ILLUMINATIONS

In the pink and why so blue? A metabolic acidosis “shock-and-awe” demonstration

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Give people facts and you feed their minds for an hour. Awaken curiosity and they feed their own minds for a lifetime.
Ian Russell (4)

INTRODUCTION

The originality, spectacle, and inherent drama of “shock-and-awe” classroom demonstrations offer emotionally engaging scientific theater that stimulates significant student interest and a powerful emotional connection (2, 3). This influential “shock-and-awe” approach also exploits our primitive power of curiosity and has the potential to bring acid-base chemistry alive. Shock-and-awe demonstrations capture student attention, engage students, and help students understand, remember, and apply their knowledge in a different way. Accordingly, we used a “shock-and-awe” classroom demonstration to create scientific theater for understanding metabolic acidosis (2, 3).

Metabolic acidosis, both normal and wide anion gap metabolic acidosis, can be difficult and confusing to understand at first. However, it is crucial for nurses, physicians, respiratory therapists, physician assistants, and other healthcare providers to understand metabolic acidosis, because pH must be regulated within a narrow range, and understanding acid-base regulation can lead to a quicker and more accurate plan of care.

Background

To help students understand metabolic acidosis and acid-base regulation, we begin with a discussion of the source of acids. Students are told that metabolism of carbohydrates, proteins, and fats produce carbon dioxide (CO₂), and the CO₂ can form an acid, a base, and a proton. Specifically, the CO₂ will react with a water molecule to form carbonic acid (H₂CO₃; an acid), which will quickly dissociate into hydrogen (H⁺; a proton) and bicarbonate (HCO₃⁻; a base). Another source of acids is CO₂ in the colonic cells. CO₂ in the colonic cells will also form an acid and base. Inside the colonic cell, CO₂ and H₂O combine to form H₂CO₃, which dissociates into H⁺ and HCO₃⁻. The HCO₃⁻ is secreted into the lumen of the colon in exchange for chloride (Cl⁻), whereas the H⁺ is reabsorbed into the blood, acidifying the blood (Fig. 1). Loss of HCO₃⁻ from the colon through diarrhea forces the colonic cell to produce more HCO₃⁻, thus raising serum H⁺ levels (Fig. 1). When diarrhea occurs, normal anion gap metabolic acidosis can result (Fig. 2).

The anion gap. The anion gap is the difference between the measured serum cations (positively charged particles) and the measured serum anions (negatively charged particles). The commonly measured cation is sodium (Na⁺), and the commonly measured anions include Cl⁻ and HCO₃⁻.

According to the principle of electrical neutrality, the number of cations and anions should be equal. However, not all ions are routinely measured. The calculated anion gap result represents the unmeasured ions and primarily consists of anions. The anion gap formula is:

$$\text{Anion gap} = [\text{Na}^+] - ([\text{Cl}^-] + [\text{HCO}_3^-])$$

where brackets denote concentration.

Nonvolatile acids. We then move to a discussion of nonvolatile acids. Nonvolatile acids in the human body are described as any acid not produced from CO₂. We start with nonvolatile acids that increase the anion gap and discuss lactic acid. The source of protons (H⁺) from lactic acid is incomplete oxidation of carbohydrates (in hypoxic conditions). The source of the elevated and unmeasured anion is lactate, the base. Lactic acid causes a high anion gap metabolic acidosis in both children and adults (Fig. 3). The following equation summarizes the dissociation of lactic acid:

$$\text{Lactic acid} \rightleftharpoons \text{H}^+ + \text{Lactate}^-$$

After discussing lactic acid, we describe ketoacids. Specifically, excessive breakdown of fatty acids in the liver (in fasting states or uncontrolled diabetes) produces ketone bodies (ketoacids). Ketone bodies include acetoacetate, β-hydroxybutyrate, and their spontaneous breakdown product, acetone. Acetoacetate can be detected in the urine, whereas acetone can be detected in the breath that smells “sweet” or “fruity.” The source of the elevated and unmeasured anion is acetocetate. Acetoacetate also causes a high anion gap metabolic acidosis in both children and adults (Fig. 3). The dissociation of ketoacids is shown below:

$$\text{Acetoacetic acid} \rightleftharpoons \text{H}^+ + \text{Acetoacetate}^-$$

Several drugs and toxins can directly or indirectly cause a high anion gap metabolic acidosis. The three most common are methanol, ethylene glycol, and salicylate. Accordingly, we discuss aspirin (acetylsalicylic acid) overdose, which also causes a high anion gap metabolic acidosis in both children and adults (Fig. 3). The dissociation of acetylsalicylic acid is shown below:
Acetylsalicylic acid ⇌ H⁺ and Acetylsalicylate⁻

Finally, we note that renal failure can cause high anion gap acidosis by decreased acid excretion and decreased HCO₃⁻ reabsorption. Accumulation of sulfates, phosphates, urate, and hippurate accounts for the high anion gap.

Metabolic acidosis. At this point, we describe metabolic acidosis, both wide anion gap and normal anion gap metabolic acidosis. Metabolic acidosis is described as any process that acidifies the blood by adding a nonvolatile or other organic acid to the blood or removing HCO₃⁻ from the blood. Specifically, with metabolic acidosis, H⁺ is added to the blood, which acidifies the blood. Chemical buffers (bicarbonate buffer system, the phosphate buffer system, and the protein buffer system) minimize pH changes. These chemical buffers do not correct pH deviations, but serve to reduce the extent of the change. In addition, the hydration equation shifts to the left (Fig. 3). A leftward shift would be expected to reduce H⁺ and HCO₃⁻ and increase CO₂. However, normal ventilation reduces the CO₂ produced from the initial addition of acid. Furthermore, the acidic environment stimulates chemoreceptors, causing hyperventilation. Hyperventilation reduces CO₂. Students are told that they can expect arterial PCO₂ to decrease to a value (in Torr) = (1.5 × [HCO₃⁻]) + 8 ± 2 [Winters formula (1)]. Subsequently, the kidney produces “new HCO₃⁻,” which compensates for the increased [H⁺].

Wide anion gap metabolic acidosis. As mentioned earlier, incomplete oxidation of carbohydrates (in hypoxic conditions) produces lactic acid (lactic acid ⇌ H⁺ and lactate⁻), excessive breakdown of fatty acids in the liver (in fasting state or uncontrolled diabetes) produces ketone bodies (ketoads; acetoacetic acid ⇌ H⁺ and acetoacetate⁻), and consumption of aspirin produces acetylsalicylate (acetylsalicylic acid ⇌ H⁺ and acetylsalicylate⁻). Lactate, acetoacetate, and acetylsalicylate are unmeasured anions that increase the anion gap (Fig. 3). In each of these situations, H⁺ is added to the blood, which acidifies the blood. Again, chemical buffers (bicarbonate buffer system, the phosphate buffer system, and the protein buffer system) minimize pH changes. In addition, the hydration equation shifts to the left. A leftward shift would be expected to reduce H⁺ and HCO₃⁻ and increase CO₂. However, normal ventilation reduces the CO₂ produced from the initial addition of acid. Furthermore, the acidic environment stimulates chemoreceptors, causing hyperventilation. Hyperventilation reduces CO₂. The kidneys produce new HCO₃⁻ to compensate for the added H⁺ (Fig. 3).

Normal anion gap metabolic acidosis. As mentioned above, diarrhea causes a loss of HCO₃⁻. This leads to normal anion gap metabolic acidosis. The source of protons (H⁺) is from the production and secretion of HCO₃⁻ by the colonic cells. The mechanism of HCO₃⁻ secretion by the colonic cells is shown in Fig. 1. Inside the colonic cell, CO₂ and H₂O combine to form H₂CO₃, which dissociates into H⁺ and HCO₃⁻. The HCO₃⁻ is secreted into the lumen of the colon in exchange for Cl⁻, while
the H+ is reabsorbed into the blood, acidifying the blood. Loss of HCO3- from the colon through diarrhea forces the colon to produce more HCO3-, thus raising serum H+ levels (normal anion gap metabolic acidosis, Fig. 2).

Role of the kidney. The role of the kidney is then briefly discussed. The students are told that one way that the body can excrete a H+ is to combine it with HCO3- to form CO2 and H2O and then to exhale the CO2 and micturate out the water. However, using this strategy alone would rapidly deplete the body’s store of HCO3-. The lost HCO3- cannot be replaced by hydrating more CO2, because hydrating a CO2 would yield a new HCO3- as well as another H+, resulting in no net excretion of H+. This leaves the task of the kidney to produce new HCO3-. To do this, the renal tubular epithelial cells hydrate CO2 to synthesize H+ and HCO3-. The kidneys excrete this “new H+” in the urine, but the “new HCO3-” gets added to the body fluid. In body fluid, this “new HCO3-” combines with H+ that was formed from nonvolatile acids, and the resulting CO2 is exhaled by the lungs (Figs. 2 and 3).

The Demonstration

To demonstrate metabolic acidosis and the compensatory response to metabolic acidosis, we presented a shock-and-awe demonstration (2, 3). Before class, we prepared two solutions. For the first solution, 5 mL of universal indicator solution were added to a 1-liter sample of distilled water. The solution immediately displayed a yellow color, with a pH ~ 5.5. Tap water may be used; however, the color of the solution will be yellow or green/blue, depending on the acidity of the water. Next, 4 g of sodium bicarbonate (baking soda) were added to the solution and mixed on a stir plate. The color of the solution turned blue-violet (Fig. 4A). For the second solution, 20 g of sodium bicarbonate and 1–2 mL of indicator solution were added to 200 mL of distilled water and mixed on a stir plate (not shown). Finally, 5 mL of 1 N hydrochloric acid were drawn into a 5- or 10-mL syringe.

On arriving to class, the students observed the blue-violet solution and were told that the solution could be considered a surrogate for the arterial blood (Fig. 4A). The students were also told that the 5 mL of 1 N hydrochloric acid could be considered a surrogate for lactic acid, ketoacid, or aspirin (all three would add a H+ as well as conjugate base that is not measured and thus increase the anion gap), or H+ from the hydration of CO2 by the colonic cells (CO2 + H2O ⇌ H2CO3 ⇌ H+ + HCO3-). The HCO3- is secreted into the lumen of the colon in exchange for Cl-, while the H+ is reabsorbed into the blood, acidifying the blood. The addition of Cl- does not increase the anion gap. Specifically, the anion gap is the difference between the measured serum cations and the measured serum anions. The commonly measured cation is sodium,
and the commonly measured anions include Cl\(^–\) and HCO\(_3\)\(^–\). Thus, since Cl\(^–\) was added, the anion gap does not change.

At this point, a 10-in. balloon, filled with confetti, was placed over the opening of the bottle (Fig. 4A). Next, the hydrochloric acid was injected into the solution through the side port (Fig. 4B). As expected, the solution rapidly turned bright pink/red, and the balloon quickly filled with CO\(_2\) (Fig. 4B). The solution turned pink/red because the addition of H\(^+\) reduced the pH (pH = negative log (activity H\(^+\)). Ion activity is also known as the effective ion concentration. Solutions with a high effective concentration of H\(^+\) ions have a low pH, and solutions with a low effective concentration of H\(^+\) ions have a high pH. The balloon rapidly filled with CO\(_2\) because the H\(^+\) combined with the HCO\(_3\)\(^–\) and produced CO\(_3\)\(^2–\) (Figs. 2 and 3).

The balloon expansion, therefore, represents CO\(_2\) generation in a closed system and H\(^+\)/H\(_1\) buffering. The bottle’s HCO\(_3\)\(^–\) stores were on the way to becoming depleted (Figs. 3 and 4). Next the students were reminded of the role of ventilation (respiratory compensation) in acid-base regulation. Specifically, the acidic environment would stimulate chemoreceptors, causing hyperventilation. Hyperventilation would reduce CO\(_2\). Accordingly, at this point, the balloon was “popped,” making a loud noise and flying confetti about the room, contributing to the shock-and-awe appeal. Furthermore, CO\(_2\) was released, simulating hyperventilation (Fig. 4C).

A discussion of closed- and open-buffering systems is informative and important at this point, because open- and closed-buffering systems differ in their ability to buffer fixed and volatile acids. Popping the balloon represents a switch from a closed- to an open-buffering system. Specifically, popping the balloon released CO\(_2\) into the atmosphere. Similarly, in the body, CO\(_2\) is directly linked to the environment via the lungs (ventilation), and H\(^+\) is directly linked to the environment via the kidneys (urination). In this open system, CO\(_2\) and H\(^+\) are continuously removed from the body at rates that exactly match production. Thus, under normal circumstances, [CO\(_2\)] and [H\(^+\)] do not build up. Accordingly, with acid-base disturbances, changes in pH are much smaller in an open system than in a closed system. This is a major strength of the CO\(_2)/\text{HCO}_3^-\) buffering system.

Next, the students were reminded of the role of the kidney. Specifically, renal tubular epithelial cells hydrate CO\(_2\) to synthesize H\(^+\) and HCO\(_3\)\(^–\). The kidneys excrete this “new H\(^+\)” in the urine, but the “new HCO\(_3^-\)” gets added to the body fluid. In body fluid, this “new HCO\(_3^-\)” combines with H\(^+\) that was formed from acids, and the resulting CO\(_2\) is exhaled by the lungs. Thus, at this point, the second solution containing HCO\(_3^-\) was slowly added to the first solution through the side port using a 60-mL syringe (Fig. 4D). As expected, the solution slowly changed from red to orange to yellow to green and finally approached blue as the HCO\(_3^-\) combined with the H\(^+\) and produced more CO\(_2\) (Fig. 4D).

This simple, easy-to-perform demonstration offered emotionally engaging scientific theater, provoked intense interest, and provided a memorable learning experience. The success of this demonstration may be attributable, in part, to a powerful emotional connection. Basic emotions are shared by all humans. When we experience emotion in our lives, we tend to remember the experience. In fact, the more emotional impact an experience has, the more intensely we remember its details, and the more likely it will be stored in long-term memory (2, 3).

**Abbreviated Procedure of Metabolic Acidosis**

**Preparation.** The procedure is as follows:

1. Add 1 liter of distilled water to a 2.5-liter bottle with a side port.
2. Add 5 mL of universal indicator solution to the bottle. The solution should display a yellow appearance, indicating a pH of ~5.5.
3. Add 4 g of baking soda to the solution. The solution should display a blue-violet color.
4. In a second bottle or beaker, add ~20 g of sodium bicarbonate and 1–2 mL of indicator solution into 200 mL of distilled water. Withdraw the solution into a 60-mL syringe.
5. Place 5 mL of 1 N hydrochloric acid solution into a 5- to 10-mL syringe.

**Demonstration.** The procedure is as follows:

1. Place a balloon, filled with confetti, over the opening of the 2.5-liter bottle (Fig. 4A).
2. Using a 5- to 10-mL syringe, add 5 mL of 1 N hydrochloric acid through the bottle’s side port and observe the rapid change of color and the expansion of the balloon as it fills with CO\(_2\) (Fig. 4B).
3. Pop the balloon using an 18-gauge needle or similar sharp object, releasing all of the CO\(_2\) and confetti. Popping the balloon simulates hyperventilation (Fig. 4C).
4. Using a 60-mL syringe, add the second baking soda solution through the side port and observe the rapid change of color (Fig. 4D).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

H.L.L. and S.E.D. conceived and designed research; H.L.L., S.A.P., N.C.L., and S.E.D. performed experiments; H.L.L., S.A.P., N.C.L., and S.E.D. interpreted results of experiments; H.L.L., S.A.P., N.C.L., and S.E.D. prepared figures; H.L.L., S.A.P., N.C.L., and S.E.D. drafted manuscript; H.L.L., S.A.P., N.C.L., and S.E.D. edited and revised manuscript; H.L.L., S.A.P., N.C.L., and S.E.D. approved final version of manuscript.

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