the stimulator from the spectrometer's computer (Aspect 3000 of a Bruker AM400). Scans were acquired at 5 (rat) or 9 (cat) s into the train and at 20, 60, 90, 120, 180, and 240 s after the trains. After an additional 15-s delay, the cycle was typically repeated to a total of 8 scans per spectrum (Fig. 1). It should be emphasized that although spectra are the average from several scans, the effective time resolution of the gated spectra is equal to the acquisition time per scan, i.e., 145 ms. Free-induction decays were zero-filled to 4K and multiplied by an exponential corresponding to 25-Hz line broadening before Fourier transformation. Insofar as the minimum interpulse delay used in this protocol was 20 s, the spectra are fully relaxed (12) and no saturation corrections are necessary. PCr, ATP, and P_i peaks were integrated by an iterative Lorentzian fitting routine (13), and integrals were scaled to micromoles per gram, with the assumption of prestimulation ATP levels of 7.2, 7.0, and 3.8 μmol/g for rat gastrocnemius (4) and cat biceps and soleus, respectively (12). Because of the relatively lower signal-to-noise ratio and a tendency toward non-Lorentzian line shape of the P_i peak in spectra acquired during stimulation, the P_i content of muscles during stimulation was estimated from the P_i content of resting muscle plus the P_i expected from the observed PCr hydrolysis. Intracellular pH was estimated from the chemical shift of the P_i peak as described previously (12). The apparent buffer capacity in intact muscle was calculated according to *Eq. 1*, assuming α_p = 0.4 near pH 7 (6).

Buffer capacity of muscle homogenates. Animals were anesthetized as above, and the muscles of interest were dissected free. In each case, a 0.5- (rat) or 1- (cat) g sample roughly corresponding to the area in the sensitive volume of the NMR coils was removed and homogenized in 20 ml of 0.9% NaCl/g muscle. To avoid the possibility of variable metabolic changes and, in particular, variable hydrolysis of phosphate metabolites, all homogenates were incubated at 37°C for 45 min before titration. A 1-ml portion of each homogenate was then frozen in liquid N_2 and extracted in perchloric acid for measurement of P_i (12) and protein (10) content. In addition, two perchlorate extracts of each muscle type were examined by ^{31}P-NMR (162 MHz, 4K complex data, 8,000-Hz sweep width, 45° pulse, 1-s pulse interval, 800–2,400 scans) in a standard broad-band probe. The remaining homogenate was acidified to pH 6 with HCl and titrated at 37°C to pH 8 with 0.2 N NaOH in 10-μl steps. The resulting mean titration curves were fit to a fourth-order polynomial from which the slope (total β, slykes) over the pH range 6–8 was computed by differentiation.

RESULTS

Buffer capacity of muscle homogenates. Titration curves of the homogenates of each muscle type appear in Fig. 2. These curves were remarkably reproducible within a muscle type. For example, the pH after addition of 30 μmol of base to rat homogenates initially adjusted to pH 6 was 7.02 ± 0.04. The total buffer capacity over the range pH 6–8 computed from the slopes of polynomial fits to the mean titration data appears in Fig. 3. At pH 7 the total buffer capacities of the fast-twitch muscles (rat gastrocnemius and cat biceps) are similar, whereas that of the soleus is somewhat less (Table 1). These results appear to be consistent with previous studies that found buffer capacities in highly glycolytic muscles of ~50–60 slykes, with somewhat lower capacities in red muscle (2, 3).

However, all the homogenates contained very high levels of P_i (Table 1), which was not present in intact unstimulated muscle (Table 2) and therefore represents hydrolysis of PCr, ATP, and other phosphate metabolites. This was confirmed by examination of ^{31}P-NMR spectra of perchlorate extracts of the homogenates after incubation. In soleus extracts, P_i was the only peak resolved in the spectra (peak signal-to-root mean square noise ratio of P_i > 30:1). In the two fast muscles, one

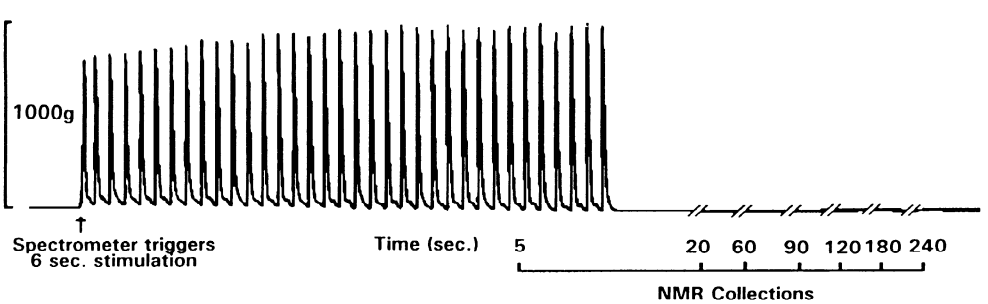

FIG. 1. Twitch force record from rat gastrocnemius muscle illustrating protocol for gated NMR data collection at 5 s after initiation of 5-Hz stimulation and at indicated intervals during recovery. Cycle of stimulation and recovery was repeated 8 times, with each NMR collection being added to appropriate computer memory block.

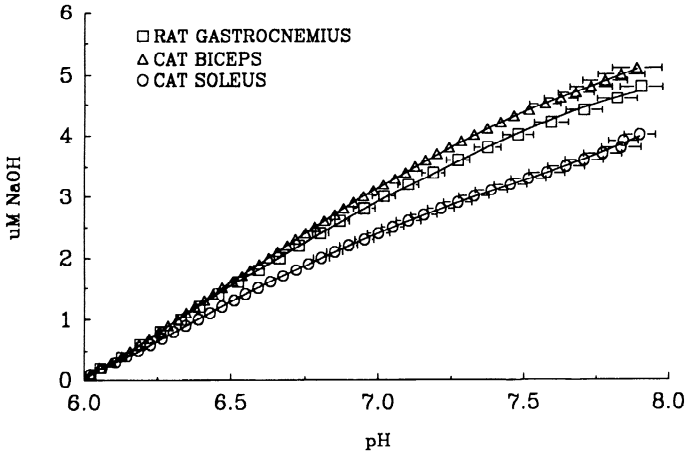

FIG. 2. Titration of muscle homogenates from pH 6 to 8 in 0.2-μM steps with NaOH (means ± SE; $n = 6$ for each muscle type).

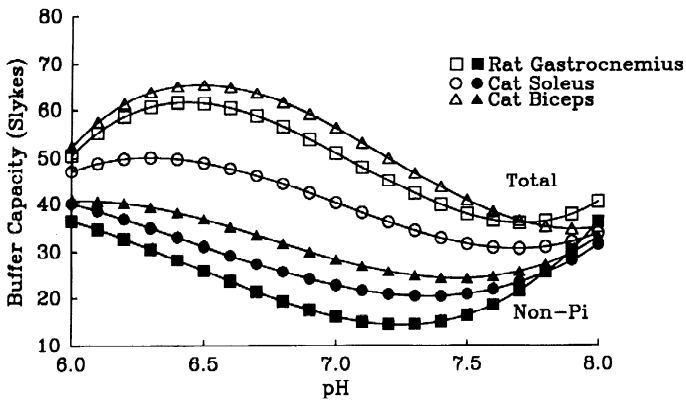

FIG. 3. Total buffer capacity (open symbols) calculated from slope of mean titration curves in Fig. 2 and nonphosphate buffer capacity (filled symbols) calculated by subtracting contribution of P_i (Table 1) to total buffer capacity of cat and rat skeletal muscle.

TABLE 1. *Measurements in muscle homogenates*

Muscle	Muscle Protein, mg/g muscle	Phosphate, μmol/g muscle	Total β (pH 7), slykes	Non-P_i β (pH 7), slykes
Cat biceps	185±16	29.6±1.3	56	28
Cat soleus	174±5	18.1±1.8	40	23
Rat gastrocnemius	159±7	35.6±0.9	51	16

Values for muscle protein and phosphate are means ± SE; $n = 6$. β, Buffer capacity.

TABLE 2. *Metabolite contents of unstimulated muscle*

Muscle	n	Ratios of NMR Integrals		Contents, μmol/g		
		P_i/ATP	PCr/ATP	ATP	P_i*	PCr*
Cat biceps	7	0.38±0.04	3.32±0.37	7.0	2.7	23.2
Cat soleus	4	1.20±0.29	2.75±0.39	3.8	4.6	10.5
Rat gastrocnemius	5	0.40±0.07	3.48±0.18	7.2	2.9	25.1

Values for ratios of NMR integrals are means ± SE. ATP values are from Refs. 4 and 12. * NMR integrals for P_i and PCr are normalized to ATP.

additional peak with area 20–25% of the area of the P_i peak was resolved. This peak resonated coincidently with inosine monophosphate added to the extract.

The acid dissociation constant (pK_a) of P_i titrated under conditions identical to those used for the homogenates was 6.75. Therefore, if the high levels of P_i in the homogenates (Table 1) are taken into consideration, P_i must make a major contribution to the total buffer capacity of the homogenates in the range pH 6.5–7.0. The solid curves in Fig. 3 are the buffer capacity of the homogenates after the calculated contribution due to P_i is subtracted, i.e., the nonphosphate buffer capacity of the homogenates (Table 1). After this correction, the apparent correlation of buffer capacity with muscle fiber type is lost, inasmuch as the rat muscle has the lowest buffer capacity whereas the two cat muscles are not markedly different.

Buffer capacity calculated from pH transients at onset of stimulation. Most muscles in these studies performed eight serial trains of contraction with a total of 36 min between the first and last bouts. There was no significant decrease in peak twitch force over the course of the experiment in any muscle [e.g., in rat muscle mean peak force was 2.22 ± 0.3 (SE) g/g body wt during the first train and 2.32 ± 0.3 g/g during the last train]. In one experiment on a large cat biceps, a sufficient signal-to-noise ratio was obtained to acquire useful spectra in a single scan (Fig. 4*A*), allowing complete data collection during and after a single 10-s train of stimuli. The results from this muscle were similar to those obtained from other muscles in which spectra were averaged over eight cycles of stimulation (Fig. 4*B*). These results demonstrate that the response to a brief train of stimuli is not altered by application of previous trains separated by a 4.5-min rest period.

Figure 5 is a set of representative spectra from a rat

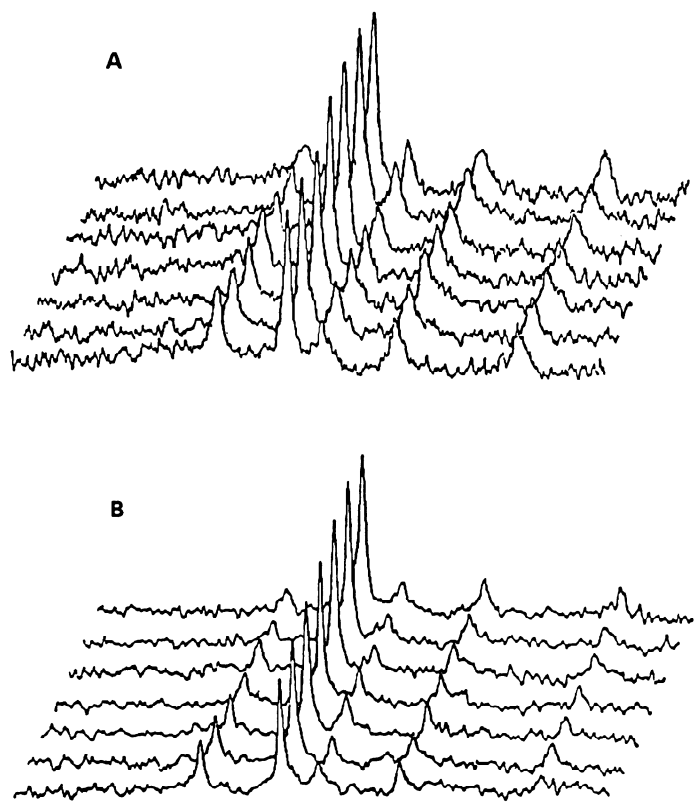

FIG. 4. Sets of ^{31}P-NMR spectra acquired by protocol of Fig. 1. *A*: from a large biceps muscle from which signal-to-noise ratio was sufficient to obtain good spectra with a single scan. *B*: representative of 8 scan spectra.

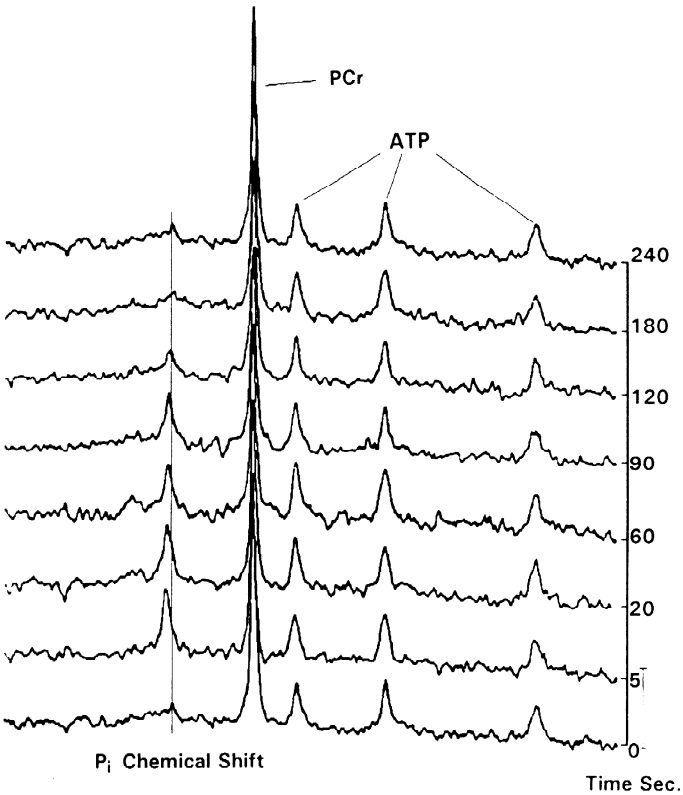

FIG. 5. Series of gated ^{31}P-NMR spectra (each 8 scans) from rat gastrocnemius collected as described in METHODS and in Fig. 1. Zero-time spectrum is identical to 240-s spectrum and is plotted twice so that alkaline shift of P_i is apparent. Bar on time scale represents 6-s contraction.

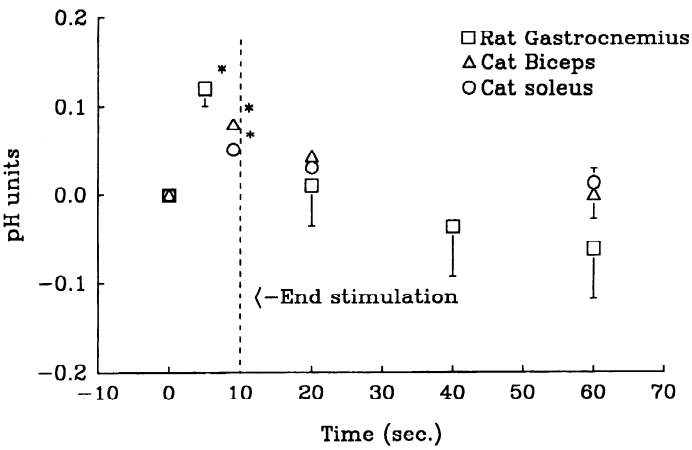

FIG. 6. pH changes relative to final recovery spectrum during and after muscle stimulation at 5 Hz for 6 (rat) or 10 (cat) s. * Significantly different from final recovery spectrum ($P < 0.05$).

experiment demonstrating the downfield (alkaline) shift of the phosphate peak after 5 s of 5-Hz stimulation relative to the spectrum acquired 4.25 min later. In all muscles there was a significant increase in pH during the stimulation train, which was reversed during the subsequent recovery period (Fig. 6). The pH in the last spectrum of the recovery period (6.92 ± 0.04, 7.03 ± 0.01, and 7.06 ± 0.01 for rat gastrocnemius and cat biceps and soleus, respectively) was not significantly different from pH before any stimulation. The changes in pH during stimulation were coincident with significant decreases in PCr in all muscle types (Table 3). The apparent buffer capacities (Table 3) calculated from the changes in PCr

TABLE 3. *Calculation of buffer capacity from in vivo measurements*

Muscle	ΔPCr, μmol/g	ΔpH	Apparent β, slykes	Predicted β,* slykes
Cat biceps	7.3±0.6	0.08±0.01	38±9	38
Cat soleus	2.6±0.7	0.05±0.01	21±7	30
Rat gastrocnemius	8.0±2.0	0.12±0.02	30±6	27

Values are means ± SE; $n = 3$. β, Buffer capacity. * From non-P_i β plus P_i.

and pH using *Eq. 1* lie intermediate between the total buffer capacities and the nonphosphate buffer capacities of the muscle homogenates. However, if the estimated P_i content in the muscles after 5 (rat) or 9 (cat) s of stimulation is added to the nonphosphate buffer capacity, then the predicted buffer capacities (Table 3) from the homogenate data agree nicely with the apparent buffer capacities calculated from PCr and pH changes in the intact muscles.

DISCUSSION

The major conclusion to be drawn from this study is that the transient alkalinization that occurs at the onset of stimulation in mammalian skeletal muscles can be quantitatively accounted for by proton consumption due to net PCr hydrolysis. If there were another quantitatively significant source or sink for protons during the first few seconds of contraction, then the buffer capacity calculated from the transient alkalinization accompanying PCr hydrolysis would differ from that predicted from titration of the homogenates. In fact, these two estimates of buffer capacity are similar in all three muscle types. Thus it appears unlikely that there is a quantitatively significant shift of protons [or of strong ions (7)] either extracellularly or into subcellular organelles at the onset of muscle stimulation (5).

The fact that the observed pH changes can be fully accounted for by PCr hydrolysis also suggests that little accumulation of lactic acid had occurred in these muscles during the first few seconds of contraction. Accumulation of lactic acid during the stimulation would oppose the alkalinizing effect of PCr hydrolysis and thus result in higher buffer capacities calculated via *Eq. 1*. Although little lactic acid formation would be expected in the highly aerobic soleus muscle (1, 12), stimulation of rat or cat fast-twitch muscle at 5 Hz for longer periods is well known to result in lactic acid accumulation (13, 14). Apparently, full activation of glycolysis is not achieved in these muscles until sometime after the first 5–10 s of stimulation.

The alkalinization observed in this study at 5 and 9 s is much less than that calculated by Connett (5) from metabolic data in dog gracilis muscle after 5 s. In that study an alkalinization from pH 7.05 to 7.8 was calculated with no significant change in PCr. Even with a PCr change of 10 μmol/g, the buffer capacity of dog muscle would have to be <6 slykes if this alkalinization were due to PCr hydrolysis. It seems unlikely that the buffer capacity of dog muscle would be so dramatically lower than that of rat and cat muscles (2, 3). A more likely explanation for the high pH calculated in Connett's study after 5 s is that one or more of the glycolytic reactions

used to calculate pH was not near equilibrium at that time. On the other hand, the pH calculated for unstimulated muscle in Connett's study is very similar to that observed in this and previous ^{31}P-NMR studies, suggesting that these reactions are near equilibrium in muscle at rest. Furthermore, the agreement between pH measurements calculated from metabolic equilibria and ^{31}P-NMR in unstimulated muscle suggests that there is no complicating subcellular compartmentation of the metabolites used in the calculations [i.e., PCr, creatine, P_i, lactate, pyruvate, dihydroxyacetone phosphate, and 3-phosphoglycerate (5)].

Titration of muscle homogenates is the conventional method for measuring muscle buffer capacity. Unfortunately, these titrations have been conducted under widely varying conditions of temperature, presence of metabolic inhibitors, and CO_2 content. The most quantitatively significant reactions likely to occur in muscle homogenates are lactate production and hydrolysis of high-energy phosphates. Of these, the former is of no concern, because neither glycogen nor lactic acid contributes significantly to buffering above pH 6. Our results also indicate that accumulation of phosphorylated glycolytic intermediates is also of little concern, inasmuch as significant levels of these were not observed in spectra of homogenate extracts. The conversion of ATP [pK 6.5 (6)] to inosine monophosphate and/or AMP [pK 6.2 (12)] observed in homogenates of fast muscle would reduce their buffer capacities compared with intact muscles at pH 7 by only 1.0 slyke, if we assume 7 μmol ATP converted/g muscle and ignore the effect of the P_i liberated. However, as our results demonstrate, the generation of P_i from ATP and PCr in the homogenates has a major impact on the measured buffer capacity. For example, in the rat muscle, 68% of the buffering capacity of the homogenates at pH 7 was due to P_i, most of which is not present in the intact muscle. Thus it seems quite possible that variable hydrolysis of high-energy phosphates at some time during preparation of the homogenates is responsible for much of the variation in buffer capacities reported using the titration method (2, 3, 15). In contrast, we incubated our homogenates without metabolic inhibitors before titration. The high levels of phosphate measured after incubation (Table 1) are due to the nearly complete hydrolysis of PCr and ATP. Titration of these homogenates was extremely reproducible (Fig. 2), and after correction for phosphate content the results are in good agreement with the estimate from intact muscles.

Our results have two additional implications. First, because the nonphosphate buffer capacity, particularly of the rat muscle, is relatively low, the phosphate released by PCr hydrolysis during muscle stimulation contributes significantly to intracellular buffer capacity. This may be a physiologically important role of the creatine kinase reaction in highly glycolytic fast-twitch muscles (2, 15, 16). Second, the agreement between buffer capacities predicted from homogenate titrations and those calculated from the intact muscle data confirms that CO_2-bicarbonate is not quantitatively important for intracellular buffering in muscle, inasmuch as the homogenates were titrated at ambient CO_2 levels (2, 7). Similarly, this agreement confirms that extracellular fluid makes an insignificant contribution to buffer capacity of muscle homogenates. As reviewed by others (15), the major contributors to the nonphosphate buffer capacity around pH 7 are probably proteins and peptides such as anserine and carnosine.

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