The following is the abstract of the article discussed in the subsequent letter:

Robergs, Robert A., Farzenah Ghiasvand, and Daryl Parker. Biochemistry of exercise-induced metabolic acidosis. Am J Physiol Regul Integr Comp Physiol 287: R502–R516, 2004.—The development of acidosis during intense exercise has traditionally been explained by the increased production of lactic acid, causing the release of a proton and the formation of the acid salt sodium lactate. On the basis of this explanation, if the rate of lactate production is high enough, the cellular proton buffering capacity can be exceeded, resulting in a decrease in cellular pH. These biochemical events have been termed lactic acidosis. The lactic acidosis of exercise has been a classic explanation of the biochemistry of acidosis for more than 80 years. This belief has led to the interpretation that lactate production causes acidosis and, in turn, that increased lactate production is one of the several causes of muscle fatigue during intense exercise. This review presents clear evidence that there is no biochemical support for lactate production causing acidosis. Lactate production retards, not causes, acidosis. Similarly, there is a wealth of research evidence to show that acidosis is caused by reactions other than lactate production. Every time ATP is broken down to ADP and Pi, a proton is released. When the ATP demand of muscle contraction is met by mitochondrial respiration, there is no proton accumulation in the cell, as protons are used by the mitochondria for oxidative phosphorylation and to maintain the proton gradient in the intermembranous space. It is only when the exercise intensity increases beyond steady state that there is a need for greater reliance on ATP regeneration from glycolysis and the phosphagen system. The ATP that is supplied from these nonmitochondrial sources and is eventually used to fuel muscle contraction increases proton release and causes the acidosis of intense exercise. Lactate production increases under these cellular conditions to prevent pyruvate accumulation and supply the NAD+ needed for phase 2 of glycolysis. Thus increased lactate production coincides with cellular acidosis and remains a good indirect marker for cell metabolic conditions that induce metabolic acidosis. If muscle did not produce lactate, acidosis and muscle fatigue would occur more quickly and exercise performance would be severely impaired.

Lactate accumulation, proton buffering, and pH change in ischemically exercising muscle

By reviewing theory (6, 11, 12, 14) and reanalyzing data (3, 15, 19, 24, 25), Robergs and colleagues argue (21), among other things, for a particular approach to the generation and disposal of protons in exercising skeletal muscle. They rightly insist that interpreting muscle cell pH changes requires an appropriate analysis of proton stoichiometry. I would add two points. First, one may be misled by neglecting, even for illustrative purposes, the pH dependence of this stoichiometry. Second, careful accounting is needed to assess how physicochemical buffering contributes to cellular acid-base balance. To argue this, I will consider only ischemic exercise (exercise without blood flow), which has the simplifying advantages that mitochondrial proton uptake and cellular proton efflux (21) are negligible, and the only glycolytic substrate is glycogen (although for completeness, results for glucose will also be given).

What might be called the traditional analysis criticized by Robergs et al. takes the increase in cytosolic lactate concentration as a measure of the proton load arising from glycolysis, which is disposed of in two ways (14, 16). The first is physicochemical buffering [also called static (23) or structural (21) buffering], predominantly by cytosolic imidazole groups and inorganic phosphate (1, 2, 16, 23, 26). The second is the consumption of protons by the Lohmann reaction (17); this term refers to the hydrolysis of ATP and its simultaneous regeneration at the expense of phosphocreatine, catalyzed by creatine kinase (7, 17, 21) and is stoichiometrically equivalent to the splitting, or hydrolysis, of phosphocreatine to inorganic phosphate [all these terms can be found in the literature, but note that this reaction does not occur (21), being only a name for the sum of two enzyme-catalyzed processes]. This proton consumption is sometimes called dynamic (23) or metabolic (21) buffering. Taking the fall in cytosolic pH (to use a physical analogy) as the strain resulting from the glycolytic proton-load stress, the ratio of lactate increase to pH fall has been used as an apparent buffer capacity, a measure of both these components of proton handling (23) More properly, the true physicochemical buffer capacity is the ratio to the pH fall of the net proton load, that is, the total glycogenolytic proton load less that consumed by the Lohmann reaction (16).

In criticizing this approach, Robergs et al. (21) note that the glycogenolytic proton load is generated by the hydrolysis of ATP rather than ionization of lactic acid (6, 12, 14), while the reduction of pyruvate to lactate actually consumes protons, mitigating acidification rather than causing it. Thus the naïve notion that glycolysis adds lactic acid to the cell is untenable (21). Robergs et al. develop this criticism to argue for a different understanding of acid-base balance, one implication of which is that for biopsy and 31P MRS studies of muscle energetics, the traditional approach yields gross underestimations of the true muscle buffer capacity (21).

I will argue that while this analysis of proton generation is broadly correct at resting pH, it is much less so at low pH values commonly found in exercising muscle. Furthermore, because this analysis of muscle cell buffering capacity adds together components of proton generation that are partially cancelled by processes of proton consumption, it may be physiologically misleading.

**BIOCHEMICAL BACKGROUND**

To understand this, we must consider the stoichiometry (Table 1) and then examine its implications for the analysis of cellular buffering (Table 2). In ischemic exercise ATP is produced by glycolysis to lactate and by the Lohmann reaction. The effects on cytosolic pH (which can vary from ~7 at rest to ~6 in intense exercise) depend on the pH-dependent proton stoichiometries of the component processes (1, 2, 6, 7, 12, 14, 16, 17). Table 1 shows (centered) the biochemical equations in a form that takes account of this pH dependence and cites the corresponding expressions or figures in Robergs et al. (21) where, for a simpler exposition, this complication is ignored. (I follow Robergs et al. in not using the alternative strong ion difference formalism, which is not very useful when lactate is the only strong ion measured.) Table 1 also shows algebraic expressions for the proton stoichiometries, along with the numerical values stated or implicit in Robergs et al. (21) and as calculated from the present analysis for three pH values spanning the physiological range. Table 2 gives expressions for the estimation of various versions of calculated buffer capacity. Figure 1 illustrates the application to a data set (16).

Glycolysis from glucose to pyruvate (Table 2 in Ref. 21) generates about one proton per pyruvate at resting pH, increasing as pH falls (Eq. 1b); this is not very different for glycolysis from glucose (although in the simplified Robergs analysis the
Table 1. Components of proton (H⁺) generation and consumption in ischaemically exercising muscle

<table>
<thead>
<tr>
<th>Process and Analysis*</th>
<th>Component</th>
<th>Substrate</th>
<th>Robergs pH 7</th>
<th>pH 6.4</th>
<th>pH 6</th>
<th>Equation and Derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycolysis to pyruvate:</strong> Eqs. 1 and 2 in Table 2 in Robergs et al. (21)</td>
<td>X + 2oADP + 2oPi → Y + 2pyr + 2oATP + 2NADH + 2oH₂O + 2u(a + b - c) + 2H⁺</td>
<td>ATP production = ω per pyruvate = ωΔLa</td>
<td>Eq. 1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H⁺ production</strong> = [ω(a + b + c) + 2]ΔLa</td>
<td>H⁺ produced per lactate:</td>
<td>glycerol (ω = 3/2)</td>
<td>0.5</td>
<td>0.91</td>
<td>1.42</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>glucose (ω = 1)</td>
<td>1</td>
<td>1.27</td>
<td>1.62</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td><strong>Reduction of pyruvate to lactate Fig. 9 in Robergs et al. (21)</strong></td>
<td>Pyr + NADH + H⁺ → La⁻ + NAD⁺</td>
<td>H⁺ consumption = ΔLa</td>
<td>H⁺ consumed per lactate</td>
<td>glycerol and glucose</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Hydrolysis of ATP: Fig. 10 and Eq. 7 in Robergs et al. (21)</strong></td>
<td>ATP⁺ + H₂O → ADP⁺ + Pi⁺ + (c - a - b)H²⁺</td>
<td>H⁺ production: in general = (c - a - b)</td>
<td>per ATP</td>
<td>H⁺ produced per ATP</td>
<td>glycerol and glucose</td>
<td>1</td>
</tr>
<tr>
<td>from glycolytic ATP = ω(c - a - b)ΔLa</td>
<td>H⁺ produced per lactate</td>
<td>glycerol and glucose</td>
<td>1</td>
<td>1.09</td>
<td>0.58</td>
<td>0.07</td>
</tr>
<tr>
<td>from ATP via CK = (c - a - b)ΔPCr</td>
<td>H⁺ produced per PCr</td>
<td>glycerol and glucose</td>
<td>1</td>
<td>0.73</td>
<td>0.38</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Glycolysis to lactate coupled with ATP hydrolysis:</strong> Eqs. 5 and 6 in Robergs et al. (21)</td>
<td>X⁻ → Y + 2La⁻ + 2H⁺</td>
<td>H⁺ production = ΔLa</td>
<td>H⁺ produced per lactate</td>
<td>glycerol and glucose</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fraction of this H⁺ derived from ATP hydrolysis = ω(c - a - b)</td>
<td>H⁺ produced per lactate</td>
<td>glycerol and glucose</td>
<td>1</td>
<td>1.09</td>
<td>0.58</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.73</td>
<td>0.38</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>ATP regeneration by phosphocreatine, catalysed by CK: Fig. 4 in Robergs et al. (21)</strong></td>
<td>PCr⁺ + ADP⁺ + (c - a - g)H⁺ → Cr⁺ + ATP⁺</td>
<td>ATP production = 1 ATP per PCr</td>
<td>H⁺ consumption = (c - a - g)ΔPCr</td>
<td>H⁺ consumed per ATP</td>
<td>glycerol and glucose</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Loehmann reaction:</strong> ATP hydrolysis plus ATP regeneration: Figs. 4 and 11 in Robergs et al. (21)</td>
<td>PCr⁺ + (b - g)H⁺ + H₂O → Pi⁺ + Cr⁺</td>
<td>H⁺ consumption = (b - g)ΔPCr</td>
<td>H⁺ consumed per ATP</td>
<td>glycerol and glucose</td>
<td>0.20</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Pyr, pyruvate; La, lactate; PCr, phosphocreatine; Cr, creatine; ADP, adenosine diphosphate. In the biochemical equations (centered) ATP, ADP, PCr, and Pi refer to the sum of all relevant chemical species, and the superscripts a, b, c, g are the generally nonintegral average charges of ADP, Pi, ATP and PCr (Cr is unchanged), respectively. In the algebraic equations, concentration brackets are omitted for simplicity; ΔLa denotes an increase and ΔPCr and ΔpH a decrease during exercise, and the stoichiometric coefficients a, b, c, g are simply the charges in the biochemical equations. The proton stoichiometry of these equations follows from the electroneutrality condition. The conclusions here follow directly from this, relatively independent of the numerical values of the charges. The equations apply to both glycolytic substrates: for glycerol (the predominant substrate in ischemic exercise), the symbols X and Y refer to glycerolₐ and glycerolₐ⁻¹ and the ATP stoichiometric factor ω = 3/2; when X refers to glucose, Y is nothing and ω = 1. β is the physicochemical cytosolic buffer capacity. Eqs. 1–7 in Table 1 are based mainly on Refs. 12, 14, 17, Eqs. 8–12 in Table 2 mainly on Refs. 1, 23, 26; see Ref. 16 for more details. Equations 13–15 in Table 2 from Ref. 21 are translated into the present terminology using the analysis presented here. One complication ignored in these expressions, although not in the illustrative calculations in Fig. 1, is that the pH dependence of both stoichiometric coefficients and buffer capacity requires that, for example, (b - g)ΔPCr and βΔpH should be (b - g)ΔPCr and βΔpH, or in practice their finite-sum equivalents, Σ[(b - g)ΔPCr] and Σ[βΔpH]. *Reference is made to the numbered equations and figures in Robergs et al. (21), where for simplicity, pH effects on stoichiometry are ignored, which are explicit here. Equations 1–4 (21) ignore the charges on ADP, ATP, Pi, lactate, and pyruvate, and Figs. 4, 10, and 11 in (21) show only ADP⁺, ATP⁺, Pi⁺ and PCr⁺ (see footnote†). †These stoichiometric coefficients are calculated using the analysis of Kushmerick (17), in which α, −β, and γ are equivalent to c - a - b, c - a - g and b - g here (this β is not that used here for buffer capacity); these coefficients can be calculated empirically from pH using γ = -27.239 - 13.593(pH)² + 2.1440(pH)⁴ - 0.10887(pH)⁶, β = -11.869 + 5.6685(pH) - 0.92213(pH)² + 0.047950(pH)⁴ and α = 39.108 - 19.262(pH) + 3.0662(pH)³ - 0.15682(pH)⁴ (17). Values are given for pH 7 (rest), pH 6.5 (end of exercise in Figure 1) and pH 6 (an extreme pH which can be reached in intense exercise); they are near-linear with pH over this range. The values of the coefficients implicit in Robergs’ analysis are calculated by setting a = -3, b = g = -2, c = -4, so that c - a - b = c - a - g = 1 and b - g = 0 (see footnote*). Glycerol and glucose consumption are distinguished where necessary. The conclusions presented here are not very sensitive to the exact values chosen for these coefficients. In Eq. 1 the product is pyruvate, but the expression is given for protons per lactate, because what is usually measured (24, 25) or inferred (16) is accumulation of lactate, which in ischemic exercise is very close to lactate + pyruvate.
assumed stoichiometric coefficients differ by a factor of 2). Next, reduction of pyruvate to lactate consumes one proton, independent of pH (Eq. 2). At resting pH, because this disposables of protons roughly equivalent to the protons generated earlier in glycolysis, both glycogenolysis and glycolysis to lactate (Eq. 3) generate few protons (21) in the simplified Robergs analysis, glycolysis from glucose to lactate produces none, while glycogenolysis consumes one proton per glucosyl (21)]. The ATP generated by glycolysis does not accumulate, being simultaneously hydrolyzed by ATPases, resulting in the generation of almost one proton per ATP at resting pH, decreasing as pH falls (Eq. 4a). Although two of the products of ATP hydrolysis, inorganic phosphate and ADP, are recycled by glycolysis, the protons accumulate to acidify the cell (12, 21). The result is that glycogenolysis or glycolysis to lactate coupled to simultaneous ATP hydrolysis generates exactly one proton per lactate, independent of pH (Eq. 5a) (12).

It is the main premise of Robergs et al. (21) that the principal source of the proton arising from coupled glycolysis is ATP hydrolysis rather than lactic acid synthesis per se. Table 1 shows that this is true at resting pH (Eq. 5b); however, as pH falls, this proportion is reversed as proton production by ATP hydrolysis decreases (6, 12, 14); until pH 6, most of the glycolytic protons actually do come from glycolysis to lactate (Eq. 5b). Thus this claim (21) is a significant oversimplification as a result of neglecting the correct values of the stoichiometric coefficients. Note that this argument is quite robust. To calculate the protons produced by glycolysis coupled to ATP hydrolysis (Eq. 5a), we simply add the protons produced by glycolysis to lactate (Eq. 3) to those produced by the balancing hydrolysis of the ATP so formed (Eq. 4b); the pH-dependent proton stoichiometry cancels out, so the result is one proton per lactate at all pH values, independent of substrate, and also using the coefficients assumed by Robergs et al. (21) (Table 1). The fraction of glycolytic protons supplied by coupled ATP hydrolysis (Eq. 5b) is therefore numerically equal to the proton load from hydrolysis of glycolytic ATP (Eq. 4b): only in the Robergs analysis for glucose is this fraction unity (21) (Table 1).

Now, we must introduce creatine kinase. In muscle, over a wide range of ATP turnover, the regeneration of ATP at the expense of phosphocreatine (Eq. 6a) buffers ATP against temporary imbalance between ATP use and glycogenolytic ATP production (9). This, the Lohmann reaction (see previous section), consumes a number of protons equal to the difference in charge between phosphocreatine and inorganic phosphate (Eq. 7). On the simplest analysis in which the phosphocreatine has charge −2, this is just the monovalent fraction of inorganic phosphate, $1/[1 + 10^[pH-6.8]] \approx 0.4$ at resting pH (1, 14, 16, 26), but more recent analysis, taking account of hitherto unconsidered potassium binding, suggests a smaller value of −0.2 at rest, increasing as pH falls (7, 17). There is some disagreement (16) about which value should be used (compare e.g., Refs. 1, 4, 10, 20, and 22), but this is not important to the present argument. However, it is a feature of the illustrative stoichiometry in the Robergs analysis (21), where this whole approach is rejected, that the implied value of the Lohmann coefficient is zero (see Table 1, footnote).

**“TRADITIONAL” APPROACHES TO INTRACELLULAR PROTON BUFFERING**

This is summarized in Table 2. A standard interpretation (7, 14, 16, 23, 25, 26) is that glycogenolysis generates protons, which are buffered by passive processes and consumed by the Lohmann reaction (Eq. 7). What the Lohmann reaction leaves behind is the net proton load (Eq. 8) that produces a pH change depending on the physicochemical buffer capacity (Eq. 9) (16); this can, therefore, be estimated as their ratio (Eq. 10), for comparison with ex vivo measurements (1, 5, 16, 17). Following Sahlin's terminology for the components of buffering (23),
I call this the physicochemical approach. The underlying concept is of a net proton-generator (or, in early exercise, proton-consumer) comprising glycolysis, ATPase and creatine kinase added to a cytosol containing only passive buffers. The argument is spelled out by Kemp and colleagues (16).

An alternative version of this approach is to regard glyco-
genolysis as a source of protons (Eq. 5a) that are dealt with both by physicochemical buffering and metabolically by the Lohmann reaction (Eq. 11). The ratio of the glycogenolytic proton load (i.e., the lactate increase) to the pH fall (Eq. 12a) can conveniently be taken as a functional or apparent buffer capacity (23, 25), which exceeds the true physicochemical buffer capacity (Eq. 12b) (there is some ambiguity about this relationship in the older experimental literature). Following Robergs’ terminology (21) for the components of buffering, I call this the metabolic approach. The underlying concept is of a net proton-generator comprising glycolysis plus ATPase added to a cytosol containing passive buffers and the net proton-consuming creatine kinase system. Thus the conceptual difference between the apparent and true buffer capacities is where the boundary is drawn between the generation and disposal of protons, whether creatine kinase is lumped with the
ATP-generating enzymes of glycolysis or with the proton-buffers of the cytosol.

The importance of these distinctions is that the balance between proton consumption and proton buffering depends on metabolic regulation (23), specifically, the relationship between glycogen phosphorylase flux and the concentration of its substrate, phosphate (16). Because of the stoichiometric Lohmann-reaction relationship between phosphocreatine and phosphate, this can be seen as a negative feedback mechanism, whereby phosphate is an error signal responding to the time-integrated mismatch between ATP usage and glycolgenolytic ATP production (16). Although its role in the regulation of glycolgenolysis is controversial (7, 8, 18), this phosphate dependence of phosphorylase is important in the control of pH, and therefore in the relationship (Eq. 12b) between apparent (Eq. 12a) and true buffer capacity (Eq. 10). One reason for the early popularity (23) of the apparent buffer capacity was that the system operates so that the relationship between pH and lactate is remarkably linear and invariant [see Fig. 3 in (21)]; the constraints on this, and its implications for metabolic control, are discussed elsewhere (16).

**PROPOSED NEW APPROACH TO INTRACELLULAR PROTON BUFFERING**

This is also summarized in Table 2. Robergs et al. (21) argue that the traditional analysis is wrong because the protons produced during glycolgenolysis arise from ATP hydrolysis (Eq. 4a) not lactic acid. However, we have seen that the premise is only approximately true and progressively less so as pH falls (Eq. 5b). Furthermore, the traditional analysis makes no necessary assumption about the ultimate source of the protons in Eq. 5a; references in the literature to protons arising from lactic acid can safely be replaced by the more conceptually accurate protons accompanying lactate.

More fundamentally, Robergs et al. argue for a new analysis of proton generation and consumption, with implications for the quantification of muscle buffer capacity. Their argument (21) is supported (in their Fig. 15) by the observation that efflux of protons during nonischemic exercise exceeds that of lactate (15) and by calculations comparing the two approaches applied to published data from exercising human quadriceps (3, 19, 24, 25). In particular, they argue (in their discussion of their Figs. 16 and 17) that 1) given the known cytosolic buffer capacity, the proton balance equations make sense only for the new model; and 2) the true buffer capacity in vivo is much greater than the traditional view allows (21). However, comparison of estimates of buffer capacity inferred in vivo with those obtained by titration of muscle homogenate in vitro is by no means straightforward (1, 16, 17), and cannot therefore settle this question. Insufficient detail is given of these calculations (21) to test them directly against the analysis above (see Appendix). However, the main issue is more fundamental, and can be argued on theoretical grounds.

Robergs et al. suggest that total proton generation (Eq. 13) has two components (Fig. 1A). The first is proton generation by ATP hydrolysis (Eq. 4a), in which one can distinguish the protons generated by hydrolysis of ATP produced from phosphocreatine via creatine kinase (Eq. 4c) from those generated from ATP produced by glycolgenolysis (Eq. 4b). The second main component of proton generation (21) is glycolgenolysis to pyruvate (Eq. 1b). Total proton generation is independent of substrate (Eq. 13). Robergs et al. (21) divide total proton consumption (Eq. 14) into three components. The first (Fig. 1A) is the traditional passively buffered component (Eq. 9). The second represents the buffering of glycolytic protons by lactate (Eq. 2). The third represents protons consumed in phosphocreatine breakdown; as the acid-base effects of ATP hydrolysis matching phosphocreatine breakdown are already taken into account in Fig. 8, this must be the unidirectional reaction (Eq. 6b) rather than the net Lohmann reaction (Eq. 7). Robergs et al. argue that the correct, or at least most physiologically meaningful, calculation of buffer capacity is the ratio of total proton production to pH fall (Eq. 15a). This gives a value (Fig. 1A) much larger (Eq. 15b) than both the traditional physicochemical calculation (Eq. 9) and indeed larger than the traditional concept (the metabolic version, in the present terminology) of apparent buffer capacity (Eq. 15a; Fig. 1B).
However, by setting the total proton generation of Robergs et al. (Eq. 13) equal to their total proton consumption (Eq. 14), we obtain precisely the traditional expression (Eq. 8). Thus whatever detailed calculations underlie Figs. 16 and 17 in Robergs et al. (21), these calculations cannot influence whether or not the implied value of cytosolic buffer capacity matches that obtained by titration of muscle homogenate in vitro.

**COMPARISON OF THESE TWO APPROACHES**

The conceptual difference between these approaches is a matter of labeling the components of proton balance (compare Figs. 1A and 1B) but with real consequences for the concept of cellular buffer capacity. Robergs et al. take a broad view of the proton load, including all the protons produced by ATP hydrolysis (Eq. 4a) and by glycolysis to pyruvate (Eq. 1b), and also of proton consumption, including the protons accounted for by reduction of pyruvate to lactate (Eq. 2) and by ATP synthesis at the expense of phosphocreatine (Eq. 6b). However, much of this proton generation is cancelled by proton consumption, so only a fraction remains to be buffered in the cytosol.

The key point is what proton buffering means. To traditionalists, it is physicochemical buffering (Eq. 9), sometimes expanded to include proton consumption in the Lohmann reaction (Eq. 7; 23) [although I prefer to distinguish these (16)]. The traditional view takes account of both the protons consumed by phosphorylation of ADP by phosphocreatine (Eq. 6) and the protons produced by the hydrolysis of the ATP supplied at the expense of phosphocreatine (Eq. 4c) and combines them into the net proton consumption by the Lohmann reaction (Eq. 7). What remains after this is subtracted from the glycolytic proton load in the physicochemical version of this approach is the net proton load, which is buffered in the cytosol (16); in the metabolic version, the ratio of total proton load to pH fall is a measure of the combined effect of physicochemical and metabolic buffering (16, 23), which may or may not be useful (see below). In both versions of the traditional approach, metabolic buffering means the Lohmann reaction.

In the proposed new view, by contrast, the concept of metabolic buffering is expanded (21) to include two processes of proton consumption: reduction of pyruvate to lactate (Eq. 2) and ATP regeneration at the expense of phosphocreatine (Eq. 6b). However, these are partially cancelled out by two processes of proton generation: hydrolysis of ATP arising from phosphocreatine breakdown (Eq. 4c) and glycolysis to pyruvate (Eq. 1b). Thus the ratio of this expanded total proton load (Eq. 13) to pH fall, proposed as total muscle buffer capacity (21), has no obvious physiological interpretation (Eq. 15b). It has a complicated relationship to physicochemical cytosolic buffer capacity: when acidification is substantial, total muscle buffer capacity considerably exceeds physicochemical cytosolic buffer capacity (Fig. 1A); in the special case of no lactate accumulation (Fig. 1C), which poses no problem to the traditional approach (4), total cellular buffering capacity is negative, a reductio ad absurdum.

The apparent buffer capacity (Eq. 12a), which Robergs et al. criticize has similar defects: it too overestimates physicochemical buffer capacity, although in a more algebraically straightforward way (Eq. 12b), which is related to the metabolically important relationship between glycogen phosphorylase flux, phosphate concentration, and creatine kinase (16), insofar as this influences the relative proportion of protons buffered and consumed. It has the technical advantage of being equal to the true physicochemical buffer capacity in the hypothetical absence of phosphocreatine breakdown but the disadvantage of being zero in the absence of lactate production. This concept might be seen as carrying useful information about metabolic regulation (16), or else as fatally flawed (21), its experimental simplicity outweighed by conceptual obscurity.

The relative proportion of protons buffered and consumed features also in the relationship between total and physicochemical buffer capacities (Eq. 15b) but in a way that makes the total buffer capacity exactly twice the true buffer capacity in the absence of phosphocreatine breakdown and negative in the absence of lactate production. I suggest that total buffer capacity is no more useful than apparent buffer capacity and physiologically more obscure.

**CONCLUSION**

Robergs et al. have usefully called attention to some ambiguities and confusions in the literature on extra- and intracellular acidification by glycolysis. In the application to exercising muscle, however, they have reached a potentially misleading conclusion about muscle cell buffer capacity. The traditional view criticized by Robergs et al. (21) certainly appears to neglect the roles of lactate synthesis in consuming the protons formed earlier in glycolysis and of ATP hydrolysis as the main source of the protons that accompany lactate. However, their presentation, which ignores for simplicity the pH dependence of the stoichiometry (6, 12, 14, 26), does not show how the importance of this point decreases as cell pH falls during exercise and that it does not in any case affect the logic of the traditional calculations. Additionally, Robergs et al. (21) make what is essentially a labeling error in the analysis of cellular proton buffering. Focusing attention on the individual reactions, they do not take account of how some of the processes of proton generation are canceled out by those of proton consumption. Thus their total muscle buffer capacity does not seem to identify a useful property of muscle physiology.

**APPENDIX**

There are some possible inconsistencies in Figs. 16 and 17 of Robergs et al. (21), and the relationship to the experimental papers cited (3, 19, 24, 25) is not entirely clear. For example, the 125 mmol proton/kg muscle used as the denominator to estimate total muscle buffer capacity (Eq. 15 here) as 208 slykes (evidently mmol/kg wet wt) appears inconsistent with the ~100 mmol proton/kg muscle shown in Fig. 17. The 72 mmol proton/kg muscle used to calculate the metabolic buffering of 120 slykes appears to be the sum of the components labeled Pi, CrP and lactate in Fig. 17, which I take to be the protons buffered by the inorganic phosphate component of β and the protons consumed by phosphocreatine hydrolysis (Eq. 6b) and the reduction of pyruvate to lactate (Eq. 2), respectively. However, the first of these should presumably be considered structural or static buffering: even though phosphate increases during exercise, the proton-binding effect of phosphate requires a fall in pH (to drive the ionic equilibrium $\text{Pi}^{2-} + \text{H}^+ \leftrightarrow \text{HPi}^{-}$ to the right) but no enzyme action; in this it differs from the indubitably metabolic Lohmann reaction, which requires enzyme activity but not a
change in pH; indeed, it can consume protons when pH is rising (Fig. 1C) and the static buffers are giving protons up. The remaining structural (physicochemical) buffer capacity of 88 (= 208 – 120) slykes is not, as claimed, close to the structural buffer capacity reported in (23); the 42 slykes they cite in Fig. 16 (21) as a value obtained by homogenate titration appears to be a value predicted from measured and estimated cytosolic concentrations (23) and is anyway at the low end of the published range of homogenate-titration values (16, 23), which are often overestimates (1). Because of these uncertainties, in this letter, I have taken a theoretical approach supported by illustrative data from another source.

REFERENCES


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