

## Acid-base analysis: a critique of the Stewart and bicarbonate-centered approaches

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**Kurtz I, Kraut J, Ornekian V, Nguyen MK.** Acid-base analysis: a critique of the Stewart and bicarbonate-centered approaches. *Am J Physiol Renal Physiol* 294: F1009–F1031, 2008. First published January 9, 2008; doi:10.1152/ajprenal.00475.2007.—When approaching the analysis of disorders of acid-base balance, physical chemists, physiologists, and clinicians, tend to focus on different aspects of the relevant phenomenology. The physical chemist focuses on a quantitative understanding of proton hydration and aqueous proton transfer reactions that alter the acidity of a given solution. The physiologist focuses on molecular, cellular, and whole organ transport processes that modulate the acidity of a given body fluid compartment. The clinician emphasizes the diagnosis, clinical causes, and most appropriate treatment of acid-base disturbances. Historically, two different conceptual frameworks have evolved among clinicians and physiologists for interpreting acid-base phenomena. The traditional or bicarbonate-centered framework relies quantitatively on the Henderson-Hasselbalch equation, whereas the Stewart or strong ion approach utilizes either the original Stewart equation or its simplified version derived by Constable. In this review, the concepts underlying the bicarbonate-centered and Stewart formulations are analyzed in detail, emphasizing the differences in how each approach characterizes acid-base phenomenology at the molecular level, tissue level, and in the clinical realm. A quantitative comparison of the equations that are currently used in the literature to calculate  $H^+$  concentration ( $[H^+]$ ) is included to clear up some of the misconceptions that currently exist in this area. Our analysis demonstrates that while the principle of electroneutrality plays a central role in the strong ion formulation, electroneutrality mechanistically does not dictate a specific  $[H^+]$ , and the strong ion and bicarbonate-centered approaches are quantitatively identical even in the presence of nonbicarbonate buffers. Finally, our analysis indicates that the bicarbonate-centered approach utilizing the Henderson-Hasselbalch equation is a mechanistic formulation that reflects the underlying acid-base phenomenology.

acid-base; SID; bicarbonate; kidney; lung

OUR UNDERSTANDING OF CLINICAL acid-base chemistry is based primarily on concepts derived from the studies of several investigators including Arrhenius (5), Sørensen (141), Henderson (57), Hasselbalch (55), Brønsted and Lowry (13, 85), Van Slyke (153), Lewis (81), Severinghaus (131, 133), Astrup (6, 7, 133), Siggard-Anderson (133–138), Schwartz (129), and Relman (119, 129). What evolved from these studies of was a consensus that the activity of  $H^+$  within a given compartment was determined by 1) the mass balance of  $H^+$ ; 2) proton transfer reactions mediated by proton donors (weak acids) and proton acceptors (weak bases) that buffer the change in  $H^+$  activity within a certain pH range; and 3) the mass balance of proton donors and proton acceptors.

In 1978, Stewart (144) questioned the traditional accepted approach used to analyze acid-base chemistry. He modeled a solution which contained a complex mixture of ions of constant

charge over the physiological pH range ( $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ), nonvolatile proton donors/acceptors which transfer  $H^+$  within the physiological pH range (albumin, phosphate, hemoglobin, metabolizable organic compounds), and the volatile bicarbonate- $CO_2$  buffer system composed of  $CO_2$ ,  $HCO_3^-$ ,  $H_2CO_2$ , and  $CO_3^{2-}$ . Accounting for requirements of electroneutrality, the law of conservation of mass and certain equilibrium constraints, Stewart solved a fourth-order polynomial equation for calculating the  $H^+$  concentration. His analysis did not depart from the traditional approach to acid-base chemistry as a result of the equations that were derived, which were based on physicochemical considerations and sound algebra (144–146). Pivotal to the Stewart formulation was the categorization of certain species as being dependent or independent variables in relationship to their purported role in determining and modifying the  $H^+$  concentration ( $[H^+]$ ) of a solution. Specifically,  $H^+$ ,  $OH^-$ ,  $HCO_3^-$ , and  $CO_3^{2-}$  were categorized as dependent variables on which he based the novel stipulation that the mass balance of these species (influx minus efflux) in a solution or specific body fluid compartment could not per se affect the  $[H^+]$ . Furthermore, Stewart stipulated that proton

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transfer reactions involving these species could not be responsible for changes in  $[H^+]$ . Finally, he contended that  $[H^+]$  was a function of three variables: [SID], strong ion difference: the difference in the net charge of fixed cations and anions fully dissociated in solution;  $[A_{TOT}]$ : partially dissociated weak acids (albumin, phosphate); and the  $P_{CO_2}$  of the solution. Subsequent investigators have 1) expanded the Stewart conceptual framework theoretically; 2) applied the Stewart framework in various physiological/clinical states; 3) reinterpreted cellular/whole organ transport processes that alter the acidity of body fluid compartments; and 4) recategorized the diagnosis of clinical acid-base disorders (3, 4, 21–31, 40–42, 45–47, 52, 54, 61–68, 70, 74, 79, 82–84, 89, 93, 98, 101–103, 105, 110, 113, 116, 117, 120, 121, 123, 125, 128, 140, 142–151, 156, 158–163). In addition, the question of the utility of Stewart framework in clinical situations has also been reported (16, 32–35, 37, 39, 126, 138).

In our view, the differences between the Stewart and traditional formulations is not mathematical in nature. Rather, the departure that Stewart and subsequent refinements of his theory engendered is related to epistemological differences. Some of these differences that will be analyzed in this review are whether 1) acid-base theories should have an underlying mechanistic underpinning as a criterion for their validity and specifically whether Stewart's formulation falls into this category; 2) Stewart's original categorization of certain chemical species into dependent and independent variables was valid, and based on this categorization, whether a new mechanistic understanding of acid-base phenomenology was uncovered; and 3) Stewart's formulation confuses calculation based on macroscopic electroneutrality requirements with causation.

Insufficient analysis of these considerations in our view accounts for the current confusion in the literature as it relates to a comparison of the traditional and Stewart formulations of acid-base phenomena. In what follows, the traditional and Stewart formulations will be examined in detail as they relate to 1) the acid-base behavior of simple and complex solutions in vitro, 2) the cellular transport of ions between body fluid compartments, 3) whole body acid-base chemistry, and 4) diagnosis of acid-base disorders.

#### *Bicarbonate-Centered Clinical Acid-Base Interpretation: Comparative $\Delta HCO_3^-/\Delta P_{CO_2}$ and Base Excess Approaches*

The traditional approach to clinical acid-base interpretation (sometimes referred to as the "bicarbonate-centered" approach) is based on 1) Lowry-Brønsted theory, wherein acids are defined as substances capable of donating protons and bases as substances capable of accepting protons; and 2) the centrality of the bicarbonate buffer system in whole body acid-base homeostasis, given that it is composed of a volatile and nonvolatile buffer pair. Quantitatively, this approach starts with the assumption that the components of the  $HCO_3^-$ - $CO_2$  equilibrium reaction are in equilibrium with nonbicarbonate buffers (albumin, phosphate, hemoglobin) and that the property of interest is the relationship between the total concentration of bound and unbound proton binding (acceptor) sites in relation to the activity of free protons in an aqueous solution.

The total concentration of proton binding sites,  $C_B$ , in a solution or body fluid compartment, is calculated as (51)

$$C_B = C + \sum_i C_i \bar{e}_i - D \quad (1)$$

where  $C$  is the total concentration of carbonate proton acceptor sites:  $HCO_3^-$ ,  $CO_3^{2-}$ , and  $PrNHCOO^-$  (carbamate);  $C_i$  is the concentration of noncarbonate buffer species,  $i$ ,  $e_i$  is the average number of proton acceptor sites per molecule of species  $i$ , and  $D$  is Ricci's difference function ( $D = [H^+] - [OH^-]$ ).

At physiological pH, the contribution of  $CO_3^{2-}$ , and  $PrNHCOO^-$  is negligible, Eq. 1 simplifies to

$$C_B = [HCO_3^-] + \sum_i C_i \bar{e}_i \quad (2)$$

Assuming the solution does not contain noncarbonate buffers,  $C_B = [HCO_3^-]$ , and from the Law of Mass action, Henderson (57) derived the following equation

$$[H^+] = \frac{K'_1 \times [CO_2]}{[HCO_3^-]} \quad (3)$$

Using the convention of Sørensen (141), where pH is the negative logarithm of the hydrogen ion activity (base 10), Hasselbalch (55) expressed the Henderson equation as

$$pH = pK'_1 + \log \frac{[HCO_3^-]}{S \times P_{CO_2}} \quad (4)$$

the Henderson-Hasselbalch (H-H) equation, where  $S$  is the solubility of  $CO_2$ .

According to traditional concepts, as with any proton donor/acceptor pair, changes in the  $HCO_3^-$  concentration and the  $P_{CO_2}$  can occur either because of an alteration in the mass balance of each species, or as a result of a shift in the  $HCO_3^-$ - $CO_2$  equilibrium reaction



By the 1960s, the bicarbonate buffer reaction and those factors that modulated its parameters became the basis for the interpretation and categorization of clinical acid-base disorders. Two bicarbonate-centered approaches evolved: the comparative  $\Delta HCO_3^-/\Delta P_{CO_2}$  approach (129) and the base excess (BE) approach (135). BE is traditionally calculated from the Van Slyke equation as developed by Siggard-Anderson (136) where

$$BE = (HCO_3^- - 24.4 + [2.3 \times Hb + 7.7] \times [pH - 7.4]) \times (1 - 0.023 \times Hb) \quad (6)$$

and ( $HCO_3^-$  and Hb are in mmol/l).

In the bicarbonate-centered formulations, primary changes in the  $P_{CO_2}$  were termed respiratory acid-base disorders, reflecting an abnormality in the mass balance of  $CO_2$  due to abnormal alveolar  $CO_2$  excretion. Acid-base disorders not due to primary changes in the  $P_{CO_2}$  were termed metabolic disturbances and had in common a change in the  $HCO_3^-$  concentration ( $[HCO_3^-]$ ) either because of 1) alterations in the mass balance of  $HCO_3^-$ ; or 2) participation of bicarbonate in internal  $H^+$  transfer reactions resulting from alterations in the mass balance nonbicarbonate buffers (albumin, phosphate, ketone bodies, etc.) in their protonated and/or deprotonated forms.

It was recognized that primary changes in the  $P_{CO_2}$  (respiratory acid-base disorders) caused secondary changes in the plasma  $[HCO_3^-]$  due to 1) a shift in the equilibrium of the

$\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium (acute respiratory acid-base disorders), and 2) mass balance changes associated with the renal excretion of  $\text{NH}_4^+$  and  $\text{HCO}_3^-$  and titrateable acid (36, 72, 76). Moreover, it was also appreciated that primary changes in the  $[\text{HCO}_3^-]$  in metabolic acid-base disorders caused secondary changes in the  $\text{Pco}_2$  not only because of an acute shift in the  $\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium reaction but also because of a change in alveolar  $\text{CO}_2$  excretion (72, 87). Determining the initiating event, e.g., change in the  $\text{Pco}_2$  vs. a change in  $[\text{HCO}_3^-]$ , became the diagnostic challenge in using the comparative  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  approach. Fortunately, each of the cardinal acid-base disorders was found to have a characteristic  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  value (36, 72, 129), and therefore an acid-base diagnosis could be made by comparing the actual  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  value in a given patient to the expected  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  value.

The comparative  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  approach has been criticized (130, 131, 133, 135–137) for being 1) qualitative in nature and 2) incapable of quantifying acid or base loads that result in metabolic acid-base disorders. In particular, because body compartments consist of multiple buffers, it is argued that the  $\text{HCO}_3^-$  buffer is only one of several buffers that were protonated by an  $\text{H}^+$  load and therefore the  $\Delta\text{HCO}_3^-$  would underestimate the actual total body acid burden as, for example, in a patient with ketoacidosis. Practitioners of the comparative  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  approach acknowledged its inability to quantify acid or base loads but argued from a purely utilitarian standpoint that the practical underlying goal in the clinical setting is to diagnose the acid-base abnormality correctly and that quantifying the magnitude of a proton/base load is of potential importance therapeutically but is not essential diagnostically. An additional criticism of the use of the comparative  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  approach in diagnosing metabolic acid-base disorders is the fact that a component of the change in the  $[\text{HCO}_3^-]$  is due to a shift in the  $\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium reaction as a result of the compensatory ventilatory response (altered  $\text{Pco}_2$ ) that occurs in patients with metabolic acid-base disturbances. Moreover, the compensatory ventilatory-induced alteration in the  $\text{Pco}_2$  causes a change in renal  $\text{NH}_4^+$  and  $\text{HCO}_3^-$  and titrateable acid excretion, which results in a further change in the  $[\text{HCO}_3^-]$  (independent of the acid/base load and independent of the change due to the shift in the  $\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium reaction) (72, 87). Therefore, in a compensated metabolic acid-base disorder the  $\Delta\text{HCO}_3^-$  is due to 1) the primary alteration in acid or base mass balance; 2) ventilatory (compensatory) changes in the  $\text{Pco}_2$  which alter the  $[\text{HCO}_3^-]$  further via a shift in the  $\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium reaction; and 3) ventilation-induced changes in renal mechanisms ( $\text{NH}_4^+$  and  $\text{HCO}_3^-$  and titrateable acid excretion). Finally, an additional criticism is that the  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  expected in acute respiratory acid-base disorders ( $\sim 0.15$ ) depends on the number of proton binding sites on nonbicarbonate buffers (e.g., albumin, hemoglobin, phosphate).

Currently, the most widely accepted approach for correcting the fact that an acute shift in the  $\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium reaction can change the  $[\text{HCO}_3^-]$  is the BE formulation, where one quantifies the amount of acid or base that must be added to whole blood in vitro to restore the pH to 7.40 while the  $\text{Pco}_2$  is kept at 40 mmHg (130, 131, 133–137). More recent BE formulas, termed standard base excess (SBE), titratable hydrogen ion concentration difference (THID), and corrected standard base excess (CSBE), take into consideration the fact that

whole blood is composed of multiple buffers (hemoglobin, phosphate, albumin) and that the in vitro whole blood titration must be corrected for the fact that in patients, acid or base loads are not only titrated in the blood compartment (38, 68, 112, 137, 162). Furthermore, as in the  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  approach each of the cardinal acid-base disorders has been shown to have a characteristic  $\Delta\text{SBE}/\Delta\text{Pco}_2$  value (127).

Like the  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  approach, the BE analysis also has been criticized (38, 129, 153). It is an approach that extrapolates results in vitro to the more complex multicompartmental real-life situation (extracellular and intracellular compartments). Specifically, the  $\Delta\text{HCO}_3^-$  induced by acutely normalizing the  $\text{Pco}_2$  in vitro differs from acute whole body  $\text{CO}_2$  titration (8, 11, 48, 49, 78, 114, 122). In addition, the result obtained from returning the pH of blood to 7.40 in vitro will differ from whole body titration with an acid or base (38, 129, 153). In a compensated metabolic acidosis, the compensatory ventilatory alteration in the  $\text{Pco}_2$  induces a further decrement in the blood  $[\text{HCO}_3^-]$  that is included in the BE value even though this component of the  $\Delta\text{HCO}_3^-$  results from a renal mechanism independent of the acid or base load  $\Delta\text{HCO}_3^-$  component (87). In chronic respiratory acid-base disorders, the renal compensatory mechanisms result in changes in BE which, if diagnosed as a separate metabolic acid-base disorder, potentially result in inappropriate therapy.

Despite these complexities, the comparative  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  and BE approaches evolved historically as two alternative “bicarbonate-centered” approaches for diagnosing clinical acid-base disturbances (Fig. 1). Either the  $\Delta\text{HCO}_3^-/\Delta\text{Pco}_2$  or BE approaches, when interpreted properly, suffice to diagnose and treat the cardinal acid-base disorders. In practice, practitioners of the BE approach need to be more aware of the effect of

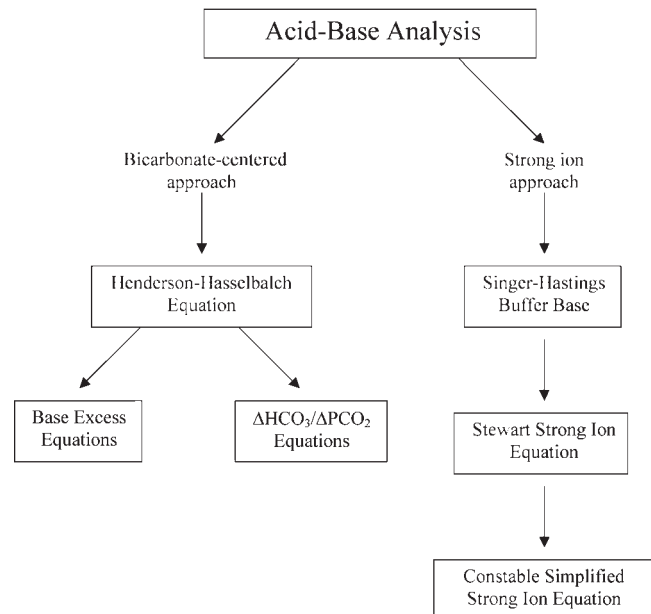


Fig. 1. Historical overview of acid-base analysis. In the past  $\sim 100$  years, 2 general approaches to quantitative acid-base analysis have evolved. The bicarbonate-centered approach utilizes the Henderson-Hasselbalch equation to interpret acid-base changes in aqueous bicarbonate-buffered solutions. The strong ion approach currently utilizes the Stewart equation or the simplified Constable equation to interpret acid-base changes in aqueous bicarbonate-buffered solutions.

chronic changes in the P<sub>CO<sub>2</sub></sub> (chronic respiratory acidosis and alkalosis) on the BE measurement to prevent misdiagnosis and inappropriate therapy with bicarbonate or HCl (127).

*Stewart Equation: Historical Perspective*

According to the Lowry-Brønsted acid-base theory based on the transfer of protons between H<sup>+</sup> donors (acids) and H<sup>+</sup> acceptors (bases), changes in either the mass balance of H<sup>+</sup> or the donation/release of H<sup>+</sup> between a donor-acceptor pair within a given compartment are responsible for an alteration in the [H<sup>+</sup>] (13, 85). Stewart’s formulation challenged this assertion (144–146). He began his analysis by considering a simple solution of water containing H<sup>+</sup>, OH<sup>-</sup>, and H<sub>2</sub>O to highlight the quantitative aspects of the controversy. At equilibrium, [H<sup>+</sup>] × [OH<sup>-</sup>] = K<sub>w</sub> × [H<sub>2</sub>O], where K<sub>w</sub> represents the dissociation constant of water. Since [H<sub>2</sub>O] can be considered constant, K<sub>w</sub> × [H<sub>2</sub>O] can be replaced by K’<sub>w</sub>, the ion product for water. Assuming that in pure water H<sup>+</sup> and OH<sup>-</sup> are the only charged species present, their activities must be equal, i.e., [H<sup>+</sup>] = [OH<sup>-</sup>]. Solving for [H<sup>+</sup>], one obtains the following equation

$$[H^+] = \sqrt{K'_w} \tag{7}$$

Now, consider a more complex solution of water containing Na<sup>+</sup> and Cl<sup>-</sup>. According to the requirements of electroneutrality, Na<sup>+</sup> - Cl<sup>-</sup> + H<sup>+</sup> - OH<sup>-</sup> = 0 or Na<sup>+</sup> - Cl<sup>-</sup> = OH<sup>-</sup> - H<sup>+</sup>. Therefore, given that [OH<sup>-</sup>] = K’<sub>w</sub>/[H<sup>+</sup>], Stewart derived an equation in terms of [H<sup>+</sup>] such that

$$[H^+] = \sqrt{K'_w + \frac{([Na^+] - [Cl^-])^2}{4} - \frac{([Na^+] - [Cl^-])}{2}} \tag{8}$$

Stewart termed the [Na<sup>+</sup>] - [Cl<sup>-</sup>] the strong ion difference or [SID] and put forward the concept that this parameter was a determinant of the [H<sup>+</sup>]. Accordingly, in this formulation, ions with fixed charges play a central role in determining [H<sup>+</sup>]. Stewart’s use of [SID] is a reintroduction of “buffer base” (BB), originally introduced in 1948 by Singer and Hastings (139) to assess the “metabolic component” of acid-base disturbances independent of acute changes in the P<sub>CO<sub>2</sub></sub>. BB was defined as the sum of fixed cations minus fixed anions in whole blood, where [BB<sup>+</sup>] = [HCO<sub>3</sub><sup>-</sup>] + [A<sup>-</sup>], and A<sup>-</sup> represents the non-bicarbonate buffer anions (mainly hemoglobin, and in addition, albumin and phosphate). Acute changes in the P<sub>CO<sub>2</sub></sub> (acute respiratory acid-base disorders) induce equal and opposite changes in [HCO<sub>3</sub><sup>-</sup>] and [A<sup>-</sup>], and BB remains unaffected; therefore, Singer and Hastings suggested that BB is an index of metabolic acid-base disturbances. However, BB historically never gained acceptance for a number of likely reasons: 1) as in the case of BE measurement, BB is altered by changes in hemoglobin, albumin, and phosphate; 2) acid or base loads are also titrated outside the blood compartment; and 3) BB is altered by both metabolic and chronic respiratory acid-base disturbances.

Since ions with a fixed charge were always present at the concentrations at which they were originally added, remained completely dissociated, and did not participate in the reactions in the solution, they were defined by Stewart as independent variables in the system. Specifically, he stated that in a simple strong electrolyte solution, “the constraints of the water disso-

ciation equilibrium and electrical neutrality require that hydrogen and hydroxyl ion concentrations assume particular values as soon as the value of the strong ion difference has been set from outside the system.” (144). More recently, Constable has stated, “Because strong ions do not participate in chemical reactions in plasma at physiological pH, they act as a collective positive unit of charge (SID<sup>+</sup>)” (24).

By taking into consideration charge, the law of mass action, and equilibrium constraints, in a compartment such as blood containing bicarbonate and nonbicarbonate (albumin, phosphate) buffers, Stewart derived a fourth-order polynomial equation depicting [H<sup>+</sup>] as a function of SID, the P<sub>CO<sub>2</sub></sub>, and total weak acid concentration [A<sub>TOT</sub>]

$$[H^+]^4 + [H^+]^3 (K_a + [SID^+]) + [H^+]^2 (K_a ([SID^+] - [A_{TOT}]) - (K'_1 \times S \times P_{CO_2} + K'_w)) - [H^+] (K_a (K'_1 \times S \times P_{CO_2} + K'_w) + K'_1 \times S \times P_{CO_2} \times K_3) - K_a \times K_3 \times K'_1 \times S \times P_{CO_2} = 0 \tag{9}$$

These parameters were defined as the key independent variables that mechanistically alter the [H<sup>+</sup>]. OH<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>2-</sup> being categorized as parameters that cannot cause a change in the [H<sup>+</sup>] (dependent variables) were not explicitly depicted in the equation.

Given the fact that H<sup>+</sup>, OH<sup>-</sup> (in the nanomolar range) and CO<sub>3</sub><sup>2-</sup> (in the micromolar range) do not contribute significantly to the total number of charges, Constable (21) simplified Stewart’s strong ion equation by deriving the following equation, which has the same form as the H-H equation where

$$pH = pK'_1 + \log \frac{([SID] - K_a[A_{TOT}]) / (K_a + 10^{-pH})}{S \times P_{CO_2}} \tag{10}$$

Since (K<sub>a</sub>[A<sub>TOT</sub>]) / (K<sub>a</sub> + 10<sup>-pH</sup>) = [A<sup>-</sup>], Schück et al. (129) have rewritten this equation as (our symbols)

$$pH = pK'_1 + \log \frac{([SID] - [A^-])}{S \times P_{CO_2}} \tag{11}$$

that was termed the “corrected Henderson-Hasselbalch equation.”

It is clear from our analysis thus far that there currently exists a fundamental difference between the proponents of the Stewart and traditional formulations in mechanistically accounting for the changes in [H<sup>+</sup>] in an aqueous solution. These differences are highlighted in the following hypothetical conversation between a proponent of the Stewart formulation and a traditional acid-base physiologist.

Stewart: In my analysis of both acid-base chemistry and clinical acid-base physiology, I have changed the focus to alterations in the concentration (activity) of substances that historically were categorized as “strong ions.”

Traditional: I base my analysis of acid-base problems on changes in the concentration (activity) of substances excluded from the strong ion category. For example, a patient has diarrhea that causes a reduction in blood pH and a metabolic acidosis. You calculate a decrease in the [SID] and conclude that the metabolic acidosis was caused by an increase in the [Cl<sup>-</sup>] that resulted in a decreased [SID]. I would respectively ask: Why do you focus predominantly on the strong ions and [SID] since given the law of electroneutrality, there must also

be an identical change in the net charge difference of substances that you excluded from the strong ion category? I concur that the [SID] has decreased, but I note that there was a decrease in the  $[\text{HCO}_3^-]$  that was equal to the increase in the  $[\text{Cl}^-]$ . I chose the decrease in the  $[\text{HCO}_3^-]$  as the mechanism by which the pH changed since  $\text{HCO}_3^-$  represents a substance that is in equilibrium with  $\text{H}^+$  and therefore modulates the pH.

Stewart: I would counter that it is the decreased [SID] that is causing the change in pH because  $\text{HCO}_3^-$  is a dependent variable and therefore it cannot cause a change in pH by definition even though it participates in an  $\text{H}^+$  donating/binding reaction.

Traditional: I would suggest that given the choice of a change in [SID] vs. a change in  $[\text{HCO}_3^-]$ , one can explain mechanistically why a decrease in the pH is associated with a decrease in the  $[\text{HCO}_3^-]$ , whereas the mechanism by which a decreased [SID] results in a decrease pH is conjectural. I would reject as unfounded statements such as: "SID has a powerful electrochemical effect on water dissociation, and hence on  $\text{H}^+$  concentration. As SID becomes more positive,  $\text{H}^+$ , a 'weak' or 'unfixed' cation, decreases (and pH increases) to maintain electrical neutrality" (67). Specifically, water equilibrium reactions cannot result in a change in the net charge of unfixed ions since  $\text{H}^+$  and  $\text{OH}^-$  are generated or consumed equally. Moreover, although the SID concept is based on electroneutrality requirements, these requirements do not dictate a specific  $[\text{H}^+]$ .

The centrality of SID to acid-base chemistry invoked by Stewart calls to mind the confusion in the acid-base literature that existed in the early 20th century. Before the paradigm shift that occurred in the understanding of acid-base chemistry following the publication of papers by Brønsted and Lowry in 1923–1924 (13, 85), acids were synonymous with strong anions, and bases were synonymous with strong cations (18, 44, 119). Brønsted and Lowry changed the focus of acid-base chemistry by stating that the key player was the chemical potential of the hydrogen ion.

This insight removed strong cations ( $\text{Na}^+$ ) and strong anions ( $\text{Cl}^-$ ) from the underlying conceptual framework of acid-base chemistry with the implication that neither ion was primarily responsible for changes in pH. Rather, it is when  $\text{Na}^+$  is added to a solution in the form of a salt accompanied by a substance ( $\text{OH}^-$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ) that can bind protons at the initial pH value that the pH can change to a new value. Similarly,  $\text{Cl}^-$  in the form of  $\text{HCl}$  is actually a base in the Brønsted and Lowry terminology since at a specific pH it is a proton acceptor. Given that the equilibrium constant for any buffer pair is dependent on ionic strength, it was clearly recognized that by changing the ionic strength, alterations in the  $[\text{Na}^+]$  and/or  $[\text{Cl}^-]$  could modulate the final pH value at a specific temperature (56, 71, 86, 134).

Despite these early insights, Relman (119) Christensen (18), and Frazer (44) discussed the confusion in the literature that still existed in the 1950s relating to the nomenclature utilized in clinical acid-base chemistry. After a thorough discussion of the historic reasons clinical chemists considered  $\text{Na}^+$  a base, and  $\text{Cl}^-$  an acid, Relman (119) went on to discuss how this terminology "entirely neglects the central position of the hydrogen ion in acid-base reactions." Christensen (18) elaborated on the same theme and mentions that in various textbooks discussing acid-base balance "a much greater emphasis upon

other inorganic ions than has been so far included here." He stated "biological fluids contain two types of anions, buffer anions and non-buffer anions. The latter are frequently called fixed anions, i.e., anions whose state of charge is fixed over the relevant range of pH. In contrast the cations are essentially all fixed cations." The difference between fixed anions and cations in this terminology is analogous to what Stewart termed [SID]. Christensen then stated

Hence we come to recognize as acid a biological solution that has a fixed *anion* level out of the usual proportion to the cation level. When the kidney corrects this situation, the fixed anion concentration of the *plasma* is again lowered to the usual value. In the meantime the level of fixed anion of the urine has been increased, and its level of buffer anion has been lowered by reaction with the excreted  $\text{H}^+$ . Therefore it appears that the *acidity of the plasma has been assumed by the urine by the transfer of fixed anions to it*. . . . Conversely, a biological solution with a proportionately increased *cation* concentration is recognized as *alkaline*; the correction of this alkaline state is marked by a *transfer of the cation excess* to the urine. . . . Going only a step further, one may come to think of the cations, as *bases*, and the fixed anions as *acids*, but this is a long and dangerous step that beings to attribute *acidifying properties* to the chloride ion and *basic properties* to the sodium ion.....The only reason the neutrality can be described by the relationship between the fixed anion and the cation levels is that this relationship can give us indirectly the concentration of the *buffer anions*. These, together with the  $\text{H}^+$  which they tend to bind, are the real actors in the drama. . . . As long as we treat the fixed-ion levels as a *reflection* of the hydrogen ion distribution rather than the *cause*, we have gained a valuable ancillary approach.

Christensen also discussed the role of bicarbonate in this context:

Responsibility for the alkalinizing action of such an agent as  $\text{NaHCO}_3$  *must be borne by either the sodium or by the bicarbonate ion* (italics ours). Lack of understanding of the properties of the bicarbonate ion in the past has perhaps misdirected attention to the sodium ion. The modern student presents a pre-medical training in chemistry that permits him readily to grasp that the bicarbonate ion (and similar buffer anions) are really basic in that they tend to remove free  $\text{H}^+$  from solution...Furthermore, nothing he has learned about the chemistry of the sodium ion permits him to understand how it can be alkalinizing.

This prescient analysis foreshadows in some sense the current issues in the literature as they relate to the Stewart framework.

#### *Dependent and Independent Variables: Cause and Effect Relationships in Stewart's Strong Ion Theory*

As discussed, Stewart began his analysis by addressing the algebraic problem of providing an exact mathematical treatment for solutions containing fixed ions, dissociated weak acids, and the amphoteric species  $\text{HCO}_3^-$ . His analysis differed in how he collected and omitted terms in his equations based on whether he had defined them as independent or dependent variables. Since he had prior to his quantitative analysis defined  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ , and  $\text{OH}^-$  as dependent variables, these species could not therefore partake mechanistically in causing changes in the  $[\text{H}^+]$  and therefore were not explicitly referred to in his final equations. Accordingly, Stewart concluded that

$H^+$  was a function of the independent variables [SID],  $[A_{TOT}]$ , and  $PCO_2$  (144–146).

Given that Stewart's dependent/independent variable categorization scheme was the starting point of his analysis, it is of importance in analyzing his formulation to determine the validity of his assertions in this area. Although mathematical equations provide information as to how specific variables are related quantitatively, they do not provide information regarding cause and effect relationships. Let us address this further in an example where a change in a parameter  $y$  is associated with a change in  $x$ . To state that  $y = f(x)$  is a symbolic way of conveying that when a particular operation is performed on  $x$ , one can calculate the value of  $y$ . One could equally have written the following equation:  $x = f(y)$ . Mathematically, there is no information represented by the relationship of the variables in these equations which can address the question as to whether changes in  $x$  cause changes in  $y$ , or whether changes in  $y$  cause changes in  $x$ . The equations simply define the quantitative interrelationship between the variables  $x$  and  $y$  that permits the calculation of  $y$  if  $x$  is known and visa versa. As a consequence, the equations per se offer no clue as to which variable is dependent (effect) or independent (cause).

The question as to which variable is independent or dependent is not mathematical, but is rather experimental/empirical in nature. If by experimentation one determines that  $x$  is the independent variable, by convention the dependence of  $y$  on  $x$  is indicated by an equation of the form  $y = f(x)$ . Importantly, very often in experimental science, the ultimate goal in determining which variable is independent or dependent is to uncover a mechanism(s) for a particular observation. These considerations emphasize that to categorize a specific term in an equation as an independent or dependent variable, one must have obtained mechanistic experimental data. In the absence of empirical data, one must at minimum have a mechanistic model that accounts for the observed phenomena on which to base one's dependent/independent variable categorization.

As a justification for his analysis, Stewart used epistemological arguments rather than experimental results when he stated that independent variables are "imposed on a system from the outside, and are not affected by the equations which govern the system, nor by changes in the system, nor by each other. Dependent variables are internal to the system; their values are determined by the system equations, and by the values of the independent variables" (146). It was on this basis that he defined  $[H^+]$ ,  $HCO_3^-$ ,  $CO_3^{2-}$ , and  $OH^-$  as dependent variables, and  $PCO_2$ ,  $[SID^+]$ , and  $[A_{TOT}]$  as independent variables. As stated by proponents of the Stewart formulation "all other variables in the system such as pH,  $[HCO_3^-]$ , and  $[A^-]$ , are dependent variables and cannot be changed primarily or individually; they change, all simultaneously, only if one or more of the independent variables change" (41).

As a corollary, Stewart parted ways with traditional acid-base theory by stipulating that " $H^+$  movements into or out of a solution do not provide quantitative explanations for changes in  $[H^+]$  . . . "do not determine the value of  $[H^+]$ , and by themselves cannot explain changes in it" (italics ours) (146). Therefore, according to Stewart's theory, neither the whole body changes in the mass balance and intercompartmental flux of  $H^+$ ,  $OH^-$ ,  $HCO_3^-$ , and  $CO_3^{2-}$ , nor buffer reactions in a solution that consume or release  $H^+$  can mechanistically result in changes in the  $[H^+]$ . Moreover, Kowalchuk and Scheuer-

mann (70) have stated that the recovery of intracellular acid-base status after severe exercise "should not be viewed as transport of  $H^+$ 's per se out of muscle, but should be considered in terms of differing changes in  $PCO_2$ ,  $[SID^+]$ , and  $[A_{TOT}]$  between the ICF and ECF, resulting in an *apparent efflux*" (italics ours).

Although Stewart's strong ion model has been called "mechanistic" and the H-H equation "descriptive" (24), this characterization is questionable for several reasons. First, epistemological arguments ought not to be the basis for a mechanistic understanding of acid-base chemistry. Second, Stewart's epistemology is questionable since there is no basis in statistical theory for his contention that that independent variables are "imposed on a system from the outside, and are not affected by the equations which govern the system, nor by changes in the system, nor by each other"; and that "dependent variables are internal to the system; their values are determined by the system equations, and by the values of the independent variables" (italics ours) (146).

Several facts are worth noting regarding the  $PCO_2$ - $HCO_3^-$ -proton transfer reaction in regard to the derivation of the Stewart equation. First, Stewart contended that internal  $H^+$ ,  $HCO_3^-$ ,  $CO_3^{2-}$ , proton transfer reactions could not be responsible for changes in  $[H^+]$  in the same system. Despite this, as discussed, the equilibrium rate constants for the  $H^+$  transfer reactions involving  $HCO_3^-$  and  $CO_3^{2-}$  are explicit parameters in Stewart's equation. Furthermore, although Stewart felt it was justified to omit  $HCO_3^-$  explicitly from his formulation as it was classified as a dependent variable,  $CO_2$  (the proton donor) was not excluded as it was defined as an independent variable. This distinction is unclear given that the concentration of both  $HCO_3^-$  and  $CO_2$  are altered by changes in mass balance and partake in the same aqueous proton transfer reaction. The  $HCO_3^-$ - $CO_2$  reaction can per se generate or consume  $CO_2$  without a change in whole body  $CO_2$  mass balance. In this sense the  $CO_2$  concentration is also a dependent variable making its assignment as an independent variable according to the Stewart formulation confusing. In a similar vein, since HA and  $A^-$  are in equilibrium, assigning  $A_{TOT}$  to be an independent variable results in similar semantic confusion.

#### Electroneutrality Considerations

Stewart's strong ion formulation (144–146) and Constable's subsequent simplified strong ion model (21–28, 142, 143) are based on the categorization of substances according to their charge characteristics. As outlined, substances that have the same charge at physiological pH are placed in the strong ion or "fixed charge" category, and substances that partake in  $H^+$  donating/binding reactions at a given pH are excluded from the strong ion category since their charge varies. Examples of the latter are volatile buffer ions (bicarbonate) and nonvolatile buffer ions (albumin, phosphate, hemoglobin). Whether one includes a substance in the strong ion category depends on the pH. For example the charge contributed by the lactate/lactic acid pair is pH dependent. Specifically, the pK of the lactate/lactic acid pair is 3.6 such that at pH 7.4, the solution contains essentially all lactate. However, at pH 3.6, 50% of the lactate has been converted to lactic acid and the charge is 50% less than at pH 7.4.

Substances in the strong ion category include:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Cl}^-$ , sulfate, nonmetabolizable organic anions ( $\text{OANM}^-$ ), and nonmetabolizable organic cations ( $\text{OCNM}^+$ ). Substances whose activity and therefore net charge varies due to proton transfer reactions include  $\text{H}^+$ ,  $\text{OH}^-$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ , albumin $^{x-}$ ,<sup>1</sup> phosphate $^{y-}$ , metabolizable organic anions ( $\text{OAM}^-$ ), and metabolizable organic cations ( $\text{OCM}^+$ ). According to the law of electroneutrality:  $\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} + \text{OCNM}^+ + \text{OCM}^+ + \text{H}^+ + \text{HCO}_3^- + \text{CO}_3^{2-} + \text{OH}^- + \text{Cl}^- + \text{sulfate}^{2-} + \text{albumin}^{x-} + \text{phosphate}^{y-} + \text{OANM}^- + \text{OAM}^- = 0$ , where  $\text{OCNM}^+$  nonmetabolizable organic cations,  $\text{OCM}^+$  metabolizable organic cations;  $\text{OANM}^-$  nonmetabolizable organic anions; and  $\text{OAM}^-$  metabolizable organic anions; and albumin $^{x-}$  + phosphate $^{y-}$  represent the net charges on albumin and phosphate, respectively (73). Calculations such as the anion gap (AG), SID gap, and strong ion gap (SIG) are based on macroscopic electroneutrality requirements (22, 30, 33, 47, 66).

Given the constraint of macroscopic electroneutrality, the net charge represented by the [cation -anion] difference of substances included in the strong ion (SID) category is always equal to the net charge represented by the [cation - anion] difference of substances that are not included in the strong ion category (volatile and nonvolatile buffers, e.g.,  $\text{HCO}_3^-$  and  $\text{A}^-$ , respectively) where  $[\text{SID}^+] = [\text{HCO}_3^-] + [\text{A}^-]$ . Although mathematically it makes no difference which parameter is used, the traditional acid-base physiologist utilizes volatile and nonvolatile buffers in acid-base analysis since these substances are involved in aqueous proton transfer reactions at physiological pH. Moreover, to the traditional acid-base physiologist, SID is a mathematical construct based on electroneutrality considerations, rather than a fundamental physicochemical property that is deterministic of acid-base behavior.

#### Thought Experiments Examining the Role of [SID] Per Se in Determining $[\text{H}^+]$

At the macroscopic level, the law of electroneutrality applies in all body fluid compartments. The term electroneutrality has been associated with two separate physical requirements: 1) that the total charge  $Z$  should vanish at every point in solution and 2) that no electrical current  $I$  should run through the solution if none is applied (10, 50). In a compartment where the change in the mass balance of various charged substances is occurring, insufficient consideration has been given as to how macroscopic electroneutrality of an aqueous solution (open system) is achieved. Two possibilities are apparent. Either the sum of all charges (fixed or strong ions, and unfixed) on the input and/or output side is zero, and therefore the solution remains electroneutral; or 2) the input (or output) of positive charges are unequal to the input (or output) of negative charges, thereby imposing a change in the net charge of the solution. Regarding in vitro or biological systems, macroscopically the sum of the positive charges equals the sum of the negative charges (input or output) whether the ions are all fixed (strong ions), or a combination of fixed and unfixed. The

second possibility does not occur macroscopically in nature; however, it can occur microscopically (1, 164).

According to the Stewart formulation, SID is a variable that can be modulated independently. We address whether this is indeed possible in vitro by performing certain thought experiments that could be performed in the lab. We start with an aqueous solution that only contains  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{H}^+$ , and  $\text{OH}^-$ . To create a change in the [SID], given the constraints of macroscopic electroneutrality, one could 1) add NaOH, 2) add HCl, 3) exchange  $\text{H}^+$  for  $\text{Na}^+$  with a cation exchange resin, or 4) exchange  $\text{Cl}^-$  for  $\text{OH}^-$  with an anion exchange resin. Notice that for the [SID] to change (add or remove  $\text{Na}^+$  or  $\text{Cl}^-$ ), there is always by necessity, a simultaneous change in either  $\text{H}^+$  or  $\text{OH}^-$ . Adding or removing  $\text{Na}^+$  with equimolar  $\text{Cl}^-$ , two fixed ions, does not change the [SID]. This simple experiment demonstrates that when changes in [SID] occur, to satisfy the constraints of macroscopic electroneutrality, there is always an associated change in the  $[\text{OH}^- - \text{H}^+]$  of equal magnitude (Fig. 2). Therefore, the concept that SID changes independently cannot occur based on constraints of electroneutrality. One could also change the [SID] by the addition or removal of water (Fig. 2); again, however, the value of  $[\text{OH}^- - \text{H}^+]$  would change identically such that

$$\Delta[\text{SID}] = \Delta[\text{OH}^- - \text{H}^+].$$

Can one change SID without a concomitant equal change in  $[\text{OH}^- - \text{H}^+]$  and in this way test Stewart's assertion that SID is an independent variable that modulates  $[\text{H}^+]$ ? As

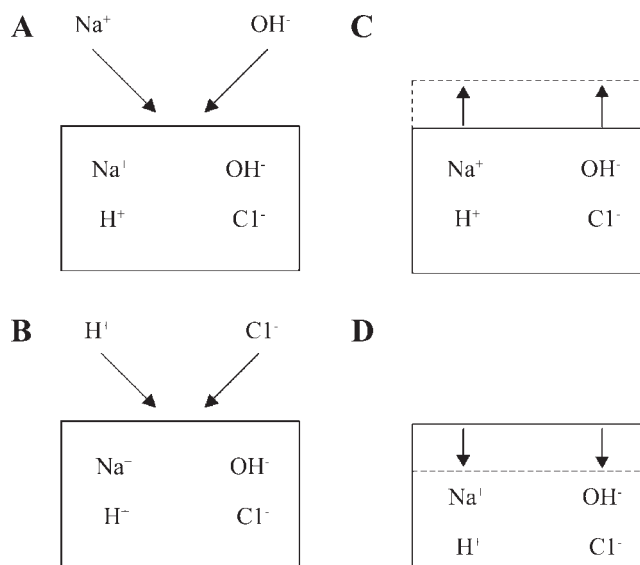


Fig. 2. Mechanisms by which strong ion difference ([SID]) can change in a single compartment containing  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{H}^+$ , and  $\text{OH}^-$ . *A* and *B*: changes in mass balance. *A*: for [SID] to change, the mass balance of  $\text{Na}^+$  must be accompanied by a weak (unfixed) anion such as  $\text{OH}^-$ . [SID] could also be changed by removing NaOH from the solution (not shown). *B*: a change in the mass balance of  $\text{Cl}^-$  must be accompanied by a weak (unfixed) cation such as  $\text{H}^+$ . [SID] could also be changed by removing HCl from the solution (not shown). *C* and *D*: changes in the volume of the compartment. *C*: during an expansion of the compartment volume, the concentration of each ion decreases by the same percentage, and the difference between the concentration of specific ions also decreases by the identical percentage. *D*: similarly, during the contraction of the compartment volume, the concentration of each ion increases by the same percentage, and the difference between the concentration of specific ions also increases by the identical percentage.

<sup>1</sup> Albumin contains fixed charges that are pH independent over the physiological pH range and imidazole groups that are protonated-deprotonated over the physiological pH range (4, 9, 41, 42, 158).

suggested above, this would require that the sum of the positive charges is not equal to the negative charges (input or output side), thereby imposing a change in the charge of the solution. Suppose that instead of adding  $H^+$  and  $Cl^-$  or  $Na^+$  and  $OH^-$  simultaneously to our hypothetical *in vitro* system, these ions were added separately and that macroscopic electroneutrality was not maintained. By adding these ions individually, one could conceivably in a straightforward way experimentally change [SID] and  $[OH^- - H^+]$  individually.<sup>2</sup> In fact, this does occur in biological systems (see below, channels, electrogenic ion transporters), where electroneutrality is violated microscopically for a predictable distance from biological membranes calculated as the Debye length (1, 164). *In vitro*, our experimentalist considers performing ion beam experiments, where an aqueous solution is bombarded with a charged ion beam, e.g., an  $Na^+$  ion beam, or a proton beam.<sup>3</sup> In regard to the  $Na^+$  ion beam experiment, the Stewart model would predict that the addition of  $Na^+$  per se by increasing [SID] would increase the  $[H^+]$ . The traditional acid-base physiologist would point out that adding  $Na^+$  would not result in a significant change in  $[H^+]$  (other than a minor effect on  $K_w$  due to a change in ionic strength, and a minor shift in the equilibrium reaction because  $Na^+$  can combine to a small extent with  $OH^-$ ). The proton beam experiment is an example of  $H^+$  flux into an aqueous solution from a source external to the system that according to the Stewart formulation cannot alter the  $[H^+]$  significantly<sup>4</sup>; an argument based on epistemological considerations. The traditional acid-base physiologist would predict that the  $[H^+]$  will indeed increase because of the change in the mass balance of  $H^+$ .

#### *Proof That a Change in SID Does Not Determine a Specific $[H^+]$*

Although the previous thought experiments have yet to be performed, using the following simple example, it can be shown that the concept that changes in SID specify a given  $[H^+]$  is incorrect (see Table 1). Start with a one-liter solution of 150 mM (0.15 M) NaCl in water with an initial [SID] = 0,  $[H^+] = 1 \times 10^{-7}$  M,  $[OH^-] = 1 \times 10^{-7}$  M, and  $[OH^-] - [H^+] = 0$ . If one were to add 100 mM (0.1 M) HCl, the SID would decrease to -100 mM (-0.1 M), and the  $[OH^-] - [H^+]$  difference would also decrease to -100 mM (-0.1 M). The excess  $H^+$  will then be consumed by  $OH^-$  to form  $H_2O$ . Since  $H^+$  and  $OH^-$  are consumed equally to form  $H_2O$ , the  $[OH^-] - [H^+]$  difference remains unchanged. The  $H^+$  and  $OH^-$  will continue to be consumed to form  $H_2O$  until the product of  $[OH^-] \times [H^+]$  is equal to  $K_w'$ . Therefore, the  $[H^+]$  can be any value for a given change in SID, and the  $[H^+]$  at equilibrium is only determined by the dissociation constant of water,  $K_w'$ . This holds true regardless of the absolute value of  $K_w'$  (effects of ionic strength).

<sup>2</sup> Strictly speaking, the Stewart formulation does not apply if electroneutrality is not maintained since the assumption of electroneutrality was used to define [SID].

<sup>3</sup> As far as we are aware, there are no reported experiments where pH measurements using ion beam bombardment of aqueous solutions have been reported.

<sup>4</sup> A proponent of the Stewart formulation might argue that  $H^+$  can combine with  $Cl^-$  to a minor extent and lower the  $[Cl^-]$  slightly, thereby altering the SID.

#### *Comparison of the H-H Equation, Stewart's Strong Ion Equation, and Constable's Simplified Strong Ion Equations: Mathematically They are Identical in Calculating the pH of a Solution*

*Equilibrium and solubility constants.* The H-H equation has historically played an important role in the interpretation and diagnosis of clinical acid-base disorders and is widely utilized in the comparative  $\Delta HCO_3^-/\Delta P_{CO_2}$  and  $\Delta BE$  approaches (36, 72, 129, 135); however, the H-H equation has been criticized (21, 23, 24, 29) in that the equilibrium ( $K_1'$ ) and solubility ( $S$ ) constants are not fixed but vary with various environmental factors, including temperature, ionic strength, protein concentration, and pH (56, 71, 86, 134, 135).<sup>5</sup> Clearly, Constable's simplified strong ion equation contains the same constants. Stewart's equation is based on these constants also but in addition utilizes the water autoionization equilibrium constant ( $K_w'$ ), and a single effective equilibrium constant for all weak acids ( $K_a$ ). Therefore the practical use of these formulas is dependent on the accuracy of the constants that are inserted into the respective formulas. Various nomograms and formulas have been derived to correct equilibrium and solubility constants for these environmental effects (56).

Can changes in [SID] be thought of as a surrogate for changes in ionic strength ( $\mu$ ) in correcting  $pK_1'$ , as is sometimes purported by the proponents of the Stewart formulation, to justify its use? In this regard, Rana et al. (117) have converted empirical data concerning the effect of ionic strength on  $pK_1'$  of the  $[HCO_3^-]-CO_2$  reaction to calculate the relationship between  $K_1'$  and SID in the presence of strong ions and bicarbonate. Importantly, changes in  $\mu$  affect  $[H^+]$  by altering the value of equilibrium constants in acid-base reactions (56, 71, 86, 135). In contrast, changes in SID do not dictate a specific  $[H^+]$  (Table 1). If one were to add NaCl to a solution, the SID will not change and yet the ionic strength of the solution will change. Therefore, SID is not equivalent to ionic strength. This is not unexpected given that SID is a mathematical construct similar to AG and SIG based on macroscopic electroneutrality and can theoretically be positive or negative. In contrast, in quantitating the ionic strength of a solution, the valence ( $z$ ) of a specific ion is squared (1, 80), and therefore, mathematically, ionic strength ( $\mu$ ) always has a positive value. Second, in the Stewart and Constable equations, SID excludes bicarbonate whereas the ionic strength is determined by all ions present in a solution. Unlike SID,  $\mu$  plays an important theoretical role in the Debye-Hückel theory used to calculate the activity coefficient ( $\gamma$ ) of an ion in solution (1, 80).

*Relationship between  $\log P_{CO_2}$  and pH.* An additional criticism purporting to demonstrate the incompleteness of the H-H equation is that it predicts a linear relationship between the

<sup>5</sup>  $K_1$  is the thermodynamic equilibrium constant that is used when  $[HCO_3^-]$  and  $[CO_2]$  indicate molar activities. At 38°C, in a  $HCO_3^-$  solution,  $pK_1$  is 6.328.  $pK_1'$  is affected by changes in ionic strength.  $K_1'$  is the equilibrium constant that is used when  $[HCO_3^-]$  and  $[CO_2]$  indicate molar concentrations. Therefore,  $pK_1'$  varies with ionic strength ( $\mu$ ) according to

$$pK_1' = pK_1 - 0.495 \sqrt{\mu}$$

In a pure sodium bicarbonate solution when  $[HCO_3^-]$  represents the apparent concentration measured titrimetrically, the symbol  $pK_1''$  is used ( $pK_1''$  when determined gasometrically) that is dependent on pH and the activity of  $Na^+$ . At 38°C, and pH 7.4,  $pK_1''$  is 6.10.



Table 1. Proof that a change in SID does not determine a specific  $[H^+]$ 

	[Na <sup>+</sup> ], mol/l	[Cl <sup>-</sup> ], mol/l	SID, mol/l	[OH <sup>-</sup> ], mol/l	[H <sup>+</sup> ], mol/l	[OH <sup>-</sup> ] - [H <sup>+</sup> ], mol/l	[OH <sup>-</sup> ] × [H <sup>+</sup> ], (mol/l) <sup>2</sup>
Initial State	0.15	0.15	0	1 × 10 <sup>-7</sup>	0.0000001	0	1 × 10 <sup>-14</sup>
Add 0.1 mol HCl/l of H <sub>2</sub> O							
Preequilibrium	0.15	0.25	-0.1	1 × 10 <sup>-7</sup>	0.1000001	-0.1	1 × 10 <sup>-8</sup>
Preequilibrium	0.15	0.25	-0.1	1 × 10 <sup>-8</sup>	0.10000001	-0.1	1 × 10 <sup>-9</sup>
Preequilibrium	0.15	0.25	-0.1	1 × 10 <sup>-9</sup>	0.100000001	-0.1	1 × 10 <sup>-10</sup>
Preequilibrium	0.15	0.25	-0.1	1 × 10 <sup>-10</sup>	0.1000000001	-0.1	1 × 10 <sup>-11</sup>
Preequilibrium	0.15	0.25	-0.1	1 × 10 <sup>-11</sup>	0.10000000001	-0.1	1 × 10 <sup>-12</sup>
Preequilibrium	0.15	0.25	-0.1	1 × 10 <sup>-12</sup>	0.100000000001	-0.1	1 × 10 <sup>-13</sup>
New equilibrium	0.15	0.25	-0.1	1 × 10 <sup>-13</sup>	0.1000000000001	-0.1	1 × 10 <sup>-14</sup>

The initial solution contains 1 liter of a 150 mM (0.15 M) NaCl with a strong ion difference ([SID]) = 0,  $[H^+] = 1 \times 10^{-7}$  M,  $[OH^-] = 1 \times 10^{-7}$  M, and  $[OH^-] - [H^+] = 0$ . Following the addition of 0.1 mol HCl, the SID decreases to -100 mM (-0.1 M), and the  $[OH^-] - [H^+]$  difference decreases to -100 mM (-0.1 M). The excess  $H^+$  is consumed by  $OH^-$  to form  $H_2O$  and since  $H^+$  and  $OH^-$  are consumed equally to form  $H_2O$ , the  $[OH^-] - [H^+]$  difference remains unchanged. The  $H^+$  and  $OH^-$  continue to be consumed to form  $H_2O$  until the product of  $[OH^-] \times [H^+]$  is equal to  $K_w$ , the dissociation constant of water. Therefore, the  $[H^+]$  can be any value for a given change in SID, and the  $[H^+]$  at equilibrium is only determined by  $K_w$ . This holds true regardless of the absolute value of  $K_w$  (effects of ionic strength). Changes in  $K_w$  only affect the final  $[OH^-] \times [H^+]$ .

$\log P_{CO_2}$  and pH with an intercept of  $\log([HCO_3^-]/K'_1S)$  despite the fact that the in vitro relationship between  $\log P_{CO_2}$  and pH becomes nonlinear in markedly acidic plasma, during in vivo  $CO_2$  equilibration studies, and is displaced by changes in the protein concentration, or the addition of  $Na^+$  or  $Cl^-$  (21, 23, 24, 29). Mathematically, a linear relationship between the pH and the  $\log P_{CO_2}$  can only exist if the term  $\log([HCO_3^-]/K'_1S)$  remains constant. Given that in any bicarbonate-buffered solution, at equilibrium, changes in pH and  $CO_2$  must also be accompanied by changes in  $[HCO_3^-]$ , the  $\log([HCO_3^-]/K'_1S)$  does not remain constant as is commonly assumed, and therefore a plot of pH and the  $\log P_{CO_2}$  in fact cannot be linear.

Moreover, as shown in APPENDIXES A and B, although the H-H equation is sometimes viewed as a subset of the strong ion model, the H-H equation, Stewart equation, and the Constable simplified strong ion equation are mathematically identical in calculating pH in the presence or absence of nonbicarbonate buffers and therefore there can be no difference in their predictive capability. Referring to the Constable's equation, although it has been previously assumed that  $[HCO_3^-] = ([SID] - K_a[A_{TOT}])/(K_a + 10^{-pH})$  only when solutions don't have albumin, globulin, or phosphate, i.e., when  $[A_{TOT}]$  is zero (21, 23, 24, 26, 29), in fact as shown in Table 2, Fig. 3, and APPENDIX B, the identity holds true regardless of the value of  $[A_{TOT}]$ . Accordingly, the H-H equation, Stewart's strong ion

equation, and Constable's simplified strong ion equation must yield an identical plot of pH vs.  $\log P_{CO_2}$ . As a corollary, given that under all circumstances the H-H, Stewart, and Constable's equations are mathematically identical in calculating pH, they are affected identically by environmental factors such as changes in ionic strength, pH, protein, etc.

*Accuracy in determination of  $[H^+]$ .* An additional critique of the H-H equation is that it is less accurate than the Stewart or Constable equations. The term "accuracy" has been used to denote the accuracy of the strong ion equations in calculating the value of  $[H^+]$  of a solution compared with the measured  $[H^+]$  value. There are several fallacies with the argument that the Stewart or Constable equations are a more accurate tool for calculating the  $[H^+]$  compared with equations depicting  $H^+$  as a function of individual buffer pairs such as the H-H equation. First, in a complex solution such as plasma containing multiple buffer pairs at equilibrium, any of the individual buffer pairs  $CO_2/HCO_3^-$ ,  $H^+$ -albumin/albumin,  $H^+$ -phosphate/phosphate, and their respective equilibrium constants can be used to calculate the  $[H^+]$  rather than measure it directly with a pH electrode (36, 72). One does not need to utilize the concentration of all the buffer pairs in a single equation to calculate  $[H^+]$ . Historically, the H-H equation and measurements of  $HCO_3^-$  (as  $T_{CO_2}$ ) and  $P_{CO_2}$  have been utilized to calculate the  $[H^+]$  in body fluids principally because the concentration

Table 2. The Henderson-Hasselbalch and strong ion equations are equivalent quantitatively

pH	$HCO_3^-$ , mM	$K'_1$	$S_{CO_2}$	SID, mM	$A_{TOT}$ , mM	$A^-$ , mM	$K_a$	$\log P_{CO_2}$ H-H	$\log P_{CO_2}$ Constable
7.40	5	$7.59 \times 10^{-7}$	0.0307	16.5	17.2	11.5	$8.00 \times 10^{-8}$	0.932	0.932
7.40	20	$7.59 \times 10^{-7}$	0.0307	31.5	17.2	11.5	$8.00 \times 10^{-8}$	1.53	1.53
7.40	40	$7.59 \times 10^{-7}$	0.0307	51.5	17.2	11.5	$8.00 \times 10^{-8}$	1.84	1.84
7.00	5	$7.59 \times 10^{-7}$	0.0307	12.6	17.2	7.64	$8.00 \times 10^{-8}$	1.33	1.33
7.00	20	$7.59 \times 10^{-7}$	0.0307	27.6	17.2	7.64	$8.00 \times 10^{-8}$	1.93	1.93
7.00	40	$7.59 \times 10^{-7}$	0.0307	47.6	17.2	7.64	$8.00 \times 10^{-8}$	2.23	2.23
6.50	5	$7.59 \times 10^{-7}$	0.0307	8.47	17.2	3.47	$8.00 \times 10^{-8}$	1.84	1.84
6.50	20	$7.59 \times 10^{-7}$	0.0307	23.5	17.2	3.47	$8.00 \times 10^{-8}$	2.43	2.43
6.50	40	$7.59 \times 10^{-7}$	0.0307	43.5	17.2	3.47	$8.00 \times 10^{-8}$	2.73	2.73

The Henderson-Hasselbalch and Constable's strong ion equation were used to calculate the  $\log P_{CO_2}$  under various pH and bicarbonate values in the presence of a constant  $A_{TOT}$ . It has previously been assumed that strong ion equations simplify to the Henderson-Hasselbalch (H-H) equation only when a given solution contains bicarbonate but not other buffers. The data in Table 2 illustrate the equivalency of the 2 equations even in the presence of nonbicarbonate buffers. In addition, APPENDIX B demonstrates that both equations are quantitatively equivalent irrespective of whether nonbicarbonate buffers ( $A_{TOT}$ ) are present.

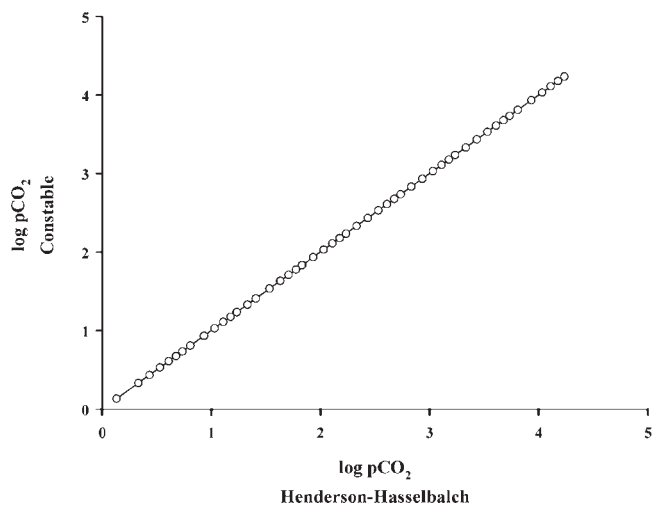


Fig. 3. The Henderson-Hasselbalch and Constable's strong ion equation were used to calculate the  $\log p\text{CO}_2$  under various pH and bicarbonate values in the presence of a constant  $A_{\text{TOT}}$ . The quantitative predictions of the Henderson-Hasselbalch and Constable's strong ion equations were identical. The bicarbonate concentration was varied from 5 to 40 mM, and pH from 5.0 to 8.5.

of  $\text{HCO}_3^-$  exceeds other buffer anions and the required measurements are readily available. In utilizing the  $\text{CO}_2/\text{HCO}_3^-$  pair for the purpose of calculating  $\text{H}^+$ , as long as the solubility of  $\text{CO}_2$  and the  $K_1'$  are known, it does not matter whether 1) more than one buffer pair is present; 2) the concentration of the individual components of other buffer pairs is altered following changes in mass balance, i.e., albumin or phosphate infusion once a new equilibrium is achieved; and 3) a proton load has resulted in the repartitioning of  $\text{H}^+$  among multiple buffers in the solution. Indeed, as demonstrated in APPENDIX A, since the equilibrium  $[\text{H}^+]$  is the same for all buffer pairs in a multiple buffer solution (isohydric principle), the Stewart equation can be simplified to the H-H equation. In other words, the H-H equation and the Stewart equation are identical quantitatively in terms of their accuracy in calculating the equilibrium pH even in a multiple buffer solution.

Finally, one of the motivations underlying the derivation of the Stewart equation was to account for bicarbonate as well as noncarbonate buffers in a complex solution such as plasma (144–146). The term accuracy has also been used to contrast the ability of the H-H and strong ion formulations to predict the magnitude of changes in  $[\text{H}^+]$  in a solution containing nonbicarbonate buffers such as albumin or phosphate. However, given that the H-H, Stewart, and Constable equations are “equilibrium equations,” none of these formulas can be used in a predictive manner.

*Electroneutrality and mass conservation requirements in the derivation of strong ion equations.* In his derivation, Stewart utilized the law of electroneutrality (as reflected in SID) and the law of conservation of mass (as reflected in  $A_{\text{TOT}}$ ) as factors that contribute quantitatively to the equilibrium  $[\text{H}^+]$ . As already demonstrated in Table 1, electroneutrality requirements (changes in SID) do not specify a unique  $[\text{H}^+]$  value. Moreover, the law of conservation of mass (as reflected in  $A_{\text{TOT}}$ ) does not per se participate in acid-base equilibria. Rather, it is the  $[\text{HA}]/[\text{A}^-]$  ratio that is related quantitatively to the  $[\text{H}^+]$  in plasma. Indeed, as shown in APPENDIX C, one can derive an equation that describes the quantitative relationship

between the equilibrium pH and multiple buffer pairs without utilizing concepts based on either electroneutrality (SID) or conservation of mass of weak acids ( $A_{\text{TOT}}$ ) in the derivation.

#### *Molecular Basis of Proton Hydration and Transfer in Acid-Base Reactions*

There has recently been a significant increase in the understanding of proton solvation and transfer reactions in aqueous solutions that impacts our concepts regarding acid-base phenomenology (152, 154). In contrast to the customary mass diffusion often termed Stokes or hydrodynamic diffusion, the transfer of excess protons in bulk water is driven by a topological mechanism termed “a structural diffusion process” (90). In this scenario, the excess proton is viewed as a topological defect in the hydrogen-bonded network of water molecules rather than as a free proton or a rigid hydronium ion complex ( $\text{H}_3\text{O}^+$ ). Proton transfer is mediated via spontaneous solvent fluctuations that result in an interconversion between the threefold coordinated more stable hydronium complex “Eigen complex”  $[\text{H}_3\text{O}^+(\text{H}_2\text{O})_3]$  and a shared proton “Zundel cation complex”  $[\text{H}_2\text{O}-\text{H}-\text{OH}_2]^+$  (12, 59, 60, 75, 88, 103, 165). Fundamental to the concept of structural diffusion of protons are the spontaneous (at 300 K) processes of the breaking and making of covalent O-H bonds and associated hydrogen bonds. The interconversion between these complexes is viewed as a spontaneous fluctuation in the hydrogen-bonded (Grotthus) network of water molecules (water wires). The pH or pK of water is intimately related to the dissociation of single water molecules and subsequent proton transfer steps. Spontaneous dissociation of water is viewed as a fluctuation-induced change in the hydrogen-bonded Grotthus network. It has been estimated that at the ambient pH of water, once every 10 h, a single water molecule will succeed in dissociating (90). The finite concentration of protons following the spontaneous dissociation of water also requires that the proton and hydroxyl ions separate sufficiently to prevent their rapid reassociation. Key to this concept is that their failure to reassociate results in a break in the Grotthus network and a finite activity of  $\text{H}^+$  (and therefore pH) in the form of Eigen and Zundel complexes.

The familiar reaction showing the dissociation of a weak acid is deceptively simple:  $\text{HA}_{(\text{aq})} + \text{H}_2\text{O} \rightarrow \text{A}^- + \text{H}_3\text{O}^+_{(\text{aq})}$ . This reaction summarizes a large body of microscopic behavior with complex intermediate steps that in part remain unexplained despite a wealth of theoretical and experimental studies. Recent detailed spectroscopic studies have characterized the molecular processes involved in the transfer of protons between acids and bases on a pico- and femtosecond timescale (58, 95–97). These studies have shown that the proton can stop at several intermediate water molecules before finally binding to a base, a process called solvent switching. Moreover, only weak acids are likely to directly (without intermediary water molecules) transfer a proton to a base. For the latter to occur, the acid and base have to form an ion pair not surrounded by water molecules (a process called desolvation). Strong and medium-strength acids likely transfer their protons initially to water molecules that have formed either a loose complex with the acid and base involving either one water molecule (loose complex), or several water molecules (solvent switch). In a solution containing a strong acid (HA) and a different weak base ( $\text{B}^-$ ), the transfer steps include almost complete desolva-

tion to form a loose complex ( $\text{HA-H}_2\text{O-B}^-$ ) followed by the rapid transfer of the proton to form  $\text{A}^-\text{-H}_3\text{O}^+\text{-B}^-$ , and a subsequent slower transfer of the proton to the base  $\text{B}^-$  to form  $\text{A}^-\text{-H}_3\text{O}^+\text{-BH}$  (picosecond timescale) with subsequent formation of the free products  $\text{A}^-$ ,  $\text{H}_2\text{O}$ , and  $\text{BH}$ . The  $\text{HA-H}_3\text{O}^+\text{-B}^-$  encounter complex resembles the proton solvation core of the Eigen cation  $\text{H}_9\text{O}_4^+$ . This transfer of protons is a von Grothuss-like mechanism through a hydrogen-bonding network of one (loose complex) or more (solvent switch) water molecules. Until it reacts with a base, the proton likely exists in a nonlocalized form that is similar to bulk solvation. Recent theoretical studies involving hydrofluoric acid dissociation in water have shown the importance of Eigen-like contact ion pairs, and Zundel-like contact ion pairs and water-separated ion pairs (59). These findings suggest that the characteristics of the base (proton acceptor) play an important role in the kinetic stability of the hydrated proton intermediate complex.

Unlike the detailed molecular analysis of kinetics and intermediate complexes involved in proton transfer reaction between an acid and a base, the Stewart formulation lacks a molecular/theoretical basis whereby the charge difference between strong ions per se is able to alter the  $[\text{H}^+]$  at equilibrium. It is known that the net electrostatic attraction exerted on a given ion varies with the average distribution of charges surrounding it according to the Debye-Hückel theory (1). Accordingly, the Debye-Hückel equation is used to calculate the activity coefficients of specific ions and provides a basis for relating the apparent dissociation constant  $K'$  (expressed in terms of concentration) of acids and bases with the actual thermodynamic dissociation constant  $K$  (expressed in terms of activity). As an example, the Debye-Hückel equation provides us with a theoretical means for determining the effect of changes in ionic strength ( $\mu$ ) on the  $\text{p}K'_1$  of the  $\text{HCO}_3^-$ - $\text{CO}_2$  buffer reaction:  $\text{p}K'_1 = \text{p}K_1 - 0.495$  ( $\sim \Delta\text{p}K'_1/\Delta\mu = -0.47$ ) (71). Note that the sign in front of the ionic strength correction term depends only on whether the acid vs. the base is charged. We therefore have an example where the same change in ionic strength has the opposite effect on the activity of  $[\text{H}^+]$  in the solution. In terms of current molecular understanding of proton transfer reaction intermediates, this phenomenon can easily be explained. Specifically, as the ionic strength increases by adding  $\text{Na}^+$  and  $\text{Cl}^-$ , the effective activity of  $\text{HCO}_3^-$  decreases, resulting in an increased stabilization of protonated water intermediate complexes. In the case of  $\text{NH}_4^+$ , its activity decreases, resulting in less proton donation to form protonated water complexes at equilibrium.

According to the Stewart formulation, SID has been hypothesized mechanistically to have a powerful electrochemical effect on water dissociation and  $\text{H}^+$  concentration (67). Various reasons have been suggested as to how, according to the Stewart formulation, changes in [SID] mechanistically alter the  $[\text{H}^+]$ . Given the variable interaction of specific ions with water (15, 77), the concept has evolved that certain ions are able to break the hydrogen-bonded structure of liquid water and other ions weaken it (94). For example,  $\text{Na}^+$  is purported to stabilize the structure of water (kosmotrope) whereas  $\text{K}^+$  is suggested to disrupt the structure of water (chaotrope). Accordingly, in regard to the purported effect of SID, it has been proposed that charged ions (and, by inference, SID) may affect the hydrogen-bonded water structure, thereby affecting  $[\text{H}^+]$  (29). What is not readily apparent from this formulation is that equimolar

changes in the concentration of  $\text{Na}^+$  and  $\text{K}^+$  affect SID identically mathematically, and yet each ion, according to the kosmotrope-chaotrope categorization, is predicted to have the opposite effect on the structure of water. However, the results of direct studies at the molecular level using femtosecond pump-probe spectroscopy have shown that the presence of ions does not alter the hydrogen-bond network in liquid water at all (110). It has also been suggested that changes in [SID] per se might affect  $[\text{H}^+]$  by altering the structural diffusion of  $\text{H}^+$  (29). Given the known lack of an effect of ions on the hydrogen-bonded network, this hypothesis also becomes unlikely. Paradoxically, according to Stewart's epistemology one needs to place less focus on the molecular details of proton hydration and transfer reactions as the primary mechanistic underpinning of macroscopic acid-base phenomenology, since these reactions are internal to the system and therefore inherently mediated by dependent variables.

#### *Changing the Charge of an Aqueous Solution Without Changing SID: Additions of Electrons*

Without investigating a mechanism, Stewart implicated the [SID] or "net fixed charge" as one of the proximate causes for changes in  $[\text{H}^+]$  in an aqueous solution. Based on this hypothesis, an alteration in the net charge of an aqueous solution by a method independent of a change in [SID] would be predicted to alter the  $[\text{H}^+]$ . At first instance, it might be predicted that the electrolytic dissociation of water would be informative (109). The reaction at the cathode is as follows:  $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$ ; indeed, the pH decreases due to the release of  $\text{H}^+$ . At the anode,  $\text{OH}^-$  are generated with a concomitant increase in pH according to  $2\text{e}^- + 2\text{H}_2\text{O} + \rightarrow \text{H}_2 + 2\text{OH}^-$ . However, it is clear that unlike Stewart's formulation, the electrons that are being added (cathode) and removed (anode) from the solution are partaking in the reaction and in this sense do not simulate a change in the net ionic charge as formulated by Stewart. Despite this difference, it should be pointed out that these oxidation-reduction reactions (107) are examples of reactions that according to the Stewart formulation are "internal" to the system and accordingly ought not to be responsible for the change in  $[\text{H}^+]$  that is measured at each electrode. According to modern mechanistic theory, the addition/removal of electrons alters the likelihood of charge ( $\text{H}^+/\text{OH}^-$ ) separation following fluctuation-induced changes in the hydrogen-bonded Grothus water network, resulting in a finite change in the  $[\text{H}^+]$  at each electrode (90). Stewart's formulation does not provide a readily apparent mechanistic understanding of the  $[\text{H}^+]$  changes detected at each electrode. Similarly, an explanation for the mechanistic working of fuel cells, and proton transfer- $[\text{H}^+]$  changes in the electron transport chain, is lacking.

#### *Physiological Applications of the Stewart and Traditional Formulations*

*Proteins that transport  $\text{H}^+$ /base equivalents: Stewart and traditional formulations.*  $\text{H}^+/\text{BASE}$  TRANSPORTERS. In the last 20 years, various membrane  $\text{H}^+/\text{base}$  transport proteins have been cloned that play an important role in transepithelial transport and intracellular pH regulation. These transporters include  $\text{Na}^+/\text{H}^+$  exchangers (NHE proteins) (111), sodium bicarbonate cotransporters (NBC proteins) (115), sodium-dependent anion exchangers (NDCBE proteins) (115), and anion exchangers

(AE and SLC26 proteins) (104, 115). The ability of these transporters to modify intracellular and extracellular pH and transport bicarbonate vectorially has been attributed to  $H^+$ ,  $HCO_3^-$ , or  $CO_3^{2-}$  transport. According to proponents of the Stewart formulation, the transfer of  $H^+$ ,  $HCO_3^-$  or  $CO_3^{2-}$  from one compartment to another cannot per se change the  $[H^+]$ , but rather a change in [SID] is invoked. As Fencl (40a) states, "A change in  $[H^+]$  (pH) in the intracellular compartment results from changes in values of the compartment's independent variables; it cannot be *physically* (our italics) achieved by simply adding or removing  $H^+$  across the membrane. When one describes the phenomenon as 'active transport' of  $H^+$ , one is applying a metaphor. This may be useful for shorthand communication, but it does not give the complete picture of the processes involved." Furthermore, the increase in intracellular pH following the addition of  $NH_4Cl$  to a cell is not viewed as being due to the rapid diffusion of  $NH_3$  intracellularly and subsequent binding of intracellular protons to form  $NH_4^+$ , but rather the increase in the  $[NH_4^+]$  per se increases the intracellular [SID], causing the intracellular pH to increase (40). The subsequent slower decrease in intracellular pH is not as traditionally thought due to the slower entry of  $NH_4^+$  but rather the entry of  $Cl^-$ , which decreases the intracellular [SID]. Similarly, as Sirker (140) has stated: "Directly adding or removing  $H^+$  to or from one of the compartments, will not alter the value of any of the independent variables present and hence  $[H^+]$  will be maintained at the same value as previously by a change in the dissociation of water to reverse any  $[H^+]$  fluctuations. The water dissociation equilibrium is able to provide an essentially inexhaustible source or sink for  $H^+$  ions." The traditional acid-base physiologist would question this formulation emphasizing that 1) transported  $H^+$  ions combine with  $HCO_3^-$  and other buffer anions; 2) SID does not contribute to the determination of a specific  $[H^+]$  value; and 3) water dissociation equilibrium reaction cannot alter the net charge of unfixated ions.

**BACTERIORRHODOPSIN.** Bacteriorhodopsin (bR) is a membrane protein in *Halobacterium salinarium* that converts the energy from photons of a given wavelength into a proton flux that generates a proton gradient. Although many details are yet to be uncovered, hydrogen-bonded water clusters (WLANs) that interact with specific amino acid residues are thought to play an essential role in the storage, transport, and release of excess protons, resulting in the generation of an  $[H^+]$  gradient (90, 92). It is not clear how according to the Stewart formulation bR generates a proton gradient given that proton flow cannot by definition be causally involved with generation of an  $H^+$  gradient.

**ATPASES.** According to the Stewart formulation, vacuolar proton pumps ( $H^+$ -ATPase and  $H^+$ - $K^+$ -ATPase), for example, do not mediate the acidification (and on the opposite compartment, alkalization) of specific fluid compartments (29, 31, 120). Rather, it is the associated transport of chloride by other processes [transport proteins (CLC5 chloride channel; paracellular transport via claudins)] via a change in [SID] that results in an alteration in  $[H^+]$ . Furthermore, according to the Stewart formulation 1) the transport of protons during oxidative phosphorylation does not per se lead to a change in mitochondrial matrix  $[H^+]$ ; 2) the flow of protons down a concentration gradient although coupled to ATP synthesis does not per se collapse the  $H^+$  gradient; and 3) ionophores do not collapse  $H^+$  gradients by increasing the flow of protons down a con-

centration gradient. A mechanism to account for these phenomena according to the Stewart formulation is not readily apparent.

**SLC4 AND SLC26 MUTATIONS.** Various proteins belonging to the SLC4 and SLC26 gene families transport bicarbonate either in exchange for chloride or coupled to the transport of sodium (104, 115). Mutations in several of these proteins result in abnormal bicarbonate transport across epithelia in various organs. According to traditional acid-base theory, defective electrogenic sodium bicarbonate absorption via NBCe1-A (SLC4 family) transport in the basolateral membrane of the proximal tubule results in proximal renal tubular acidosis, and mutations in DRA (SLC26 family) lead to congenital chloridorrhea with subsequent retention of bicarbonate and subsequent metabolic alkalosis. The systemic acid-base changes in these disorders are caused by defective bicarbonate transport.

According to the Stewart formulation, however, it is abnormal sodium transport in patients with NBCe1-A mutations, and abnormal chloride transport in patients with DRA mutations via changes in [SID] that result in the changes in blood  $[H^+]$  (29, 31, 120). In this sense, it makes no difference whether, for example, defective sodium is coupled to bicarbonate transport (NBCe1-A mutations) vs. abnormal sodium transport via a channel such as ENaC mutations, or whether defective chloride transport is coupled to bicarbonate transport (DRA mutations) vs. abnormal chloride transport via a channel such as CFTR, causing cystic fibrosis. In all cases, the acid-base changes are said to be accounted for by a change in SID. Accordingly, any protein whose transport potentially leads to a change in  $[Na^+]$  or  $[Cl^-]$ , for example, independent of whether  $H^+$ /bicarbonate/ $OH^-$  is a cotransported species is predicted according to the Stewart formulation to change the aqueous  $[H^+]$ .

**Whole body acid-base balance: Stewart and traditional formulations.** The production of  $H^+$  and bicarbonate by metabolism is an integral component of acid-base balance (20, 72, 76). In an average North American diet, metabolism of absorbed dietary constituents generates both  $H^+$  and bicarbonate. Alterations in the quantity of  $H^+$  and bicarbonate produced can have a marked impact on the level of blood bicarbonate concentration in individuals with normal and compromised renal function. In general, according to the traditional formulation, protons are generated when metabolism of a nutrient yields a product with a greater net negative charge and  $H^+$ :  $A^0 \rightarrow H^+ + B^-$  (where  $A^0$  is an amino acid such as cysteine or methionine) or  $A^+ \rightarrow H^+ + B^0$  (where  $A^+$  is an amino acid such as lysine, arginine, or histidine). Bicarbonate is generated, as  $H^+$  is consumed, when metabolism of a nutrient yields a product with a greater positive charge:  $C^- + H^+ \rightarrow D$  (where  $C^-$  is an organic anion such as citrate).

Important dietary substances whose metabolism leads to  $H^+$  generation include the neutral sulfur-containing amino acids methionine and cysteine, which are converted to sulfate and  $H^+$  ions, and the cationic amino acids lysine, arginine, and some histidine residues, which are converted into neutral products and  $H^+$ . It has been estimated that the net total  $H^+$  load generated by these sources approximates 200 meq/day. Substances which generate bicarbonate during their metabolism, i.e., consume protons, include the amino acids glutamate and aspartate, and organic anions such as citrate, gluconate, malate, acetate, and lactate. Approximately 150 meq/day of bicarbonate are generated from metabolism of these sub-

stances. Therefore, net proton generation each day is  $\sim 50$  meq. However, there is often great variability among individuals in acid generation given the wide differences in dietary intake (range 20–120 meq/day).

In what is now relevant to those who support the Stewart formulation, Christensen (19) pointed out years ago that if we limit ourselves to the “cation-anion” point of view, it will be impossible to interpret the metabolic production and consumption of  $H^+$  when there is “no net change in the fixed ions of the body”. Moreover, it is known that the metabolic production of sulfate is acidifying, and proponents of the “cation-anion” or [SID] approach would potentially attribute the acidifying properties to an increase in fixed anions (sulfate). However, as Christensen (18) stated, “One cannot readily imagine why sulfate should be any more acidifying if not quite all is excreted”.

*Gastric  $H^+$  secretion-mechanism of change in luminal  $[H^+]$ : Stewart and traditional formulations.* According to the traditional model for acidification of the lumen of the stomach (124), gastric parietal cells secrete  $H^+$  via an apical  $H^+-K^+$ -ATPase that generates a concentration of  $H^+$  ions  $10^6$  times greater in the stomach lumen than in the cell cytosol (pH = 1.0 vs. pH = 7.0). The action of the  $H^+-K^+$ -ATPase, which exports one  $H^+$  ion and imports one  $K^+$  ion for each ATP hydrolyzed, produces no net movement of electric charge. If parietal cells simply exported  $H^+$  ions in exchange for  $K^+$  ions, a rise in the concentration of  $OH^-$  ions and thus a marked rise in intracellular pH would occur. The excess cytosolic  $OH^-$ , generated by exporting of protons, combines with  $CO_2$ , forming  $HCO_3^-$  in a reaction catalyzed by cytosolic carbonic anhydrase. The  $HCO_3^-$  ion is transported across the basolateral membrane of the cell to the gastric vein in exchange for an incoming  $Cl^-$  via the anion exchanger AE2 and the  $Cl^-$  channel (SLC26A7) (69). The  $Cl^-$  ions imported into the cell exit through  $Cl^-$  channels in the apical membrane. To preserve electroneutrality, each  $Cl^-$  ion that moves into the stomach lumen across the apical membrane is accompanied by a  $K^+$  ion that moves outward through a separate  $K^+$  channel. In this way, the excess  $K^+$  ions pumped inward by the  $H^+-K^+$ -ATPase are returned to the stomach lumen, thus maintaining the intracellular  $[K^+]$ . The net result is accumulation of both  $H^+$  and  $Cl^-$  ions (HCl) in the stomach lumen.

According to the Stewart formulation,  $H^+$  secretion via the apical  $H^+-K^+$ -ATPase coupled to basolateral bicarbonate efflux is not responsible for acidification of the gastric contents with commensurate alkalization of the gastric vein. Rather, changes in [SID] are considered the mechanism for generating pH changes in these compartments. In this regard, the parietal cells lower the [SID] in the gastric lumen via an increase in luminal  $[Cl^-]$  while raising the [SID] in the gastric vein (via a decrease in  $[Cl^-]$ ). Again, Christensen's (18) comments in analyzing the role of the stomach in acid-base physiology are relevant

One may say that vomiting of gastric juice tends to cause alkalosis because of the excess chloride loss and that loss of intestinal secretions is acidifying because excessive proportions of  $Na^+$  and  $K^+$  are lost. One may say that a solution of physiological saline placed in the high  $PCO_2$  of the physiological environment is acid because it has an excessive (relative) chloride content. To permit it to become neutral, a gap is needed between the  $Na^+$  and  $Cl^-$  concentrations, which may

be filled with bicarbonate ion. This temporarily overlooks the fact that forming  $HCO_3^-$  from  $H_2CO_3$  requires the removal of  $H^+$ , so that we must remove  $H^+$  and  $Cl^-$ , not just  $Cl^-$  from physiological saline to make of it a solution neutral in the physiological environment.

Based on the Stewart model, there are various factors which would predictably alter the luminal [SID] and enhance the  $[H^+]$ . The gastric  $H^+-K^+$ -ATPase by absorbing  $K^+$  would decrease the luminal [SID], resulting in an increase in  $[H^+]$ . The concomitant outward  $K^+$  movement through a separate  $K^+$  channel has an opposite effect in that it raises the [SID], thereby decreasing the  $[H^+]$ . If one assumes that  $K^+$  absorption via the  $H^+-K^+$ -ATPase and  $K^+$  flux through the  $K^+$  channels are equal, there will be no net change in luminal [SID] and therefore no expected change in  $[H^+]$  overall. The movement of  $Cl^-$  from the cytosol to the lumen is responsible for lowering the [SID] (67, 140). It remains to be explained why the cellular machinery to acidify the lumen would utilize ATP to secrete protons, when, according to the Stewart formulation,  $H^+$  flux per se has no effect on gastric acidity. The coupling of  $K^+$  channel flux to  $H^+-K^+$ -ATPase activity has no net effect on SID but results in the loss of ATP. Given that according to the Stewart formulation, it is the secretion of  $Cl^-$  that lowers the [SID], thereby acidifying the lumen, one might ask why nature didn't simply couple the transport  $Cl^-$  to ATP hydrolysis rather than the utilization of three separate processes each of which can change the [SID] and alter lumen  $[H^+]$ . In addition, although according to the Stewart formulation internal  $H^+$  transfer reactions cannot per se be responsible for changes in  $[H^+]$ , it is the internal proton transfer reaction that generates intracellular bicarbonate via  $H^+-K^+$ -ATPase-mediated  $H^+$  efflux that drives basolateral  $Cl^-$  influx and subsequent luminal  $Cl^-$  secretion that causes a decrease in luminal [SID]. In the absence of the intracellular proton transfer reaction generating bicarbonate, basolateral-to-luminal transcellular  $Cl^-$  secretion would not occur.

*Renal proximal tubule bicarbonate transport and collecting duct acidification: Stewart and traditional formulations.* The traditional view as to how the kidney modulates systemic acid-base balance focuses on the mechanistic understanding of membrane  $H^+$ /base transport processes between plasma, tubule cell, and urine compartments (53), which the Stewart formulation does not accept a priori (29, 31, 67, 120, 140). According to the traditional view, the proximal tubule absorbs  $\sim 60\%$  of the filtered bicarbonate load and generates additional bicarbonate predominantly from the metabolism of  $\alpha$ -ketoglutarate derived from glutamine. Proximal renal tubular acidosis is a genetic or acquired abnormality of proximal tubule bicarbonate absorption. In renal failure, glutamine uptake is decreased with a concomitant decrease in new bicarbonate generation from  $\alpha$ -ketoglutarate (53, 72, 76). Either decreased bicarbonate absorption or decreased new renal bicarbonate generation can cause a metabolic acidosis. The proximal tubule and collecting duct also generates new bicarbonate by secreting protons that are excreted in the urine as titratable acid ( $H_2PO_4$ ). In addition, collecting duct acidification converts luminal  $NH_3$  into  $NH_4^+$ . Approximately 50% of the  $NH_4^+$  produced in the proximal tubule via glutamine metabolism is excreted in the urine (53, 72, 76). This process prevents excess  $NH_4^+$  from returning to the systemic circulation via the renal vein that results in the hepatic consumption of bicarbonate and

$\text{NH}_4^+$  in the urea cycle. Defective collecting duct acidification due to genetic or acquired causes results in a decrease in collecting duct new renal bicarbonate generation from titratable acid excretion and inappropriate shunting of  $\text{NH}_4^+$  to the renal vein, resulting in excessive bicarbonate consumption in the urea cycle.

Bicarbonate absorption by the proximal renal tubule is thought to be mediated by apical  $\text{Na}^+/\text{H}^+$  exchange via NHE3 (an indirect process) and basolateral sodium bicarbonate efflux via NBCe1-A (53, 111, 115). It is known that the luminal  $[\text{H}^+]$  increases axially down the proximal tubule. According to traditional acid-base theory, apical  $\text{H}^+$  efflux via NHE3 acidifies the lumen, resulting in the protonation of luminal bicarbonate and its conversion to  $\text{CO}_2$ , which is absorbed across the apical membrane. According to the Stewart formulation, apical  $\text{H}^+$  efflux via NHE3 cannot per se acidify the lumen. It is known, however, that  $\text{Na}^+$  transport in the early proximal tubule is isonatric, and therefore [SID] cannot change via a change in  $[\text{Na}^+]$  or the function of NHE3. Rather, [SID] decreases due to an increase in the luminal  $[\text{Cl}^-]$ . The increase in luminal  $[\text{Cl}^-]$  is mediated by water absorption in excess of chloride absorption because of the relatively low rate of chloride transport in the early proximal tubule. Therefore according to the Stewart formulation, water absorption in excess of chloride absorption by decreasing [SID] accounts for the increase in luminal  $[\text{H}^+]$ . As a correlate, according to this formulation, inhibition of any apical sodium-coupled cotransport process that drives osmotic water flow would be predicted to diminish the increase in luminal  $[\text{Cl}^-]$ , resulting in an increase in [SID] and a decrease in the luminal  $[\text{H}^+]$ . Transcellular chloride transport in the proximal tubule is accompanied by the transport of other species. Specifically, chloride is absorbed in the proximal tubule via  $\text{Cl}^-/\text{formate}^-$ ,  $\text{Cl}^-/\text{oxalate}^-$ , and  $\text{Cl}^-/\text{OH}^-$  exchangers (53).  $\text{Cl}^-/\text{formate}^-$  and  $\text{Cl}^-/\text{oxalate}^-$  exchange would not be predicted to change [SID] at physiological pH since formate and oxalate are at physiological pH fixed anions. However, since  $\text{OH}^-$  transport per se cannot according to the Stewart formulation change the  $[\text{H}^+]$  of a solution,  $\text{Cl}^-/\text{OH}^-$  exchange (viewed as identical to  $\text{Cl}^-$  transport via a channel) would result in an increase in [SID] and tend to alkalinize the luminal pH.

Examining the cellular transport processes responsible for urine acidification in the medullary collecting duct, is instructive in providing further insight into the differences between the traditional understanding of these physiological mechanisms vs. the Stewart theory. It is known that the apical membrane of  $\alpha$ -intercalated cells in this nephron segment possesses an electrogenic vacuolar  $\text{H}^+$ -ATPase that utilizes ATP and is responsible for hyperpolarizing the apical cell membrane and generating a lumen-positive transepithelial voltage (53). The excess cytosolic  $\text{OH}^-$ , generated by exporting protons, combines with  $\text{CO}_2$ , forming  $\text{HCO}_3^-$  in a reaction catalyzed by cytosolic carbonic anhydrase. The  $\text{HCO}_3^-$  ion is transported across the basolateral membrane of the cell to the renal vein in exchange for an incoming  $\text{Cl}^-$  via the anion exchanger AE1 (53, 115). The  $\text{Cl}^-$  ions imported into the cell via AE1 exit through  $\text{Cl}^-$  channels in the apical membrane. Apical  $\text{Cl}^-$  secretion is a passive diffusional process and not a process that directly utilizes ATP. In contrast, according to the proponents of the Stewart formulation, the transport of  $\text{Cl}^-$  by  $\alpha$ -intercalated cells and the commensurate change in [SID]

acidifies the luminal fluid. Given that according to the Stewart formulation, it is the secretion of  $\text{Cl}^-$  that lowers the [SID], thereby acidifying the lumen, similar to in the stomach, one might ask why nature did not simply couple the transport of  $\text{Cl}^-$  to ATP hydrolysis. In addition, although according to the Stewart formulation internal  $\text{H}^+$  transfer reactions cannot per se be responsible for changes in  $[\text{H}^+]$ , as in parietal cells, it is the internal proton transfer reaction that generates intracellular bicarbonate via  $\text{H}^+$  efflux that drives basolateral  $\text{Cl}^-$  influx via AE1 and subsequent luminal  $\text{Cl}^-$  secretion that causes a decrease in luminal [SID]. In the absence of the intracellular proton transfer reaction generating bicarbonate, basolateral-to-luminal transcellular  $\text{Cl}^-$  secretion would not occur unless  $\text{Cl}^-$  secretion were active. Therefore, as in the stomach, according to the Stewart formulation, the secretion of protons consumes ATP without being directly involved in luminal acidification.

The proponents of the Stewart theory view the kidney as an organ that regulates systemic acid-base balance primarily via changes in the excretion of the strong ion  $\text{Cl}^-$ , thereby modulating the urine [SID]. Acetazolamide, for example, is thought to alter blood pH by increasing the urinary [SID] (105). Recently, it has been stated that hemodialysis alters systemic pH by altering the dialysate  $\text{Cl}^-$  and [SID] (82) and that blood  $\text{Cl}^-$  alters blood pH and correlates with bone markers (83). The role of  $\text{NH}_4^+$  excretion in the urine as suggested by the Stewart formulation is a permissive one in that it allows  $\text{Cl}^-$  to be excreted in the absence of  $\text{Na}^+$  (29, 67, 120, 140). Sirker et al. (140) suggest that according to the Stewart theory, urine  $\text{NH}_4^+$  allows  $\text{Cl}^-$  to be excreted "without the loss of any strong cations." According to the Stewart formulation,  $\text{NH}_4^+$  excretion accompanied by  $\text{Cl}^-$  is thought to decrease urinary [SID], thereby increasing plasma [SID] (29, 67, 120, 140). This formulation is questionable given that over the physiological urinary pH range,  $\text{NH}_4^+$  is a "strong cation" equivalent to  $\text{Na}^+$  or  $\text{K}^+$  and these ions are interchangeable as far as their effect on urine [SID]. Moreover, urine organic anion excretion accompanied by  $\text{Na}^+$  is also thought to increase urinary [SID], thereby decreasing plasma [SID]. However, organic anions in the urine have a fixed negative charge (to the extent that the urine pH is significantly above their respective pK values), and their effect is equivalent to  $\text{Cl}^-$  in regard to the urine [SID]. Finally, it needs to be stressed that examining the urine [SID] cannot predict the net effect on blood [SID] if the input (ingested/absorbed) [SID] is ignored as is the case in examining the mass balance of any substance (45, 108).

#### *Clinical Acid-Base Disturbances*

*Critique of the diagnostic categories.* In the  $\Delta\text{HCO}_3^-/\Delta\text{PCO}_2$  approach to clinical acid-base diagnosis, each of the six cardinal acid-base disorders has a characteristic value (36, 72). These relationships have also been expressed in terms of standard base excess (127). According to the strong ion formulation, there are also six primary acid-base disturbances (23, 24, 29). The diagnostic categories differ in the following manner: 1) in the  $\Delta\text{HCO}_3^-/\Delta\text{PCO}_2$  approach, respiratory acid-base disorders are subdivided into acute and chronic disorders because they have a different characteristic  $\Delta\text{HCO}_3^-/\Delta\text{PCO}_2$  value; 2) according to the strong ion formulation, strong ion acidosis (decreased SID) and alkalosis (increased SID) and

nonvolatile buffer ion acidosis (increased  $A_{TOT}$  or  $A^-$ ) and alkalosis (decreased  $A_{TOT}$  or  $A^-$ ) are acid-base disorders that independently change the blood pH. The categorization is based on changes in the parameters on the right side of the following equality where  $[HCO_3^-] = [SID^+] - [A^-]$ .

The proponents of the Stewart formulation, by creating the categories of strong ion and nonvolatile acid-base disorders, implicitly assume that  $[SID]$  and/or  $[A^-]$  is individually regulated in normal and pathological states. Accordingly, the bicarbonate-centered approach is not capable of making the acid-base diagnosis in a case, for example, when  $\Delta[SID^+] = \Delta[A^-]$ , i.e., strong ion acidosis and nonvolatile buffer ion alkalosis (hypoalbuminemia) of equal magnitude, since the  $\Delta HCO_3^- = 0$ . However, the traditional acid-base physiologist would not have any difficulty in diagnosing a mixed acid-base disorder in this case despite the normal  $[HCO_3^-]$ , pH, and  $PCO_2$  because, importantly, the AG must have increased, reflecting an organic acid-induced metabolic acidosis (AG variant after having accounted for the decreased albumin). A metabolic alkalosis of equal magnitude is also present accounting for the normal  $[HCO_3^-]$ . Regarding the diagnostic categories utilized by the Stewart formulation, proponents of the bicarbonate-centered approach would also point out that  $[SID]$  can never change in a macroscopic system in isolation without an equivalent change in the difference in unfixated ions. Moreover, whether changes in  $[A_{TOT}$  or  $A^-]$  should be categorized as a separate acid-base disturbance has also been questioned (67, 138).

In the bicarbonate-centered approach primary changes in  $PCO_2$  are associated with predictable changes in  $[HCO_3^-]$  both acutely (acute respiratory  $\Delta HCO_3^-/\Delta PCO_2$  rules) and chronically (chronic respiratory  $\Delta HCO_3^-/\Delta PCO_2$  rules). Furthermore, primary changes in  $[HCO_3^-]$  are associated with predictable changes in  $PCO_2$  (so-called compensatory changes in metabolic acid-base disorders). It might be predicted that were  $[SID^+]$  and  $[A_{TOT}$  or  $A^-]$  to have an individual effect on acid-base balance, that the respiratory center would in strong ion and "nonvolatile" acid-base disorders respond with predictable compensatory ventilatory changes. A correlation between changes in  $A_{TOT}$  and the  $PCO_2$  has not been observed (159). Mathematically, one can replace all the six diagnostic  $\Delta HCO_3^-/\Delta PCO_2$  rules used in the bicarbonate-centered approach with  $\Delta([SID^+] - [A^-])/\Delta PCO_2$  rules that will have the identical values because  $HCO_3^-$  is always equal to  $([SID^+] - [A^-])$ . In this regard, the traditional acid-base physiologist would point out that in all acid-base disorders, changes in  $([SID^+] - [A^-])$  are indeed associated with changes in  $PCO_2$  simply because of macroscopic electroneutrality requirements where  $\Delta HCO_3^- = \Delta([SID^+] - [A^-])$ . The traditional acid-base physiologist would also point out that there is no known physiological mechanism that can cause a predictable change in  $([SID^+] - [A^-])$  following chronic changes in the  $PCO_2$  (chronic respiratory acid-base disturbances), nor is there a known mechanism in the respiratory center that can cause the  $PCO_2$  to change predictably following an alteration in  $([SID^+] - [A^-])$ .

**SIG vs. AG.** Gilfix et al. (47) initially proposed the term "SID gap" and Kellum et al. (66) subsequently proposed the calculation of the SIG as a refinement of the classic AG measurement  $[Na^+] - [Cl^-] + [HCO_3^-]$ , used to assess the concentration of organic anions in metabolic acidosis. According to the Stewart formulation, the organic anions (acetoace-

tate, lactate) per se, rather than the accompanying proton by decreasing SID, result in an increase in  $[H^+]$ . The motivation for modifying the classic AG calculation was that it did not routinely correct for changes in 1) albumin concentration; 2) charge contributed by albumin; 3) effect of pH on the charge of albumin; 4) phosphate concentration; 5) charge contributed by phosphate; and 6) effect of pH on the charge of phosphate. Importantly, the charge on albumin at pH 7.4 contributes ~66% of the total net charge calculated by the AG, with the remainder composed of phosphate, urate, lactate, ketone bodies, and sulfate. Unlike the classic parameters used to calculate the AG, in calculating SIG, the effect of albumin, phosphate, urate, and lactate is "subtracted out." Therefore, SIG compared with the AG is composed of a shorter list of unmeasured ions including ketone bodies, sulfate, and uremic anions (22–24, 66, 163). Given the low concentration of urate and lactate in most clinical situations, SIG can be calculated as  $[albumin^{x-}] + [phosphate^{y-}] - AG$ . The charge on albumin and phosphate is corrected for pH as discussed below (30, 40–43). Although the proponents of the Stewart formulation have been the major users of SIG, the calculation per se is not theoretically based on concepts that are specific to the Stewart formulation. The SIG calculation simply reflects an attempt to make the estimate of unmeasured organic anions that accumulate in certain types of metabolic acidosis more accurate by including in the calculation, the charge on albumin and phosphate and as many other measured anions/cations as is clinically feasible. As recently reported by Dubin et al. (35), in intensive care unit patients, the Stewart/SIG approach did not offer any diagnostic or prognostic advantage over the bicarbonate-centered-corrected AG method.

In what simply amounts to careful bookkeeping of each of the positive and negative charges that contribute to the macroscopic electroneutrality, SIG can be calculated according to  $SIG = SID_m - SID_e$ .  $SID_m$  (measured SID, also called  $SID_a$  for apparent) is the net charge on strong ions whose charge is constant (strong or fixed ions) over the physiological pH range and is calculated as  $[Na^+] + [K^+] + 2[Ca^{2+}] + 2[Mg^{2+}] - [Cl^-] - lactate^- - urate^-$  (22–24, 42, 66, 106, 163).  $SID_e$  (effective SID) is composed of those substances whose charge is modified over the physiologic pH range as a result of proton transfer reactions,  $[HCO_3^-] + [albumin^{x-}] + [phosphate^{y-}]$ , where the charge on albumin and phosphate is corrected for pH as discussed below. What remains unmeasured and therefore contributes to SIG is sulfate, and organic ions composed of  $OC_{NM}^+$ ,  $OC_M^+$ ,  $OA_{NM}^-$ , and  $OA_M^-$ . Caveats in any calculation of net charge include 1) in the presence of weak bases ( $R-NH_3^+$ ), which can undergo proton transfer reactions in the physiologic range,  $SID_e$  must be calculated as:  $[HCO_3^-] + [albumin^{x-}] + [phosphate^{y-}] - [R-NH_3^+]$  (79); 2) SIG has been corrected for water excess/deficit based on the change in plasma  $[Na^+]$  with the assumption that the dilution factor for all charges is the same and that the change in  $[Na^+]$  is solely due to mass balance changes in water balance (35, 40); 3) the  $SID_m$  is calculated using the measured values of selected ions rather than being measured directly, and since not all strong ions are measured, there is always a difference between the actual or true  $SID_m$  and the calculated  $SID_m$ ; and 4) because of the curvilinear relationship between  $[SID]$  and  $[H^+]$ , at lower  $[SID]$ , differences in the measured and true  $SID_m$  will result in a greater error in the calculated  $[H^+]$

using the Stewart equation. The error is also greater when the calculated  $SID_m$  is less than the true value (70).

**Oxidation of organic acids.** It is well known that organic anions such as lactate, acetate, and citrate are metabolized to  $HCO_3^-$  (2, 17, 157). Clinicians take advantage of this to prevent (peritoneal dialysis, total parenteral nutrition) or treat (citrate) metabolic acidosis without requiring bicarbonate administration. For example,  $Na^+ + CH_3CHOHCOO^-$  (lactate) +  $3O_2 \rightarrow 2CO_2 + 2H_2O + Na^+ + HCO_3^-$ . The  $CO_2$  that is generated is excreted by the lungs and if the generation of  $HCO_3^-$  exceeds its excretion rate, the blood pH increases. According to traditional concepts, the oxidation of lactate and other organic anions to bicarbonate involves an  $H^+$  transfer reaction from carbonic acid. According to the Stewart formulation, the addition of sodium lactate to blood should not change the  $[SID]_a$  because a strong cation and anion (lactate) are added in equimolar concentrations.<sup>6</sup> Subsequent lactate conversion to bicarbonate is viewed as an alkalinizing process because of the increase in  $[SID]_a$  (as [lactate] decreases) and not because the  $[HCO_3^-]$  increases. In predicting the effect on systemic acid-base status of infused metabolizable organic anions, the calculation of  $[SID]_a$  should not include the metabolizable organic anion (i.e., lactate) concentration. Therefore, the so-called "theoretical maximum"  $SID_a$  (the  $SID_a$  that will be potentially measured after lactate is metabolized) can be quantitated simply by ignoring the [lactate] in calculating the  $SID_a$  of the infusate (37).

Several questions remain for the proponent of the Stewart formulation. Specifically, if an internal  $H^+$  transfer reaction is not involved, by what mechanism chemically does the decrease in the [lactate] result in an increase in blood pH and  $[HCO_3^-]$ ? Second,  $[SID]$  is not increasing as lactate is metabolized because of a process imposed on a system from the outside (all independent variables are initially unchanged by the addition of sodium lactate to the system); therefore, there should be no mechanism for any dependent variables to change.

**Hypoproteinemic alkalosis and hyperproteinemic acidosis.** In addition to the  $PCO_2$  and  $SID$ , Stewart indicated that the total concentration of nonvolatile weak acids ( $A_{TOT}$ ) is a third independent variable that determines the  $[H^+]$ . Stewart had used a single dissociation constant ( $K_A$ ) to represent the role of all weak acids in plasma (144–146).  $A_{TOT}$  in human plasma is primarily composed of albumin, with the contribution of phosphate and citrate being minor (25). The potential role of hypoalbuminemia in causing metabolic alkalosis was first described by Ystgaard (166) and subsequently by McAuliffe et al. (93). McAuliffe et al. presented a group of patients diagnosed as having hypoproteinemic alkalosis. These patients had no other apparent causes of metabolic alkalosis and had a normal plasma  $Na^+$ ,  $K^+$ ,  $Cl^-$ , and an AG that was decreased commensurate with the decreased plasma albumin (mean value 2.7 g/dl). The mean  $[HCO_3^-]$ ,  $PCO_2$ , and pH in these patients were 31.3 mM, 46.8 mmHg, and 7.44, respectively. No data

were provided regarding urine chloride excretion or concentration (and not all known causes of metabolic alkalosis were ruled out), and the authors suggested that the decreased albumin concentration per se resulted in the increase in pH. Rossing et al. (123) subsequently showed that changes in the [albumin] in human blood in vitro are associated with inverse changes in bicarbonate and pH and invoked the Stewart formulation to account for this effect. As stated by Rossing et al. "if interpretation of acid-base data is limited to evaluation of the variables in the H-H equation, the source of the metabolic alkalosis in hypoproteinemia cannot be revealed." However, the above assertion by Rossing et al. deserves more critical analysis. The in vitro study performed by Rossing et al. was performed in a two-compartmental model (blood) rather than plasma. Specifically, hypoalbuminemia can lead to an increase in the  $[HCO_3^-]$  due to a decrease in the Gibbs-Donnan effect exerted by the negatively charged albumin. Moreover, the solubility of  $CO_2$ ,  $S$ , and the dissociation constant  $K'_1$  in the H-H equation are dependent on ionic strength and protein concentration. Therefore, alterations in the albumin concentration will result in a change in the  $CO_2$  concentration due to a change in the solubility of  $CO_2$  ( $S$ ) and a change in the dissociation constant  $K'_1$ , thereby leading to an alteration in the  $[HCO_3^-]$ . Furthermore, changes in the albumin concentration will also lead to alterations in all ionic activities due to changes in Gibbs-Donnan equilibrium, resulting in a change in the ionic strength that will also alter the  $CO_2$  concentration due to a change in both the solubility of  $CO_2$ ,  $S$ , and the dissociation constant  $K'_1$ .

From these studies, the concept of hypoproteinemic alkalosis and hyperproteinemic acidosis evolved. However, Wilkes (159) reported a weak inverse association between  $[A_{TOT}]$  and  $[Cl^-]$  in hypoproteinemic critically ill patients, and no relationship was detected between  $[A_{TOT}]$  and  $[HCO_3^-]$  or blood pH, suggesting that changes in  $[SID]$  rather than the  $PCO_2$  compensate for the effect of changes in  $[A_{TOT}]$  on pH. Furthermore, Corey (30) recently reported patients with nephrotic syndrome (mean albumin  $\sim 2.3 \pm 0.66$  g/dl) had a normal plasma bicarbonate (mean  $25.5 \pm 2.9$  mM). Story and Kellum (148) have concluded that "although the loss of weak acid from the plasma is an alkalinizing process, there is no evidence that the body regulates albumin to maintain acid-base balance and there is no evidence that clinicians should treat hypoalbuminemia as an acid-base disorder." In contrast Wang et al. (155) have suggested that hyperalbuminemia secondary to dehydration in patients with cholera may induce a metabolic acidosis. Bruegger et al. (14) have reported a small but significant decrease in systemic pH following the infusion of 20% albumin.

Figge et al. (41) pointed out that because Stewart had represented the polyprotic proteins and phosphate as having a single dissociation constant,  $K_a$ , the titration curve should be nonlinear over a range of  $\pm pH$  unit. However, in plasma, the titration curves are known to be linear (135). This prompted these authors to undertake a more detailed study of the acid-base behavior of albumin. Figge et al. studied the treatment of plasma proteins as weak acids and predicted a net charge of  $-11.4$  meq/l on albumin (41, 42), which compared well with Van Leeuwen's data,  $-12.2$  meq/l. However, as shown by Watson (158), the concentration of negatively charged albumin does not follow typical weak-acid chemistry where  $[A^-] =$

<sup>6</sup> In vitro the addition of sodium lactate to an aqueous solution will increase the concentration of  $Na^+$  and lactate equally and  $[SID]$  will not change. In vivo, the situation is more complex because the plasma water sodium concentration,  $[Na^+]_{pw}$ , is kept constant by changes in whole body water mass balance. However, because of macroscopic electroneutrality, assuming the increment in  $[lactate^-]$  equals the decrement in  $[Cl^-]$ ,  $SID$  remains unchanged before lactate conversion to bicarbonate.



$K_a[A_{TOT}]/(K_a + H^+)$ . Specifically, albumin contains  $\sim 99$  residues with fixed negative charges (mainly aspartate, and glutamate), and  $\sim 77$  fixed positive charges (lysine and arginine) that are independent of pH in this range. Therefore there are net  $\sim 22$  eq/l fixed negative charges on albumin. Albumin has 16 histidine residues that react with  $H^+$ . The Watson model for the concentration of the net charge on albumin in the physiological range is  $[A^-] = [A^-_{FIXED}] - [H^+][A_{H,TOT}]/(K_H + H^+)$ . Based on the data of Reeves (119), the average  $K_H$  of the histidine residues in albumin is  $1.77 \times 10^{-7}$  eq/l at 37.5 degrees. Albumin is then modeled as a protein with a fixed net negative charge (21–22 eq/l) and a variable positive charge on the 16 histidine residues: albumin charge (meq) =  $21 - [16 \times (1 - \alpha_{pH}) \times 10,000/66,500 \times [\text{albumin g/dl}]]$ , where  $\alpha_{pH}$  is the ratio of unprotonated to total histidine residues at a given pH (30). Staempfli et al. (142) have estimated the fixed charge on albumin is 3.7 meq/l (assuming the concentration of albumin is 4.1 g/dl) and the pH-dependent charge is 9.3 meq/l at pH 7.4 (total charge 13 meq/l). Fogh-Andersen et al. (43) have pointed out that the effective net charge on albumin is also dependent on pH-dependent ion binding. In a comparison of the Stewart, Figge, and Watson models of the behavior of human albumin and plasma, the predicted log $P_{CO_2}$ -pH relationship was not statistically different (4). Although an area of controversy, more recent estimates of  $[A_{TOT}]$  and  $K_A$  of human plasma are  $\sim 17.2$  mmol/l (or 0.378 mmol/g albumin) and  $\sim 0.8 \times 1.77 \times 10^{-7}$ , respectively (142). Astrup had previously demonstrated that albumin is a weak acid and that the addition of albumin to human plasma lowered the pH (at all  $P_{CO_2}$  values) and increased its buffer capacity (6). Although  $[A^-]$  is a nonlinear function of pH, it can be approximated over the physiological range by  $C_{alb}(1.2pH - 6.15) + C_{Phos}(0.097pH - 0.13)$ , where  $C_{alb}$  and  $C_{Phos}$  are plasma albumin and phosphate concentrations (mg/dl), respectively (30, 41, 42). When  $C_{alb}$  and  $C_{Phos}$  are expressed in milliequivalents per liter, the equation becomes  $C_{alb}(8.0pH - 41) + C_{Phos}(0.30pH - 0.4)$  (163).

Unlike plasma, the urine pH can decrease to a value of 4.5 in certain settings (76). Although albumin is essentially absent from urine in normal individuals, various glomerular abnormalities are associated with nephrotic ( $>3.5$  g/day) range albuminuria. One can estimate the effect of excess urinary albumin excretion on titratable acid excretion and concomitant renal bicarbonate generation. For example, in a patient with an albumin excretion rate of 10 g/day in 1 liter of urine (albumin concentration 1 g/dl), at a urine pH of 5.0, the charge on albumin is  $\sim 0$ . At a plasma albumin concentration of 3.0 g/dl and pH 7.4, the charge on albumin is  $-8.2$ . Therefore, 8.2 meq/day of new bicarbonate were generated in association with urinary protonation and excretion of albumin. Whether the plasma  $[HCO_3^-]$  increases is dependent on the ability of the kidney (factors include GFR and tubular absorption) to excrete the additional bicarbonate load.

**Dilution acidosis and contraction alkalosis.** Following acute infusion of D5W or 0.9% saline, the plasma  $[HCO_3^-]$  and  $P_{CO_2}$  will decrease as might be predicted simply by a dilutional mechanism (34, 91, 132). The opposite effect is expected following loss of plasma water in dehydration. The final  $P_{CO_2}$  level will vary depending on the ventilatory response to the changes in blood chemistry. If the  $P_{CO_2}$  in the steady state is decreased in proportion to the decrease in  $[HCO_3^-]$ , the pH

remains constant. Were the ventilatory system to keep the  $P_{CO_2}$  at 40 mmHg, according to traditional concepts, a diagnosis of metabolic (dilutional) acidosis and respiratory acidosis would be made. The  $P_{CO_2}$  could also have an intermediary value if the ventilatory system adjusts the  $P_{CO_2}$  to a level expected in a compensated metabolic acidosis. According to the Stewart formulation, in dilutional acidosis,  $[H^+]$  increases because of a strong ion acidosis (decreased SID) that exceeds in magnitude the effect of a decrease in  $A_{TOT}$  (nonvolatile buffer ion alkalosis) (3, 37, 84, 99, 100, 102, 156). Moreover, it has been suggested that the Stewart formulation predicts less of a decrease in  $[HCO_3^-]$  than the H-H equation for a given volume of infusate because the former takes into consideration the alkalinizing effect of a decrease in  $A_{TOT}$  and that an experimental confirmation of this prediction would favor the Stewart formulation (37). However, given that the H-H equation and the Constable simplified strong ion equations are equivalent (APPENDIX A), they must predict the same decrease in plasma  $[HCO_3^-]$  and the same change in pH. Simply stated, in dilutional acidosis  $\Delta[HCO_3^-] = \Delta([SID^+] - [A^-])$  because of electroneutrality requirements.

### Conclusion

Is the Stewart formulation a stately horse without legs? Given that the sometimes maligned H-H equation and the Stewart equation (Stewart, Constable) are interchangeable, it is clear that their quantitative predictions must also be equivalent under all circumstances without exception. In this regard, the Stewart formulation does not provide any diagnostic or prognostic advantage (35). Simply stated, given the precision of the bicarbonate (or  $T_{CO_2}$ ) assay and the complexity and potential inaccuracies in correctly calculating  $[SID] - K_a[A_{TOT}]/(K_a + 10^{-pH})$ , which is numerically equal to the bicarbonate concentration, the assessment of the latter parameter becomes redundant. Importantly, the lack of a mechanistic basis to justify the use of the Stewart formulation is critical. In this regard, the hypothesis that in a given compartment, SID (a mathematical construct and not a physicochemical property) mechanistically influences  $[H^+]$  to maintain electroneutrality has no experimental basis. Those valuing the Stewart formulation would be wise to follow Occam's Razor, which asserted that "entities are not to be multiplied without necessity." Translated, this means that the equations one utilizes should include only those terms that are necessary to characterize the underlying phenomenology mechanistically. Although the pursuit of simplicity can sometimes be misguided, regarding the current state of our understanding of acid-base phenomenology, the moral of the story appears to be rather straightforward: "If its not broken, don't fix it!"

### APPENDIX A

*Proof that the Henderson-Hasselbalch and Stewart Equations are Quantitatively Identical*

Derivation of the Stewart Equation

$$[H^+] \times [OH^-] = K'_w \quad (A1)$$

$$[H^+] \times [A^-] = K_a \times [HA] \quad (A2)$$

$$[H^+] \times [HCO_3^-] = K'_1 \times S \times P_{CO_2} \quad (A3)$$

$$[H^+] \times [CO_3^{2-}] = K_3 \times [HCO_3^-] \quad (A4)$$

Summing the four equations together

$$[H^+]( [OH^-] + [A^-] + [HCO_3^-] + [CO_3^{2-}] ) \quad (A5)$$

$$= K'_w + K_a \times [HA] + K'_1 \times S \times P_{CO_2} + K_3 \times [HCO_3^-]$$

$$[SID^+] + [H^+] = [HCO_3^-] + [A^-] + [CO_3^{2-}] + [OH^-] \quad (A6)$$

$$[H^+]( [SID^+] + [H^+] ) = K'_w + K_a \times [HA] \quad (A7)$$

$$+ K'_1 \times S \times P_{CO_2} + K_3 \times [HCO_3^-]$$

$$[HA] + [A^-] = [A_{TOT}] \quad (A8)$$

Rearranging

$$[A^-] = [A_{TOT}] - [HA] \quad (A8a)$$

Substituting Eq. A8a into Eq. A6

$$\text{Since } [SID^+] + [H^+] = [HCO_3^-] + [A_{TOT}] \quad (A9)$$

$$- [HA] + [CO_3^{2-}] + [OH^-]$$

Rearranging

$$[HA] = -[H^+] - [SID^+] + [OH^-] \quad (A10)$$

$$+ [A_{TOT}] + [HCO_3^-] + [CO_3^{2-}]$$

Substituting Eq. A10 into Eq. A7

$$[H^+]( [SID^+] + [H^+] ) = K_a ( -[H^+] - [SID^+] \quad (A11)$$

$$+ [OH^-] + [A_{TOT}] + [HCO_3^-] + [CO_3^{2-}] )$$

$$+ K'_w + K'_1 \times S \times P_{CO_2} + K_3 \times [HCO_3^-]$$

Equations A1, A3, and A4 can be rearranged as follows:

$$[OH^-] = \frac{K'_w}{[H^+]} \quad (A1a)$$

$$[HCO_3^-] = \frac{K'_1 \times S \times P_{CO_2}}{[H^+]} \quad (A3a)$$

$$[CO_3^{2-}] = \frac{K_3 \times [HCO_3^-]}{[H^+]} = \frac{K'_1 \times S \times P_{CO_2} \times K_3}{[H^+]^2} \quad (A4a)$$

Substituting Eqs. A1a, A3a, and A4a into Eq. A11 and rearranging

$$[H^+]( [SID^+] + [H^+] + K_a ) = K_a ( [A_{TOT}] - [SID^+] \quad (A12)$$

$$+ \frac{K'_w}{[H^+]} + \frac{K'_1 \times S \times P_{CO_2}}{[H^+]} + \frac{K'_1 \times S \times P_{CO_2} \times K_3}{[H^+]^2} )$$

$$+ K'_w + K'_1 \times S \times P_{CO_2} + \frac{K'_1 \times S \times P_{CO_2} \times K_3}{[H^+]}$$

Multiplying both sides of Eq. A12 by  $[H^+]^2$  and rearranging

$$[H^+]^3 ( [SID^+] + [H^+] + K_a ) = [H^+]^2 ( K_a \times [A_{TOT}] \quad (A13)$$

$$- K_a \times [SID^+] ) + [H^+]( K'_w \times K_a + K'_1 \times S \times P_{CO_2}$$

$$\times K_a ) + K'_1 \times S \times P_{CO_2} \times K_3 \times K_a + [H^+]^2 ( K'_w + K'_1$$

$$\times S \times P_{CO_2} ) + [H^+]( K_3 \times K'_1 \times S \times P_{CO_2} )$$

Rearranging

$$[H^+]^4 + [H^+]^3 ( K_a + [SID^+] ) = [H^+]^2 ( K_a ( [A_{TOT}] - [SID^+] ) \quad (A14)$$

$$+ ( K'_1 \times S \times P_{CO_2} + K'_w ) ) + [H^+]( K_a ( K'_1 \times S \times P_{CO_2}$$

$$+ K'_w ) + K'_1 \times S \times P_{CO_2} \times K_3 ) + K_a \times K_3 \times K'_1 \times S \times P_{CO_2}$$

Rearranging into the Stewart equation

$$[H^+]^4 + [H^+]^3 ( K_a + [SID^+] ) + [H^+]^2 ( K_a ( [SID^+] - [A_{TOT}] \quad (A15)$$

$$- ( K'_1 \times S \times P_{CO_2} + K'_w ) ) - [H^+]( K_a ( K'_1 \times S$$

$$\times P_{CO_2} + K'_w ) + K'_1 \times S \times P_{CO_2} \times K_3 ) - K_a \times K_3 \times K'_1 \times S \times P_{CO_2} = 0$$

Proof that the Stewart Equation Can be Simplified to the Henderson-Hasselbalch Equation

As demonstrated above, the Stewart equation is derived from Eq. A5

$$[H^+]( [OH^-] + [A^-] + [HCO_3^-] + [CO_3^{2-}] ) \quad (A15)$$

$$= K'_w + K_a \times [HA] + K'_1 \times S \times P_{CO_2} + K_3 \times [HCO_3^-]$$

$$[H^+]( [OH^-] + [A^-] + [HCO_3^-] + [CO_3^{2-}] ) = K'_w \left( \frac{[OH^-]}{[OH^-]} \right) \quad (A5a)$$

$$+ K_a \times [HA] \left( \frac{[A^-]}{[A^-]} \right) + K'_1 \times S \times P_{CO_2}$$

$$\times \left( \frac{[HCO_3^-]}{[HCO_3^-]} \right) + K_3 \times [HCO_3^-] \left( \frac{[CO_3^{2-}]}{[CO_3^{2-}]} \right)$$

At equilibrium, the  $[H^+]$  is equal for all the buffer pairs

$$[H^+] = \frac{K'_w}{[OH^-]} = \left( \frac{K_a \times [HA]}{[A^-]} \right) \quad (A5a)$$

$$= \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right) = \left( \frac{K_3 \times [HCO_3^-]}{[CO_3^{2-}]} \right)$$

Substituting

$$\frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]}$$

for each buffer pair ratio into Eq. A5a

$$[H^+]( [OH^-] + [A^-] + [HCO_3^-] + [CO_3^{2-}] ) = [OH^-] \quad (A5b)$$

$$\times \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right) + [A^-] \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right)$$

$$+ [HCO_3^-] \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right) + [CO_3^{2-}] \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right)$$

Rearranging

$$[H^+]( [OH^-] + [A^-] + [HCO_3^-] + [CO_3^{2-}] ) \quad (A5c)$$

$$= \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right) ( [OH^-] + [A^-] + [HCO_3^-] + [CO_3^{2-}] )$$

Therefore

$$[H^+] = \left( \frac{K'_1 \times S \times P_{CO_2}}{[HCO_3^-]} \right)$$

or

$$pH = pK'_1 + \log \frac{[HCO_3^-]}{S \times P_{CO_2}} \quad (\text{Henderson-Hasselbalch Equation})$$

#### APPENDIX B

Proof that the Henderson-Hasselbalch and Constable Simplified Strong Ion Equation are Quantitatively Identical

The dissociation reaction for a weak acid-conjugate base pair, HA and  $A^-$ , is



$$[\text{H}^+] = K_a \times \frac{[\text{HA}]}{[\text{A}^-]} \quad (\text{B2})$$

Since there is conservation of mass

$$[\text{HA}] + [\text{A}^-] = [\text{A}_{\text{TOT}}] \quad (\text{B3})$$

where  $[\text{A}_{\text{TOT}}]$  is the total concentration of weak acids.  
Rearranging Eq. B3

$$[\text{HA}] = [\text{A}_{\text{TOT}}] - [\text{A}^-] \quad (\text{B3a})$$

Substituting Eq. B3a for  $[\text{HA}]$  in Eq. B2

$$[\text{H}^+] = K_a \times \frac{[\text{A}_{\text{TOT}}] - [\text{A}^-]}{[\text{A}^-]} = K_a \times \left( \frac{[\text{A}_{\text{TOT}}]}{[\text{A}^-]} - 1 \right) \quad (\text{B4})$$

Due to electroneutrality

$$[\text{SID}^+] - [\text{HCO}_3^-] - [\text{A}^-] = 0 \quad (\text{B5})$$

Rearranging Eq. B5

$$[\text{SID}^+] - [\text{HCO}_3^-] = [\text{A}^-] \quad (\text{B5a})$$

Substituting Eq. B5a for  $[\text{A}^-]$  in Eq. B4

$$[\text{H}^+] = K_a \times \left( \frac{[\text{A}_{\text{TOT}}]}{[\text{SID}^+] - [\text{HCO}_3^-]} - 1 \right) \quad (\text{B6})$$

Rearranging Eq. B6

$$[\text{H}^+] + K_a = \frac{K_a \times [\text{A}_{\text{TOT}}]}{[\text{SID}^+] - [\text{HCO}_3^-]} \quad (\text{B6a})$$

Rearranging Eq. B6a

$$([\text{H}^+] + K_a) \times ([\text{SID}^+] - [\text{HCO}_3^-]) = K_a \times [\text{A}_{\text{TOT}}] \quad (\text{B6b})$$

Rearranging Eq. B6b

$$([\text{H}^+] + K_a) \times [\text{SID}^+] - (K_a \times [\text{A}_{\text{TOT}}]) = ([\text{H}^+] + K_a) \times [\text{HCO}_3^-] \quad (\text{B6c})$$

Rearranging Eq. B6c

$$[\text{HCO}_3^-] = [\text{SID}^+] - \frac{K_a \times [\text{A}_{\text{TOT}}]}{[\text{H}^+] + K_a} \quad (\text{B7})$$

Since

$$\text{pH} = -\log [\text{H}^+] \quad (\text{B8})$$

$$-\text{pH} = \log [\text{H}^+] \quad (\text{B8a})$$

$$10^{-\text{pH}} = [\text{H}^+] \quad (\text{B9})$$

Substituting Eq. B9 for  $[\text{H}^+]$  in Eq. B7

$$[\text{HCO}_3^-] = [\text{SID}^+] - \frac{K_a \times [\text{A}_{\text{TOT}}]}{10^{-\text{pH}} + K_a} \quad (\text{B10})$$

The  $\text{HCO}_3^-$ - $\text{CO}_2$  buffer system is defined by the Henderson-Hasselbalch equation

$$\text{pH} = \text{p}K'_1 + \log \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \quad (\text{B11})$$

Substituting Eq. B10 for  $[\text{HCO}_3^-]$  in Eq. B11

$$\text{pH} = \text{p}K'_1 + \log \frac{[\text{SID}^+] - \left( \frac{K_a \times [\text{A}_{\text{TOT}}]}{10^{-\text{pH}} + K_a} \right)}{S \times \text{PCO}_2} \quad (\text{B12})$$

Therefore, the Constable simplified strong ion equation (Eq. B12) is

simply another mathematical expression of the Henderson-Hasselbalch equation where

$$[\text{HCO}_3^-] = [\text{SID}^+] - \frac{K_a \times [\text{A}_{\text{TOT}}]}{10^{-\text{pH}} + K_a}$$

## APPENDIX C

### Derivation of the Nguyen-Kurtz Multiple Buffer Equation

For any weak acid-conjugate base buffer pair

$$\text{pH} = \text{p}K'_1 + \log \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \quad (\text{C1})$$

$$\text{pH} = \text{p}K'_2 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \quad (\text{C2})$$

$$\text{pH} = \text{p}K'_3 + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad (\text{C3})$$

where HA and  $\text{A}^-$  represent any nonbicarbonate nonphosphate weak acid-conjugate base buffer pair (for example albumin).  
Combining the three equations together

$$n \times \text{pH} = \text{p}K'_1 + \text{p}K'_2 + \text{p}K'_3 + \log \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad (\text{C4})$$

where  $n$  = number of buffer pairs in solution.  
Therefore

$$n \times \text{pH} = -\log K'_1 - \log K'_2 - \log K'_3 + \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right) \quad (\text{C5})$$

Therefore

$$n \times \text{pH} = -(\log K'_1 + \log K'_2 + \log K'_3) + \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right) \quad (\text{C6})$$

Therefore

$$n \times \text{pH} = -\log (K'_1 \times K'_2 \times K'_3) + \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right) \quad (\text{C7})$$

Multiplying  $1/n$  on both sides of the equation

$$\text{pH} = -\frac{1}{n} \log (K'_1 \times K'_2 \times K'_3) + \frac{1}{n} \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right) \quad (\text{C8})$$

Therefore

$$\text{pH} = -\log (K'_1 \times K'_2 \times K'_3)^{1/n} + \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{PCO}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right)^{1/n} \quad (\text{C9})$$

Let

$$K'_T = K'_1 \times K'_2 \times K'_3$$

Therefore

$$\text{pH} = -\log (K'_T)^{1/n} + \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{Pco}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right)^{1/n} \quad (C10)$$

Therefore

$$\text{pH} = \text{p}K'_T{}^{1/n} + \log \left( \frac{[\text{HCO}_3^-]}{S \times \text{Pco}_2} \times \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \times \frac{[\text{A}^-]}{[\text{HA}]} \right)^{1/n} \quad (C11)$$

where

$$\text{p}K'_T{}^{1/n} = -\log (K'_T)^{1/n}$$

When there are more than three buffer pairs, Eq. C11 can be generalized as follows

$$\text{pH} = \text{p}K'_T{}^{1/n} + \log B^{1/n} \quad (C12)$$

$K'_T$  = product of dissociation constants of all buffer pairs

$B$  = product of ratios of all weak acid-conjugate base buffer pairs

$n$  = total number of buffer pairs

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