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Experimental Determination of Net Protein Charge and A_{tot} and K_a of Nonvolatile Buffers in Canine Plasma

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Acid-base abnormalities frequently are present in sick dogs. The mechanism for an acid-base disturbance can be determined with the simplified strong ion approach, which requires accurate values for the total concentration of plasma nonvolatile buffers (A_{tot}) and the effective dissociation constant for plasma weak acids (K_a). The aims of this study were to experimentally determine A_{tot} and K_a values for canine plasma. Plasma was harvested from 10 healthy dogs; the concentrations of quantitatively important strong ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, L-lactate) and nonvolatile buffer ions (total protein, albumin, phosphate) were determined; and the plasma was tonometered with CO_2 at 37°C. Strong ion difference (SID) was calculated from the measured strong ion concentrations, and nonlinear regression was used to estimate values for A_{tot} and K_a, which were validated with data from an in vitro and in vivo study. Mean (±SD) values for canine plasma were A_{tot} = (17.4 ± 8.6) mM (equivalent to 0.273 mmol/g of total protein or 0.469 mmol/g of albumin); $K_a = (0.17 \pm 0.11) \times 10^{-7}$; pK_a = 7.77. The calculated SID for normal canine plasma (pH = 7.40; P_{CO_2} = 37 mm Hg; [total protein] = 64 g/L) was 27 mEq/L. The net protein charge for normal canine plasma was 0.25 mEq/g of total protein or 0.42 mEq/g of albumin. Application of the experimentally determined values for A_{tot}, K_a, and net protein charge should improve understanding of the mechanism for complex acid-base disturbances in dogs.

Key words: Anion gap; Metabolic acidosis; Respiratory acidosis; Strong ion gap.

Acid-base balance traditionally has been evaluated by means of the Henderson-Hasselbalch equation[1,2] to characterize 4 primary acid-base disturbances (ie, respiratory acidosis and alkalosis, metabolic acidosis and alkalosis),[3–5] and by calculating the anion gap to estimate the unmeasured anion concentration.[6–8] However, the clinical use of the Henderson-Hasselbalch equation to guide treatment is hampered by the fact that the approach is more descriptive than mechanistic.[9] Moreover, because the anion gap reflects the net anionic charge on serum proteins as well as unmeasured strong anion concentration, the traditional Henderson-Hasselbalch approach fails to provide an accurate method for quantifying the unmeasured strong ion charge in animals with abnormal serum protein concentrations.[4,5,7,8] A mechanistic approach to acid-base balance that provides a method to quantify unmeasured strong anion charge would therefore be clinically useful.

Two mechanistic physicochemical approaches have been developed to evaluate acid-base balance: Stewart's strong ion model[10] and the simplified strong ion model.[9] The strong ion approach states that 3 independent variables (plasma strong ion difference [SID], P_{CO_2}, and plasma nonvolatile buffer ion concentration) directly determine plasma pH. The strong ion approach therefore differs in 3 important areas from the traditional focus of the Henderson-Hasselbalch equation on bicarbonate[11]: (1) acid-base balance is examined with a systems approach, (2) a clear conceptual distinction is made between dependent variables (such as pH and $[HCO_3]$) and the 3 independent variables, and (3) the effects of protein and phosphate concentration on acid-base balance are considered.[4,5,9,10] In contrast to the Henderson-Hasselbalch equation, the strong ion approach characterizes 6 primary acid-base disturbances (ie, respiratory acidosis and alkalosis, strong ion acidosis and alkalosis, non volatile buffer ion acidosis and alkalosis), and the unmeasured strong anion concentration is quantified by calculating the strong ion gap (SIG).[8,12,13] The SIG attributes a value to the net anionic charge on serum proteins and phosphate and more accurately quantifies the unmeasured strong anion charge in animals with abnormal serum protein concentrations than does the anion gap.[5,8,14]

The strong ion approach requires species-specific values for the total plasma concentration of nonvolatile weak acids (A_{tot}—ie, the total concentration of plasma nonvolatile buffers—albumin, globulin, and phosphate) and the effective dissociation constant (K_a) for plasma nonvolatile buffers.[9] Values for A_{tot} and K_a have been experimentally determined in the plasma of horses,[9,15] humans,[16] cats,[17] and calves,[b] and theoretically determined for the plasma of humans[18] and adult cattle.[14] Values for A_{tot} and K_a of dog plasma presently are unavailable. All of the studies that have applied the strong ion approach to acid-base disturbances in dogs assumed that values for A_{tot} and the net protein charge were similar in canine plasma to human plasma[19,20] or horse plasma,[21] or assumed that $K_a = 4 \times 10^{-7}$,[22] or $K_a = 3 \times 10^{-7}$.[23] It is highly probable that canine plasma proteins have a greater net negative charge than human and bovine plasma proteins because albumin provides the greatest contribution to net protein charge and because canine albumin has a different amino acid composition[24] and carries a greater net negative charge at physiological pH than do human, bovine, or equine albumin.[25,26] We therefore hypothesized that values for net protein charge, A_{tot}, and K_a of canine plasma differed from those of other species. Accordingly, the objective of the study reported here was to experimentally determine A_{tot} and K_a values for canine plasma; to compare these values to A_{tot} and K_a values for the plasma of horses, calves, cats, and humans; and to calculate the net protein charge in canine plasma.

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Materials and Methods

Blood and Plasma Collection

Twenty milliliters of venous blood was collected into lithium-heparin tubes from the jugular vein of 10 healthy adult dogs (5 female and 5 male). One milliliter of whole venous blood was immediately analyzed, and plasma was harvested from the remaining 19 ml by centrifugation that was completed within 30 min of collection. CO_2 tonometry was performed on all samples within 24 hours.

CO_2 Tonometry of Plasma

Plasma samples were tonometered[c] for 20 minutes at 37°C over a P_{CO_2} range of 10 to 141 mm Hg and a pH range of 6.89 to 7.88 with a mixture of humidified 20% CO_2 and 100% O_2. A total of 142 CO_2 tonometered plasma samples were analyzed, representing 7 to 20 tonometered samples from each dog.

Blood and Plasma Analyses

The jugular venous blood sample was analyzed in duplicate,[d] and all tonometered plasma samples were analyzed[d] once for blood/plasma gas analysis (pH, P_{CO_2}) and determination of $[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Cl^-]$, and [L-lactate$^-$] simultaneously and at 37°C. An untonometered plasma sample was analyzed[e] in duplicate for determination of strong cation (Mg^{2+}) and nonvolatile buffer ion (total protein, albumin, and inorganic phosphate) concentrations.

Calculation of SID

All strong cation (Na^+, K^+, Ca^{2+}, Mg^{2+}) and strong anion (Cl^-, L-lactate) concentrations were assumed to be constant during CO_2 tonometry and an ionic equivalency assigned to those variables not measured with ion selective potentiometry (Mg^{2+}). Accurate measurements of SID are difficult to obtain in plasma because of cumulative measurement error, presence of unknown strong anions,[12,13] and differences in equipment and methodology used to measure strong ion concentrations.[27] Accordingly, SID was initially calculated by 3 methods—$SID_3 = [([Na^+] + [K^+]) - ([Cl^-])]$, $SID_4 = [([Na^+] + [K^+]) - ([Cl^-] + [L\text{-lactate}])]$, $SID_6 = [([Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}]) - ([Cl^-] + [L\text{-lactate}])]$—and a fixed value for SID_3, SID_4, and SID_6 assigned with the mean value for all CO_2 tonometered samples from each dog. A fixed value for SID during in vitro CO_2 tonometry is one of the assumptions of the strong ion approach; SID does not vary over the physiological range of pH because strong ions are fully dissociated at physiological pH.[9,10]

Estimation of A_{tot} and K_a

Measured values for pH and P_{CO_2}; calculated values for SID_3, SID_4, and SID_6; the 6-factor simplified strong ion electroneutrality equation[9]; and the Marquardt nonlinear regression procedure[28,f] were used to simultaneously estimate values for A_{tot} and K_a. This required application of the 6-factor simplified strong ion electroneutrality equation,

$$SID - [HCO_3^-] - [A^-] = 0, \quad (1)$$

in which the net nonvolatile buffer ion concentration in plasma ($[A^-]$) was evaluated. The 6-factor simplified strong ion model[9] was used instead of Stewart's 8-factor strong ion model[10] because reducing the number of variables in a nonlinear regression model leads to more precise estimates[28] and because changes in 2 of the 8 factors in Stewart's electroneutrality equation do not produce changes in pH and are therefore redundant.[9,14,18]

To assist in estimating values for A_{tot} and K_a, Equation 1 was expressed in this form:

$$[HCO_3^-] = S \times P_{CO_2} \times 10^{(pH - pK'_1)} = SID - (A_{tot} \times K_a)/(K_a + 10^{-pH}), \quad (2)$$

with known values for S (0.0307 [mM]/mm Hg)[29] and pK'_1 (6.120 at [NaCl] = 0.16 mM).[30] With the value of 6.120 for pK'_1 we can calculate actual plasma $[HCO_3^-]$ in (mM) at 37°C[31]; likewise, the methods used to calculate SID provide a value in terms of concentration. This means that Equation 2 estimates a value for A_{tot} in terms of concentration (mM).[9] The form of the simplified strong ion electroneutrality equation used in Equation 2 was selected because it provided the narrowest confidence intervals for the estimated values of A_{tot} and K_a. Initial estimates for A_{tot} of 5 to 30 mM in increments of 5 mM and initial estimates for K_a of 0.1×10^{-7} to 3.0×10^{-7} in increments of 0.1×10^{-7} were used for the nonlinear regression procedure.[28] For each nonlinear regression procedure, the accuracy of the estimated values for A_{tot} and K_a were evaluated with the number of iterations required to converge to a solution, calculating the R^2 value, comparison of actual versus predicted values for $[HCO_3]$, and examination of residual plots. A value of $P < .05$ was regarded as significant.

The true value for SID was unknown. Therefore, a 4th nonlinear regression procedure was performed to simultaneously estimate values for A_{tot}, K_a, and SID (called $SID_{estimated}$),[16] with initial estimates for $SID_{estimated}$ of 25 to 50 mEq/L in increments of 5 mEq/L. R^2 values were calculated for the 4 nonlinear regression models (on the basis of SID_3, SID_4, SID_6, $SID_{estimated}$),[28] with the values obtained during CO_2 tonometry of each plasma sample. The calculated A_{tot} values were evaluated as the A_{tot} indexed to the total protein concentration ($A_{tot\text{-}tp}$) and the A_{tot} indexed to the albumin concentration ($A_{tot\text{-}alb}$). Mean (±SD) values for A_{tot}, $A_{tot\text{-}tp}$, $A_{tot\text{-}alb}$, and K_a were determined.

Validation of A_{tot} and K_a Values

In vitro. The estimated A_{tot} and K_a values were applied to data obtained from an in vitro study[32] that examined the change in plasma pH during CO_2 tonometry at 38°C and the addition of the strong acid (HCl) and strong alkali (NaOH). Plasma pH was calculated with the simplified strong ion equation[9]:

$$pH = \log_{10}\Big(2 \times SID \Big/ \Big\{K'_1 \times S \times P_{CO_2} + K_a \times A_{tot} - K_a \times SID + \Big\{\Big(K'_1 \times S \times P_{CO_2} + K_a \times SID + K_a \times A_{tot}\Big)^2 - 4 \times K_a^2 \times SID \times A_{tot}\Big\}^{-1/2}\Big\}\Big). \quad (3)$$

The reported values for P_{CO_2}, base excess, and pH were obtained from Table 1,[32] and the total protein concentration was assumed to equal the median value (59.5 g/L). Emuakpor and his colleagues[32] did not measure SID (estimated value 35.1 mEq/L) but adjusted the base excess value so that base excess = 0 mEq/L when P_{CO_2} and pH equaled 40 mm Hg and 7.40, respectively. Accordingly, the actual values for SID_3, SID_4, SID_6, and $SID_{estimated}$ in this data set were calculated by rearranging Equation 2 to produce this:

$$SID = S \times P_{CO_2} \times 10^{(pH - pK'_1)} + (A_{tot} \times K_a)/(K_a + 10^{-pH}), \quad (4)$$

with known values at 38°C for S (0.0301 [mM]/mm Hg)[29] and pK'_1 (6.111)[30] and estimated values for A_{tot} and K_a. The estimated value for K_a (obtained at 37°C with SID_3, SID_4, SID_6, or $SID_{estimated}$) was corrected to 38°C for use in Equation 4 by applying the van t'Hoff equation.[9]

In vivo. The estimated A_{tot} and K_a values also were applied to data obtained from an in vivo study that examined the change in plasma pH in 9 anesthetized dogs during experimentally induced respiratory acidosis and alkalosis (induced by altering the rate of ventilation or the CO_2 tension in the respired mixture) and strong ion acidosis and alkalosis (induced by intravenous administration of 0.3 M HCl or 0.3 M NaOH, 0.3 M $NaHCO_3$, and 0.6 M $NaHCO_3$).[33] Plasma pH was calculated by Equation 3. The actual values for SID_3, SID_4, SID_6, and $SID_{estimated}$ were calculated from the reported values for sodium, potassium, and chloride concentration as follows: $SID_3 = [Na^+] + [K^+] - [Cl^-]$, $SID_4 = 0.947 \times SID_3$, $SID_6 = 1.051 \times SID_3$, and $SID_{estimated} =$

Table 1. Mean (±SD) biochemical values of plasma obtained from blood samples collected from the jugular vein in 10 dogs.

Variable	Mean	SD
pH	7.40	0.04
P_{CO_2} (mm Hg)	36.9	6.6
Strong ions		
$[Na^+]$ (mM)	150.7	1.4
$[K^+]$ (mM)	4.07	0.20
$[Ca^{2+}]$ (mM)	2.60	0.07
$[Mg^{2+}]$ (mM)	1.63	0.14
$[Cl^-]$ (mM)	113.6	1.3
[L-lactate] (mM)	1.7	0.5
$[SID_3]$[a] (mEq/L)	41.2	2.1
$[SID_4]$[b] (mEq/L)	39.0	1.9
$[SID_6]$[c] (mEq/L)	43.3	1.9
Volatile buffer ions		
$[HCO_3]$ (mM)	22.4	2.8
Nonvolatile buffer ions		
[Total protein] (g/L)	63.7	4.0
[Albumin] (g/L)	37.7	1.9
[Globulin] (g/L)	26.0	4.4
[Phosphate] (mM)	1.42	0.22
Other		
Anion gap[d] (mEq/L)	18.8	2.9

SID, strong ion difference.

[a] $[SID_3] = ([Na^+] + [K^+]) - [Cl]$.

[b] $[SID_4] = ([Na^+] + [K^+]) - ([Cl^-] + [$L-lactate$])$.

[c] $[SID_6] = ([Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}]) - ([Cl^-] + [$L-lactate$])$.

[d] Anion gap $= ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$.

$0.682 \times SID_3$, with the coefficients for SID_4, SID_6, and $SID_{estimated}$ reflecting the ratio of their calculated value to that of SID_3 obtained in the study reported here (Table 1). A_{tot} was calculated from the reported values for total protein concentration.

For both the in vitro and in vivo data sets, the calculated pH value (pH_{calc}) was regressed against the measured pH value (pH_{meas}) with a statistical software package.[g] The accuracy of the 4 linear regression equations was evaluated with the R^2 value and examination of residual plots. A value of $P < .05$ was regarded as significant.

Comparison of A_{tot} and K_a Values

The estimated values for A_{tot} and K_a in plasma of dogs were compared with those obtained for plasma of calves,[b] horses,[9,15] humans,[16] and cats[17] by means of an unpaired *t*-test.[h] Because 5 multiple comparisons were performed, the *P* value for significance was adjusted for the number of comparisons by the Bonferroni procedure and considering $P < .01$ as significant.

Sensitivity of Plasma pH to Changes in SID, P_{CO_2}, and A_{tot}

Sensitivity of the dependent variable (plasma pH) to the 3 independent factors SID, P_{CO_2}, and A_{tot} of the simplified strong ion model was conveyed by a spider plot,[18,34] which graphically depicted the relationship between the dependent variable and percentage change in 1 independent factor while the remaining 2 independent factors were held constant at typical values. The spider plot was created with Equation 3 and typical values for the plasma of healthy dogs (Table 1), known values at 37°C for $S = 0.0307$ [mM]/mm Hg[29] and $pK_1' = 6.120$,[30] and the estimated values for A_{tot} and K_a.

By use of the simplified strong ion model, the derivatives of pH with respect to the 3 independent factors (SID, P_{CO_2}, A_{tot}) then were calculated as described[18] to provide an index of the sensitivity of plasma pH to changes in each of the independent factors at physiological pH.

Sensitivity of Plasma pH to Changes in Plasma Total Protein Concentration

Because changes in serum total protein and phosphate concentration will change pH by altering both A_{tot} and SID,[16] the effect of change in plasma protein concentration on acid-base balance was characterized by considering the separate contribution of changes in protein concentration to changes in SID and A_{tot}, and then calculating the net result of these changes on pH.

Buffer Value of Plasma

The buffer value represents the amount of acid or alkali required to alter the acidity of the solution by 1 pH unit.[35] In plasma, a plot of HCO_3 versus pH over a pH range of 7.20 to 7.50 approximates a straight line, thereby permitting calculation of the buffer value (β, in units of [mEq/L]/pH unit) from the HCO_3 − pH relationship,[36] whereby

$$\beta = -\Delta HCO_3/\Delta pH. \quad (5)$$

We can calculate a value for β at a specific pH from the estimated values for A_{tot} and pK_a by differentiating Equation 2 with respect to pH, which produces this solution:

$$-dHCO_3/dpH = \beta = 2.303 \times A_{tot}/(10^{pH-pK_a} + 10^{pK_a-pH})^2. \quad (6)$$

Equation 6 therefore was solved for the typical pH of dog plasma (7.40; Table 1) with the estimated values for A_{tot} and pK_a. The calculated value for β then was compared to published values for canine plasma: 0.068 mmol/g total protein[37]; 0.093 mmol/g total protein (95% confidence interval, 0.079 to 0.108 mEq/g total protein)[35]; and 0.124 mmol/g total protein.[38]

Results

Blood and Plasma Analyses

The values for jugular venous blood from 10 dogs are presented in Table 1.

Calculation of SID

Small differences in the mean value for SID_3 (41.2 mEq/L), SID_4 (39.0 mEq/L), and SID_6 (43.3 mEq/L) were obtained, whereas a markedly lower value for $SID_{estimated}$ (28.1 mEq/L) was obtained (Table 2).

Estimation of A_{tot} and K_a

The R^2 value for all nonlinear regression models was >.98, indicating excellent fit to the data. The estimated values for A_{tot} were similar, with slight variations occurring depending on the value assigned to SID (Table 2). In contrast, the estimated value for K_a obtained with $SID_{estimated}$ was much lower than that determined with SID_3, SID_4, and SID_6.

Validation of A_{tot} and K_a Values

In vitro. The estimated A_{tot} and K_a values were applied to data obtained from an in vitro study involving plasma

Table 2. Summary of mean (±SD) estimated values for SID K_a, A_{tot}, $A_{tot\text{-}alb}$, and $A_{tot\text{-}tp}$ for 10 dogs. SID, strong ion difference, which is the difference in charge between plasma strong cations and anions; K_a, effective dissociation constant; A_{tot}, total plasma concentration of nonvolatile weak acids; $A_{tot\text{-}alb}$, A_{tot} indexed to the albumin concentration; $A_{tot\text{-}tp}$, A_{tot} indexed to the total protein concentration; $SID_{estimated}$, a nonlinear regression procedure was used to simultaneously estimate values for A_{tot}, K_a, and SID. See Table 1 for remainder of key.

Measurement Method	SID (mEq/L)	K_a ($\times 10^{-7}$)	A_{tot} (mM)	$A_{tot\text{-}alb}$ (mmol/g)	$A_{tot\text{-}tp}$ (mmol/g)
SID_3	41.2 ± 2.1	4.84 ± 2.82	19.6 ± 3.3	0.527 ± 0.091	0.306 ± 0.046
SID_4	39.0 ± 1.9	4.17 ± 2.45	17.5 ± 2.9	0.468 ± 0.079	0.272 ± 0.044
SID_6	43.3 ± 1.9	7.83 ± 5.88	21.2 ± 2.9	0.568 ± 0.080	0.330 ± 0.044
$SID_{estimated}$	28.1 ± 2.8	0.17 ± 0.11	17.4 ± 8.6	0.469 ± 0.239	0.273 ± 0.143

from 5 dogs. Calculated values for SID_3, SID_4, SID_6, and $SID_{estimated}$ of 40.3, 38.2, 42.1, and 28.3 mEq/L were obtained, respectively, when base excess = 0 mEq/L, P_{CO_2} = 40 mm Hg, and pH = 7.40 at 38°C. These values were similar to those obtained in the study reported here (Table 1). All 4 pairs of A_{tot} (Table 2) and temperature-adjusted K_a values produced a strong linear relationship between calculated pH and measured pH, with $R^2 > .9990$ for all regression analyses (data not shown). However, on the basis of the values for sum of squares and mean difference between calculated and measured plasma pH, the best fitting A_{tot} and K_a values were derived from $SID_{estimated}$ (mean difference in pH = 0.0009 ± 0.0067; Fig 1).

In vivo. The estimated A_{tot} and K_a values were applied to data obtained from an in vivo study involving 9 dogs. All 4 pairs of A_{tot} and K_a values (Table 2) produced a good linear relationship between calculated pH and measured pH (R^2 ranging from .62 to .73, data not shown). However, on the basis of the values for R^2, sum of squares, and mean difference between calculated and measured plasma pH, the best fitting A_{tot} and K_a values were derived from $SID_{estimated}$ (Fig 1).

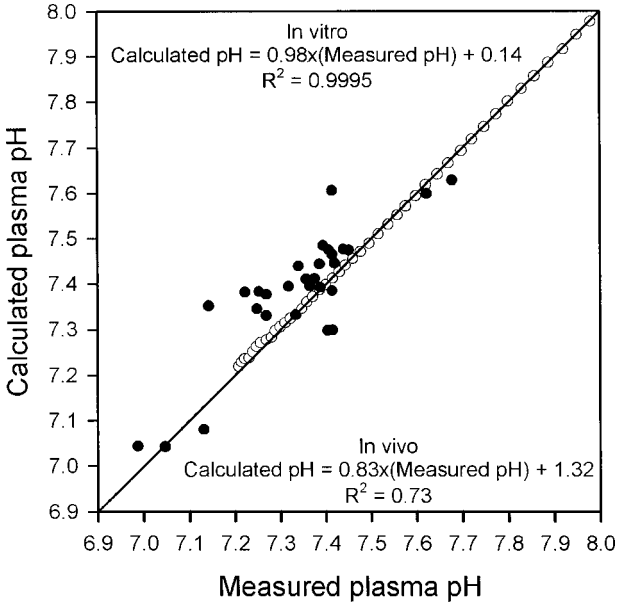

Fig 1. Relationship between calculated and measured pH. In vitro data (open circles) were obtained from a study by Emuakpor et al,[32] who measured the change in plasma pH during CO_2 tonometry and the addition of the strong acid (HCl) and strong alkali (NaOH). In vivo data (closed circles) were obtained form a study by Siggaard-Andersen,[33] who measured the change in plasma pH in 9 anesthetized dogs during experimentally induced respiratory acidosis and alkalosis (induced by altering the rate of ventilation or the CO_2 tension in the respired mixture) and strong ion acidosis and alkalosis (induced by intravenous administration of 0.3 M HCl or 0.3 M NaOH, 0.3 M $NaHCO_3$, and 0.6 M $NaHCO_3$). Plasma pH was calculated with the simplified strong ion equation.[9] The solid line is the line of identity.

Comparison of A_{tot} and K_a Values

The A_{tot} values of canine plasma were similar to those for cat, calf, human, and equine plasma (Table 3). In contrast, the K_a values of canine plasma were different ($P < .001$) from those for cat, calf, human, and horse plasma.

Sensitivity of Plasma pH to Changes in SID, P_{CO_2}, and A_{tot}

Analysis of the spider plots revealed that plasma pH was most sensitive to changes in SID (Fig 2). The tangent to each line in the spider plot reflects the sensitivity of dog plasma pH to that factor, such that at the reference value for pH in samples obtained from the jugular vein (ie, pH = 7.40), $d\text{pH}/d\text{SID} = +0.018$ $(\text{mEq/L})^{-1}$, $d\text{pH}/dP_{CO_2} = -0.010$ mm Hg^{-1}, and $d\text{pH}/dA_{tot} = -0.005$ $(\text{mM})^{-1}$. This result indicated that at a pH of 7.40, a 1 mEq/L decrease in SID will decrease pH by 0.018, and a 1 mm Hg decrease in P_{CO_2} will increase pH by 0.010.

Sensitivity of Plasma pH to Changes in Plasma Total Protein Concentration

Because SID_3 approximated the difference in strong cation and anion charge (excluding the net strong anion charge of protein and phosphate), the estimated strong ion charge of protein and phosphate = 41.2 − 28.1 (from Table 2) = 13.1 mEq/L. A phosphate concentration of 1.4 mM (Table 1) provides a strong anion charge of phosphate (HPO_4^-) of 1.4 mEq/L. Because the net strong ion charge of protein and phosphate = 13.1 mEq/L, the net strong ion charge or protein alone = 11.7 mEq/L (13.1 − 1.4 mEq/L), or 0.184 mEq/g total protein (on the basis of a total protein concentration of 63.7 g/L; Table 1).

A 10 g/L increase in total protein concentration will therefore decrease SID by 1.84 mEq/L, which will directly decrease pH by 0.033. A 10 g/L increase in total protein concentration will also increase A_{tot} by 2.73 mmol; which will directly decrease pH by 0.014. The overall effect of a

Table 3. Mean (±SD) values for A_{tot}, $A_{tot\text{-}alb}$, $A_{tot\text{-}tp}$, and K_a of plasma from different species. A_{tot}, total plasma concentration of nonvolatile weak acids; $A_{tot\text{-}alb}$, A_{tot} indexed to the plasma albumin concentration; $A_{tot\text{-}tp}$, A_{tot} indexed to the plasma total protein concentration; K_a, effective dissociation constant for plasma nonvolatile weak acids. Values for A_{tot} and K_a were determined using similar methodology (in vitro CO_2 titration) for all species.

Species	n	A_{tot} (mM)	Albumin (g/l)	$A_{tot\text{-}alb}$ (mM)	Total Protein (g/l)	$A_{tot\text{-}tp}$ (mM)	K_a ($\times 10^{-7}$)	Reference
Horse	6	15.0 ± 3.1	32	0.47	67	0.22	2.22 ± 0.32*	Constable 1997[9]
Horse	10	14.9 ± 0.8	34	0.44	71	0.21	2.11 ± 0.50*	Stämpfli et al 1999[15]
Calves	9	23.1 ± 6.1	31	0.75	57	0.41	0.85 ± 0.32*	Constable et al 2004[b]
Human	8	17.2 ± 3.5	46	0.38	77	0.22	0.80 ± 0.60*	Stämpfli and Constable 2003[16]
Cat	10	24.3 ± 4.6	33	0.76	70	0.35	0.67 ± 0.40*	McCullough and Constable 2003[17]
Dog	10	17.4 ± 8.6	38	0.47	64	0.27	0.17 ± 0.11	This study

* Value significantly different ($P < .001$) from value in plasma of dogs.

10 g/L increase in total protein concentration therefore is a decrease in pH of 0.047 (0.033 + 0.014).

Buffer Value of Plasma

The buffer value (β) of canine plasma was calculated at pH = 7.40 with Equation 6 and estimated values for A_{tot} and pK_a, producing a buffer value of 0.082 mmol/g total protein. This value was within the range of the 3 published estimates (0.068 to 0.124 mmol/g total protein) for canine plasma.

Net Protein Charge

Net protein charge has 2 components: nonvolatile buffer ion charge and SID charge. The value for $[A^-]$ at pH = 7.40 reflects the net negative buffer ion charge of protein and phosphate; $[A^-] = A_{tot}/(1 + 10^{pK_a - pH}) = 17.4$ mM/$(1 + 10^{7.77-7.40}) = 5.2$ mEq/L (assuming HA is uncharged and A^- has a valence of −1). Because the net buffer ion charge of phosphate at pH = 7.40 approximates 1.1 mEq/L (0.8 × [phosphate]; Table 1), the net buffer ion charge of protein was 5.2 − 1.1 = 4.1 mEq/L. The net strong ion charge of protein = 11.7 mEq/L (calculated previously). The net protein charge at pH = 7.40 is therefore 15.8 mEq/L (4.1 + 11.7 mEq/L), equivalent to 0.25 mEq/g of total protein or 0.42 mEq/g of albumin.

Discussion

Use of the strong ion approach to evaluate acid-base status requires species-specific values for A_{tot} and K_a. In this study, we experimentally determined mean values for A_{tot} (0.273 mmol/g of total protein or 0.469 mmol/g of albumin) and K_a (0.17×10^{-7}) in plasma of dogs. The finding that the K_a values for dog and human plasma are different is consistent with observations that the pH-P_{CO_2} coordinates for the base excess curve of dog plasma[32] differ from those for human plasma.[39]

We calculated the net protein charge of canine plasma at pH = 7.40 to be 16.0 mEq/L. We also calculated the anion gap (AG) of canine plasma at pH = 7.40 to be 18.8 mEq/L; this value was similar to mean estimates obtained previously from large numbers of dogs (AG = 18.3 mEq/L, n = 80; AG = 19 mEq/L, n = 117).[40,41] The results therefore

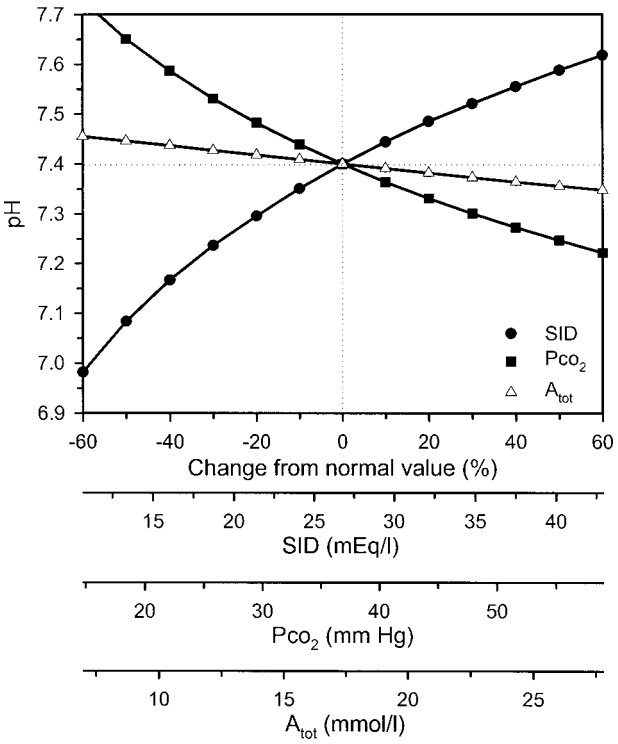

Fig 2. Spider plot revealing the dependence of plasma pH on changes in the 3 independent variables (strong ion difference, SID; P_{CO_2}, and total concentration of nonvolatile weak acids, A_{tot}). The spider plot was obtained by systematically varying 1 independent variable while holding the other 2 independent variables at their reference values for dog plasma. Reference values for the 3 independent variables were as follows: SID, 27 mEq/L (large open circle); P_{CO_2}, 37 mm Hg (open square); A_{tot}, 17.4 mM (open triangle). The dotted lines indicate that pH = 7.40 when SID, P_{CO_2}, and A_{tot} are at their reference values.

clearly indicate that the AG in healthy dogs essentially reflects the plasma protein concentration. Changes in the AG in diseased dogs may therefore result from changes in the plasma protein concentration or changes in unmeasured strong cation and anion concentrations. In other words, the AG is a nonspecific method for quantifying the unmeasured strong ion concentration in canine plasma.

The AG in dog plasma is assumed to change by 0.24 mEq/L for every 1 g/L change serum albumin concentration,[42] although this estimate approximates the net charge of human albumin (0.25 mEq/g)[43] and may not be valid for canine albumin. Suspicion has been raised previously that the net negative charge of dog plasma proteins was much greater than that of human plasma proteins.[44] An important finding of this study is that the AG of canine plasma at pH = 7.40 changes 0.42 mEq/L for every 1 g/L change in plasma albumin concentration and 0.25 mEq/L for every 1 g/L change in plasma total protein concentration; the latter value agrees completely with the estimated charge carried by canine plasma protein (0.25 mEq/g total protein) obtained by Dill and his colleagues in 1932.[35] Changes in the plasma concentrations of albumin and total protein therefore have a much greater effect on the AG in dogs than previously assumed.

A clinically useful method for adjusting the AG value in dog plasma for changes in the albumin or total protein concentration is calculation of an albumin-adjusted or total protein–adjusted AG,[43] whereby

$$\text{Albumin-adjusted AG} = \text{AG} + 0.42 \times (37.7 - [\text{albumin}]), \quad (7)$$

$$\text{Total protein–adjusted AG} = \text{AG} + 0.25 \times (63.7 - [\text{total protein}]), \quad (8)$$

where the albumin and total protein concentrations are measured in g/L and are subtracted from reference mean values for serum albumin concentration (37.7 g/L) and serum total protein concentration (63.7 g/L, Table 1). Equations 7 and 8 are formulated to provide the normal reference range when albumin or total protein concentrations are normal and therefore are familiar to the clinician.[43]

Alternatively, an albumin (or total protein) and phosphate adjusted AG can be calculated as follows:

$$\text{Albumin and phosphate-adjusted AG} = \text{AG} + 0.42 \times (37.7 - [\text{albumin}]) + 1.8 \times (1.4 - [\text{phosphate}]), \quad (9)$$

$$\text{Total protein and phosphate-adjusted AG} = \text{AG} + 0.25 \times (63.7 - [\text{total protein}]) + 1.8 \times (1.4 - [\text{phosphate}]), \quad (10)$$

where the phosphate concentration is measured in mM, the normal phosphate concentration is 1.4 mM (Table 1), and the net negative charge of phosphate at pH = 7.40 is assumed to be 1.8.[9] Equations 7, 8, 9, and 10 can be routinely programmed into automated clinical biochemistry analyzers that measure serum sodium, potassium, chloride, albumin, total protein, phosphate, and total CO_2 concentrations. An adjusted AG above the normal reference range provides a more specific indicator of the presence of unmeasured strong anions than does the traditional AG.

A useful clinical application of strong ion difference theory is calculation of the SIG instead of the AG. The SIG provides an estimate for the difference between the unmeasured strong anion charge (due to L-lactate, D-lactate, sulfate, nonesterified fatty acids, ketoacids, pH-independent protein and phosphate charge, and other strong anions) and unmeasured strong cation charge (Ca^{2+}, Mg^{2+}). Total protein or albumin can be used to calculate the SIG; however, because albumin is the most important buffer in the plasma of humans (exerting 73% of total buffering, compared with 22% for globulin and 5% for phosphate),[45] albumin may provide the best index, assuming albumin is the most important buffer in dog plasma. On the basis of the estimated values for A_{tot} and K_a, the equations below for calculating SIG (in mEq/L) in the plasma of dogs are proposed:

$$\text{SIG} = [\text{albumin}] \times (0.348 + 0.469/\{1 + 10^{[7.77 - \text{pH}]}\}) - \text{AG}, \quad (11)$$

$$\text{SIG} = [\text{total protein}] \times (0.206 + 0.273/\{1 + 10^{[7.77 - \text{pH}]}\}) - \text{AG}, \quad (12)$$

where [albumin] and [total protein] are in g/L and the AG (in mEq/L) = $([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$. Total CO_2 could be substituted for $[HCO_3^-]$ and pH could be assumed to equal 7.40 when blood gas analysis is not available. This will create an error in SIG of up to 3.0 mEq/L (for instance, SIG is overestimated by 2.7 mEq/L when pH is 7.00 instead of 7.40). The error in using a simplified formula for calculating SIG results from the effect of pH on the net anionic charge of dissociable amino-acid groups (predominantly histidine) in plasma proteins and the effect of pH on dissociation of monobasic phosphate ($H_2PO_4^-$) to dibasic phosphate (HPO_4^{2-}). However, if pH is assumed to be 7.40, Equations 11 and 12 can be simplified to these equations:

$$\text{SIG} = [\text{albumin}] \times 0.49 - \text{AG}, \quad (13)$$

$$\text{SIG} = [\text{total protein}] \times 0.29 - \text{AG}. \quad (14)$$

If we apply the reference values for jugular venous plasma of healthy dogs (pH = 7.40, [albumin] = 37.7 g/L, [total protein] = 63.7 g/L, anion gap = 18.8 mEq/L) and Equations 11, 12, 13, or 14, the SIG is approximately 0 mEq/L with either the albumin or total protein concentration, with the 95% confidence interval for SIG being −5.0 to 5.4 mEq/L for albumin and −6.0 to 6.3 mEq/L for total protein. With Equation 11, a SIG >5 mEq/L therefore indicates an increase in unidentified strong cations (a rare event), whereas a SIG <−5 mEq/L indicates an increase in unidentified strong anions, such as L-lactate. Calculating the SIG with Equation 11 has the advantage over calculating the AG with Equation 10 in that the SIG equation accounts for the effect of changes in pH, whereas the AG has been shown to decrease as plasma pH decreases in dogs.[40] Finally, Equations 11 and 12 can also be applied to blood gas and serum biochemistry values of healthy calves as an adjunct quality control for assessing measurement accuracy.[i] In this case, the 95% confidence interval for the mean SIG value should include 0.

In conclusion, application of the experimentally determined values for A_{tot}, K_a, and net protein charge of canine plasma should improve understanding of the mechanism for complex acid-base disturbances in critically ill dogs.

Footnotes

[a] Constable PD, Stämpfli HR. Clinical assessment of acid-base status in dogs: Calculation of plasma A_{tot} and K_a values for use in the strong ion and simplified strong ion models. J Vet Intern Med 2004;18:391 (abstract 24)

[b] Constable PD, Stämpfli HR. Using the simplified strong ion approach to determine the mechanism for an acid-base disturbance in calves. Med Vet Québec 2004;34:105 (abstract 026)

[c] Model IL235, Instrumentation Laboratory, Lexington, MA

[d] Statprofile 9+, Nova Biomedical, Canada Ltd, Mississauga, Ontario, Canada

[e] Dacos multi-analyzer, Coulter Electronics, Hialeah, FL

[f] PROC NLIN, SAS 8e, SAS Institute, Cary, NC

[g] PROC REG, SAS 8e, SAS Institute, Cary, NC

[h] PROC TTEST, SAS 8e, SAS Institute, Cary, NC

[i] Stämpfli HR, Stevenson AJ, Brooks L, et al. The use of physicochemical calculations for additional quality control of automated multi-analyzer and blood gas measuring systems. Proceedings XI Congress International Society of Animal Clinical Biochemistry. Chile 2004;9:66.

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