Dilutional acidosis was first described in 1931 as a metabolic acidosis resulting from the rapid expansion of the extracellular space with a non–bicarbonate-containing solution.\(^1\) The description has since been broadened to include fluid administration with a concentration of a bicarbonate or bicarbonatelike anion (ie, lactate, acetate, gluconate, or citrate) that is less than the physiologically normal bicarbonate concentration of ECF.\(^2,3\) Despite major advances in information about acid-base physiology, there is still controversy regarding the mechanism behind dilutional acidosis. Traditional acid-base analysis suggests that direct dilution of the bicarbonate concentration while maintaining a constant P\(\text{CO}_2\) is responsible for the acidosis. However, bicarbonate concentration does not decrease to the degree that would be predicted from simple dilution.\(^2,4,5\) It has been suggested that new bicarbonate is generated during the rapid IV infusion of large volumes of fluids. The total quantity of extracellular bicarbonate is increased in the new volume, and the measured concentration is not reduced as much as would be expected from the dilution.\(^2,4,5\) In contrast to the traditional approach, the Stewart approach to acid-base analysis attributes dilutional acidosis to a concurrent decrease in SID and \(A_{\text{TOT}}\).\(^6\)

The impact of the in vitro addition of water to, and the removal of water from, plasma and whole blood has been evaluated in other studies\(^4,6\) conducted by our laboratory group. Results of those experiments revealed

**Effects of acute dilutional hyponatremia on acid-base changes and electrolyte concentrations in rats with bilateral renal pedicle ligation**

Kate Hopper, BVSc, PhD, and Steve C. Haskins, DVM

**Objective**—To describe the effects of increasing the extracellular fluid (ECF) volume by approximately 20% on acid-base changes and electrolyte concentrations in anesthetized rats.

**Animals**—18 adult male Sprague-Dawley rats.

**Procedures**—Rats were assigned to a control group (\(n = 6\) rats) and a treatment group (12). All rats were anesthetized, and instrumentation and bilateral renal pedicle ligation were performed. The treatment group was infused IV with sterile water throughout a 30-minute period. Acid-base variables and concentrations of electrolytes, lactate, albumin, phosphorus, and hemoglobin were measured before (baseline) and 30 and 60 minutes after onset of infusion. Anion gap, strong ion difference, strong ion gap, and contributions of sodium, chloride, albumin, phosphorus, and lactate concentrations to base excess were calculated at each time point.

**Results**—Infusion of sterile water led to an increase in ECF volume of approximately 18%. This had no effect on acid-base balance, compared with that in control rats. Infusion of sterile water caused a significant decrease in sodium, chloride, ionized calcium, lactate, and albumin concentrations, compared with concentrations in the control group. Anion gap and calculated effects of sodium, chloride, albumin, and lactate concentrations on base excess at 60 minutes differed significantly between infused and control rats.

**Conclusions and Clinical Relevance**—Infusion of sterile water did not cause clinically relevant dilutional acidosis. The acidic impact of water administration was offset by generation of new bicarbonate via carbonic acid equilibration and intracellular buffering in combination with the alkalotic effects of decreases in albumin, phosphorus, and lactate concentrations. (Am J Vet Res 2010;71:967–975)

\(^{\text{AG}}\) Anion gap

\(^{\text{ATOT}}\) Total quantity of weak acids

\(^{\text{ECF}}\) Extracellular fluid

\(^{\text{pHadj}}\) pH value adjusted to a P\(\text{CO}_2\) of 40 mm Hg at a predetermined standardized base excess

\(^{\text{SBE}}\) Standardized base excess

\(^{\text{SID}}\) Strong ion difference

\(^{\text{SIDapp}}\) Apparent strong ion difference

\(^{\text{SIDeff}}\) Effective strong ion difference

\(^{\text{SIG}}\) Strong ion gap

Abbreviations

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that neither simple dilution of the bicarbonate concentration nor a decrease in calculated SID provided a sufficient explanation for the changes detected. In contrast to in vitro experiments, the effect of water administration in vivo is complicated by fluid and electrolyte redistribution as well as interstitial and intracellular buffering. The purpose of the study reported here was to analyze via both the traditional and Stewart methods the acid-base effects of water administration in vivo in rats. It was our hypothesis that the acid-base effect of water administration in vivo in the absence of renal function could not be explained simply by dilution of bicarbonate concentrations or changes in the SID and that the magnitude of acid-base changes would be less than that detected for in vitro experiments, given the greater buffering potential available.

**Materials and Methods**

**Animals**—Eighteen adult male Sprague-Dawley rats were used in the experiments. Rats weighed between 260 and 350 g. The experimental protocol was approved by the University of California-Davis Institutional Animal Care and Use Committee.

**Procedures**—Anesthesia was induced in each rat by administration of pentobarbital sodium (50 mg/kg, IP). A 25-gauge catheter then was inserted into the coccyegeal vein, and a constant rate infusion of pentobarbital (1 to 4 mg/kg/h) was used to maintain anesthesia. Tracheostomy was performed, and the left jugular vein and right carotid artery were each cannulated with a 22-gauge catheter. Rats had spontaneous respiration throughout the experiment. Rectal temperature was monitored continuously and maintained at 37°C, and a Doppler crystal was placed over the ventral aspect of the base of the tail to monitor heart rate. Midline laparotomy and bilateral ligation of the renal artery and vein were performed in all rats, and the abdominal cavity was closed in a routine manner.

Rats were assigned into 2 groups; there were 6 rats in the control group and 12 rats in the treatment group. An arterial blood sample was obtained at time 0 (ie, baseline), and blood gas and electrolyte concentrations (including the serum sodium concentration) were measured. The 12 treatment rats were infused with sterile water via a syringe pump during a 30-minute period with the intention to reduce the baseline serum sodium concentration by 20%. The volume of water infused was calculated as follows: volume of water administered (in L) = 0.6 X body weight (in kg) X 0.2. No water was administered to the 6 control rats.

In addition to the arterial blood sample collected from all rats at time 0 (ie, before onset of the infusion of sterile water), arterial blood samples were also collected at 30 and 60 minutes after onset of the sterile water infusion, which corresponded to the completion of the 30-min infusion period and 30 minutes after completion of the infusion, respectively. Partial pressures of blood gases and concentrations of lactate, sodium, potassium, chloride, and ionized calcium were measured by use of an automated analyzer. Hemoglobin concentration was measured by photometric determination, and phosphorus and albumin concentrations were measured by use of an automated analyzer. The PCV was also measured at all time points. Values for SBE and bicarbonate concentration as calculated by the analyzer were recorded. The pHadj was derived with an alignment nomogram. The AG, SIDapp, SIDeff, and SIG; specific contributions of sodium, chloride, lactate, phosphorus, and albumin; the sum of quantitative contributions; and contributions of unmeasured anions were calculated by use of equations (Appendix). Mean baseline albumin and phosphorus concentrations were used as the value for the calculation of the contributions of albumin and phosphorus, respectively.

**Statistical analysis**—Mean and SD of the data were calculated. Differences between values at baseline, 30 minutes, and 60 minutes were analyzed by use of a repeated-measures ANOVA. Significant differences were identified by use of the Bonferroni (all pairwise) multiple comparison test. All statistical tests were performed by use of statistical software. Values of P < 0.05 were considered significant.

**Results**

**Control group**—Mean body weight of rats in the control group was 318 g. From baseline to 60 minutes, there was a significant decrease in pHadj (from 7.435 to 7.380), SBE (from 2.2 to –0.2 mEq/L), PO4 (from 38 to 31 mm Hg), sodium concentration (from 145 to 143 mEq/L), and bicarbonate concentration (from 26 to 23 mEq/L). Potassium, chloride, ionized calcium, lactate, and phosphorus concentrations did not change over time. Albumin concentration, hemoglobin concentration, and PCV all decreased significantly over time in the control rats (Table 1).

The AG and SIG did not change significantly over time in the control rats, whereas both SIDapp and SIDeff decreased significantly (from 4.7 to 4.18 mEq/L and from 4.15 to 3.66 mEq/L, respectively). At 60 minutes, the control rats had an acidotic change in the calculated sodium contribution (–0.7 mEq/L) and the calculated chloride contribution (–2.8 mEq/L). These changes were offset by an alkalotic change in the albumin contribution (1.7 mEq/L) and an extremely small alkalotic change in the lactate and phosphorus contributions (0.6 mEq/L and 0.3 mEq/L, respectively). The net sum of these contributions at 60 minutes was zero, and there was an acidotic change in the unmeasured anion contribution from 1.3 mEq/L at baseline to –0.2 mEq/L at 60 minutes (Table 2).

**Treatement group**—Mean body weight of the rats in the treatment group was 303 g. Mean ± SD volume of sterile water administered was 42 ± 7.0 mL, which was a mean dose of 139 mL/kg for each treated rat. The pHadj decreased significantly from baseline to 60 minutes. The pHadj decreased from 7.411 to 7.374, the PO4 decreased from 39 to 32 mm Hg, the SBE decreased from 0.5 to –1.0 mEq/L, and the bicarbonate concentration decreased from 25 to 22 mEq/L. Sodium, potassium, chloride, ionized calcium, lactate, phosphorus, albumin, and hemoglobin concentrations and PCV all decreased significantly from baseline to 60 minutes (Table 1).
The AG, SIDapp, and SIDeff decreased significantly from baseline to 30 minutes. The AG decreased from 16.9 to 14.1 mEq/L, the SIDapp decreased from 40.4 to 37.7 mEq/L, and the SIDeff decreased from 40.1 to 35.4 mEq/L. Only the SIDeff was significantly different from the baseline value at 60 minutes. The SIG increased significantly from 0.3 mEq/L at baseline to 3.7 mEq/L at 60 minutes. At 60 minutes, the rats infused with sterile water had an acidic change in calculated sodium contribution of –6.7 mEq/L and had alkalotic changes in the chloride contribution (3.8 mEq/L), lactate contribution (1.8 mEq/L), phosphorus contribution (0.9 mEq/L), and albumin contribution (2.7 mEq/L). These contributions combined to cause an overall alkalotic change (sum, 2.5 mEq/L) from baseline to 60 minutes. Contribution of the unmeasured anions decreased significantly from 3.9 mEq/L at baseline to 0 mEq/L at 60 minutes (Table 2).

Treatment group versus control group—At baseline, most values did not differ significantly between the treatment and control groups for any variables; however, chloride concentration, calculated chloride contribution, sum of the quantitative contributions, SIDapp, and SIG did differ significantly (P = 0.01). Mean chloride concentration at baseline for the treatment group was 107 mEq/L, which was significantly higher than the concentration of the control group (103 mEq/L) at baseline. The calculated chloride contribution at baseline for the treatment group was an acidic effect of –0.6 mEq/L, compared with an alkalotic chloride contribution of 3.4 mEq/L at baseline for the control group. The sum of the quantitative contributions was significantly lower at baseline for the treatment group (~3.5 mEq/L) than for the control group (0.9 mEq/L), and both SIDapp and SIG were significantly lower at baseline for the treatment group (40.4 mEq/L and 0.3 mEq/L, respectively), compared with values for the control group (44.7 mEq/L and 3.2 mEq/L, respectively).

At 30 minutes, there was a significant decrease in 

Table 1—Mean ± SD values for acid-base analysis; concentrations of electrolytes, lactate, phosphorus, albumin, and hemoglobin; and PCV in untreated (control) rats and rats infused IV with sterile water.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 6 rats)</th>
<th>Treatment (n = 12 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 minutes</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.44 ± 0.03</td>
<td>7.48 ± 0.04</td>
</tr>
<tr>
<td><strong>pHadj</strong></td>
<td>7.435 ± 0.03a</td>
<td>7.405 ± 0.04ab</td>
</tr>
<tr>
<td><strong>PCO2 (mm Hg)</strong></td>
<td>38 ± 0.50</td>
<td>33 ± 0.53</td>
</tr>
<tr>
<td><strong>Bicarbonate (mEq/L)</strong></td>
<td>26 ± 1.9</td>
<td>24 ± 2.1</td>
</tr>
<tr>
<td><strong>SBE (mEq/L)</strong></td>
<td>2.2 ± 1.6</td>
<td>1.6 ± 1.9</td>
</tr>
<tr>
<td><strong>Sodium (mEq/L)</strong></td>
<td>145 ± 1.9a</td>
<td>143 ± 2.5a</td>
</tr>
<tr>
<td><strong>Potassium (mEq/L)</strong></td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td><strong>Chloride (mEq/L)</strong></td>
<td>103 ± 1.8</td>
<td>104 ± 2.6</td>
</tr>
<tr>
<td><strong>Ionized calcium (mmol/L)</strong></td>
<td>0.86 ± 0.03</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td><strong>Lactate (mmol/L)</strong></td>
<td>2.7 ± 0.5</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td><strong>Phosphorus (mg/dL)</strong></td>
<td>8.2 ± 0.9</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td><strong>Albumin (mg/dL)</strong></td>
<td>3.8 ± 0.4a</td>
<td>3.4 ± 0.2c</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dL)</strong></td>
<td>14.1 ± 1.5a</td>
<td>13.2 ± 1.3c</td>
</tr>
<tr>
<td><strong>PCV (%)</strong></td>
<td>44 ± 2.6</td>
<td>38 ± 4.2</td>
</tr>
</tbody>
</table>

Baseline is time 0 (ie, before onset of the infusion of sterile water); 30 and 60 minutes correspond to the completion of the 30-minute infusion period and 30 minutes after completion of the infusion, respectively.

*Within a row, value differs significantly (P < 0.05; 1P = 0.01) from the value for the control group at the same time point.

Table 2—Mean ± SD values for AG, SID variables, and specific contributions to SBE in untreated (control) rats and rats infused IV with sterile water.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 6 rats)</th>
<th>Treatment (n = 12 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 minutes</td>
</tr>
<tr>
<td><strong>AG (mEq/L)</strong></td>
<td>19.8 ± 2.8</td>
<td>18.8 ± 2.7</td>
</tr>
<tr>
<td><strong>SIDapp (mEq/L)</strong></td>
<td>44.7 ± 2.8a</td>
<td>42.7 ± 2.7b</td>
</tr>
<tr>
<td><strong>SIDeff (mEq/L)</strong></td>
<td>41.5 ± 2.9a</td>
<td>38.6 ± 2.0b</td>
</tr>
<tr>
<td><strong>SIG (mEq/L)</strong></td>
<td>3.2 ± 3.4</td>
<td>4.1 ± 2.3</td>
</tr>
<tr>
<td><strong>Sodium contribution (mEq/L)</strong></td>
<td>0 ± 0.5a</td>
<td>0.4 ± 0.7b</td>
</tr>
<tr>
<td><strong>Chloride contribution (mEq/L)</strong></td>
<td>3.4 ± 2.2a</td>
<td>1.3 ± 2.2b</td>
</tr>
<tr>
<td><strong>Albumin contribution (mEq/L)</strong></td>
<td>0.1 ± 1.9</td>
<td>1.2 ± 0.6a</td>
</tr>
<tr>
<td><strong>Phosphorus contribution (mEq/L)</strong></td>
<td>0.1 ± 0.5</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td><strong>Lactate contribution (mEq/L)</strong></td>
<td>-2.7 ± 0.5</td>
<td>-2.1 ± 0.7</td>
</tr>
<tr>
<td><strong>Sum of quantitative contributions (mEq/L)</strong></td>
<td>0.9 ± 3.0</td>
<td>0.5 ± 2.5</td>
</tr>
<tr>
<td><strong>Unmeasured anion contribution (mEq/L)</strong></td>
<td>1.3 ± 3.2</td>
<td>0.6 ± 2.0</td>
</tr>
</tbody>
</table>

See Table 1 for key.
utes, compared with values in the control group, and a significantly \( P = 0.01 \) larger acidotic sodium contribution was evident at 30 minutes for the treatment group, compared with the value for the control group (Table 2).

At 60 minutes, the sodium, chloride, ionized calcium, lactate, and albumin concentrations were all significantly lower in the treatment group than in the control group (Table 1). The AG was significantly \( P = 0.01 \) lower in the treatment group at 60 minutes, compared with the AG in the control group at that same time. An acidotic sodium contribution, alkaliotic chloride contribution, and alkaliotic albumin contribution were all significantly greater at 60 minutes in the treatment group, compared with those contributions in the control group, whereas the lactate contribution was significantly less acidotic at 60 minutes in the treatment group, compared with that in the control group at the same time (Table 2).

**Discussion**

The purpose of the study reported here was to increase the ECF volume of anesthetized rats by approximately 20% via infusion of sterile water to generate dilutional acidosis without administration of any electrolytes or organic anions. This degree of fluid dilution was chosen to create a substantial and clinically relevant decrease in sodium concentration. To increase the ECF volume by 20% via the infusion of sterile water, it is necessary to increase total body water by 20% because water will equilibrate across both the extracellular and intracellular compartments. Initial volume of total body water was estimated at 60% of body weight, as has been reported for young Sprague-Dawley rats. The accuracy of estimation of the volume of total body water in sexually intact animals is affected by age and fat content and is expected to be somewhat variable among individual animals. Hence, our calculations for the volume of water to infuse were based on an assumption of a typical rat and may have led to some variation for specific rats in the present study.

The degree of expansion of the ECF volume achieved in this study was estimated from the change in serum sodium concentration, which decreased from 145 to 119 mEq/L after infusion of sterile water. This represents an approximate increase in ECF volume of 18% and was achieved by the administration of a mean of 139 mL of sterile water/kg. We assumed that the intracellular-to-extracellular volume ratio was 2:1 in the rats used for this study; therefore, effects on expansion of the ECF volume for this dose of sterile water would approximate those for administration of 46 mL of an isotonic crystalloid solution/kg. Administration of water was performed over a 30-minute period in the treatment group to allow time for fluid redistribution to avoid severe lysis of erythrocytes and acute cerebral edema. Acid-base status was evaluated immediately after the completion of the water infusion (30 minutes) and again at 60 minutes to ensure sufficient time was provided for complete equilibration and buffering.

We chose to perform these experiments in rats with bilateral renal pedicle ligation to allow evaluation of the acid-base effects of the administration of water to the body and the subsequent extracellular and intracellular buffering in a static system, without the influence of renal acid-base manipulations. Bilateral renal pedicle ligation is a recognized method for removing renal function in research animals and has been used in other acid-base studies.

The rats in the control group developed respiratory alkalosis marked by an increase in measured pH and a decrease in \( \text{PACO}_2 \). The cause of the respiratory alkalosis in these spontaneously breathing anesthetized rats was not obvious. Potential causes of hyperventilation in these rats include collapse of small airways and alveoli or airway irritation as a result of the tracheostomy tube. The metabolic contribution to acid-base balance can be evaluated by adjusting the pH (ie, pHadj). In the control group, the pHadj and SBE both decreased by a small but significant amount, which indicated metabolic acidosis. The cause of this metabolic acidosis was attributed, at least in part, to retention of hydrogen ions following renal pedicle ligation. There was also likely to be some degree of ECF dilution as indicated by decreases in albumin and phosphorus concentrations and PCV in the control group. These dilutional effects are likely to have been the result of fluids we were obligated to administer to flush the catheters, despite our best efforts to minimize the volume used for the flushes. Plasma water may have been further increased as a result of increases in ECF volume and redistribution of fluid from the interstitial space. These effects can be attributable to decreases in capillary hydrostatic pressure as a consequence of anesthesia as well as the hyperosmolar nature of pentobarbital. The magnitude of the metabolic acidosis detected in the control group (pHadj decreased from 7.435 to 7.380, and SBE decreased by \(-2 \text{mEq/L}\) is considered clinically unimportant. Similar to the control animals, the treatment group also developed respiratory alkalosis and metabolic acidosis. There was no difference in the magnitude of this metabolic acidosis between the 2 groups.

The rapid expansion of ECF volume as a result of administration of fluids with a bicarbonate or bicarbonate-atlike anion concentration less than that of the ECF can cause metabolic acidosis. This has been reported in research animals and human clinical patients. Dilutional acidosis has been generated with the administration of free water, isotonic crystalloid solutions, and hypertonic crystalloid solutions. The reported doses of isotonic crystalloid solutions associated with dilutional acidosis vary from 30 to 300 mL/kg. In other studies conducted by our laboratory group, we found that a 20% dilution of plasma or whole blood in vitro via administration of sterile water causes significant metabolic acidosis. It was our hypothesis that a similar degree of dilution in vivo with sterile water would also cause significant metabolic acidosis, but that the magnitude of this acidosis would be less than that for the in vitro studies because of interstitial dilution and intracellular buffering.

In contrast to the aforementioned in vitro studies, a dilution of approximately 20% in the ECF volume via the administration of sterile water in the present study had no significant acid-base effects at 30 or 60 minutes, compared with results for the control group.
The magnitude of dilutional acidosis will depend on the volume of fluid administered and the time provided for interstitial and intracellular fluid redistribution and buffering reactions. Reports of dilutional acidosis are associated with rapid administration of large fluid volumes with minimal time for fluid equilibration. For example, dogs (body weight not reported) receiving saline (0.9% NaCl) solution at a rate of 300 mL/min had substantial acidosis during the 5 minutes of the infusion. Human cardiopulmonary bypass patients can develop a significant decrease in pH and bicarbonate concentration within the first 2 minutes after beginning infusion of isotonic saline solution at a dosage of approximately 20 mL/kg. When adequate time is provided for acid-base buffering, the degree of acidosis may be minimal. The human cardiopulmonary bypass patients in the aforementioned study regained a physiologically normal metabolic acid-base balance by the end of the surgical procedure. In a study comparable to the study reported here, isotonic saline solution (50 mL/kg) was administered to nephrectomized dogs over a 30-minute period to increase ECF volume by 24%; however, no significant change in bicarbonate or hydrogen ion concentration was evident 60 minutes after the infusion. In another study of sexually intact dogs, ECF expansion was achieved by administration of an isotonic saline solution over a 2-hour period, and an additional 3 hours was allowed for equilibration. Despite generating increases in ECF volume of 24% to 38%, there was a minimal decrease in pH and bicarbonate concentration.

If volume dilution of the ECF is large enough, it will overwhelm the interstitial and intracellular buffering ability. Significant dilutional acidosis has been reported following administration of isotonic crystalloid solutions at a dosage of approximately 100 to 250 mL/kg. The administration of an equivalent volume of sterile water was precluded in the present study because it would have caused lethal cerebral edema. For this reason, it is unlikely that administration of sterile water alone will cause clinically relevant dilutional acidosis. In contrast, there is a potential to administer large volumes of isotonic crystalloid solutions, and this may be associated with metabolic acidosis.

In addition to redistribution of fluid and buffering reactions, dilution of albumin concentrations will further minimize the acidotic effect of ECF dilution. Albumin acts as a weak acid, and it has many hydrogen ion binding sites associated with the imidazole group of the amino acid histidine. Consequently, a decrease in serum albumin concentration will have an alkalotic influence on metabolic acid-base balance.

In the present study, the administration of sterile water caused a decrease of approximately 20% in the concentration of many ECF constituents, such as sodium, chloride, and albumin; however, there was not a significant decrease in bicarbonate concentration or SBE, compared with results for the control group. This can be explained by the generation of new bicarbonate. This new bicarbonate is the product of numerous extracellular and intracellular buffering reactions. A buffer will resist changes in pH by binding or releasing hydrogen ions. Quantitatively, the most important buffering system in the ECF of mammals is the carbonic acid system. Following ECF dilution with water, the carbon dioxide in ECF would rapidly re-equilibrate, and the law of mass action would result in shifting of the reaction toward the generation of hydrogen and bicarbonate ions. The hydrogen ions would then be buffered by movement into cells or by binding to plasma proteins or bone. The result is an increase in extracellular bicarbonate content and minimal decreases in pH. In other in vitro studies conducted by our laboratory group, a 20% dilution of plasma or whole blood by the administration of sterile water caused a significant metabolic acidosis, in contrast to the present study in which there was no significant change in pHadj. The in vivo buffering of the interstitial and intracellular compartments far exceeded that for the in vitro studies; hence, a significant acidosis was prevented despite a similar degree of dilution.

The administration of sterile water to the rats in the present study caused an 18% decrease in the serum sodium concentration, which was attributed to dilution. Intracellular buffering of an acid involves movement of hydrogen ions into cells. To maintain electroneutrality, these ions need to be exchanged for an intracellular cation (such as sodium or potassium), or they can be cotransported with an anion (such as chloride), as happens in RBCs via the band-3 protein. Another major buffer is bone because hydrogen ions bind to bone in exchange for surface sodium and potassium ions. As a consequence, intracellular and bone buffering reactions are associated with changes in electrolyte concentrations. In a study of nephrectomized dogs, extracellular sodium content increased by approximately 3.6 mEq/L following IV administration of hydrochloric acid at a dosage of 10 mmol/kg. The effective buffering of the acidosis associated with ECF dilution that was detected in the present study would also have been expected to cause an increase in the ECF sodium concentration. For this reason, the decrease in sodium concentration recorded in the present study may have been less than that attributable to dilution alone.

In contrast to sodium and chloride concentrations, the concentration of potassium increased following administration of sterile water to rats in the study reported here. The increase of 10% in potassium concentration despite dilution is attributed to movement of potassium ions out of cells in response to intracellular buffering of hydrogen ions. In addition, although there was no visible hemolysis in the blood samples collected, administration of a hypotonic fluid is likely to have caused some lysis of RBCs, which may have further increased the potassium concentration.

The lactate concentration decreased a small amount over time in the control group, which was attributed to a decrease in anaerobic metabolism in the unstimulated, anesthetized rats. The lactate concentration at baseline may have remained slightly elevated as a consequence of the muscle activity associated with anesthetic induction. Following administration of sterile water, there was a decrease of >20% in the lactate concentration, which was thought to have been attributable to the combined effects of a decrease in the rate of lactate production as well as dilution.
Both albumin and hemoglobin concentrations decreased over time in the control group, which was attributed to the combined effects of blood loss as a consequence of collection of blood samples and the dilutional effects from the flushing of catheters and fluid redistribution. Pentobarbital-induced anesthesia in dogs has been reported to increase ECF volume and cause a gradual decrease in Hct and protein concentration over time. Despite limiting collection of blood samples to a minimum, it was impossible to avoid a certain degree of blood loss in the rats of the present study. In combination with fluid redistribution, this would have further contributed to the decreases in albumin and hemoglobin concentrations detected. There was a greater decrease in albumin and hemoglobin concentrations following administration of sterile water than that detected in the control group, although this was not a significant difference. This pattern can be attributed to the effects of dilution in addition to the effects of collection of blood samples and anesthesia. In contrast to hemoglobin concentrations, the PCV decreased similarly in both the control and treatment groups. This was attributed to RBC swelling subsequent to administration of hypotonic fluid in the treatment group.

A small but significant decrease was detected in the AG at 30 and 60 minutes after administration of sterile water in the treatment group. There was no change in the AG over time in the control group. Because administration of sterile water caused a quantitatively greater decrease in the sodium concentration than in the chloride and bicarbonate concentrations, the calculated result was a lower number than that determined for the control rats.

According to traditional acid-base analysis, dilutional acidosis is attributable to decreases in bicarbonate concentration. In the present study, extensive buffering by non–bicarbonate-buffering systems prevented bicarbonate depletion despite dilution and prevented the development of acidosis. The Stewart method of acid-base analysis does not consider bicarbonate concentration as a determinant of pH. Instead, the 3 independent determinants of pH for the Stewart method are $PCO_2$, SID, and $A_{TOT}$. The SID and $A_{TOT}$ are proposed to alter pH by virtue of their net charge exerting a force to alter water dissociation, which (according to the Stewart approach) ultimately determines the hydrogen ion concentration of the body. There is no evidence that these variables influence water dissociation to any clinically relevant degree. In many clinical situations, SID and $A_{TOT}$ may be used as markers of certain acid-base processes. The SID can be used to identify changes in bicarbonate concentration that develop in association with changes in sodium or chloride (or both) concentration. For example, when hydrochloric acid is added to a blood sample, the quantity of added hydrogen ions can be quantitated by measuring the change in chloride concentration. The change in SID in this situation would accurately represent the acidotic influence that was attributable to the addition of the hydrochloric acid. This does not mean that the SID caused the change in acid-base balance. The measure referred to as $A_{TOT}$ is actually a measure of the influence of phosphorus and albumin concentrations on pH. Both of these substances are weak acids and can have a direct effect on pH, although not by changing water dissociation as suggested by supporters of the Stewart method, but rather by causing increases or decreases in hydrogen ion concentration associated with increases or decreases in albumin or phosphorus (or both) concentration.

According to the Stewart method, dilutional acidosis is attributable to decreases in SID most commonly resulting from the administration of fluids (eg, saline solution) with a high chloride concentration. The magnitude of dilutional acidosis will be offset by concurrent dilutional decreases in $A_{TOT}$. There are 2 methods for the calculation of SID. The SID can be estimated by subtracting the total measured strong anion concentration from the total measured strong cation concentration. Because there is usually a greater number of measured strong cations than strong anions, there is a calculated SID. This calculated difference is the SIDapp. In the present study, the SIDapp decreased a small but significant amount in both groups but there was no significant difference between the groups. The pHadj also decreased significantly in each group, but the changes did not differ significantly between the 2 groups. In the control group, the chloride concentration increased (but not significantly), whereas the sodium concentration decreased a small but significant amount; thus, the result was a decrease in SIDapp. In the treatment group, the chloride concentration decreased to a greater extent than did the sodium concentration and the potassium concentration increased. The slightly disproportionate changes in electrolyte concentrations detected after administration of the sterile water accounted for the extremely small decrease in SIDapp. When the major electrolytes associated with an acid-base process (eg, buffering) are included in the SID calculation, it can be an effective marker of acid-base status, as described in the aforementioned example for hydrochloric acid and as indicated by the results of the present study.

The SIDapp is an estimate of the unmeasured (not included in the calculation) anions and cations. Anions not included in the SIDapp equation (Appendix) are mostly bicarbonate, albumin, and phosphorus. An alternative method for estimating SID is to calculate the bicarbonate, albumin, and phosphorus contributions. This is known as the SIdEff. Similar to SIDapp, there was no difference in the SIdEff at any time point in the treatment group of the present study, compared with results for the control group. Again, this is consistent with the overall acid-base effects observed.

If all the anions and cations in the system were accounted for in these 2 calculations, SIDapp minus SIdEff would equal zero. In reality, all the cations and anions are rarely accounted for, and the difference between SIDapp and SIdEff (ie, the SIdEff) represents unmeasured anions and cations. Increases in the SIdEff in clinically affected patients are considered a marker of unmeasured metabolic acids in a manner similar to an increase in AG. But in contrast to AG, SIdEff is not influenced by hypoalbuminemia. In the study reported here, SIdEff increased a small but significant amount in the treatment group but not in the control group. This
is suggestive of a small increase in the number of unmeasured anions as a result of water administration. This finding is in contrast to results of other studies conducted by our laboratory group in which the dilution of plasma and whole blood in vitro with sterile water was associated with little change or a small decrease in SIG. The origin of the unmeasured anions is not obvious; they cannot represent lactate because lactate concentration decreased after administration of sterile water. In the treatment group, the SIG increased but the AG decreased.

For the traditional method, SBE is a quantitative estimate of the metabolic contribution to acid-base balance. However, SBE does not provide insights to the specific mechanisms responsible for that overall balance. Equations have been developed on the basis of the Stewart principles of acid-base chemistry for use in estimating the quantitative impact of specific acid-base processes on the overall base excess. These equations can be used to calculate contributions of sodium, chloride, phosphate, and albumin to base excess (Appendix). When lactate concentration is measured, investigators can also account for its impact on SBE. The sodium contribution is a misnomer because the sodium concentration has no acid-base effects. In clinically affected patients, changes in sodium concentration most frequently reflect changes in free water content. Changes in free water content have acid-base effects by virtue of dilution or concentration of ECF bicarbonate concentration. When there is a decrease in sodium concentration as a result of a gain in free water content, the sodium concentration may be a useful clinical marker of an acidotic process. In quantitative acid-base analysis, the magnitude of a sodium effect on base excess can be calculated for a given change in sodium concentration. In the present study, the addition of water was associated with a substantial, acidotic sodium effect on the SBE of approximately –7 mEq/L. This effect was counteracted by alkalotic processes associated with administration of the sterile water.

The chloride contribution is another misnomer because chloride has no acid-base effects. Rather, it is a marker for changes in bicarbonate concentration that are a result of exchange for chloride. There are 2 main mechanisms by which bicarbonate concentration in the body can change: indirectly via titration or buffering reactions with nonvolatile acids (eg, sterile water, albumin, phosphoric acid, or lactic acid) or directly via alterations in the bicarbonate concentration. These direct mechanisms are often associated with reciprocal changes in the chloride concentration. For example, gastric secretion of hydrochloric acid, intestinal secretion of bicarbonate, renal handling of acids, and many intracellular shifts of acids are associated with a 1-to-1 exchange of chloride for bicarbonate. Because changes in water content will change the chloride concentration in a manner similar to that for the sodium concentration, the measured chloride concentrations must be adjusted for changes in water content prior to calculation of the chloride contribution. This is an attempt to ascertain actual changes in the chloride concentration independent of changes in the water concentration. In the present study, there was an alkalotic chloride effect of 3.8 mEq/L on SBE as a result of sterile water administration, compared with an acidic chloride effect of –2.8 mEq/L in the control group. The alkalotic effect identified in the treatment group is a result of a proportionally greater decrease in chloride concentration than in sodium concentration (21% vs 18%, respectively). The acidic chloride effect detected in the control group was the combined result of a small increase in the chloride concentration and a small decrease in the sodium concentration.

Albumin is a weak acid, and changes in albumin concentration can cause acid-base effects. The decrease in albumin concentration in both the control and treatment groups had a significant alkalotic effect on the SBE. The alkalotic impact of hypoalbuminemia was significantly greater in the treatment group, and this helped offset the acidic effect of the sterile water administration. Phosphorus is also a weak acid, and a small alkalotic effect on SBE was calculated in the treatment group, which reflected the small decrease in phosphorus concentration.

The lactate effect is based on the assumption that changes in lactate concentration are associated with equivalent changes in hydrogen ions (lactic acid). In a patient, lactic acidosis is most commonly associated with an equimolar increase in both lactate and hydrogen ions; hence, the basis for this equation is that lactate has a 1-to-1 effect on the SBE. The lactate anion has no acid-base effects. When lactate enters the body accompanied by hydrogen ions, it can be a useful marker of metabolic acidosis. In the present study, there was a decrease in lactate production over time in both groups and the lactate concentration in the treatment group was further reduced by dilution. Consequently, the acidotic lactate effect on SBE was significantly smaller in the treatment group, compared with that in the control group. This further attenuated the acid-base effects of the administration of sterile water.

If all the acid-base processes that influence the SBE were identified and accurately accounted for with these 5 calculations (ie, sodium, chloride, albumin, phosphorus, and lactate effects), then the sum total of these effects would equal the SBE. Any difference between the sum of the calculated effects and the SBE is considered an estimate of unmeasured acidic or basic substances. When the sum of effects is greater than the SBE, it suggests the presence of unidentified acids, whereas when the sum of effects is less than the SBE, it suggests the presence of unidentified bases. In the present study, there was an extremely small decrease in the sum of effects in the control group at 60 minutes, compared with the value at baseline, and there was an increase in the sum of effects at 60 minutes after administration of sterile water in the treatment group; however, these changes were not significant. In the control group, the SBE decreased by 2 mEq/L, whereas the sum of effects decreased by only 0.9 mEq/L, which resulted in an acidotic change in unmeasured anion effect of 1.1 mEq/L. In the treatment group, the SBE decreased by 2 mEq/L, whereas the sum of effects increased by 2.5 mEq/L, which resulted in a change in unmeasured acid effect of 3.9 mEq/L. The origin of the unmeasured acids in this study was not obvious.
Administration of sterile water is unlikely to cause a clinically relevant change in acid-base balance. This is because the acidic impact of water administration is offset by generation of new bicarbonate via carbonic acid equilibration and intracellular buffering in addition to the alkalotic effects of decreases in albumin and phosphorus concentrations.

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References

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Appendix

Equations used to calculate values for acid-base variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>Sodium concentration + potassium concentration – chloride concentration – bicarbonate concentration</td>
</tr>
<tr>
<td>SIDapp</td>
<td>Sodium concentration + potassium concentration + (2 × ionized calcium concentration) – chloride concentration</td>
</tr>
<tr>
<td>SIDeff</td>
<td>Bicarbonate concentration + albumin contribution + phosphate contribution</td>
</tr>
<tr>
<td>SIG</td>
<td>SIDapp – SIDeff</td>
</tr>
<tr>
<td>Albumin contribution to AG, SIDeff, and SIG</td>
<td>10 × albumin concentration* × ((0.123 × pH) – 0.631)</td>
</tr>
<tr>
<td>Phosphorus contribution to AG, SIDeff, and SIG</td>
<td>Phosphorus concentration† × (10/30.97) × ((0.309 × pH) – 0.469)</td>
</tr>
<tr>
<td>Sodium contribution to standardized base excess</td>
<td>0.237 × (measured sodium concentration – 144)</td>
</tr>
<tr>
<td>Corrected chloride concentration</td>
<td>Measured chloride concentration × (144/measured sodium concentration)</td>
</tr>
<tr>
<td>Chloride contribution to standardized base excess</td>
<td>106 – corrected chloride concentration</td>
</tr>
<tr>
<td>Lactate contribution to standardized base excess</td>
<td>–1 × measured lactate concentration‡</td>
</tr>
<tr>
<td>Phosphorus contribution to standardized base excess</td>
<td>(8.4 – phosphorus concentration†) × (10/30.97) × ((0.309 × pH) – 0.469)</td>
</tr>
<tr>
<td>Albumin contribution to standardized base excess</td>
<td>10 × (3.8 – albumin concentration*) × ((0.123 × pH) – 0.631)</td>
</tr>
<tr>
<td>Sum of quantitative contributions</td>
<td>Sodium contribution + chloride contribution + albumin contribution + phosphorus contribution + lactate contribution</td>
</tr>
<tr>
<td>Contribution of unmeasured anions on standardized base excess</td>
<td>Standardized base excess – sum of quantitative contributions</td>
</tr>
</tbody>
</table>

Measurement units for all variables are mEq/L unless stated otherwise.
*Measured as g/dL. †Measured as mg/dL. ‡Measured as mmol/L.