Serum proteins and acid-base equilibria: a follow-up

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A mathematic model that described the acid-base behavior of blood plasma has been revised to incorporate pK values of individual histidine residues on human serum albumin determined by nuclear magnetic resonance spectroscopy. With the insights derived from the model a method for evaluation of the strong ion difference has been developed. Thus if pH, Pco_2, and the concentrations of serum albumin and phosphate are measured, all independent variables, which physically determine "acid-base balance" in plasma, can be quantified. New ways to evaluate "unidentified anions" in metabolic acidosis can be explored with this approach. (J Lab Clin Med 1992;120:713-9)

Abbreviations AG = anion gap; [Alb] = concentration of serum albumin (g/dl); [Alb^{x-}] = concentration of serum albumin (mEq/L); BB_{p1} = buffer base in plasma; NMR = nuclear magnetic resonance; Pi = inorganic phosphate; [Pi_{tot}] = total concentration of inorganic phosphorus-containing species (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} [mmol/L]); [Pi^{y-}] = concentration of inorganic phosphorus-containing species (mEq/L); pHc = calculated pH; pHm = measured pH; Pr^- = negative electric charges on a protein molecule; SID = strong ion difference; SID_{app} = apparent SID; SID_e = effective SID; [XA] = concentration of unidentified anions (mEq/L)

Extending the work of Stewart,[1] we recently developed a mathematic model that accurately described acid-base states in artificial solutions resembling plasma, quantified the decisive role of serum albumin in acid-base equilibria, and showed that globulins have a negligible role in this respect.[2] The model did not use available data[3] on pK values of individual histidine residues in human serum albumin.[4] The contribution of histidine to the acid-base behavior of albumin is indeed decisive over the range of pH values of biologic interest.[5] Accordingly, we have revised the model to incorporate that information.

The model. The acid-base state in biologic fluids is determined by several independent variables[1,2]: Pco_2, SID {SID = Σ (all strong cations) − Σ (all strong anions)}, and the [Alb] and [Pi_{tot}]. Our model is a mathematic function that relates pH, a dependent variable, to the values of the independent variables:

$$pH = f_{pH}\{Pco_2, SID, [Alb], [Pi_{tot}]\} \quad (1)$$

The contribution of a protein to the chemical equilibria in plasma depends on the number of dissociating groups on the macromolecule (e.g., histidine residues on serum albumin) and on the pK of each such group. The function f_{pH} treats albumin as a complex polyprotic acid, with multiple dissociating groups defined according to the known amino acid composition of the molecule.[6,7] In the original model,[2] all 16 histidine residues in serum albumin were characterized by a least-squares optimization as having effective pK values in the range of 7.2 to 7.3. However, in a macromolecule, the pK of a dissociable group is affected by the surrounding microenvironment. Microenvironmental pK values measured by NMR spectroscopy are available for 13 of the 16 histidine residues on human serum albumin.[3] We incorporated these in the revised model, and determined values for the remaining three histidine residues by a least-squares optimization

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Table I. Equilibrium constants

Ion product for water	K'w = 4.4E-14 (Eq/L)^2
Carbonic acid system	Kc1 = 2.46E-11 (Eq/L)^2/torr
	Kc2 = 6.0E-11 (Eq/L)
Phosphoric acid system	K1 = 1.22E-2 (mol/L)
	K2 = 2.19E-7 (mol/L)
	K3 = 1.66E-12 (mol/L)

All values apply to blood plasma at 37° C and are identical to the values used in Figge et al.[2] *Kc1* and *Kc2* are equilibrium constants governing the carbonic acid–bicarbonate-carbonate system. *K1* through *K3* are the equilibrium constants for the phosphoric acid-$H_2PO_4^-$-HPO_4^{2-}-PO_4^{3-} system.

method using our data on artificial albumin–containing solutions.[2]

We also used the revised model to derive formulas for characterization of the various "metabolic" acid-base disturbances and for estimation of unmeasured anions ([XA]), taking into account the acid-base effects of primary changes of [Alb]. This approach can be of help in evaluating complex acid-base disturbances, in which the traditional AG is often unreliable.[8,9]

METHODS

We followed the general approach described for our original model,[2] employing the same two sets of experimental data used in that study. First, the new model was fitted to data from artificial solutions simulating acid-base disturbances in plasma, but with human serum albumin as the only protein. We then applied the new model to the second data base, on artificial solutions containing all proteins of normal human sera, with values of SID, $[Pi_{tot}]$, [Alb], and Pco_2 imposed (by repeated ultrafiltration and tonometry) to simulate complex acid-base disturbances.

Table I shows equilibrium constants for water, and the bicarbonate and phosphate systems. The pK values for the titratable residues on albumin are shown in Table II. Microenvironmental pK values for 13 of the 16 histidine residues in human albumin were originally determined by NMR spectroscopy at 25° C.[3] We have applied an approximate temperature correction factor to adjust these pK values to 37° C; the temperature-corrected values are shown in Table II. To derive the approximate temperature correction factor, we used 4-methyl-imidazole as a model compound since it is substituted at the same position as the imidazole ring in the histidyl residue.[10] From the data of Nozaki et al.,[10] the temperature correction coefficient (dpK/dt) for 4-methyl-imidazole at an ionic strength of 0.16 is −0.0226 per degree Celcius. Thus we applied a correction factor of (37 − 25) × −0.0226 = −0.27 to adjust the histidine pK values from 25° C to 37° C. The remaining three histidine pK values that could not be determined by NMR were estimated empirically, as follows.

For two of these 3 pK values boundaries were determined by NMR[3]: one pK was between 7 and 8, another was <5.5. Accordingly, after applying the above-noted temperature correction factor, we set up the following search ranges to optimize the values of these two unknown histidine pK values:

$$6.7 \leq N1 \leq 7.7 \quad (2)$$

$$4.8 \leq N2 \leq 5.2 \quad (3)$$

No data were available on the pK of the third histidine residue. It was optimized within the arbitrary range of 4.8 to 7.7:

$$4.8 \leq N3 \leq 7.7 \quad (4)$$

A computer program (see Appendix A) explored each of these search intervals at 0.1 pK unit increments to find the values that optimized the least-squares fit of the calculated pH values with those measured in our artificial albumin–containing solutions.[2]

The pK values for tyrosine, aspartic acid, and glutamic acid were taken from the data of Tanford,[11] based on his titration of human serum albumin. For cysteine, and the amino and carboxy termini, pK values standard for globular proteins were applied.[12]

Each titratable lysine and arginine residue was assigned either a "low" pK value (pK = 9.4) or a "high" pK value (pK = 11). The total number of titratable lysine and arginine residues with a low pK value (N4) was optimized as follows:

$$0 \leq N4 \leq 83 \quad (5)$$

The total number of titratable lysine and arginine residues with a high pK value (N5) was optimized within the search range of:

$$0 \leq N5 \leq 83 \quad (6)$$

The sum of low- and high-titrating lysine and arginine residues (N4 + N5) was constrained as follows:

$$75 \leq (N4 + N5) \leq 83 \quad (7)$$

The maximum number for all titratable groups conforms to the known amino acid composition of human serum albumin.[6,7] Equations 5 to 7 allow for the possibility that not all arginine and lysine residues are titratable at pH values of biologic interest; some might be buried within the interior of the protein. A reasonable lower limit for the number of these titratable groups was empirically found to be 75, as shown in equation 7.

The mathematic model was optimized to fit our experimental data on albumin solutions. Two criteria defined a successful fit. First, the absolute value of the sum of differences of calculated pH (pHc) values and measured pH values (pHm) had to be near zero:

$$|\Sigma\ (pHc - pHm)| \leq 0.05 \quad (8)$$

Second, the computer selected from all mathematic solutions satisfying the above criterion the single one that minimized the value of S^2, the sum of the squares of the differences between pHm and pHc:

$$S^2 = \Sigma\ (pHc - pHm)^2 \quad (9)$$

Table II. Ionizable groups in human serum albumin

Residue	Number present*	pK (K, Eq/L)	Reference
Cysteine	1	8.50 (3.1623E-09)	12
Aspartic acid + glutamic acid	98	4.00 (1.0000E-04)	11
Tyrosine	18	9.60 (2.5119E-10)	11
Arginine + Lysine			
Low pK	N4	9.40 (3.9811E-10)	2; see text
High pK	N5	11.00 (1.0000E-11)	2; see text
Histidine #1	1	7.12 (7.5858E-08)†	3
#2	1	7.22 (6.0256E-08)†	3
#3	1	7.10 (7.9433E-08)†	3
#4	1	7.49 (3.2359E-08)†	3
#5	1	7.01 (9.7724E-08)†	3
#6	1	7.31 (4.8978E-08)†	3
#7	1	6.75 (1.7783E-07)†	3
#8	1	6.36 (4.3652E-07)†	3
#9	1	4.85 (1.4125E-05)†	3
#10	1	5.76 (1.7378E-06)†	3
#11	1	6.17 (6.7608E-07)†	3
#12	1	6.73 (1.8621E-07)†	3
#13	1	5.82 (1.5136E-06)†	3
#14	1	N1	See text
#15	1	N2	See text
#16	1	N3	See text
Amino terminus	1	8.00 (1.0000E-08)	12
Carboxy terminus	1	3.10 (7.9433E-04)	12

*From Takahashi et al.[6] and Meloun et al.[7] N1 through N5 are parameters to be optimized by the computer program (see Appendix A).
†Temperature corrected to 37° C by subtracting 0.27 pK units from the values given by Bos et al.,[3] as described in Methods.

The computer program incorporating the above considerations and using a least-squares procedure[2] was run to optimize N1 to N5. Details of the program are in Appendix A.

RESULTS

Human serum albumin solutions. The pH values calculated with the new model (pHc) are in good agreement with the values measured (pHm) in the 65 samples of human albumin solutions (Fig. 1, *A*). A least-squares linear regression of pHc *(y)* versus pHm *(x)* is described by the equation $y = 1.01\ x - 0.083$ ($r = 0.99$), which is statistically not distinguishable from the line of identity shown in the graph; the mean of the differences (pHc − pHm) = +0.00039 (±0.034, SD) is not statistically different from zero ($t = 0.093$, $0.90 < p < 0.95$, $d.f. = 63$).

The negative electric charges contributed by albumin, calculated with the mathematic model ($[Pr^{-}c_{alb}] = y$) agree well with those experimentally determined ($[Pr^{-}m_{alb}] = x$) (where $Pr^{-}c_{alb}$ = calculated value of Pr^{-} for albumin); $Pr^{-}m_{alb}$ = measured value of Pr^{-} for albumin). The plot fits the straight line $y = 0.90x + 1.46$ ($r = 0.94$), which is statistically not distinguishable from the line of identity shown in the graph (Fig. 1, *B*); the mean difference ($[Pr^{-}c_{alb}] - [Pr^{-}m_{alb}]$) = 0.063 mEq/L (±2.16, SD) is statistically not different from zero ($t = 0.24$, $0.80 < p < 0.90$).

Solutions with human serum proteins. The new model also gave accurate predictions of pH in solutions containing all proteins present in normal human sera (i.e., both albumin and globulins), which is in keeping with our previous results.[2] The plot of pHc *(y)* against pHm *(x)* yielded a best-fit line $y = 1.08x - 0.60$ ($r = 0.99$), which is statistically not distinguishable from the line of identity (Fig. 1, *C*); the mean of the differences (pHc − pHm) = −0.0024 (±0.033 SD) is not statistically different from zero ($t = 0.63$, $0.50 < p < 0.60$, $d.f. = 70$).

A plot of negative charges contributed by the proteins, calculated with the supposition that albumin was the only reacting moiety ($[Pr^{-}c_{alb}] = y$) against the values measured in the solutions with serum proteins ($[Pr^{-}m_{serum}] = x$) fits the straight line $y = 0.77x + 2.72$ ($r = 0.87$), with a good fit around the line of identity (Fig. 1, *D*); the mean of the differences ($Pr^{-}c_{alb} - Pr^{-}m_{serum}$) = −0.17 mEq/L (±2.20 SD) is statistically not different from zero ($t = 0.66$, $0.5 < p < 0.6$).

Titration curve of human serum albumin. Fig. 2 shows the titration curve for human serum albumin at 37° C calculated with the new model, the charge on albumin

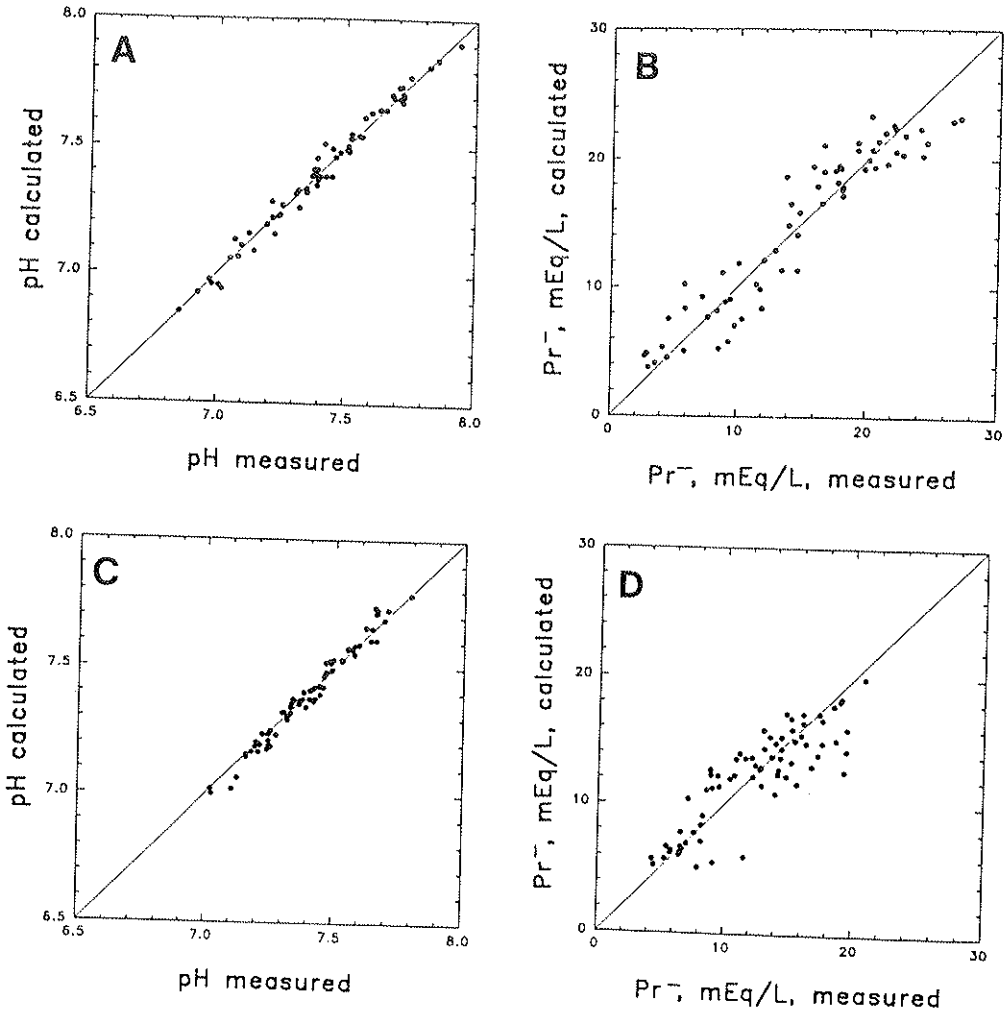

Fig. 1. A and **B**, Albumin solutions (n = 65). pH and Pr^- contributed by albumin were calculated with the mathematic model (see Appendix A) and plotted against those measured in the solutions. **C** and **D**, Filtrands of human sera (n = 72). pH and Pr^- contributed by the serum proteins were calculated with the mathematic model and plotted against those measured in the filtrands. Lines of identity are shown in all panels.

(mEq/gm) plotted as a function of pH. As with the previous mathematic model,[2] there is good agreement with the data of Tanford[11] and van Slyke et al.[13] As noted previously,[2] the data of Tanford and van Slyke et al. were obtained in solutions without the divalent cations or phosphate; P_{CO_2} was ambient (<1 torr) in all Tanford's samples and in some samples of van Slyke et al.

DISCUSSION

Our published mathematic model that successfully described the acid-base behavior of blood plasma[2] has been criticized[4] for not incorporating all the information from an NMR study on pK values of histidine residues in human serum albumin.[3] The revised model (see Appendix A for details) incorporates the information derived from the NMR study on the effect of the microenvironments within the macromolecule of albumin on the pK values of the histidine residues. As with the previous model, the predictions of the acid-base equilibria in plasma are in satisfactory agreement with the experimental data. Our current results also agree with the previous observation that the globulins contribute negligibly to the acid-base equilibria in plasma, over the pH range of biologic interest.

Calculation of SID from routinely measured quantities. All acid-base disturbances in plasma can be viewed as arising from a perturbation of SID, P_{CO_2}, or the concentrations of the nonvolatile weak acids ([Alb], $[Pi_{tot}]$), or any combination of these four independent variables.[1,2] For evaluation of the acid-base status, it is therefore desirable to quantify all the independent variables. For this purpose, P_{CO_2}, [Alb], and $[Pi_{tot}]$ can be readily measured; conversely, SID cannot.

From the routinely measured serum electrolytes an "apparent SID" (SID_{app}) can be calculated:

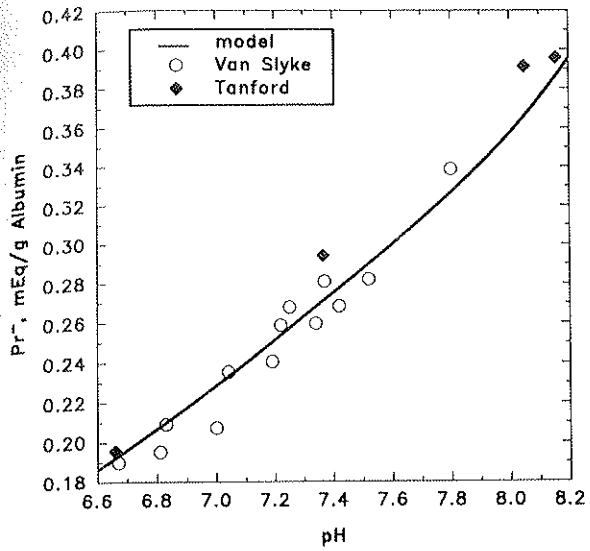

Fig. 2. Titration curve of human serum albumin at 37° C, over the range of pH values of biologic relevance, as predicted by the mathematic model (see Appendix A) for the system containing Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, Pi, SO_4^{2-}, and P_{CO_2} varying between 21.9 and 91.2 torr, with ionic strength ~0.15. Experimental data cover the range of pH 6.85 to 7.94; beyond these limits the curve is an extrapolation. *Open circles* (○) are data points taken from van Slyke et al.,[13] who titrated horse serum albumin at 38° C in solutions with only Na^+ and Cl^- as strong ions, with P_{CO_2} varying from <1 torr to 128.8 torr. *Diamonds* (◆) are data points taken from Tanford,[11] who titrated human serum albumin at 37.8° C with ionic strength 0.150, the solution containing only Na^+ and Cl^- as strong ions, and P_{CO_2} < 1 torr.

$$SID_{app} = [Na^+] + [K^+] + [Mg^{++}] + [Ca^{++}] - [Cl^-] \quad (10)$$

(All values are expressed in mEq/L). However, in body fluids, there are always some anions present ("unidentified" or "undetermined" anions, [XA]) that are not routinely measured. All these acidic anions of clinical interest (lactate, keto acids, formate, salicylate, sulfate, and other anions of renal failure) have pK values at least three orders of magnitude lower than the pH compatible with life. In blood plasma, all these acids are therefore always more than 99.9% dissociated. Their anions can thus be operationally considered as co-determinants of SID (together with all truly nonreacting ions). Therefore a quantity called "effective SID" (SID_e) can be defined as follows:

$$SID_e = [Na^+] + [K^+] + [Mg^{++}] + [Ca^{++}] - [Cl^-] - [XA] \quad (11)$$

Combining equations 10 and 11:

$$[XA] = SID_{app} - SID_e \quad (12)$$

With the help of our mathematic model, the independent variable SID_e can be calculated if all the remaining independent variables (P_{CO_2}, [Alb], $[Pi_{tot}]$) and pH, a dependent variable, are known: if, as stated in equation 1, $pH = f_{pH}\{P_{CO_2}, SID, [Alb], [Pi_{tot}]\}$, then

$$SID_e = f_{SID}\{pH, P_{CO_2}, [Alb], [Pi_{tot}]\} \quad (13)$$

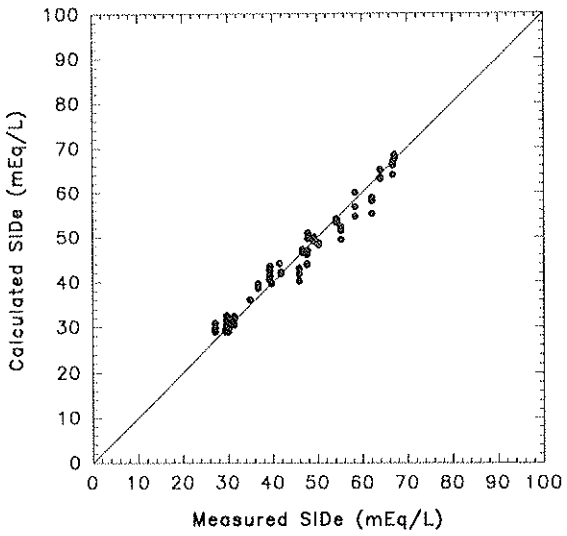

Fig. 3. Solutions containing serum proteins (n = 72). Plot of the effective SID_e calculated with the mathematic model (see Appendix A) from the measured pH, P_{CO_2}, [Alb], and $[Pi_{tot}]$, versus the value of SID_e directly derived (with equation 11) from the measured values of all strong ions present in the solutions.

We applied this approach to our published data base[2] on artificial solutions containing serum proteins. In these solutions, the concentrations of all ions that determined the SID_e were known and measured. They included the commonly measured Na^+, K^+, Mg^{++}, Ca^{++}, Cl^-, and also 1.5 mEq/L of SO_4^{2-}, which is not routinely determined and therefore classifies as [XA] (see equation 11). In Fig. 3, values of SID_e have been calculated with the new mathematic model from the measured pH, P_{CO_2}, [Alb], and $[Pi_{tot}]$ and are plotted (y axis) against SID_e values that were directly derived (with equation 11) from the measured values of all strong ions present in the solutions (x axis). The plot fits the straight line $y = 0.92x + 3.68$ (r = 0.98), which is statistically not different from the line of identity shown in the figure; the mean of the differences (SID_e calculated) − (SID_e measured) was −0.15 mEq/L (±2.37 SD), which is statistically not distinguishable from zero (t = 0.54, $0.50 < p < 0.60$, $d.f.$ = 70).

Calculation of SID_e with the mathematic model requires access to a microcomputer. However, with the insights derived from the model, an alternate simple estimate of SID_e in plasma can be derived, based on the principle of electroneutrality. This was first explored in 1948 by Singer and Hastings,[14] when they defined

Table III. Mathematic model for acid-base equilibrium in plasma*

$$
\begin{aligned}
SID_e + 1000 \times ([H^+] - K'w/[H^+] - Kc1 \times Pco_2/[H^+] \\
- Kc1 \times Kc2 \times Pco_2/[H^+]^2) - [Pi_{tot}] \times Z \\
+ \{ \quad -1 \times 3.1623E\text{-}09/(3.1623E\text{-}09 + [H^+]) \\
-98 \times 1.0000E\text{-}04/(1.0000E\text{-}04 + [H^+]) \\
-18 \times 2.5119E\text{-}10/(2.5119E\text{-}10 + [H^+]) \\
-1 \times 7.9433E\text{-}04/(7.9433E\text{-}04 + [H^+]) \\
+ 1 \quad -1 \times 1.0000E\text{-}08/(1.0000E\text{-}08 + [H^+]) \\
+16 \quad -1 \times 7.5858E\text{-}08/(7.5858E\text{-}08 + [H^+]) \\
-1 \times 6.0256E\text{-}08/(6.0256E\text{-}08 + [H^+]) \\
-1 \times 7.9433E\text{-}08/(7.9433E\text{-}08 + [H^+]) \\
-1 \times 3.2359E\text{-}08/(3.2359E\text{-}08 + [H^+]) \\
-1 \times 9.7724E\text{-}08/(9.7724E\text{-}08 + [H^+]) \\
-1 \times 4.8978E\text{-}08/(4.8978E\text{-}08 + [H^+]) \\
-1 \times 1.7783E\text{-}07/(1.7783E\text{-}07 + [H^+]) \\
-1 \times 4.3652E\text{-}07/(4.3652E\text{-}07 + [H^+]) \\
-1 \times 1.4125E\text{-}05/(1.4125E\text{-}05 + [H^+]) \\
-1 \times 1.7378E\text{-}06/(1.7378E\text{-}06 + [H^+]) \\
-1 \times 6.7608E\text{-}07/(6.7608E\text{-}07 + [H^+]) \\
-1 \times 1.8621E\text{-}07/(1.8621E\text{-}07 + [H^+]) \\
-1 \times 1.5136E\text{-}06/(1.5136E\text{-}06 + [H^+]) \\
-1 \times 10^{-N1}/(10^{-N1} + [H^+]) \\
-1 \times 10^{-N2}/(10^{-N2} + [H^+]) \\
-1 \times 10^{-N3}/(10^{-N3} + [H^+]) \\
+N4 \ -N4 \times 3.9811E\text{-}10/(3.9811E\text{-}10 + [H^+]) \\
+N5 \ -N5 \times 1.0000E\text{-}11/(1.0000E\text{-}11 + [H^+])\} \\
\times 1000 \times 10 \times [Alb]/66{,}500 = 0
\end{aligned}
$$

N1 through N5 are parameters to be determined by the least-squares method. N1, N2, and N3 are pK values for the three histidine residues not determined by NMR (reference 3). N4 equals the number of titratable lysine plus arginine residues with a pK of 9.4; N5 equals the number of titratable lysine plus arginine residues with a pK of 11.0; $[H^+] = 10^{-pH}$; 66,500 is the molecular weight of albumin.

*Microenvironmental pK values for all histidine residues in the molecule of human serum albumin are incorporated.

$Z = (K1 \times [H^+]^2 + 2 \times K1 \times K2 \times [H^+] + 3 \times K1 \times K2 \times K3)/([H^+]^3 + K1 \times [H^+]^2 + K1 \times K2 \times [H^+] + K1 \times K2 \times K3)$ (see reference 2). See Tables I and II and text for definition of other constants.

BB_{pl}. On one hand, they defined it as the difference between the sum of concentrations of the "fixed bases and acids" (in current terminology, chemically nonreacting cations and anions); in this sense, BB_{pl} is synonymous with SID in plasma,[15] i.e., $BB_{pl} = SID_e$. Alternately, Singer and Hastings defined BB_{pl} as the sum of all "plasma buffer anions": bicarbonate plus negative charges contributed by plasma proteins (i.e., albumin[2]) and by inorganic phosphate:

$$BB_{pl} \approx [HCO_3^-] + [Alb^{x-}] + [Pi^{y-}] \approx SID_e \quad (14)$$

If Pco_2, pH, [Alb], and $[Pi_{tot}]$ are known, the solution of equation 14 is simple and can be computed with a handheld calculator (see Appendix B).

The values of SID_e estimated with equation 14 agree well with those directly measured. The mean difference between the estimated and measured values of SID_e was −0.073 mEq/L (±2.39, SD), which is statistically not different from zero ($t = 0.26$, $0.80 < p < 0.90$, $d.f. = 70$).

With this approach one can explore new ways to evaluate [XA] in metabolic acidosis. The customary approach to acid-base balance relies on the evaluation of AG for this purpose.[16] AG is strongly influenced by the concentration of serum proteins,[2,15,16] specifically albumin. Moreover, when Pco_2 and SID vary widely, it is difficult to establish a normal value for AG at any given level of [Alb].[2] This is so because AG is a *dependent* variable and is actually determined by several independent variables (e.g., Pco_2, SID, [Alb], $[Pi_{tot}]$) in addition to [XA]; therefore an increase in AG is not always a simple stoichiometric reflection of an increase in unidentified acidic anions as the customary use of AG tacitly assumes. This is why, although of some usefulness in fairly simple acid-base disturbances, AG is equivocal in estimating accumulation of unidentified acidic anions when several independent variables change widely and divergently[2]; in critically ill patients, this is rather common.[17] On the other hand, since SID is an *independent* variable, the value of $(SID_{app} - SID_e)$ defined earlier should give an estimate of [XA] that is free of the theoretic limitations of AG.

The model presented in this report may serve as a useful theoretic framework for studies into the role of serum proteins in acid-base equilibria. Further questions that need to be addressed include the effects of: (1) variation in ionic strength and temperature, (2) covalent modifications of the albumin molecule (e.g., genetic variants, carbamino compounds, glycosylation in diabetic patients, (3) binding of various ligands (e.g., drugs, lipids) that might alter the conformation and/or titrating behavior of albumin, and (4) the presence of cationic paraproteins. In the future, it might be possible to use a derivative of the mathematic model to help classify and interpret acid-base disorders in clinical settings.

We thank David E. Leith for useful discussions.

APPENDIX A

We followed the general approach developed by Stewart[1] and previously used by Figge et al.[2] to design a mathematic model of an open system consisting of water, strong ions, CO_2, and the nonvolatile weak acids inorganic phosphate and albumin. In this model (Table III), we have improved on the previous version[2] by including microenvironmental pK values for the histidine residues of human serum albumin as determined by NMR.[3] The resulting model simultaneously satisfies requirements for: (1) electrical neutrality in the system; (2) the dissociation equilibria for water, the carbonic acid system, and the phosphoric acid system (see Table I for values of these dissociation constants); and (3) the dissociation equilibria for

all ionizable groups on the albumin molecule (see Table II).

As shown in Table III, five parameters (N1 to N5) are to be optimized by a least-squares procedure to fit our experimental data on albumin solutions.[2] N1 through N3 represent pK values of three albumin histidine residues that were not determined by NMR.[3] N4 represents the total number of titratable lysine and arginine residues with a low pK (pK = 9.4), and N5 represents the total number of titratable lysine and arginine residues with a high pK (pK = 11). Equations 2 through 7 in the Methods section describe constraints on the values of N1 through N5. A computer program was written in QuickBASIC (Microsoft Corp., Bellevue, Wash.) to determine the optimal values of the parameters N1 through N5 (within the ranges defined by the constraints) by using a strategy similar to that previously described.[1,2] The set of parameters that satisfied the least-squares criteria given in equations 8 and 9 (see Methods) were then considered to represent the optimal model. The computer program was compiled and run on an Epson 80386/20 MHz computer (Epson America, Inc., Torrance, Calif.) equipped with an 80387 math coprocessor using double-precision floating-point arithmetic. A hard copy of the program may be obtained from one of the authors (J. F.).

The final results yielded the following optimized values: N1 = 7.3, N2 = 5.2, N3 = 7.3, N4 = 77, N5 = 0. The corresponding minimum value for S^2 was 0.0728 as defined by equation 9 in the Methods section. The value of S^2 from the previous model[2] was 0.0675; thus the fit of the experimental data to the present model is nearly as good as the fit to the original model.

APPENDIX B

A simple formula for the calculation for SID_e can be derived from a statement of electrical neutrality:

$$SID_e + [H^+] = [OH^-] + [HCO_3^-] + [CO_3^{--}] + [Alb^{x-}] + [Pi^{y-}]$$

In the physiologic pH range of 6.9 to 7.9, several terms are negligible and can be omitted, giving the simplified equation (see Discussion, equation 14):

$$SID_e = [HCO_3^-] + [Alb^{x-}] + [Pi^{y-}]$$

Over the stated pH range, the negative charge displayed by albumin is nearly linearly related to the pH (see Fig. 2). Thus a linear function can be used to approximate the value of $[Alb^{x-}]$ from the measured values of [Alb] and pH. Similarly, by solving function z (Table III), it is possible to approximate $[Pi^{y-}]$ from the measured values of $[Pi_{tot}]$ and pH by a linear fit over the same pH range. The value of $[HCO_3^-]$ can be determined from the measured values of pH and Pco_2. By performing the appropriate least-squares fits and substituting into the above equation, the following formula is derived:

$$SID_e = 1000 \times Kcl \times Pco_2/(10^{-pH}) + 10 \times [Alb] \times (0.123 \times pH - 0.631) + [Pi_{tot}] \times (0.309 \times pH - 0.469)$$

This formula is simple to apply. Note that $[Pi_{tot}]$ is in mmol/L, whereas routine measurements of phosphate are usually in milligrams per deciliter of phosphorus. To convert to mmol/L, multiply the measured value by the factor (10/30.97).

REFERENCES

1. Stewart PA. Modern quantitative acid-base chemistry. Can J Physiol Pharmacol 1983;61:1444-61.
2. Figge J, Rossing TH, Fencl V. The role of serum proteins in acid-base equilibria. J Lab Clin Med 1991;117:453-67.
3. Bos OJM, Labro JFA, Fischer MJE, Wilting J, Janssen LHM. The molecular mechanism of the neutral-to-base transition of human serum albumin. J Biol Chem 1989;264:953-9.
4. Reeves RB. Error proved and corrected: net anionic charge on serum albumin. J Lab Clin Med 1991;117:437.
5. Edsall JT, Wyman J. Biophysical chemistry, vol. I. New York, Academic Press, 1958.
6. Takahashi N, Takahashi Y, Blumberg BS, Putnam FW. Amino acid substitutions in genetic variants of human serum albumin and in sequences inferred from molecular cloning. Proc Natl Acad Sci USA 1987;84:4413-7.
7. Meloun B, Morávek L, Kostka V. Complete amino acid sequence of human serum albumin. FEBS Lett 1975;58:134-37.
8. Rossing TH, Boixeda D, Maffeo N, Fencl V. Hyperventilation with hypoproteinemia. J Lab Clin Med 1988;112:553-9.
9. Iberti TJ, Liebow AB, Papadakos PJ, Fischer EP. Low sensitivity of the anion gap as a screen to detect hyperlactatemia in critically ill patients. Crit Care Med 1990;18:275-7.
10. Nozaki Y, Gurd FRN, Chen RF, Edsall JT. The association of 4-methylimidazole with the ions of cupric copper and zinc; with some observations on 2,4-dimethylimidazole. J Am Chem Soc 1957;79:2123-9.
11. Tanford C. Preparation and properties of serum and plasma proteins. XXIII. Hydrogen ion equilibria in native and modified human serum albumins. J Am Chem Soc 1950;72:441-51.
12. Stryer L. Biochemistry. 1st ed. San Francisco, WH Freeman, 1975:86.
13. van Slyke DD, Hastings AB, Hiller A, Sendroy J Jr. Studies of gas and electrolyte equilibria in blood. XIV. Amounts of alkali bound by serum albumin and globulin. J Biol Chem 1928;79:769-80.
14. Singer RB, Hastings AB. An improved clinical method for the estimation of disturbances of the acid-base balance of human blood. Medicine (Baltimore) 1948;27:223-42.
15. Rossing TH, Maffeo N, Fencl V. Acid-base effects of altering plasma protein concentration in human blood in vitro. J Appl Physiol 1986;61:2260-5.
16. Emmet M, Narins RG. Clinical use of anion gap. Medicine (Baltimore) 1977;56:38-54.
17. Fencl V, Rossing TH. Acid-base disorders in critical care medicine. Annu Rev Med 1989;40:17-29.