

Crystalloid strong ion difference determines metabolic acid-base change during *in vitro* hemodilution

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Objectives: To determine the relationship between the strong ion difference (SID) of a diluting crystalloid and its metabolic acid-base effects on *in vitro* blood dilution.

Design: Prospective *in vitro* study.

Setting: University research laboratory.

Subjects: Normal human blood.

Interventions: Three solutions were prepared, each with $[Na^+] = 140$ mmol/L. $[Cl^-]$ for solutions 1, 2, and 3 was 120, 110, and 100 mmol/L, respectively, the other anion being HCO_3^- . SID values were thus 20, 30, and 40 mEq/L, respectively. Serial dilutions of well-oxygenated fresh venous blood were performed anaerobically by using each of solutions 1–3 as well as 0.9% saline (SID = 0 mEq/L) and Hartmann's solution (SID = -4 mEq/L).

Measurements and Main Results: Blood gas and electrolyte analyses were performed before and after each dilution. Apart from dilutions with solution 3 (crystalloid SID 40 mEq/L) during which plasma SID did not change, plasma SID decreased during hemodilution. In contrast, base excess increased during hemodi-

lution with solutions 3 and 2 (crystalloid SID 40 mEq/L and 30 mEq/L, respectively) and decreased only with the remaining three solutions. The relationships between hemoglobin concentrations and both plasma SID and whole blood base excess throughout dilution were linear, with slopes proportional to the SID of the diluent in each case. Linear regression revealed that the SID of crystalloid producing a zero base excess/hemoglobin concentration slope during blood dilution (i.e., no change in metabolic acid-base status) is 23.7 mEq/L.

Conclusions: On *in vitro* hemodilution, there is a simple linear relationship between diluent crystalloid SID and the rate and direction of change of plasma SID and whole blood base excess. Direct extrapolation to *in vivo* situations such as acute normovolemic hemodilution and large volume correction of extracellular fluid deficits requires experimental confirmation. (Crit Care Med 2002; 30:157–160)

KEY WORDS: crystalloid; hemodilution; metabolic acidosis; strong ion difference

When large volumes of intravenous saline are administered to correct extracellular fluid deficits, metabolic acidosis can result (1–3). Infusion-related metabolic acidoses have been described as “dilutional” (4–7), the concept being that extracellular $[HCO_3^-]$ is reduced by simple dilution with large volumes of non- HCO_3^- -containing fluid. Risky clinical scenarios are those where crystalloid fluids are commonly added to the vasculature in large volumes. They include normovolemic hemodilution, cardiopulmonary bypass, preload responsive shock states, multitrauma, burns, liver transplantation, diabetic ketoacidosis, and hyperosmolar nonketotic coma.

Stewart's physical-chemical approach provides further insight into the causation of infusion-related metabolic acidosis (8–10). In the Stewart analysis, pH

and $[HCO_3^-]$ are dependent variables determined by three independent variables: P_{CO_2} , strong ion difference (SID), and the total concentration of nonvolatile weak acid buffer (A_{TOT}). Strong ions such as Na^+ , K^+ , Cl^- , and lactate are essentially fully ionized *in vivo* at all physiologic concentrations and acid-base conditions, so that $SID = [strong\ cations] - [strong\ anions]$. Plasma A_{TOT} consists largely of albumin and inorganic phosphate, whereas hemoglobin is the predominant contributor to A_{TOT} in whole blood (11).

In normal plasma there is a surfeit of strong cations, so that plasma SID is approximately 40 mEq/L. This SID “space” is filled passively by the components of buffer base (12), consisting of the weak ions HCO_3^- and nonvolatile buffer anions (A^-), where $A_{TOT} = HA + A^-$. By definition, SID is numerically identical to the buffer base concentration (13). In metabolic acidosis, SID and buffer base are decreased, whereas in metabolic alkalosis they are increased. On the other hand, acute reductions in A_{TOT} cause

metabolic alkalosis, and elevations cause metabolic acidosis (14).

According to this model, crystalloid infusions can affect metabolic (nonrespiratory) acid-base balance in two ways—by altering SID and by reducing A_{TOT} . Although SID is a term normally applied to body fluids, it also seems logical to consider how the SID of a given crystalloid might determine its acid-base effects (15). All NaCl solutions have a zero SID, because Na^+ and Cl^- are present in equimolar concentrations. This is also true of dextrose in saline solutions. The SID of water, dextrose solutions, and mannitol is also zero, because no strong ions are present. Dilution of any body fluid (plasma, whole blood, or extracellular fluid) must alter the SID of that fluid toward that of the diluent. For example, large-volume infusion with zero SID fluids such as saline reduces plasma, whole blood, and extracellular SID, pushing acid-base balance in the direction of a metabolic acidosis (16, 17). Importantly, although this type of acidosis is commonly thought of as

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hyperchloremic, the phenomenon also can occur with a reduction in chloride concentrations, as demonstrated with mannitol infusions (18).

Apart from effects on SID, acute extracellular dilution with crystalloid reduces A_{TOT} , so that there is simultaneous production of a metabolic alkalosis. The final outcome is then a summation of the effects of altered extracellular SID and reduced A_{TOT} . In the case of large-volume infusions of crystalloids such as saline, SID reduction overwhelms the metabolic alkalosis of A_{TOT} dilution, and metabolic acidosis is the result. For a crystalloid not to produce acid-base disturbances during large-volume infusion, some SID reduction must be necessary to offset the metabolic alkalosis of A_{TOT} dilution, but the two processes should balance exactly.

Although these general principles can be stated, it is unclear whether the calculated SID of a crystalloid can be used in any practical sense to quantify its acid-base effects. The precise relationship between the SID of an infused crystalloid and its effect on metabolic acid-base status is difficult to determine in theory. This is because strong ions introduced into the vasculature are distributed among plasma, erythrocytes, and interstitial fluid in complex ways governed by Gibbs-Donnan equilibria and the laws of electroneutrality and of chemical equilibrium. We therefore devised an *in vitro* experiment to determine whether calculating crystalloid SID might find application as a means of quantifying its acid-base effects.

MATERIALS AND METHODS

We performed serial dilutions of oxygenated fresh blood by using five different crystalloid solutions with *in vitro* SID values ranging from -4 mEq/L to 40 mEq/L (Table 1). Solutions 1–3 were prepared fresh by admixture of 0.9% saline, 0.45% saline (Baxter Healthcare, Sydney, Australia), and 8.4% sodium bicarbonate (Astra Pharmaceuticals, Sydney, Australia) to a total of 100 mL in proportions calculated to produce the electrolyte concentrations set out in Table 1.

Venous blood was collected from two of the investigators (TJM and BV) in heparin-coated syringes. The policy of the Institutional Review Board is to allow the use of investigators' blood for experimental purposes. Each specimen then was divided into five aliquots of 6 mL. These were drawn into syringes and agitated with a gas mixture containing 95% oxygen and 5% CO_2 (Carbogen; BOC Gases Australia) until blood gas analysis at 37°C (ABL

Table 1. Electrolyte concentrations (mmol/L) and strong ion difference (SID) values (mEq/L) of five crystalloid fluids used as experimental diluents

	Hartmann's ^a	0.9% Saline ^a	Solution 1	Solution 2	Solution 3
Sodium	129	150	140	140	140
Chloride	109	150	120	110	100
Bicarbonate			20	30	40
Lactate	29				
Potassium	5				
Calcium	2				
SID	-4	0	20	30	40

^aBaxter Healthcare, Sydney, Australia.

625; Radiometer, Copenhagen, Denmark) confirmed that the oxygen tension exceeded 250 torr. The gas was expelled and the syringes were sealed with rubber stoppers and placed on ice. One experimental diluent fluid (Table 1) was allocated to each aliquot.

Four to five dilutions of each aliquot were then performed by serial anaerobic injections of 1 mL of diluent through the rubber stopper via a 23-gauge needle. After each admixture, the blood was agitated for 2 mins. Blood gas analysis at 37°C was performed before and after each admixture, and 1 mL was set aside in a plain glass vial for subsequent multichannel biochemical analysis.

Calculations. Whole blood base excess (BE) values were calculated by substituting plasma pH, blood P_{CO_2} , and oximetrically measured total hemoglobin concentrations ([Hb]) in the Van Slyke equation (19).

Plasma SID was calculated as the plasma buffer base concentration by using an adaptation by Schlichtig and colleagues (11) of the formula of Figge and coworkers (20):

$$SID = [\text{buffer base}] = [HCO_3^-] + [A^-],$$

where $[A^-] = [\text{albumin}] \times (1.16 \times \text{pH} - 5.83) + [\text{phosphate}] \times (0.42 \times \text{pH} - 1.27)$, and $[HCO_3^-] = 0.03 \times PCO_2 \times \text{antilog}(\text{pH} - 6.1)$ with [albumin] expressed in g/L, [phosphate] in mM, and PCO_2 in torr.

Data Analysis. A software program was used (Excel 97 for Windows; Microsoft, Redmond, WA). Correlations between variables were analyzed by linear regression. Significance was accepted at $p \leq .05$. Data are expressed as mean \pm sd.

RESULTS

Mean [Hb] values were 148 ± 7 g/L predilution and 65 ± 11 g/L after the final addition of diluent crystalloid. Mean predilution SID and BE values were 39.2 ± 2.0 mEq/L and -1.0 ± 0.7 mEq/L, respectively. The lowest blood PO_2 measurements were 333 torr predilution and 238 torr postdilution.

Effect of Hemodilution on SID. Apart from dilutions with solution 3 (crystalloid SID 40 mEq/L) during which plasma

SID did not change, plasma SID decreased during hemodilution in a linear relationship with [Hb] ($R^2 = .87-.91$; Fig. 1). SID reductions became more pronounced as crystalloid SID decreased.

Effect of Hemodilution on BE. BE was also linearly related to [Hb] in each series of dilutions, with R^2 values ranging from .81 to 1 (Fig. 1). However, BE increased during hemodilution with solutions 3 and 2 (crystalloid SID 40 mEq/L and 30 mEq/L, respectively) and only decreased with the remaining three solutions (Fig. 1).

The BE/[Hb] and SID/[Hb] slopes were linearly related to the diluent SID ($R^2 = 1$ and .98, respectively, Fig. 2). The equation of the BE/[Hb] slope regression was $BE/[Hb] \text{ slope} = 0.14506 - 0.00613 \times \text{diluent SID}$. By solving for a slope of zero, we thus determined that the diluent SID associated with no change in BE on dilution was 23.7 mEq/L.

DISCUSSION

In an *in vitro* blood dilution, we found simple linear relationships between the degree of hemodilution (as quantified by decreasing [Hb]) and both SID and BE, with the slopes of these relationships directly proportional to the SID of the diluent. If this also holds true *in vivo*, the SID of any given crystalloid might be used as a simple descriptor of its potential acid-base effects on large-volume infusion or during acute normovolemic hemodilution. The concept might also assist in developing crystalloid fluids designed to affect acid-base balance in precise and specific ways.

Our findings confirm that no change or a small reduction in plasma SID on hemodilution is associated with a progressive metabolic alkalosis (increasing BE) attributable to the predominance of the concurrent reduction in A_{TOT} (solutions 3 and 2; Fig. 1). The data thus

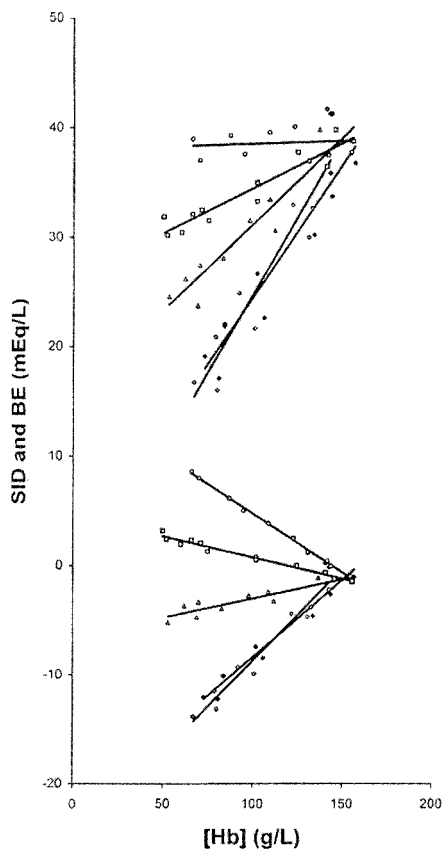


Figure 1. Concurrent effects on plasma strong ion difference (SID) and whole blood base excess (BE) of *in vitro* hemodilution by using five diluents with differing SID values. Diluents were solution 3 (SID, 40 mEq/L; open circles), solution 2 (SID, 30 mEq/L; open squares), solution 1 (SID, 20 mEq/L; open triangles), 0.9% saline (SID, 0 mEq/L; closed diamonds), and Hartmann's solution (SID, -4 mEq/L; open diamonds). Regression lines are shown (see text). Decreasing crystalloid SID increases the slopes of the SID/[Hb] and BE/[Hb] relationships. However, no solution increases plasma SID on hemodilution, whereas dilution with solutions 3 and 2 increases BE because of concurrent reduction in the total concentration of nonvolatile weak acid buffer. [Hb], hemoglobin concentration.

support the concept that hemodilution must significantly decrease plasma SID to maintain a neutral metabolic acid-base status. From our data, a crystalloid SID of 24 mEq/L is required to achieve this goal *in vitro*.

Of interest, this value is identical to the effective *in vivo* SID of Hartmann's solution, assuming that infused lactate is completely metabolized so that it no longer contributes to SID. Conversely, when considered purely as an *in vitro* preparation (i.e., without lactate metabolism), the SID of Hartmann's solution is -4 mEq/L. This is the value we assigned

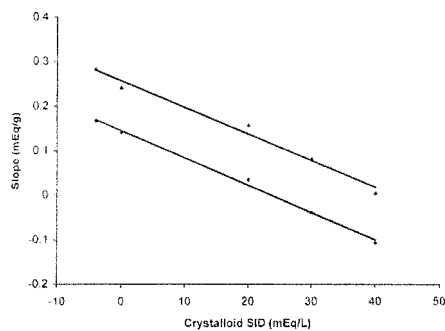


Figure 2. Linear relationship between base excess/hemoglobin concentration (diamonds) and strong ion difference (SID)/hemoglobin concentration (triangles) slopes and diluent SID. The regression lines are shown (see text).

to the solution for the *in vitro* experiment (Table 1). In all SID determinations, we followed the view that Ca^{2+} and Mg^{2+} should be categorized as weak ions because they bind reversibly to albumin in a pH-dependent manner (11). This is contrary to the widely held opinion that they are both strong ions (9).

A degree of hyperoxia was maintained throughout the period of data collection to prevent saturation-linked alterations in hemoglobin buffering capacity (21), and the blood also was cooled during this time to minimize endogenous lactate production. Changes in metabolic acid-base status during data collection thus were restricted as far as possible to the effects of hemodilution. [Hb] reduction was chosen rather than changes in [albumin] to quantify hemodilution and its relationship with BE. This is because hemoglobin is the major contributor to whole blood A_{TOT} , and BE is the relevant *in vitro* whole blood acid-base parameter. Although SID was determined in plasma rather than whole blood, there was also a clear linear relationship between plasma SID and the whole blood parameter [Hb] on hemodilution.

Direct extrapolation of these data to *in vivo* conditions is not automatic. An important disparity is that infused strong ions are distributed in the total extracellular space, whereas the components of A_{TOT} (especially hemoglobin) remain concentrated in the intravascular space. A modification of BE called standard base excess is the recommended clinical metabolic acid-base parameter because it more accurately quantifies *in vivo* metabolic acid-base changes (13). To emulate total extracellular buffering, standard base excess is BE calculated at a hemoglobin concentration of 50 g/L, which is

We conclude from this *in vitro* study that there is a simple linear relationship between diluent crystalloid strong ion difference (SID) and the rate and direction of change of plasma SID and whole blood base excess on hemodilution.

the approximate mean extracellular hemoglobin concentration. Standard base excess is demonstrably CO_2 -invariant *in vivo* (22), implying that although derived from *in vitro* data it successfully models *in vivo* buffering conditions. Standard base excess is thus the appropriate end point with which to quantify the *in vivo* acid-base effects of crystalloid hemodilution.

However, there is currently little detailed information on *in vivo* acid-base consequences of altering crystalloid SID. To our knowledge, the best available data come from an investigation into the acid-base effects of two different pump-prime fluids during cardiopulmonary bypass, one of which was a colloid-crystalloid mix and the other Plasmalyte 148 (23). Both priming fluids precipitated an immediate metabolic acidosis on commencement of bypass. With Plasmalyte 148, the acidosis was transient and attributable to unmeasured anions, presumably unmetabolized acetate and gluconate. By completion of bypass, the unmeasured anions had disappeared, and there was an overall trend toward a metabolic alkalosis (change in $\text{BE} = +1.15$ mEq/L). The effective *in vivo* SID of Plasmalyte 148 is 47 mEq/L assuming complete metabolism of acetate and gluconate. From this study it can only be said that a crystalloid SID of 47 mEq/L creates a metabolic alkalosis on hemodilution *in vivo*, and that the SID of a crystalloid with neutral acid-base characteristics must be less than this value.

Our findings relate exclusively to crystalloid fluids. We did not study the acid-base effects of *in vitro* colloid hemodilution. Albumin-containing preparations

are likely to have more complex effects, because any infused albumin contributes to A_{TOT} . Plasma expanders containing molecules such as hydroxyethyl starch may induce changes in acid-base balance that are less well explained by the Stewart approach (24). One "physiologically balanced" preparation containing hydroxyethyl starch is currently under trial (25). Information on its acid-base effects is still limited, but in an animal model of septic shock it produced less metabolic acidosis and longer survival as a resuscitation fluid compared with 0.9% saline (26).

We conclude from this *in vitro* study that there is a simple linear relationship between diluent crystalloid SID and the rate and direction of change of plasma SID and whole blood BE on hemodilution. Direct extrapolation to *in vivo* situations such as acute normovolemic hemodilution and large volume correction of extracellular fluid deficits requires experimental confirmation.

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