Intensive Care Med (2009) 35:2033–2043
DOI 10.1007/s00134-009-1653-7

ORIGINAL

Luciano Gattinoni
E. Carlesso
G. Maiocchi
F. Polli
P. Cadringher

Dilutional acidosis: where do the protons come from?

Received: 8 May 2009
Accepted: 31 July 2009
Published online: 18 September 2009

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-009-1653-7) contains supplementary material, which is available to authorized users.

L. Gattinoni
Dipartimento di Anestesia, Rianimazione (Intensiva e Subintensiva) e Terapia del Dolore, Fondazione IRCCS, "Ospedale Maggiore Policlinico Mangiagalli Regina Elena" di Milano, Milan, Italy

L. Gattinoni (✉) · E. Carlesso · F. Polli · P. Cadringher
Dipartimento di Anestesiologia, Terapia Intensiva e Scienze Dermatologiche, Università degli Studi, Milan, Italy
e-mail: gattinon@policlinico.mi.it
Tel.: +39-02-55033232
Fax: +39-02-55033230

G. Maiocchi
Unità Operativa Complessa di Farmacia, Fondazione IRCCS, "Ospedale Maggiore Policlinico Mangiagalli Regina Elena", di Milano, Italy

Abstract *Purpose:* To investigate the mechanism of acidosis developing after saline infusion (*dilutional acidosis* or *hyperchloremic acidosis*). *Methods:* We simulated normal extracellular fluid dilution by infusing distilled water, normal saline and lactated Ringer's solution. Simulations were performed either in a closed system or in a system open to alveolar gases using software based on the standard laws of mass action and mass conservation. In vitro experiments diluting human plasma were performed to validate the model. *Results:* In our computerized model with constant pKs, diluting extracellular fluid modeled as a closed system with distilled water, normal saline or lactated Ringer's solution is not associated with any pH modification, since all its determinants (strong ion difference, CO_2 content and weak acid *concentration*) decrease at the same degree, maintaining their relative proportions unchanged. Experimental data confirmed the simulation results for normal saline and lactated Ringer's solution, whereas distilled water dilution caused pH to increase. This is due to the increase of carbonic pK induced by the dramatic decrease of ionic strength. Acidosis developed only when the system was open to gases due to the increased CO_2 content, both in its dissociated (bicarbonate) and undissociated form (dissolved CO_2). *Conclusions:* The increase in proton concentration observed after dilution of the extracellular system derives from the reaction of CO_2 hydration, which occurs only when the system is open to the gases. Both Stewart's approach and the traditional approach may account for these results.

Keywords Stewart's approach · Acid–base equilibrium · Metabolic acidosis · Volume resuscitation · CO_2 content

Introduction

Over the last years, the approach to the acid–base equilibrium developed by Peter Stewart has gained large consensus [1, 2], although it has generated controversies and debates [3]. We believe that the greatest merit of Stewart's approach was to synthesize into a unique vision both the acid–base and the electrolyte equilibriums, leading to a more profound understanding of the interactions occurring within the body fluids and of their homeostasis [4]. Stewart's reasoning was triggered by two preliminary considerations: first, how is it possible for

phenomena such as the Na^+–H^+ co-transport or H^+–Cl^- exchange to occur if free protons have concentrations on the order of nanomoles as opposed to millimoles for electrolytes (i.e., each single proton compares with 1 million Na^+ atoms)? The second observation raised by Stewart with regard to the traditional acid–base approach was that the latter—according to the Henderson–Hasselbach equation [5] and the base excess concept [6–8]—implies that protons can be added or withdrawn from a system as such, while in reality H^+ movements are always associated with an accompanying anion.

Moving from these considerations, Stewart developed a physico-chemical model for the acid–base equilibrium. A comprehensive description of his approach may be found elsewhere [9, 10]. Briefly, according to Stewart's model, the concentration of free protons in a solution (since they cannot be added or withdrawn from the system, as such) must be a function of other variables. These are: (1) the *strong ion difference* (SID), i.e., the difference in the electrical charges due to the ions that are always dissociated in plasma; (2) the partial pressure of CO_2 in alveolar air (supposedly, in equilibrium with the body fluids); (3) the total concentration of weak acids ([Atot])—called "non-carbonic buffers", according to the traditional terminology—which exist in body fluids partially in their dissociated electrically charged form (A^-) and partially in their indissociated electrically neutral form (AH). A more detailed description of SID, CO_2 content, Atot and the dissociation of water, according to Stewart's approach, are reported in the Electronic supplementary material (ESM), where an outline of the common misinterpretations of that theory is also provided.

The paradigm called forth to maintain the superiority of Stewart's approach is the acidosis developing after the infusion of normal saline. In fact, where does that excess of protons come from, since no protons are added to the plasma and the added solution is characterized by equal amounts of sodium and chloride? We would like to offer our view of the problem, which differs—at least in part—both from the traditional approach (acidosis originating from the dilution of bicarbonates, *dilutional acidosis* [11–13]) and from Stewart's approach (acidosis originating as a consequence of the decrease in strong ion difference, *hyperchloremic acidosis* [14, 15]).

Our view of the problem was developed by computer-based simulations virtually diluting normal extracellular fluid either with distilled water, normal saline or lactated Ringer's solution. Simulations were then reproduced with in vitro experiments in which human plasma was diluted with the same three diluents at different PCO_2 tensions.

Materials and methods

We examined the effects of diluting normal extracellular fluid with different solutions (see Table 1) both in a closed system (i.e., a system not exchanging matter with its environment, such as venous blood before reaching the lung), and in a system open to gases (i.e., capable of equilibrating with the external carbon dioxide tension). Our analysis was performed by a custom-made simulator and by in vitro experiments. The simulator (ACBA) computes the solutions of the fourth order system of equations describing the acid–base equilibrium via iteration (see ESM for details). All the computations performed are based on concentrations rather than ion activity (common behavior in laboratory medicine) as the ionic strength remains quite constant when diluting plasma with electrolyte solution. Accordingly, in our simulations we assumed constant pKs of the buffer pairs (6.1 for carbonic buffers and 6.8 for non-carbonic buffers). We have taken into account the ionic strength only when diluting 1:1 with distilled water. The ionic strength, in fact, in these conditions cannot be ignored. Accordingly, we corrected carbonic pK for ionic strength in the validation set of simulations including distilled water. Computer-based simulations included the introduction of lactate metabolism, which, however, could not be reproduced in our in vitro experiments. Neither the computer-based simulations nor in vitro experiments included the analysis of kidney effect on the system, despite this plays a substantial role in vivo. A short comment will only be made in the discussion.

Extracellular fluid

Simulations involved examination of the effects of dilution of a solution whose composition resembles that of normal

Table 1 Intravenous fluids and electrolytes [18]

Solution	Na^+ mEq/l	Cl^- mEq/l	K^+ mEq/l	Ca^{2+} mEq/l	Mg^{2+} mEq/l	Lactate mEq/l	SID mEq/l
Distilled water							0
0.9% NaCl	154	154					0
Lactated Ringer's	130	109	4	3		28	0*

Na^+ indicates sodium ion; Cl^-, chloride; K^+, potassium; Ca^{2+}, calcium; Mg^{2+}, magnesium; NaCl, sodium chloride, and 0.9% NaCl indicates normal saline. *Lactated Ringer's solution SID is equal to zero while the system is closed to lactate metabolism

extracellular fluid. This was modeled as an aqueous solution in which strong and weak electrolytes are dissolved. Strong electrolytes are considered as a unique substance according to Stewart's concept of SID. Weak electrolytes include carbonic (bicarbonate/carbonic acid, HCO_3^-/H_2CO_3 couple) and non-carbonic buffers. Non-carbonic buffers include proteins (mainly albumin) and phosphates. The total concentration of non-carbonic buffers is referred to as ATOT, according to Stewart's terminology. The characteristics of the reference extracellular fluid solution were: pH 7.4, PCO_2 40 mmHg, ATOT 22 mmol/l and SID 42 mEq/l. The detailed composition of the reference extracellular fluid solution is reported in Table 2.

Simulations

Using ACBA, we simulated a 10% stepwise dilution (from 10 to 100% of the initial volume) of the starting solution with three diluting solutions, proportionally reducing the initial CO_2 content, SID and ATOT. Each step was modeled initially in the closed system and then after opening the system to alveolar gases (i.e., $PCO_2 = 40$ mmHg).

Simulation 1

Distilled water was used as the diluting solution. This diluent is characterized by the absence of electrolytes.

Simulation 2

Normal saline (0.9% NaCl) was used as the diluting solution. This solution, due to the presence of equal amounts (154 mEq/l) of Na^+ and Cl^-, is also characterized by a SID equal to zero.

Simulation 3

We simulated the same stepwise dilution of the reference extracellular fluid solution with lactated Ringer's. Unlike in simulations 1 and 2, the system was opened not only to alveolar gases but also to the metabolism of lactate. This means that the SID of the diluting solution, which initially is equal to zero, with lactate being almost completely dissociated (pK = 3.8), increases to 28 mEq/l when lactate disappears, being either converted into glucose or eliminated as CO_2 and water.

Table 2 Extracellular fluid composition (starting solution)

Variable	
pH	7.40
CO_2 (mMol/l)	1.22
HCO_3^- (mMol/l)	24.42
CO_2tot (mMol/l)	25.64
AH (mMol/l)	4.42
A^- (mMol/l)	17.58
ATOT (mMol/l)	22.00
SID (mEq/l)	42.00
PCO_2 (mmHg)	40

Experimental validation

Human plasma

We performed in vitro experiments using fresh frozen plasma treated with 63 ml of citrate-phosphate-dextrose (CPD) as a starting solution. Plasma composition (see Table 3) was determined by laboratory test (strong and weak electrolytes) and by blood gas analysis (pH, PCO_2, BE) (ABL800 FLEX, Radiometer). Since only standard laboratory electrolytes were measured, the strong ion difference was computed as:

$$ISID = \text{SIDnormal value} - BE.$$

Experimental protocol

The experimental protocol was characterized by three main steps: baseline, plasma dilution in a closed system; plasma dilution in an open system.

Baseline: 1.5-ml plasma samples were equilibrated for 20 min at 37°C in a tonometer (EQUILibrator, RNA Medical, Devens, MA, USA) with constant gas flow. Two

Table 3 Fresh frozen human plasma composition (starting solution)

Variable	
Laboratory test derived parameters	
Glucose (mg/dl)	362
Albumin (g/dl)	3.6
K^+ (mmol/l)	3
Na^+ (mmol/l)	169
Cl^- (mmol/l)	77
Total calcium (mg/dl)	6.9
Phosphate (mg/dl)	12.2
Magnesium (mg/dl)	1.8
Gas analysis derived parameters (mean of two analyses)	
pH	7.221
CO_2 (mMol/l)	1.29
HCO_3^- (mMol/l)	16.98
CO_2tot (mMol/l)	18.27
BE (mEq/l)*	−11.11
PCO_2 (mmHg)	42.05

The reported values refer to the analysis performed in the plasma immediately after defrosting. *The low base excess (BE) is primarily due to the citrate, which at the observed pH behaves as a strong negative ion

gas mixtures were used with two different CO_2 fractions [4.9% CO_2, complement N_2, and 12.05% CO_2, 19.98% O_2, N_2 complement, respectively (gruppo SAPIO, Carnago, MI, Italy)].

Plasma dilution in a closed system The previously equilibrated plasma samples were diluted 1:1 with one of the tested diluents.

Plasma dilution in an open system The previously diluted plasma sample was re-equilibrated with the same gas mixture as at baseline.

Immediately after each step, and avoiding any contact of the sample with room air, we performed a blood gas analysis to obtain pH, PCO_2, electrolytes and derived variables. The entire procedure was repeated with two gas mixtures at 4.9 and 12.05% CO_2 and with the same three different diluents used for simulations (distilled water, normal saline and lactated Ringer's). Each of the high CO_2 percentage/diluent combinations was repeated four times, while each of the low CO_2 percentage/diluents combinations was repeated two times. This was because our plasma unit was finished and another one with exactly the same composition was not available.

Ionic strength was computed as:

$$I = \frac{1}{2} \sum c_i \times Z_i^2$$

I = ionic strength
c_i = concentration of the ith species, mole/L
Z_i^2 = valence (or oxidation) number of the ith species.

We used only K^+, Na^+, Cl^- lactate and HCO_3^- concentrations as the only analysis available from blood gas. The carbonic pK was computed according to the formula [16] :

$$I\text{pK} = 6.33 - 0.5 \times \sqrt{\text{ionic strength}}$$

to verify possible significant errors from the constant 6.1 value used in our simulations.

ACBA validation

The experimental values obtained with the in vitro experiments were used to validate the results obtained using ACBA. Simulations resembled the experimental steps. The Atot value was iteratively determined so as to make the ACBA baseline results match the experimental baseline results.

Baseline Input variables were the PCO_2, SID and Atot of the experimental baseline step. The pKs were kept constant at 6.8 (non-carbonic) and 6.1 (carbonic).

Plasma dilution in a closed system Input variables were half of the baseline CO_2 content, half of the baseline SID and half of the baseline Atot. The non-carbonic pK was kept constant (6.8), whereas the carbonic one was corrected for ionic strength.

Plasma dilution in an open system Input variables were the PCO_2 of the corresponding experimental step, half of the baseline SID and half of the baseline Atot. The non-carbonic pK was kept constant (6.8), whereas the carbonic one was corrected for ionic strength.

Statistical analysis

A Bland–Altman analysis and a linear regression were used to assess the level of agreement between the experimentally measured pH and the pH computed by ACBA.

Mixed-design one-way ANOVA for repeated measures was used to test the effects of the step (baseline, dilution in a closed system; dilution in an open system) and of the diluents both in the in vitro experiments and in the ACBA validation simulations. Bonferroni's t-test was used to correct for multiple comparisons. Analysis was performed using SAS software, version 8.2 (SAS Institute, Cary, NC).

Results

Model

Closed system

Dilution of the extracellular fluid with distilled water The theoretical effects on the acid–base and electrolyte equilibriums of diluting a solution similar to the normal extracellular fluid from 0 to 100% is reported schematically in Fig. 1. For the sake of clarity, we will describe here a dilution 1:1 (100%) of normal extracellular fluid. After the dilution, if the system is closed, the concentration of all the substances in the system (except for protons and hydroxyl ions) will be halved. Therefore, the strong ion difference will change from 42 to 21 mEq/l, the concentration of bicarbonate will change from 24.42 to 12.21 mEq/l, and the dissolved CO_2 will change from 1.22 to 0.61 mmol/l and non-carbonic buffers from 22 to 11 mEq/l. It is easy to verify that the pH of the solution will not change (it will remain 7.4), despite a decrease in SID (Stewart's model) and a dilution of bicarbonate (traditional approach). Obviously, the dilution with distilled water is associated with a decrease in the concentration of all electrolytes and a decrease in osmolarity.

Dilution of the extracellular fluid with normal saline As for the previous simulation, we will consider here the extreme case of a 100% dilution. Hypothesizing an initial sodium concentration in the extracellular fluid equal to

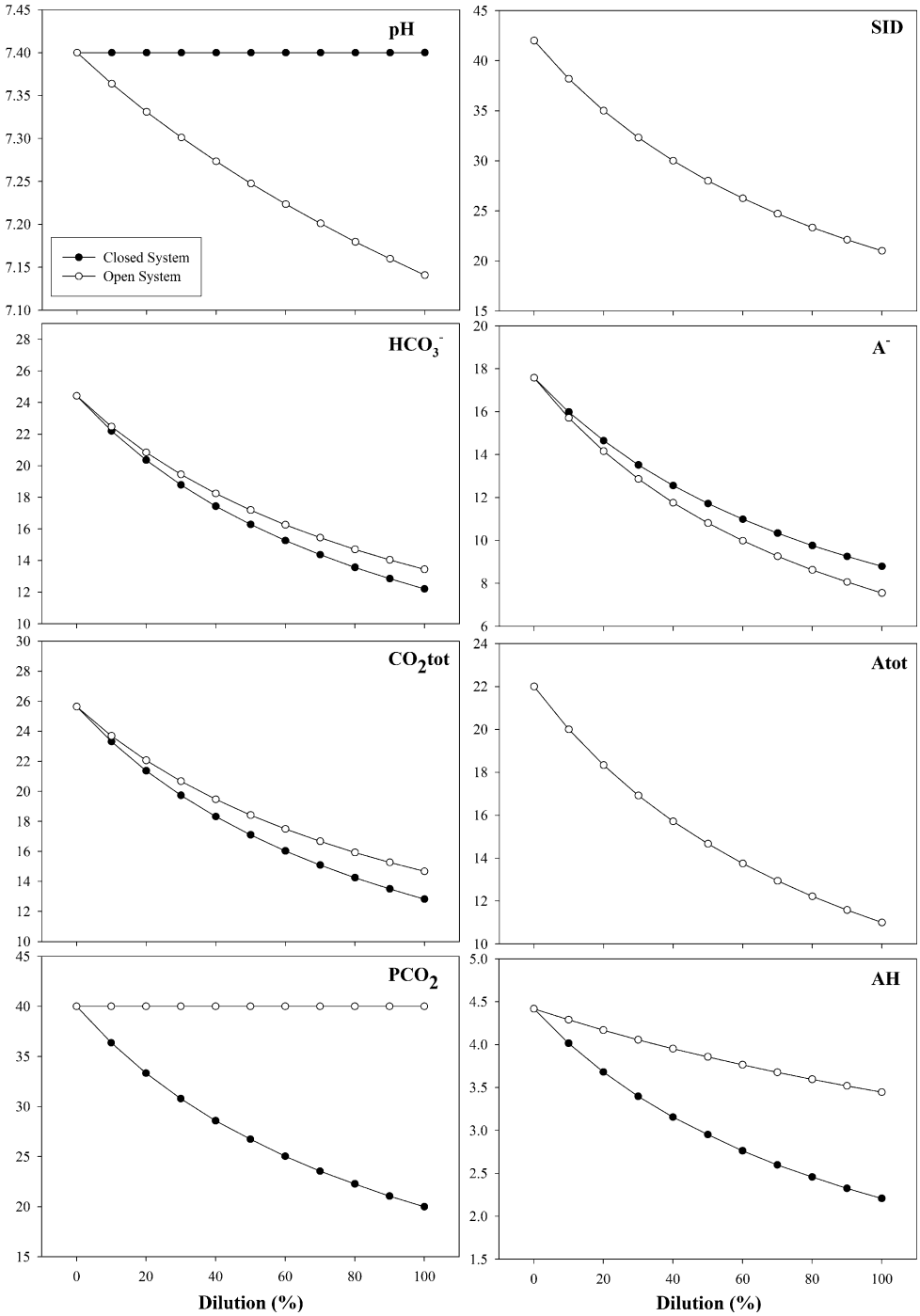

Fig. 1 Figure represents the effects on acid–base determinants of a stepwise dilution of extracellular fluid with a solution characterized by SID equal to zero (distilled water or normal saline). Each step represents a 10% volume increase with respect to initial volume. *Black dots* represent a closed system, i.e., no matter exchange with the surroundings; *white dots* represent an open system, i.e., CO_2 equilibration with alveoli. See text for description

140 mEq/l and a chloride concentration of 104 mEq/l, the final sodium concentration will increase slightly (147 mEq/l) because of the higher concentration of sodium in normal saline, whereas the final chloride concentration will increase markedly (129 mEq/l). As a consequence, the SID decreases from 42 down to 21 mEq/l. Of note, the saline composition is 154 mEq/l Na^+ and 154 Cl^- (i.e., its SID is equal to zero); this means that the decrease in SID after the dilution will be exactly the same as the decrease observed when the dilution is obtained with an equal volume of distilled water. The total CO_2 content and its two components (bicarbonate and dissolved CO_2) are halved, as are Atot in its two components, AH and A^-. If we compute the pH of this new solution, either referring to Stewart's equations or simply referring to the most familiar Henderson–Hasselbach equation, it is easy to verify that the pH of this new diluted solution is unchanged. This happens despite

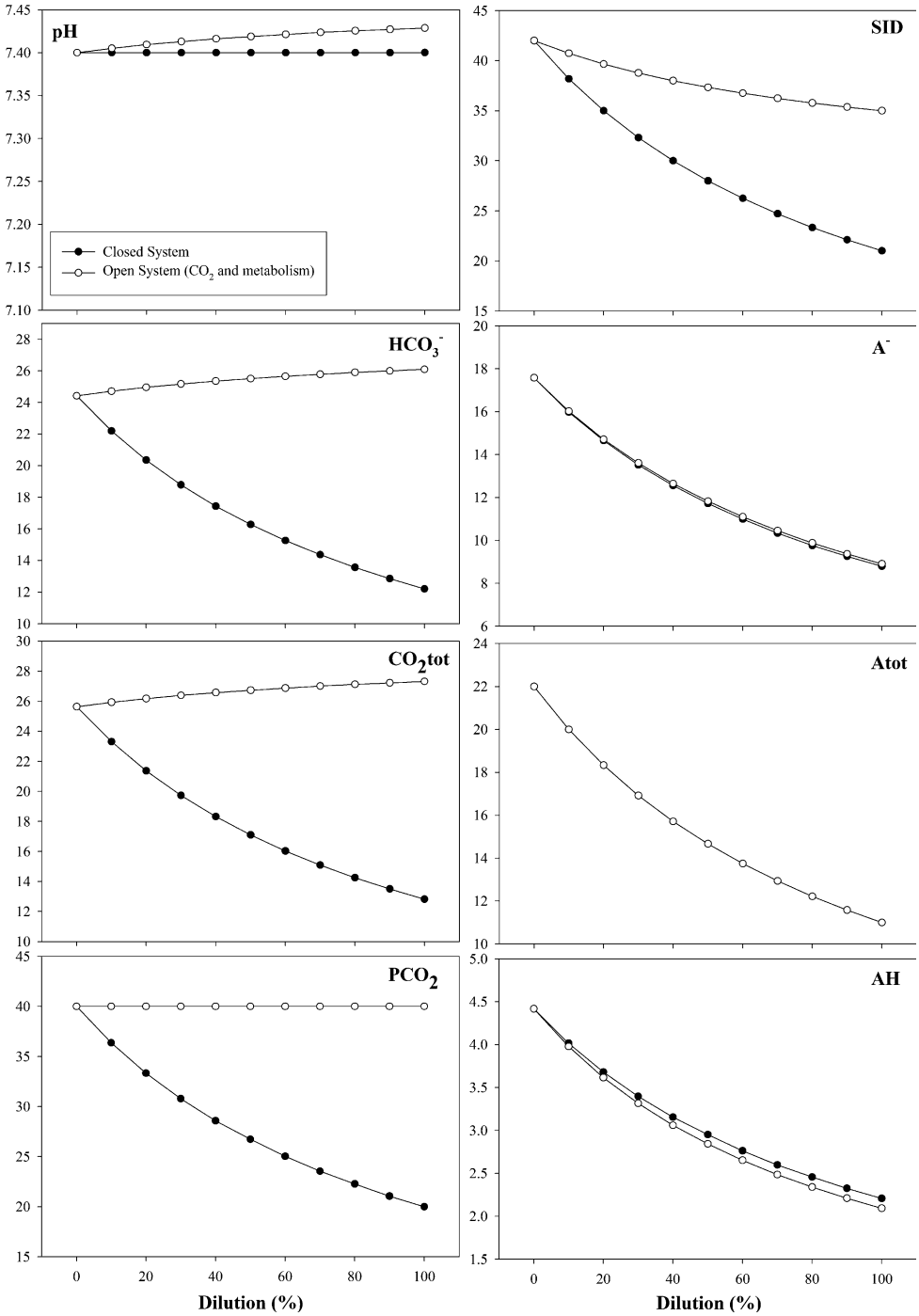

Fig. 2 Figure represents the effects on acid–base determinants of a stepwise dilution of extracellular fluid with lactated Ringer's solution. Each step represents a 10% volume increase with respect to the initial volume. *Black dots* represent a closed system, i.e., no matter exchange with the surroundings; *white dots* represent an open system, i.e., CO_2 equilibration with alveoli and lactate metabolism. See text for description

the resulting hyperchloremia, the decreased SID and the decreased bicarbonate concentration.

Dilution of the extracellular fluid with lactated Ringer's A description of the effects of diluting the normal extracellular fluid—modeled as a closed system—with lactated Ringer's solution is reported in Fig. 2. As shown, the effects are similar to the case of dilution with distilled water and normal saline. In fact, lactate—being almost completely dissociated—behaves as a strong ion. Therefore, the strong ion difference of lactated Ringer's is equal to zero, as the SID of saline.

Open system

When including the effects of alveolar ventilation (which is able to regulate the partial pressure of respiratory

gases), the extracellular fluid we are using in these simulations can be modeled as an open system. In particular, as far as the acid-basis equilibrium is concerned, what is important is the ability to regulate the CO_2 present in alveolar gases. This implies that if pulmonary venous blood has lower CO_2 tension than the alveoli, part of the CO_2 enters into the blood; vice versa, if the CO_2 tension is higher in pulmonary blood, part of the CO_2 will enter the gas phase until the same PCO_2 is reached in both phases. At equilibrium, we may assume that PCO_2 of the extracellular fluid is very close to that of the alveolar gases.

Dilution of the extracellular fluid with distilled water The diluted solution after equilibration with the gas phase (where we assume a PCO_2 of 40 mmHg) will have the following composition: SID of 21 mEq/l, A^- of 7.55 mEq/l, Atot of 11 mEq/l and HCO_3^- of 13.45 mEq/l. Figure 1 shows the differences compared to the closed system solution and relative to dissolved CO_2 (i.e., PCO_2), HCO_3^-, A^- and AH. In fact, a given amount of CO_2 molecules must enter the blood to raise its PCO_2 to 40 mmHg (i.e., the dissolved CO_2 concentration must raise from 0.61 to 1.22 mmol/l). The amount of CO_2 (L) to be added to 1 l of extracellular fluid to raise the dissolved CO_2 concentration from 0.61 to 1.22 mmol/l must satisfy the following equilibrium [17] :

$$6.1 + \log \frac{HCO_3^- + x}{CO_2 \text{ dissolved} + L - x} = 6.8 + \frac{A^- - x}{AH + x}$$

where L is total amount of CO_2 added, and x is the amount of CO_2 that dissociates into bicarbonate and protons. Note that $L-x$ is equal to 0.61 mmol/l, and the increase of HCO_3^- (by x) is equal to the decrease of A^- (by x) and to the increase of AH.

Therefore, it must be noted that the only change that occurs when the diluted system is open to the gases is the rise of the dissolved CO_2 (i.e., $PCO_2 \times 0.0306$) and the generation of new bicarbonate by the reaction between CO_2 and A^-. Since the total content of CO_2 (dissolved plus bicarbonate) increases, pH obviously decreases. Indeed, acidosis develops in the diluted solution simply because the system is open to gases and one of the independent variables identified by Stewart's approach (the CO_2 content) changes. Such acidosis, indeed, is well accounted for by Stewart's approach. Considering the traditional approach, it must be noted that, in the Henderson–Hasselbach equation, both the numerator (diluted bicarbonate plus new bicarbonate) and the denominator (dissolved CO_2 at equilibrium PCO_2) change. All these phenomena, for different degrees of dilution, are shown in Fig. 1.

Dilution of the extracellular fluid with normal saline When the solution diluted with normal saline equilibrates with the PCO_2, the same phenomenon described above will occur. The strong ion difference remains unchanged; the total CO_2 content increases as new CO_2 enters into the system, in part as dissolved CO_2 and in part as new bicarbonate. Of note, the pH changes occurring in this saline diluted solution are exactly the same as the ones observed with distilled water.

Dilution of the extracellular fluid with lactated Ringer's When a solution diluted with lactated Ringer's is open to gases, it behaves similarly to when dilution is obtained with distilled water and saline, as far as pH, bicarbonate and CO_2 are concerned. A second step, however, must be taken into account: the metabolism of lactate (either to glucose or to CO_2 and water). When lactate (28 mEq/l) disappears, the strong ion difference of the diluted solution becomes equal to 28 mEq/l, and these counteract in part the acidifying effects due to the increased CO_2 content (see Fig. 2). As a final result, the pH slightly increases.

ACBA validation

Figure 3 (upper panel) shows the linear regression between the pH predicted by ACBA simulator and all the experimentally measured pHs ($R^2 = 0.984$, $p < 0.0001$), suggesting that data derived from our model may be consistent. Figure 3 (lower panel) shows the level of agreement between the two pH measurements according to the Bland-Altman analysis. The overall mean (range) pH from experimental data and ACBA simulation was 7.034 (6.708–7.359). The overall bias from these measurements was −0.00007 (−0.0647–0.0645) and the overall limit of agreement (i.e., ±2 SD)±0.053 (−0.052:0.052). The percentage error, i.e., the ratio of 2× standard deviation to mean pH, amounted to 0.7%.

Experimental model

The results of diluting human plasma obtained in vitro and by ACBA simulator are reported in Table 4. The upper part of table refers to the setting in which a 12.05% CO_2 gas mixture was used for tonometry; the lower part of the table refers to a 4.9% CO_2 gas mixture. Atot values that best fitted baseline experimental values used for simulations were 19.1 mmol/l for the higher CO_2% gas mixture and 22.2 mmol/l for the lower CO_2% gas mixture. As shown, the baseline pH with both CO_2%s after tonometry is not different from the pH measured after dilution with saline and lactated Ringer's in the closed system. This happened despite the baseline strong ion difference; Atot and CO_2 content were halved. In contrast the pH measured after dilution with distilled water in the closed system was significantly higher than the baseline value. It must be realized, however, that when diluting a sample with distilled water (both in a closed and in an open system), the ionic strength becomes almost

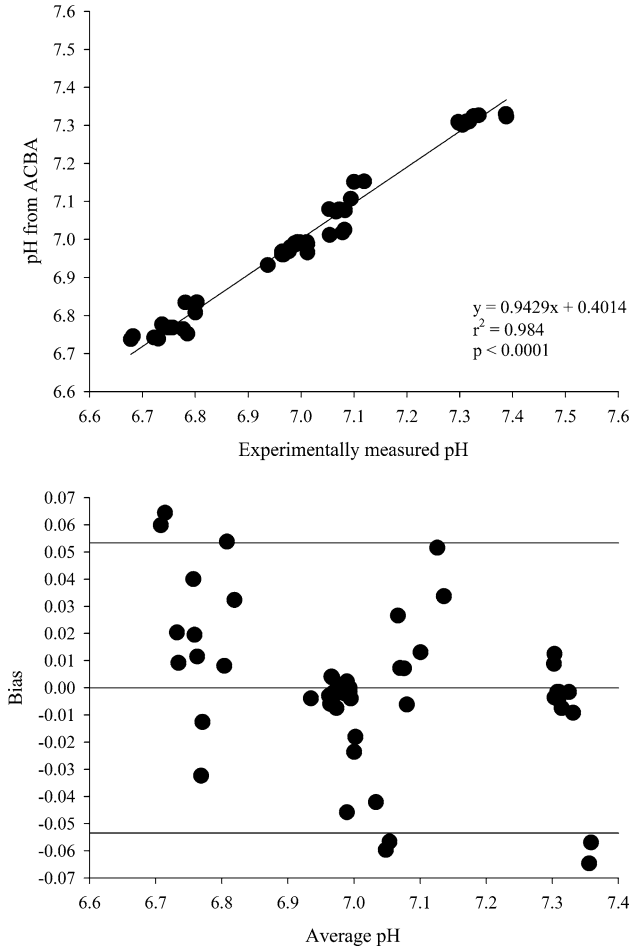

Fig. 3 Figure represents correlation and linear regression (*upper panel*) and Bland–Altman analysis (*lower panel*) using experimentally measured pH and pH resulting from simulations. All steps are represented. Bland–Altman analysis provides mean bias and lower and upper limits of agreement

halved (baseline 0.128, closed system 0.065, open system 0.065). This implies that, for the same CO_2 content and HCO_3^-/CO_2 ratio, the pH increases as the pK for this ionic strength increases from 6.1 to 6.2. When the diluted closed system underwent tonometry at the same CO_2% used at the baseline, the pH dropped significantly.

Discussion

In this paper, by simply applying the well-recognized laws of mass action and of mass conservation (see ESM), we found that the dilution of a solution similar to normal extracellular fluid (up to 100%) does not lead to any detectable change of the protons concentration (i.e., pH), if the system is closed (i.e., does not exchange with surroundings). Obviously, if the diluting volume is greatly higher than the initial volume of the solution, the final composition will be similar to the one of the diluent. This condition, of course, does not occur in clinical practice when the volume infused into the extracellular fluid is only a fraction of the initial volume. Interestingly, we found in our model that, in a closed system, the pH does not change whatever the composition of the diluting solution is. In a model in which the ionic strength is not taken into account, a solution of distilled water, normal saline and lactated Ringer's produced—despite their different electrolyte composition—the same results. Actually, in our experiments, we found that the model fits the experimental data extremely well when the dilution is performed with saline and lactated Ringer's. In this case the computed ionic strength of the diluted solution is very similar to that of the plasma (0.128 vs. 0.136 basal vs. diluted, respectively). Therefore, the pK of the buffers is likely unmodified. However, when distilled water was used as a diluent, we found a paradoxical increase of pH. This is due to the change of pK. Increasing the carbonic pK to 6.2 due to the decrease in ionic strength from 0.129 to 0.065 leads to an increase of pH, with the HCO_3^-/CO_2 ratio in solution being the same.

Halving the SID in our diluted system should lead, according to a superficial vision of Stewart, to an extreme acidosis. Analogously, halving HCO_3^- should lead to acidosis according to the "traditional approach." Therefore, it may appear, quite surprisingly at the first glance, that in our diluted closed system the pH was unchanged both when considering the model and when analyzing the experimental results. However, the explanation of this phenomenon is straightforward. When a volume with zero SID is added to normal extracellular fluid, all the determinants of the proton concentration (i.e., SID, CO_2 content and A_{TOT}) are equally diluted, and their relative proportions do not change. Actually, the decrease in SID (leading to acidosis) is exactly balanced by the decrease in CO_2 content and non-carbonic buffers (leading to alkalosis). As a consequence, pH does not change. The only difference, if the diluting solution is normal saline, is the final electrolyte (sodium and chloride) concentration. The strong ion difference, however, is the same no matter if distilled water or saline is added. In fact, the addition of saline will dilute all the determinant of the acid–base equilibrium by the same proportion, and the proton concentration will not change.

Reportedly, the strong ion difference of lactated Ringer's is 28 mmol/l [18]. This is true only if lactate disappears from the solution after metabolism. Actually, lactate at physiological pH behaves as a strong ion, its pK being equal to 3.8 (i.e., at pH 7.4, the ratio of dissociated lactate and undissociated lactic acid is 3,981 to 1). Indeed, as in the closed system, before lactate is metabolized, the SID of the diluting solution is zero and the overall effect of lactated Ringer's is identical to that of saline.

Table 4 Experimental and ACBA validation results

	Diluent	Model	Baseline	Diluted closed system	Diluted open system
Higher CO_2%					
pH	Distilled water	Experimental	6.975 ± 0.026	7.057 ± 0.032[a]	6.780 ± 0.030[ac]
		ACBA	6.973 ± 0.027	7.005 ± 0.027[a]	6.814 ± 0.028[ac]
	Saline	Experimental	6.980 ± 0.014	6.992 ± 0.023	6.734 ± 0.042[ac]
		ACBA	6.978 ± 0.015	6.979 ± 0.015	6.752 ± 0.016[ac]
	Lactated Ringer's	Experimental	6.981 ± 0.013	6.979 ± 0.017	6.737 ± 0.045[ac]
		ACBA	6.980 ± 0.014	6.980 ± 0.013	6.752 ± 0.011[ac]
PCO_2	Distilled water	Experimental	77.78 ± 2.99	38.33 ± 1.63[a]	77.4 ± 2.10[c]
		ACBA	77.78 ± 2.99	44.51 ± 1.63[a]	77.4 ± 2.10[c]
	Saline	Experimental	77.98 ± 0.61	38.7 ± 1.91[a]	74.45 ± 1.37[c]
		ACBA	77.98 ± 0.61	38.99 ± 0.30[a]	74.45 ± 1.37[c]
	Lactated Ringer's	Experimental	77.75 ± 0.19	39.23 ± 1.95[a]	74.63 ± 1.36[c]
		ACBA	77.75 ± 0.19	38.96 ± 0.07[a]	74.62 ± 1.36[c]
CO_2 content	Distilled water	Experimental	19.58 ± 0.82	11.42 ± 0.51[a]	13.22 ± 0.45[ab]
		ACBA	20.13 ± 0.83	10.07 ± 0.41[a]	12.11 ± 0.33[ac]
	Saline	Experimental	19.86 ± 0.45	10.08 ± 0.79[a]	11.68 ± 0.82[ab]
		ACBA	20.41 ± 0.49	10.20 ± 0.25[a]	12.51 ± 0.23[ac]
	Lactated Ringer's	Experimental	19.83 ± 0.58	9.98 ± 0.69[a]	11.81 ± 1.13[ab]
		ACBA	20.43 ± 0.55	10.21 ± 0.28[a]	12.54 ± 0.32[ac]
Lower CO_2%					
pH	Distilled water	Experimental	7.308 ± 0.004	7.388 ± 0.001[a]	7.110 ± 0.134[ac]
		ACBA	7.304 ± 0.005	7.327 ± 0.005[a]	7.152 ± 0.001[ac]
	Saline	Experimental	7.316 ± 0.009	7.322 ± 0.012	7.077 ± 0.021[ac]
		ACBA	7.314 ± 0.009	7.315 ± 0.011	7.088 ± 0.017[ac]
	Lactated Ringer's	Experimental	7.311 ± 0.001	7.298 ± 0.001	7.069 ± 0.004[ac]
		ACBA	7.308 ± 0.002	7.308 ± 0.002	7.076 ± 0.004[ac]
PCO_2	Distilled water	Experimental	35.05 ± 0.64	18.06 ± 0.06[a]	34.15 ± 0.21[c]
		ACBA	35.05 ± 0.64	20.74 ± 0.37[a]	34.15 ± 0.21[c]
	Saline	Experimental	35.13 ± 0.50	17.09 ± 0.21[a]	33.47 ± 0.49[c]
		ACBA	35.13 ± 0.50	17.52 ± 0.20[a]	33.47 ± 0.49[c]
	Lactated Ringer's	Experimental	34.55 ± 0.07	17.51 ± 0.02[a]	33.60 ± 0.28[c]
		ACBA	34.55 ± 0.07	17.26 ± 0.02[a]	33.60 ± 0.28[c]
CO_2 content	Distilled water	Experimental	18.12 ± 0.09	9.06 ± 0.05[a]	11.44 ± 0.28[ac]
		ACBA	18.26 ± 0.14	9.13 ± 0.07[a]	10.4 ± 0.05[ac]
	Saline	Experimental	18.48 ± 0.62	9.24 ± 0.31[a]	10.42 ± 0.34[ab]
		ACBA	18.67 ± 0.60	9.33 ± 0.29[a]	10.98 ± 0.22[ac]
	Lactated Ringer's	Experimental	17.96 ± 0.00	8.98 ± 0.00[a]	10.33 ± 0.01[ab]
		ACBA	18.12 ± 0.05	9.06 ± 0.03[a]	10.76 ± 0.00[ac]

The table shows the results of diluting human plasma obtained in vitro and by ACBA simulator. Results are grouped by diluent solutions and by step (baseline, plasma dilution in a closed system; plasma dilution in an open system). The upper part of the table refers to the higher CO_2% gas mixture (12.05%); the lower part of the table refers to the lower CO_2% gas mixture (4.9%). We have to point out that the CO_2 contents of the diluted plasma in a closed system at the lower CO_2% gas mixture were calculated halving the corresponding baseline value and then used to compute the PCO_2 values of this step. This was done since, in this step only, we obtained PCO_2 values overestimated by nearly 2 mmHg, which caused a paradoxical CO_2 content of the closed system higher than the corresponding CO_2 content of the open system. [a] $p < 0.001$ vs. baseline; [b] $p < 0.05$ vs. diluted closed system; [c] $p < 0.001$ vs. diluted closed system

Open system

The acid–base equilibrium, however, operates in systems open to lung gases, to metabolism and to kidney action.

We first considered, for simplicity, the effect of opening the system, after dilution, to alveolar gas. When the diluted solution (with pH identical to the one observed before the dilution) is open to gases with normal CO_2 tension of 40 mmHg, CO_2 enters into the system because of the different tensions between lung (40 mmHg) and the diluted solution (approximately 20 mmHg) until the CO_2 tension of the solution equals that of the gas phase. For this equilibrium to be reached, some additional CO_2 is hydrated to carbonic acid, reacts with the dissociated non-carbonic weak acids (A^-), and new bicarbonate is generated. Therefore, the "excess protons" observed in this dilutional acidosis come from CO_2 hydration. Considering Stewart's approach, since SID and A_{TOT} remain unchanged after opening the system to gases, the only determinant of the new pH is the increase in PCO_2 from 20 to 40 mmHg or, more precisely, the increase of CO_2 content (including the new bicarbonate). According to the

traditional model, the acidosis is due to the increased PCO_2 (of note, not only PCO_2, but also bicarbonate increases). When the system is open to gases, the same mechanisms are operating and observed with distilled water, saline and lactated Ringer's. This hypothesis is strongly supported by our experimental data in which the same extent of decreased pH was observed when the diluted system was open to the baseline CO_2.

In vivo, however, further changes should occur when the system is also open to metabolism. This refers to solutions in which an ion associated with the strong ions may undergo metabolism. The net positive charges of the strong ions in a solution such as lactated Ringer's are neutralized by lactate. Although in a closed system lactate behaves as a strong ion, in biological systems, it usually disappears from the solutions after metabolism either to glucose or to CO_2 and water. In this situation, two of the determinants of the acid–base equilibrium change, i.e., the strong ion difference (which increases due to lactate metabolism) and the CO_2 content (which increases due to the new equilibrium with gases). The effects of increased SID contrast the effect of increased PCO_2, resetting pH to more normal values. Similar reasoning applies to all crystalloid solutions in which the strong ions are associated with weak or metabolizeable ions, such as HCO_3^-, lactate, glutamate, malate or acetate. The SID effect will appear only after the metabolism of metabolizeable ions.

Furthermore, the system can also be opened to renal function. The kidneys deeply affect the whole equilibrium by manipulating both the strong ions and the fluid volumes. In the presence of acidosis, the kidneys usually react by excreting urine characterized by a lower SID than that of normal plasma. Typically, chloride is excreted associated with ammonium to retain sodium ions [19, 20]. According to Stewart's approach, this correction by the kidneys affects the acid–base balance by increasing plasma SID; according to the traditional approach, the excretion of chloride along with ammonium equals the excretion of a strong acid. Independently of electrolytes manipulation, a key factor is represented by the urine volume. If, for example, the kidneys excreted an infused volume at the same rate at which its infusion occurs, the SID, CO_2 content and A_{TOT} would remain unchanged, or, according to the traditional approach, PCO_2 and bicarbonate would maintain the same ratio and no acidosis would occur. Actually, in all the studies reporting "dilutional acidosis" in vivo, the infusion rate largely exceeded the urine output rate [21–23].

Therefore, if we want to approach the entire process in vivo, it is necessary to consider together endogenous production, infusion rate, urine output and quality of the infused solution, as we previously described in the summary equation [24]:

$$[\mathrm{SID}(t)] = \frac{V(0) * [\mathrm{SID}(0)] + \int_0^t \mathrm{EPR(t)dt} + \int_0^t \mathrm{IR(t)} * [\mathrm{SID_{infusion}(t)}]\mathrm{dt} * - \int_0^t \mathrm{UR(t)} * [\mathrm{SID_{urine}(t)}]\mathrm{dt}}{V(t)}$$

where $ERP(t)$ is the endogenous production rate (mEq/min), $IR(t)$ is the infusion rate, $UR(t)$ is the urine rate and $V(t)$ the volume of the extracellular fluid.

In conclusion, when adding a solution to the extracellular fluid, different aspects must be considered. First, for the acidosis to develop, the prerequisite is that the volume infused must largely exceed the urine output during the time considered. Second, the degree of acidosis is strictly related to the extent of the dilution, and the excess protons originate from CO_2 hydration. Third, the acidosis may be in part counteracted if ions such as lactate are metabolized. The final effect depends on all these interactions, and both Stewart and the traditional approach may well account for the observed results.

References

1. Kellum JA (1999) Acid–base physiology in the post-Copernican era. Curr Opin Crit Care 5:429–435
2. Corey HE (2003) Stewart and beyond: new models of acid–base balance. Kidney Int 64:777–787
3. Siggaard-Andersen O, Fogh-Andersen N (1995) Base excess or buffer base (strong ion difference) as measure of a non-respiratory acid–base disturbance. Acta Anaesthesiol Scand Suppl 107:123–128
4. Gattinoni L (2009) Foreword. In: Kellum JA, Elbers PWB (eds) Stewart's textbook of acid–base, 2nd edn. Lulu.com, Amsterdam, pp 21–23
5. Henderson LJ (1908) The theory of neutrality regulation in the animal organism. Am J Physiol 21:427–448
6. Siggaard-Andersen O, Engel K (1960) A new acid–base nomogram, an improved method for calculation of the relevant blood acid–base data. Scand J Clin Lab Invest 12:177–186
7. Grogono AW, Byles PH, Hawke W (1976) An in-vivo representation of acid–base balance. Lancet 1:499–500
8. Siggaard-Andersen O (1963) Blood acid–base alignment nomogram. Scales for pH, PCO_2, base excess of whole blood of different hemoglobin concentrations. Plasma bicarbonate and plasma total CO_2. Scand J Clin Lab Invest 15:211–217

9. Stewart PA (1981) How to understand acid–base. A quantitative acid–base primer for biology and medicine. Elsevier, New York
10. Kellum JA, Elbers PWB (2009) Stewart's textbook of acid–base. Lulu.com, Amsterdam
11. Shires GT, Holman J (1948) Dilutional Acidosis. Ann Intern Med 28:557–559
12. Asano S, Kato E, Yamauchi M, Ozawa Y, Iwasa M (1966) The mechanism of acidosis caused by infusion of saline solution. Lancet 1:1245–1246
13. Garella S, Chang BS, Kahn SI (1975) Dilution acidosis and contraction alkalosis: review of a concept. Kidney Int 8:279–283
14. Kellum JA (2002) Saline-induced hyperchloremic metabolic acidosis. Crit Care Med 30:259–261
15. Constable PD (2003) Hyperchloremic acidosis: the classic example of strong ion acidosis. Anesth Analg 96:919–922
16. Hastings AB, Sendroy J Jr (1925) The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid. J Biol Chem 65:445–455
17. Gattinoni L, Lissoni A (1998) Pathophysiology and diagnosis of respiratory acid–base disturbances in patients with critical illness. In: Bellomo R, Ronco C (eds) Critical care nephrology. Kluwer Academic Publishers, Dordrecht, pp 297–311
18. (1997) Appendix C: fluids and electrolytes. In: Civetta JM, Taylor RW, Kirby RR (eds) 2257, 3rd edn. Lippincott–Raven, Philadelphia/New York, p 2257
19. Ring T, Frische S, Nielsen S (2005) Clinical review: renal tubular acidosis–a physicochemical approach. Crit Care 9:573–580
20. Kellum JA (2000) Determinants of blood pH in health and disease. Crit Care 4:6–14
21. Scheingraber S, Rehm M, Sehmisch C, Finsterer U (1999) Rapid saline infusion produces hyperchloremic acidosis in patients undergoing gynecologic surgery. Anesthesiology 90:1265–1270
22. Takil A, Eti Z, Irmak P, Yilmaz GF (2002) Early postoperative respiratory acidosis after large intravascular volume infusion of lactated ringer's solution during major spine surgery. Anesth.Analg 95:294–298
23. Wilkes NJ, Woolf R, Mutch M, Mallett SV, Peachey T, Stephens R, Mythen MG (2001) The effects of balanced versus saline-based hetastarch and crystalloid solutions on acid–base and electrolyte status and gastric mucosal perfusion in elderly surgical patients. Anesth Analg 93:811–816
24. Gattinoni L, Carlesso E, Cadringher P, Caironi P (2006) Strong ion difference in urine: new perspectives in acid–base assessment. Crit Care 10:137

[Foreword]

Luciano Gattinoni

When I first heard a lecture on the physicochemical approach to acid-base equilibrium based on Stewart's work my first reaction was: "another useless and complicating addition to an already difficult topic". After studying it, however, I realized that the Stewart approach is the best way to understand and remember acid-base equilibrium per se, and, more importantly, its relationship with another key equilibrium of living organisms, the electrolytes.

Medical students bump into the acid-base equilibrium several times throughout their course of study, in chemistry, biochemistry, pathology, clinics and during the post-graduate courses, particularly when the acute stage of illness is the main topic. Unfortunately the acid-base equilibrium is usually forgotten as many times as it is taught. Usually, the knowledge of this topic is limited to the realization that pH may indicate alkalosis or acidosis, that Base Excess may assess the metabolic acidosis/alkalosis and that PCO_2 changes may be associated with respiratory changes. In different chapters of their physiology textbooks, these students study electrolyte equilibrium, learning about concepts such as hyper- or hypo- natremia, -chloremia, -kalemia and their clinical consequences. Acid-base equilibrium and electrolyte equilibrium are then taught, analyzed, learned and forgotten as part of two different worlds.

The great merit of Stewart's physical-chemical approach is to show that acid-base and electrolytes are part of the same picture. In a painting the details may be beautiful, but if the details are considered only by themselves one cannot appreciate the whole scene. In analogy, looking at Leonardo's famous painting "The Last Supper", the discussion between the three Apostles on the right or on the left corners does not give us the whole picture. I believe that Stewart's approach gives us the complete painting, while other approaches give us only the details. While these other approaches describe the same reality and are true, only the Stewart one provides a tool for fully understanding of the problem.

What is important in the "beautiful mind" of the physicochemical approach is, first, the distinction between independent and dependent variables, second the embedding of the acid-base equilibrium in the general equilibrium of water and electrolytes. Stewart identified three independent variables which may change the hydrogen concentration in water (i.e. the acid-base

equilibrium): the strong ion difference (SID), the total weak acid concentration (A_{TOT}) and the PCO_2 (which is nothing else than a different expression of another weak acid, the carbonic acid). The strong ion difference is regulated by the kidney, weak acid concentration primarily by liver, PCO_2 by the lung. All the independent variables are present in concentrations on the order of millimoles or milliequivalents and their interaction with water dictates the amount of free hydrogen ions, the concentration of which is in the order of nanomoles.

The best way to understand the architecture of the Stewart's model is to visualize a histogram with two columns: one including all the strong ions with positive charge, and the other one with all the strong ions with negative charge. The difference in electrical charge between these two columns is called strong ion difference. In normal plasma this amounts to about 42 mEq/L. Indeed, to reach the electroneutrality, 42 mEq of negative charged ions, different from strong ions, are required. These are basically the bicarbonate (HCO_3^-) and the negative charged form of the weak acids (A^-), primarily albumin plus an extremely small amount of hydroxyl (OH^-). The sum of $[HCO_3^-] + [A^-]$ which equals the strong ion difference was called "Buffer Base" by Singer and Hastings, and later by Siggaard-Andersen. Indeed the big difference between the Siggaard-Andersen's approach and the Stewart's approach is that the first considers what happens inside the buffer base domain. As an example, in the Siggaard-Andersen's model the normal buffer base (42 mEq/L as the normal [SID]) may decrease by 10 mEq/L if the $[A^-]$ and $[HCO_3^-]$ are consumed by adding 10 mEq of H^+. In this case the actual buffer base is 32 mEq/L and the difference between the actual buffer base and the ideal buffer base is equal to –10 mEq/L, this difference being called base excess. In the Stewart model the same problem is considered from another point of view. If a strong ion is added to the system the difference between the two columns changes. As an example, adding 10 mEq/L of lactate the strong ion difference decreases from 42 mEq/L to 32 mEq/L. The "space" available for A^- and HCO_3^- and OH^- decreases, indeed part of A^- will become AH, part of HCO_3^- will become H_2CO_3 and part of OH^- will become H_2O. As the product of H^+ and OH^- is constant, a decrease of OH^- will correspond to an increase of H^+, i.e. acidosis.

The disadvantage of Siggaard-Andersen's approach is that it implies adding or subtracting H^+ to the solution, which is impossible. The more general Stewart's approach explains the acid-base variations on a more valuable physical basis. As an example adding pure water to a solution in which the strong ion difference is 42 mEq/L will decrease [SID], while subtracting pure water will increase [SID]. In the first case acidosis develops, in the second case alkalosis. The Stewart's approach basically allows one to understand the acid-base equilibrium considering what enters the system, as an example lactate or other dietary strong ions assumed with food or infused in solution and what is eliminated from the system primarily with urine. This mass balance approach is not fully appreciated to date, but it is impossible using the Siggaard-Andersen or Henderson-Hasselbalch models.

This book includes the original chapters of Stewart's classic treatise and many other contributions relative to the acid-base disturbances in different clinical conditions. It will be clear to the reader how the physicochemical approach can be helpful in understanding mechanisms

barely understandable using the traditional approaches. Clinical acid-base equilibrium has been studied for more than 100 years. Nothing is definitive; however the physicochemical approach represents a further step in this rather complicated field -not just to compute the pH but to better understand the pathophysiology of the system.