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Applying physicochemical principles to skeletal muscle acid-base status

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AMONG PHYSIOLOGISTS, acid-base chemistry has become controversial during the past five decades. In part, this was due to a movement by physiologists away from the classical descriptions of acid-base chemistry in physiological solutions as developed by the research of Lawrence Henderson (e.g., 12), Donald van Slyke and Cullen (30) and others (15, 17, 22) in the first decades of the past century. Another contributor to the controversy was the invention and use of the proton-selective electrode: the first semiquantitative assessment of muscle $[H^+]$ was reported in 1914 by Michaelis and Kramsztyk (26), and the first reliable measures in muscle were reported by Furusawa and Kerridge in 1927 (5)—with the later mathematical conversion of $[H^+]$ to pH. The ease with which $[H^+]$ could be measured led directly to the concept of pH-dependent processes. One of these processes is skeletal muscle fatigue. As a caveat of our present treatment of muscle acid-base state, it should be recognized that the positive charge in pure water is physically represented by oxonium or hydronium ion (H_3O^+) and that it is conventional for physical chemists and physiologists to ignore the water to which the proton is attached, hence giving reference to H^+ (3).

Robergs and colleagues (27) have reopened a challenging debate on the underlying mechanisms of exercise-induced metabolic acidosis, lactic acid, and their association with skeletal muscle fatigue (4). Previous descriptions presented by Gevers (6), Jones (16), and Hochachka and Mommsen (13) appeared either to be largely ignored or not accepted. The purposes of this paper are to highlight the approach described by Robergs et al. (27), particularly at the conceptual and qualitative levels, and to demonstrate how consideration of the physical chemistry of the intracellular environment of muscle cells provides additional insight and ultimately a more complete and correct description of acid-base status. Specifically, a physicochemical approach demonstrates that the physical behavior of molecules in aqueous solutions is independent of transport and buffering mechanisms; rather, their physical behavior and the apparent proton stoichiometry of biochemical reactions depend on physicochemical interactions with water, with the added constraint that physical and chemical laws must be obeyed.

It should be very clear from the review of Robergs et al. (27), and in the response from Kemp (18), that the biochemical reactions involved in glycogenolytic and glycolytic ATP production produce pyruvate (not pyruvic acid), which can then be converted to lactate$^-$ (not lactic acid) via the lactate dehydrogenase (LDH) reaction; reasons for others disagreeing with this view are unclear (2). Furthermore, when viewed either as a series of linked reactions, or as a system of near-instantaneous and simultaneous reactions, lactate$^-$ production is not associated with a stoichiometrically equivalent net production of protons (H^+). It is unfortunate, though not surprising, that the numerical (semiquantitative) approach to count protons adopted by Robergs et al. (27) invited quantitative criticism on the part of Kemp (18). These attempts to quantitatively determine the stoichiometry of proton production/consumption by means of biochemical reactions and by proton buffering mechanisms hinders interpretation and detracts from the demonstration of lactate$^-$ production independent of increasing intracellular $[H^+]$. Kemp's (18), and earlier researchers' (6, 13, 16), descriptions of the partial ionizations of metabolic products remain useful from the perspective of understanding the biochemical reactions at different conditions of intracellular $[H^+]$. However, these descriptions represent an incomplete attempt to account for two physical laws: conservation of mass and maintenance of electroneutrality in solutions. As recognized by the classical acid-base physiologists such as Henderson and van Slyke and as detailed by Harned and Owen (8) and Edsall and Wyman (3), it is imperative that the apparent stoichiometry of proton balance within biochemical reactions be viewed within the context of the physical milieu in which the reactions occur, namely, water. The partial ionizations described in the biochemical reactions are a direct result of how reaction substrates and products alter the behavior of the aqueous solution in which the reactions occur. These partial ionizations are present within these reaction equations because charge balance (physical law of electroneutrality) must be maintained (see below). As Robergs et al. point out in their response letter (28), further work remains to be conducted on the H^+ dependence of biochemical reactions and on muscle proton buffering. Indirect approaches to estimating proton production/consumption and intracellular proton buffering provide a simplified construct with which to understand muscle acid-base alterations. However, they suffer from lack of mechanistic insight and fail to contribute significantly to increasing our understanding of muscle acid-base balance.

Both Robergs et al. (27) and Kemp (18) note the importance of physical chemistry in their approaches to describing and understanding cellular biochemistry. However, their treatments of physical chemistry incompletely account for conservation of mass within biochemical reactions and maintenance of electroneutrality within aqueous solutions. Hence these treatments are inherently inadequate both with respect to the contribution of lactate$^-$ to intracellular acid-base state and with respect to structural nonbicarbonate proton buffering due to the presence of appreciable concentrations of dissociated weak acids. Knowledge of the physical chemistry of aqueous solutions is essential to understanding the physical behavior of molecules in these solutions, irrespective of how these molecules got there (transport, biochemical reactions). This is recognized by

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modern physical chemistry texts, but we favor the more classical treatments by Harned and Owen (8) and Edsall and Wyman (3).

Peter Stewart, the physical chemist who summarized the physicochemical basis for understanding the physiology of body fluids (17), reintroduced and clarified the concept of physicochemical analysis of body fluid acid-base status from a physiological perspective (29). This work represented a return to the thinking of Henderson and van Slyke and other lesser-known investigators of acid-base balance in the early 20th century (15, 22). Stewart (29) emphasized that $[H^+]$ (or the less preferred, pH) and $[HCO_3^-]$ are dependent acid-base variables; that is, they do not cause acid-base alterations. No matter what the biochemical source of protons, these dependent acid-base variables cannot be considered as independent factors in determining their own concentration. In essence, the notion of $[H^+]$ (pH) as an independent variable dates back to Hasselbalch's (9) construct of Henderson's (11) work, which became entrenched as the Henderson-Hasselbalch equation. Furthermore, Stewart, as others before him, recognized that protons are not produced or consumed by chemical reactions—and it is incorrect to use these terms in the context of protons.

One of the keys to understanding metabolic biochemistry in aqueous solutions is that all the reactions occur in a milieu having a water concentration of about 55.5 mol/l (3, 8). In addition to this, water exists mostly in its associated state (H_2O), with dissociation into H^+ and HO^- governed, in part, by the relation:

$$K_w[H_2O] = [H^+][HO^-] \quad (1)$$

where the dissociation constant of water (K_w) is very small [$\sim 4.4 \times 10^{-14}$ (Eq/l)^2], and therefore also the concentrations of $[H^+]$ and $[HO^-]$ are very low ($\sim 10^{-7}$ Eq/l). *Equation 1* may be rewritten as

$$[H^+] = (K_w[H_2O])/[HO^-] \quad (2)$$

or

$$[H^+] = K'_w/[HO^-] \quad (2a)$$

where $K'_w = K_w [H_2O]$. It is also little recognized that individual H^+ and HO^- molecules are only fleetingly in existence (3, 8)—their rate of dissociation/association from water is on the order of $10^6/s^{-1}$ (1). This raises the question of whether any individual molecule of H^+ or HO^- is in existence long enough to substantively participate in a biochemical reaction or membrane transport process. Furthermore, because the concentration of water is so large, and that of H^+ so low, water effectively provides an infinite supply of protons, as required for any biochemical reaction that may require a proton, and similarly, protons evolving from biochemical reactions may reassociate with HO^-.

A second key to understanding metabolic biochemistry in aqueous solutions is recognizing that all ions in solution govern the dissociation of water and hence the concentration of H^+. In all aqueous solutions the concentration of H^+ is governed by three fundamental laws of mass action (8): *1)* conservation of mass; *2)* the equilibrium state between water and weak electrolytes, as defined by their equilibrium constants; and *3)* maintenance of electrical neutrality.

It is unfortunate that muscle and exercise physiologists frequently overlook one or more of these laws when applying interpretations to muscle and blood biochemistry and acid-base state. It can be demonstrated in the test tube, by measuring the $[H^+]$ of solutions, that some of the metabolic biochemical reactions associated with energy provision appear to consume or generate protons (for summaries, see 6, 13, 16, 18, 21, 27). This approach is useful because they demonstrate conservation of mass as exemplified in the equations summarizing the biochemical reactions. However, when applying the equations summarizing metabolic biochemical reactions in muscle, most researchers neglect the very important balance between water and the reaction substrates/products, such that the third law, maintenance of electrical neutrality, is violated.

According to Peter Stewart (29), the fundamental principle of maintaining electroneutrality provides the "link between the concentrations of the nonreacting strong ions and the equilibrating weak ions. Its physical basis is in Coulomb's law, which specifies the very large electrical forces that come into play whenever charge imbalance occurs". Making reference to Guggenheim's (7) monograph, Stewart (29) notes, in particular, that minute charge imbalances would result in "very large electrical forces," so charge imbalances occur only fleetingly. The required high rate of charge balance is provided by water. Our remaining discussion requires an introduction to weak and strong ions, as provided previously (10, 15, 21, 23, 24).

The maintenance of electroneutrality refers to the fact that aqueous solutions are always electrically neutral, that is, the sum of all negatively charged ion concentrations equals the sum of all positively charged ion concentrations (29):

$$\sum[\text{strong base cations}] - \sum[\text{strong acid anions}] + \sum[\text{weak base cations}] - \sum[\text{weak acid anions}] + [H^+] - [HO^-] = 0 \quad (3)$$

Acid-base status is determined by the independent effects of carbon dioxide (P_{CO_2}), the total concentration of noncarbonate weak acid anions ($[A_{tot}]$), and the charge difference between the sum of strong base cations and strong acid anions, denoted as the strong ion difference [SID]; that is, the three independent acid-base variables whose interactions ultimately determine the $[H^+]$ and $[HO^-]$ in solution. In this correct physicochemical formulation, [lactate$^-$], by way of its effect on [SID] is an important independent determinant of muscle $[H^+]$. During muscle contraction, changes in each of the three independent variables occur simultaneously. Therefore, at any point in time, $[H^+]$ must conform to the ratios of $[A_{tot}]$:dissociated weak acids, P_{CO_2}:HCO_3^-, and H^+:HO^- that all achieve instantaneous equilibrium. We will simplify our discussion by focusing the remainder of this section only on the strong ions, such that:

$$[\text{SID}] = \sum[\text{strong base cations}] - \sum[\text{strong acid anions}] \quad (4)$$

The charges reside on ions, including the dissociation products of water (HO^- and H^+), strong ions [those that are fully or nearly completely dissociated such as Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+}, lactate$^-$, pyruvate$^-$, phosphocreatine^{2-} (PCr^{2-})], and weak ions (those that are only partially dissociated such as HCO_3^- and the numerous glycolytic intermediates) summarized by Gevers (6), Hochachka and Mommsen (13), and more recently in Table 2 of Robergs et al. (27).

Within contracting skeletal muscle, increased glycolytic activity results in the net accumulation of strong acid anions

(mainly lactate$^-$ with a small amount of pyruvate$^-$) and weak acid anions (most other glycolytic intermediates). By definition, the maintenance of electroneutrality requires that the net increase in the concentrations of acid anions will affect the balance between the two most abundant weak ions in solution, namely HO^- and H^+. An increase in the concentration of acid anion molecules must be accompanied by an equal increase in the concentration of net positive charge, and this positive charge is provided in large part by the dissociation of water.

So, in an aqueous solution that contains only strong ions, *Eq. 1* may be simplified to

$$[SID] + [H^+] - [HO^-] = 0 \quad (5)$$

Substituting $K'_w/[H^+]$ for $[HO^-]$ (from *Eq. 2*) into *Eq. 5*:

$$[H^+]^2 + [SID] \cdot [H^-] - K'_w = 0 \quad (6)$$

which is the same as

$$[H^+] = (K'_w + [SID]^2/4)^{1/2} - [SID]/2 \quad (7)$$

From *Eq. 7*, it is demonstrated that an increase in the concentration of strong acid anions, which lowers [SID], directly contributes to an increase in $[H^+]$. This is cause and effect.

The main point of the preceding description is that one may take the approach of counting the protons either produced or consumed in metabolic biochemical reactions, thus determining the biochemical stoichiometry of individual reactions. However, this approach is incomplete because it fails to consider the associations of reaction substrates and products with water. Furthermore, there is also a high capacity for intracellular weak acid anions (so-called structural nonbicarbonate proton buffers) to instantaneously and simultaneously buffer protons. Therefore, it is not physically possible for the metabolic biochemical reactions per se to contribute to measured increases in intracellular $[H^+]$.

In contracting skeletal muscle, changes in the concentrations of strong ions are the most important physical and chemical contributors to the increase in $[H^+]$—this is pure physics and chemistry. While P_{CO_2} and $[A_{tot}]$ also contribute, their contributions to muscle $[H^+]$ during exercise are less than those of the strong ions (19, 22, 24) and because the focus of this paper is on the strong ion lactate$^-$, these will not be further considered here. The biochemical reactions help us to understand why the concentrations of the metabolic (organic) strong ions change; however, this understanding often has not been extended to the inorganic strong ions that also contribute to intracellular acid-base state (21, 24, 29). The primary inorganic strong ions in skeletal muscle include the strong base cations Na^+ and K^+ and the strong acid anion Cl^-. The primary organic strong ions include phosphocreatine (PCr^{2-}), itself a divalent strong acid anion ($pK' = 4.5$), and lactate$^-$ ($pK' = 3.9$). The primary effect of changes in the ion concentrations on muscle $[H^+]$ during contraction is a decrease in intracellular $[K^+]$ that reduces intracellular [SID] (since $[Na^+]$ and $[Cl^-]$ accumulate to similar degrees; see Ref. 25). With high-intensity exercise, the net reduction in $[K^+]$ occurs rapidly and, through its effect on the intracellular [SID], contributes to the increases in $[H^+]$ (*Eq. 7*). Coincident with this change, however, is the concurrent hydrolysis of PCr^{2-}. The rapid hydrolysis of PCr^{2-} reduces its concentration, which has a direct effect on increasing intracellular [SID] and thus contributes to a decrease in $[H^+]$ (*Eq. 7*). In the first seconds of the rest to work transition, the decrease in $[PCr^{2-}]$ effectively raises [SID], and thus $[H^+]$ must decrease. It should also be recognized that PCr^{2-} hydrolysis results in the production of creatine, which is electroneutral and that the subsequent hydrolysis of ATP form from the creatine kinase reaction results in the production of the weak acid inorganic phosphate. PCr^{2-} hydrolysis is thus a potent means for increasing [SID] and for reducing intracellular $[H^+]$, albeit while modestly increasing $[A_{tot}]$. With increasing glycogenolytic and glycolytic activity, the strong acid anion lactate$^-$ progressively increases in concentration, and its net accumulation is not effectively balanced by the simultaneous changes in other intracellular strong ions. Thus, concurrent decreases in intracellular $[K^+]$ and increases in [lactate$^-$] result in progressive decreases in intracellular [SID] because $[PCr^{2-}]$ either does not change further or may increase if ATP demand is reduced. The unequal accumulation and/or removal of cations and anions during exercise leads to a decrease in intracellular [SID] (altered balance of strong and weak ions in solution), which directly and physicochemically contributes to the increase in $[H^+]$ during moderate- to high-intensity exercise (19, 21, 24, 29). The magnitude of this increase is, as noted by Robergs et al. (27), also proportional to *1)* the concentrations of dissociated weak acids (i.e., structural nonbicarbonate proton-buffering capacity of muscle); *2)* the rate at which acid equivalents (strong acid anions such as PCr^{2-}, pyruvate$^-$, and lactate$^-$) accumulate or are removed from muscle or consumed within muscle; and *3)* the rate at which strong base cations are added to or removed from muscle (25).

Many physiologists and medical scientists use the term lactic acidosis because of an entrenched sloppy nomenclature within the literature and not an inherent misunderstanding of the mechanism of lactate$^-$ production. Nonetheless, in dispelling the myth of lactic acidosis, Robergs et al. (27) take a force-feeding approach and an overt rejection of constructs in general. We feel that this is not warranted and, in fact, is inconsistent within their review. For example, Robergs et al. (27) define a construct as an "unproven, nonfactual interpretation that has mistakenly been accepted as fact." While constructs may help our understanding of difficult concepts, it should be recognized that usually they are simply qualitative interpretations, which may or may not be accurate, and, for some, they may or may not enhance understanding. In debunking the construct of lactic acid production, Robergs et al. (27) themselves use a number of other constructs that are also not founded on fact to support their arguments. These include the construct of metabolic intracellular nonbicarbonate proton buffering and the construct of cotransport of lactate$^-$ and H^+. With respect to the latter, consideration of the physical chemistry of water reveals that it is highly improbable that H^+ is physically transported and that movement of lactate$^-$ alone from one side of a membrane to the other is sufficient to produce the measured H^+ responses. Indeed, as long ago as 1920, Jacobs (14) demonstrated that H^+ ions do not cross the cell membrane. And yes, pH is also a construct, albeit founded on fact and it can be useful.

In conclusion, it is useful and instructive to accurately follow the path of protons in metabolic pathways. We agree with the data as summarized by others (18, 27, 28) that lactic acid is not produced in muscle and that it is not present in meaningful concentrations. There is merit in providing a useful

summary of the underlying biochemical reactions involved in energy production within muscle and in identifying the correct species of metabolic substrates and products. However, failure to apply the entirety of physicochemical principles leads to the incorrect and misleading conclusion that lactate$^-$ is unrelated to the metabolic acidosis of exercise. We do contend, therefore, that the accumulation of lactate$^-$ within skeletal muscle directly contributes to intracellular acidosis, by virtue of the fact that it is a strong acid anion that fundamentally alters the behavior of water. With respect to acid-base balance, it is inappropriate to consider each biochemical reaction independently, and it is similarly inappropriate to try to link them temporally or in biochemical sequence. Acid-base balance changes instantaneously; therefore, a more complete understanding of the acidosis of exercise considers the simultaneous biochemical, transport, and proton buffering reactions, as well as their instantaneous and simultaneous physicochemical interactions with water, at any point in time. As stated by Norman Jones in 1980 (16): "The simple biochemical relationships above yield only a shortsighted view of proton release because the ionic state of the reactants is ignored. As they may exist in either acidic or basic forms, the net charges need to be taken into account. Reference to a biochemistry text (20) will show that the equations may be written more accurately and the source of protons is not what it seemed at first."

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Corrigendum

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Pages R891–R910: In a discussion consisting of an Editorial Focus and three Letters to the Editor, an incorrect author list was presented in a reference that appeared in two of the three letters. The correct reference is Böning D, Strobel G, Beneke R, and Maassen N. Lactic acid still remains the real cause of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 289: R902–R903, 2005.