Acid-base disturbance during hemorrhage in rats: significant role of strong inorganic ions

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Alfaro, V., J. Pesquero, and L. Palacios. Add-base disturbance during hemorrhage in rats: significant role of strong inorganic ions. J. Appl. Physiol. 86(5): 1617–1625, 1999.—The present study tests the hypothesis that changes in the strong inorganic ion concentrations contribute significantly to the acid-base disturbance that develops during hemorrhage in the arterial plasma of rats in addition to lactate concentration ([Lac⁻]). The physicochemical origins for this acid-base disorder were studied during acute, graded hemorrhage (10, 20, and 30% loss of blood volume) in three groups of rats: conscious, anesthetized with ketamine, and anesthetized with urethan. The results support the hypothesis examined: strong-ion difference (SID) decreased in the arterial plasma of all groups studied because of an early imbalance in the main strong inorganic ions during initial hemorrhagic phase. Moreover, changes in plasma [Lac⁻] contributed to SID decrease in a later hemorrhagic phase (after 10% hemorrhage in urethan-anesthetized, after 20% hemorrhage in ketamine-anesthetized, and after 30% hemorrhage in conscious group). Inorganic ion changes were due to both dilution of the vascular compartment and ion exchange with extravascular space and red blood cells, as compensation for blood volume depletion and hypocapnia. Nevertheless, anesthetized rats were less able than conscious rats to preserve normal arterial pH during hemorrhage, mainly because of an impaired peripheral tissue condition and incomplete ventilatory compensation.

strong-ion difference; ion imbalance; metabolic acidosis; anesthesia; ketamine; urethan

METABOLIC ACIDOSIS IN BLOOD is common during hemorrhage (8, 17). Reduction in blood volume during hemorrhagic shock results in decreased cardiac output and decreased O₂ delivery to tissues (8, 10, 17, 29). This latter may increase the activity of the anaerobic energy-producing systems or decrease aerobic energy-producing systems during hemorrhagic shock, thus raising lactic acid concentration of extracellular fluid and reducing plasma HCO₃⁻ concentration ([HCO₃⁻]) (17). However, hemorrhage also results in changes in the main plasma inorganic ions and proteins (6, 10, 16, 18, 37–39, 41).

In 1983, Stewart (35) designed an approach for the study of acid-base changes in body fluids, which assumes that ion and protein changes influence the acid-base balance in a physiological compartment, arterial plasma in the present study. Several authors have used this physicochemical approach to quantify mixed acid-base disorders (3–5, 21, 25, 27, 40). The physicochemical analysis is done by combining the state of electroneutrality with the state of equilibrium for all incompletely dissociated substances and the solvent, water. Three sets of variables that are relevant to the acid-base balance can be changed primarily and/or individually in vivo. They can be regarded as independent variables and are the Pco₂, the strong-ion difference (SID), and the total concentration of weak acids ([A⁻])). Pco₂ represents the respiratory component of an acid-base disorder, whereas SID describes the acid-base interrelations on the basis of the difference in charge between the main strong cations and the main strong anions. A decrease or increase in plasma SID is usually recognized as a decrease or increase in [HCO₃⁻] or as a base deficit or base excess, respectively (11). To maintain the constraint of electroneutrality within a body fluid compartment, the charge resulting from SID must balance the charge of the weak acid systems (mainly due to albumin and Pi) as well as the charge derived from the acid-base respiratory component (Pco₂), i.e., the anionic charges of HCO₃⁻ and CO₃⁻. For practical purposes, charge from CO₃⁻ is minimal and not considered. Thus SID = [HCO₃⁻] – [A⁻] (weak acids) must be equal to 0 (35).

The main purpose of the present study was to test the hypothesis that changes in strong inorganic ions (mainly Na⁺ and Cl⁻) during acute graded hemorrhage in rats affect the acid-base status of arterial plasma together with the changes in lactate concentration ([Lac⁻]). A short-term hypotensive hemorrhagic shock was considered a suitable model to study the effects of inorganic ion imbalance on acid-base changes because of the relevance of ion changes in the consideration of fluid therapy for resuscitation in hemorrhage (34).

Another frequent finding during hemorrhage is that anesthetic agents are often associated with a significant deterioration in microvascular control mechanisms, which leads to a reduction in the O₂ extraction capacity of tissues (14, 33, 35). Several studies have addressed questions on the peripheral circulatory actions of anesthetics during hemorrhage, but there is little specific information about their acid-base effects during hemorrhagic shock in rats. Ketamine is widely used in studies on hemorrhage in several species (14, 15, 26, 36), whereas urethan has been used to a lesser extent (33). An additional goal of the present study was to compare the evolution of acid-base changes during hemorrhage in rats anesthetized with ketamine or urethan with respect to that found in conscious hemorrhaged rats.

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METHODS

Animals. Male Sprague-Dawley rats (300–400 g) were housed individually in propylene cages (Technoplast, Milano, Italy) in a temperature-controlled room (23 ± 1°C) and exposed to a 12:12-h light-dark cycle. Animals had free access to tap water and Purina laboratory chow pellets (A04, Panlab, Barcelona, Spain). They were not starved overnight before experiments. The experimental procedures were performed according to the European Community regulations for the use and handling of experimental animals and were approved by the Ethics Committee of the University of Barcelona.

Surgical procedures. Under ether anesthesia, an indwelling polyethylene cannula (PE-50, Clay Adams, Sparks, MD) was inserted through the left carotid artery into the aorta, and a thermocouple probe (KPi/45, Kane May, London, UK) was advanced close to the aortic arch to obtain continuous body temperature (Tb) measurements (2). Both the cannula and the probe were exteriorized at the back of the neck, and the animals were allowed to recover at least 24 h before the hemorrhage protocol started. The cannula was periodically flushed with heparinized isotonic saline (100 U/ml) to prevent clotting. Lithium heparin (Aulabor, Barcelona, Spain) was used to prevent interference with the ion analysis. At the end of the study, the rats were killed by an overdose of anesthetic (urethan for the control group).

Hemorrhage protocol and blood sampling. Animals were randomly divided into three groups: one control group [conscious unrestrained rats (C); n = 7], one group anesthetized with ketamine hydrochloride (KA; 125 mg/kg body wt im plus 30 mg/kg as required; Sigma Chemical, Leverkusen, Germany; n = 7), and one group anesthetized with urethan (UA; 1.5 g/kg body wt ip; Fluka Chemie, Dresden, Germany; n = 7). Total blood volume was estimated as 7% of body weight (22). Graded hemorrhage was induced in all groups by anaerobic withdrawal of blood. Three successive 10% reductions in blood volume were achieved by drawing blood through the arterial cannula at a rate of 2 ml/min. Blood samples were taken immediately, and a 5-min period was allowed between each blood withdrawal and further blood sampling. Therefore, hemorrhage was studied as a non-steady-state condition during a total period of 13 min: three periods of ~1 min for bleeding and further blood sampling plus two intermediate periods of 5 min. This non-steady-state disorder represents bleeding and further blood sampling plus two intermediate periods of 5 min. This non-steady-state disorder was advanced close to the aortic arch to obtain continuous polyethylene cannula (PE-50, Clay Adams, Sparks, MD) coupled to a polygraph (model 2006, Letica, Barcelona, Spain) and connected to the arterial cannula through a three-way tap system. MAP was noted before each blood withdrawal.

PO2, PCO2, and pH were analyzed immediately after sampling (ABL5 Blood Gas System, Radiometer, Copenhagen, Denmark). Plasma concentrations of Na+ ([Na+]1), K+ ([K+]1), Ca2+ ([Ca2+]), and Cl− ([Cl−]) were measured by ion-specific electrodes (EM 100 Electrolyte Metabolite Laboratory, Radiometer). All electrodes were maintained at 37°C, and all measurements were performed in duplicate. Hb concentration (Hb) and Hb O2 saturation (SO2) were also measured (OSM3 Hemoximeter, Radiometer).

The remaining blood sample was immediately collected into tubes containing dry lithium heparin, which were stored on ice for further analysis. Hematocrit was determined by centrifugation for 10 min at 15,000 revolutions/min (Hae-mofuge, Haeraeus, Hamburg, Germany). Analysis of [Lac−] was performed in additional deproteinized 20-liter samples of whole blood by using a standard spectrophotometric procedure (Boehringer Mannheim, Mannheim, Germany). Whole blood [Lac−] values were later corrected to plasma [Lac−] values (5). The remaining blood was centrifuged at 5,000 g for 15 min (J oyau CR411, Saint Nazaire, France). Plasma was removed and frozen in Eppendorf vials, which were stored at −30°C for further analysis.

Plasma concentration of Mg2+ ([Mg2+]) was measured by using a standard commercial kit (Magnesium 60s, Menagent, Menarini, Milano, Italy). Plasma concentration of Pi was determined by the ammonia-molybdate technique using a commercial kit (Fosfofix, Menarini). Total protein was measured by using the Biuret technique (Total Protein HF, Menarini). Plasma albumin was determined by using the green bromocresol technique (Albumin, Menarini). Plasma osmolality was measured with a microsmometer, based on the freezing-point depression method (3MO, Advanced Instruments, Needham Heights, MA).

Calculations. Blood gases and pH values were corrected for the appropriate Tb by using values found for the same rat strain in vitro (3). Plasma [HCO3−] was calculated from a microsmometer, based on the equation described by Stewart (35), with plasma [H+] calculated from the dissociation equilibrium equation: arterial HCO3− concentration ([HCO3−]) × [H+] = acid dissociation constant of carbonic acid and CO2 solubility coefficient corrected to Tb (30).

Plasma SID was calculated as the sum of the main strong cations minus the main strong anions (expressed in meq/l) (35)

\[
\text{SID} = ([\text{Na}^+] + [\text{K}^+] + [\text{Ca}^{2+}] + [\text{Mg}^{2+}]) - ([\text{Cl}^-] + [\text{Lac}^-])
\]

The magnitude and sign of plausible free-water abnormalities in arterial plasma and their expected effect on SID were assessed by reference to the measured [Na+] changes (24).

The [A(car)] values in arterial plasma were calculated from their mass action equilibrium as the concentration of anionic forms plus the concentration of acidic forms of weak acids ([HA]) ([A(car)] = [A-] − [HA]). [A-] was calculated from the contribution of changes in plasma albumin and P, negative charges, as described by Figge et al. (13). [HA] was then calculated from the dissociation equilibrium equation: arterial H+ concentration ([H+]) × [A-] = acid dissociation constant of albumin and P, assuming the dissociation constants for albumin and P, reported by Figge et al. (12).

Validation of the physicochemical approach applied was carried out by comparing plasma [H+] calculated by using a computer program (ACIDBASES I, Insight Services) based on the equations described by Stewart (35), with plasma [H+] directly measured by a pH electrode. The results obtained were consistent with each other (Fig. 1).

Statistics. Values were assessed by a one-way repeated-measures ANOVA for the evaluation of the effects of hemorrhage on the variables studied (SigmaStat v. 1.0, Jandel Scientific, San Rafael, CA). The Student-Newman-Keuls test was applied within groups to compare values during hemorrhage with respect to the baseline value. Values were assessed by a one-way ANOVA for comparisons between groups at the same degree of hemorrhage. An unpaired Student’s t-test was used for post hoc comparisons between means when a significant F value was obtained. Linear regressions were done by the least squares method. The results were expressed as means ± SD, and they were considered significant at the P < 0.05 level.
ACID-BASE STATUS DURING ACUTE HEMORRHAGE

RESULTS

The successive withdrawal of blood caused significant decreases in MAP, although C rats always showed higher MAP than did anesthetized rats (Fig. 2). The concomitant decrease in [Hb] and hematocrit during hemorrhage was similar in all groups (Table 1).

Significant changes were found for the acid-base-dependent variables, pH, and [HCO₃⁻] (Fig. 3). Whereas pH remained almost unchanged in C hemorrhaged rats, significant decreases were found in the anesthetized groups, with the lowest pH values being found in the UA group. Moreover, [HCO₃⁻] decreased with hemorrhage in all groups, although the higher [HCO₃⁻] values were found in C rats.

In respect to the acid-base-independent variables, all groups showed a decrease in PO₂, SID, and [Atot] as hemorrhage progressed (Fig. 4). PO₂ decrease was accompanied by an increase in Po₂, although So₂ and mean blood O₂ content were lower in the anesthetized groups (Table 1). Plasma SID decreased with hemorrhage in all groups but in different ways: after 10% hemorrhage in UA and after 20% hemorrhage in KA and C groups (Fig. 4). Significant decreases in SID corresponded in all groups with significant decreases in [HCO₃⁻] (Figs. 3 and 4), with SID changes being related to changes in both strong organic and inorganic ions.

Hemorrhage notably altered the profile of strong inorganic ion concentrations in plasma (Table 2), although some of these changes were not present or were always minor in C rats. [Na⁺] decreased while [K⁺], [Ca²⁺], and [Mg²⁺] increased. [Cl⁻] was more stable during the hemorrhagic shock and only decreased significantly in the UA group after 30% hemorrhage. As a result of these unbalanced changes, the [Na⁺]/[Cl⁻] ratio and the plasma osmolality decreased after 30% hemorrhage in all groups (Table 2). A significant, although minor, deficit of base related to free-water abnormalities was quantified from [Na⁺] decreases at 20 and 30% hemorrhage (Fig. 5). With respect to strong organic ion concentrations in plasma, [Lac⁻] increased with hemorrhage, but the progression of [Lac⁻] changes
was different in each group studied (Fig. 6). [Lac⁻] increased significantly after 10% hemorrhage in UA group, after 20% hemorrhage in KA group, and after 30% hemorrhage in C rats. UA group always showed the higher [Lac⁻] values at the baseline and at all levels of hemorrhage studied, whereas [Lac⁻] values were intermediate in KA and lower in C groups.

As mentioned previously, SID decrease was related to changes in strong inorganic and organic ion concentrations, but when SID changes were examined without including [Lac⁻], we also found significant SID decreases in all groups (Fig. 7). Figure 8 shows the percentage of SID decrease due to changes in strong inorganic ions. In all groups, the increase in plasma [Lac⁻] was an increasingly important contributor to the decrease in SID as hemorrhage progressed. However, SID decrease in C and KA groups was initially mainly due to inorganic ion imbalance rather than to [Lac⁻] increase (Figs. 7 and 8). In fact, after 30% hemorrhage, the contribution of ions other than [Lac⁻] to a decrease in SID was 43.7% in C rats, 21.6% in KA group, and 17.4% in UA group (Fig. 8). In absolute terms, SID decrease was ~4.5 meq/l for C rats, 2.1 meq/l for KA group, and 1.9 meq/l for UA group after a 30% hemorrhage (Fig. 7).

The third independent variable, [Acol], decreased in a similar and significant way (~2 meq/l) (Fig. 4) as a result of a significant albumin decrease and a minor P1 increase. Plasma proteins and albumin decreased as a consequence of hemorrhage, although this decrease did not generally produce changes in the albumin-to-globulin ratio (Table 3). Moreover, plasma P1 was stable during hemorrhage in C rats but increased in KA and UA groups (Table 3).

**DISCUSSION**

Acid-base response to hemorrhage in C rats. The acute acid-base response to hemorrhage found in the
Table 2. Inorganic ion changes found in the arterial blood of C, KA, and UA rats during acute, graded hemorrhage

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Hemorrhage 10%</th>
<th>Hemorrhage 20%</th>
<th>Hemorrhage 30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na⁺], mmol/l</td>
<td>C</td>
<td>140 ± 1</td>
<td>138 ± 2</td>
<td>137 ± 1*</td>
<td>134 ± 2*</td>
</tr>
<tr>
<td></td>
<td>KA</td>
<td>139 ± 2</td>
<td>136 ± 3*</td>
<td>131 ± 3†</td>
<td>128 ± 2†</td>
</tr>
<tr>
<td></td>
<td>UA</td>
<td>138 ± 1</td>
<td>131 ± 3†</td>
<td>131 ± 3†</td>
<td>128 ± 2†</td>
</tr>
<tr>
<td>[K⁺], mmol/l</td>
<td>C</td>
<td>4.34 ± 0.40</td>
<td>4.73 ± 0.44</td>
<td>4.41 ± 0.70</td>
<td>4.76 ± 0.57</td>
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<tr>
<td></td>
<td>KA</td>
<td>4.37 ± 0.22</td>
<td>4.49 ± 0.23</td>
<td>4.99 ± 0.32†</td>
<td>6.15 ± 0.07†</td>
</tr>
<tr>
<td></td>
<td>UA</td>
<td>3.87 ± 0.16†</td>
<td>4.03 ± 0.43†</td>
<td>4.97 ± 0.99†</td>
<td>5.67 ± 0.38†</td>
</tr>
<tr>
<td>[Ca²⁺], mmol/l</td>
<td>C</td>
<td>1.35 ± 0.04</td>
<td>1.35 ± 0.04</td>
<td>1.32 ± 0.04</td>
<td>1.26 ± 0.06†</td>
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<td>1.32 ± 0.03</td>
<td>1.37 ± 0.02</td>
<td>1.38 ± 0.03 †</td>
<td>1.47 ± 0.06†</td>
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<td></td>
<td>UA</td>
<td>1.24 ± 0.01†</td>
<td>1.29 ± 0.04 †</td>
<td>1.34 ± 0.04 †</td>
<td>1.43 ± 0.10 †</td>
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<tr>
<td>[Mg²⁺], mmol/l</td>
<td>C</td>
<td>0.53 ± 0.03</td>
<td>0.52 ± 0.03</td>
<td>0.57 ± 0.05</td>
<td>0.72 ± 0.08†</td>
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<tr>
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<td>KA</td>
<td>0.62 ± 0.12</td>
<td>0.61 ± 0.06†</td>
<td>0.73 ± 0.14 †</td>
<td>1.02 ± 0.01 †</td>
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<td></td>
<td>UA</td>
<td>0.57 ± 0.07</td>
<td>0.75 ± 0.17†</td>
<td>1.00 ± 0.25 †</td>
<td>1.26 ± 0.17 †</td>
</tr>
<tr>
<td>[Cl⁻], mmol/l</td>
<td>C</td>
<td>103 ± 2</td>
<td>104 ± 3</td>
<td>105 ± 4</td>
<td>102 ± 3</td>
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<td>103 ± 2</td>
<td>103 ± 2</td>
<td>100 ± 1*</td>
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<tr>
<td>[Na⁺]/[Cl⁻]</td>
<td>C</td>
<td>3.15 ± 0.02</td>
<td>3.13 ± 0.04</td>
<td>3.13 ± 0.04*</td>
<td>3.13 ± 0.04*</td>
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<td>3.30 ± 0.03</td>
<td>3.12 ± 0.02</td>
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<td>3.12 ± 0.02*</td>
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<td>UA</td>
<td>3.33 ± 0.01</td>
<td>3.13 ± 0.01</td>
<td>3.13 ± 0.01*</td>
<td>3.13 ± 0.01*</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH₂O</td>
<td>C</td>
<td>284 ± 2</td>
<td>282 ± 2</td>
<td>280 ± 2</td>
<td>270 ± 3*</td>
</tr>
<tr>
<td></td>
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<td>283 ± 3</td>
<td>282 ± 3</td>
<td>278 ± 6</td>
<td>271 ± 4*</td>
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<tr>
<td></td>
<td>UA</td>
<td>280 ± 2</td>
<td>277 ± 4</td>
<td>272 ± 5*</td>
<td>267 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7 rats in each group. Brackets indicate concentration. *Significantly different from baseline value, P < 0.05. †Significantly different from conscious rats, P < 0.05.

Arterial plasma of C rats was characterized by a compensated primary metabolic acid-base disorder. Hemorrhaged C rats preserved arterial pH within a normal range, although a significant metabolic acid component was found in the arterial plasma, as reflected by significant decreases in SID. Nevertheless, plasma SID decreased significantly in hemorrhaged C rats after 20% hemorrhage, whereas [Lac⁻] increased significantly only after 30% hemorrhage. Hemorrhaged C rats showed a respiratory compensation to the acid component in plasma after 20% hemorrhage (Fig. 9), coincident with decreases in MAP and SID and reflected by decreased arterial PCO₂ and increased arterial PO₂. Therefore, plasma SID reflected the development of a primary metabolic acid component in the arterial plasma of hemorrhaged C rats more accurately than did plasma [Lac⁻] alone. [Lac⁻] induced a minor SID change after 10 and 20% hemorrhage (~10–15% of total SID change) and was not responsible for the initial respiratory compensation. Only after 30% hemorrhage did [Lac⁻] changes have a significant effect on SID (about one-half of the total change in SID). Hence, changes in ions other than [Lac⁻] (strong inorganic ions) had a relevant effect on the initial development of the metabolic acid-base disorder in hemorrhaged C rats. Apart from the effect of changes in [Lac⁻], two general mechanisms can change the SID value in arterial plasma.
biological fluids. The first involves changes only in the water content of plasma, without any imbalance in the content of strong ions. The strong cations and anions are diluted or concentrated in the same proportion, and SID decreases or increases, respectively, by the same proportion. Metabolic acid-base disturbances of this nature can be classified as dilutional acidosis and concentration alkalosis, respectively (11). In the pure form of these derangements, [Na\(^+\)] and [Cl\(^-\)], the major strong cation and anion, respectively, deviate from their normal values in the same direction and proportion. The second way in which SID values change is through an isotonic imbalance of strong ions. If the water content in plasma is normal (reflected as normal [Na\(^+\)]), then the SID may decrease and acidosis can result if inorganic anions such as [Cl\(^-\)] or anions of some strong organic acids (lactate, formate, keto acids) accumulate in plasma (11). Nevertheless, in most situations in vivo, these two general mechanisms are often combined (19).

In the present study, decreases in plasma osmolality, [Na\(^+\)], and plasma protein concentration indicated hemodilution during acute hemorrhage. It is well known that a rapid phase of blood volume restoration occurs concurrently with hemorrhage. This phase involves entry of extravascular fluid lower in protein into the vascular space (6, 16, 28, 38). The short-term nature of the present study did not allow enough time to observe further compensatory mechanisms, including hormonal and renal effects (28, 38). However, [Na\(^+\)] and [Cl\(^-\)] did not change in the same direction and proportion, as may be the case in a pure dilutional acidosis. Thus [Na\(^+\)] decreased, whereas [Cl\(^-\)] was almost stable. We may consider that, as mentioned above, plasma

Fig. 7. Changes in SID with and without including [Lac\(^-\)] in C rats (A) and in KA (B) and UA (C) groups during acute, graded hemorrhage; n = 7 rats in each group. Data are means ± SE. *Significantly different from baseline value, **significantly different from SID without Lac\(^-\), P < 0.05.

Fig. 8. Percent change in plasma SID in C rats and KA and UA groups due to changes in main strong ions other than [Lac\(^-\)] (inorganic ions: [Na\(^+\)], [K\(^+\)], [Ca\(^{2+}\)], [Mg\(^{2+}\)], and [Cl\(^-\)]; brackets indicate concentration). Data are means ± SE.

Table 3. Protein and Pi changes found in the arterial blood of C, KA, and UA rats during acute, graded hemorrhage

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
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<tbody>
<tr>
<td>[Protein], g/dl</td>
<td>C</td>
<td>5.5 ± 0.9</td>
<td>5.4 ± 0.9</td>
<td>4.9 ± 0.8</td>
<td>4.3 ± 0.8*</td>
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<td>KA</td>
<td>5.1 ± 0.5</td>
<td>5.0 ± 0.4</td>
<td>4.4 ± 0.3*</td>
<td>3.9 ± 0.2*</td>
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<td>UA</td>
<td>5.3 ± 0.4</td>
<td>4.8 ± 0.5</td>
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<td>4.0 ± 0.1*</td>
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<tr>
<td>[Alb], g/dl</td>
<td>C</td>
<td>3.9 ± 0.6</td>
<td>3.8 ± 0.7</td>
<td>3.3 ± 0.6</td>
<td>3.2 ± 0.7</td>
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<tr>
<td></td>
<td>KA</td>
<td>3.9 ± 0.4</td>
<td>3.7 ± 0.3</td>
<td>3.2 ± 0.3*</td>
<td>2.8 ± 0.2*</td>
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<td></td>
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<td>3.9 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>3.1 ± 0.6*</td>
<td>2.7 ± 0.1*</td>
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<tr>
<td>[Alb]/[Glob]</td>
<td>C</td>
<td>0.72 ± 0.06</td>
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<td>0.68 ± 0.02</td>
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<td>[Pi], mmol/l</td>
<td>C</td>
<td>2.03 ± 0.15</td>
<td>1.90 ± 0.25</td>
<td>1.89 ± 0.20</td>
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<td>2.23 ± 0.25</td>
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<td>1.87 ± 0.16</td>
<td>2.01 ± 0.31</td>
<td>2.44 ± 0.83</td>
<td>2.77 ± 0.64†</td>
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</table>

Values are means ± SD; n = 7 rats in each group. [Alb]/[Glob], albumin-to-globulin ratio. *Significantly different from baseline value, P < 0.05. †Significantly different from conscious rats, P < 0.05.
SID reflects not only the metabolic component but also the respiratory component. As a matter of fact, hypocapnia decreases SID in plasma, because changes in PCO$_2$ cause HCO$_3^-$-Cl$^-$ exchange, i.e. Hamburger shift between red blood cells and plasma (32). Then, part of the nonlactate change found in SID could be produced as a compensation to the hypocapnia produced during hemorrhage. Nevertheless, hypocapnia may not explain all the nonlactate decrease in SID, because in vivo the plasma also interacts with the extravascular space (32). Hepatic and skeletal muscle alterations in ion transport have been observed during shock via hemorrhage. These alterations include impairment of active cellular K$^+$ accumulation, increased permeability to Cl$^-$, membrane depolarization, and failure of the electrogenic Na$^+$ pump (31). Such alterations of ion homeostasis could also contribute to the uncompensated inorganic ion changes found in the arterial plasma during our study. In fact, uncompensated changes in main plasma ions during hemorrhage, as found here, have been previously reported (39, 41). However, the effect of these ion changes on acid-base status has not been quantified up until the present study. The fluid homeostasis during hemorrhage depends on the nutritional state of the animal (9, 39), which could also explain the different results found in ion and osmolality changes in other hemorrhage studies. The decrease found in the other metabolic acid-base-independent variable ([A$_{tot}$]) was probably a consequence of hemodilution. Previous studies have found decreases in plasma proteins during hemorrhage (18). However, the decrease was insufficient (~2 meq/l) to have a relevant effect on [H$^+$] changes that could significantly counteract SID changes.

Acid-base response to hemorrhage in anesthetized rats. At baseline, i.e., after 30 min of anesthesia, KA and UA rats already had mild metabolic acidosis, reflected by significantly low pH, [HCO$_3^-$], and SID values. This means that, from an acid-base point of view, both groups of anesthetized rats started hemorrhagic shock from an impaired state. Moreover, the acid-base response found during hemorrhage in KA rats was intermediate between that found in C and UA rats. Indeed, the finding in hemorrhaged C rats of a normal blood pH differs from the low blood pH usually found in previous studies performed in anesthetized hemorrhaged animals (17, 20, 37) and from the low blood pH found in anesthetized hemorrhaged rats in the present study. Unlike C rats, KA rats showed a primary metabolic acidosis reflected by a decrease in SID. SID changes were initially attributed mainly to strong inorganic ions but, in contrast with C rats, after 20% hemorrhage more than one-half of the SID decrease was attributed to [Lac$^-$] increase. Furthermore, KA rats were not able to compensate for the primary metabolic acidosis by decreasing PCO$_2$ values, the respiratory compensation being less successful than in hemorrhaged C rats (Fig. 9). As a consequence of all these changes, pH values were lower after 20 and 30% hemorrhage. On the other side, UA rats showed an uncompensated metabolic acidosis during hemorrhage, the pH values being the lowest of all groups (Fig. 9). Moreover, in contrast with the other experimental groups, SID changes were already mainly attributed to a [Lac$^-$] increase after 10% hemorrhage (~70% of the total SID changes). With respect to the other acid-base-independent variable, no significant differences in [A$_{tot}$] were found between the three experimental groups, with the decrease in [A$_{tot}$] in anesthetized groups being a consequence of compensatory hemodilution. Thus UA animals showed the highest deficit of base due to free-water increase in plasma, parallel to the low [A$_{tot}$] values.

The present results suggest that ketamine and urethane have different effects on the ratio of O$_2$ supply to O$_2$ demand during hemorrhage, this ratio deviating from that expected in C rats, thus leading to a more injurious state. O$_2$ supply may be influenced by a number of factors, including arterial hypoxemia, alterations in the affinity of Hb for O$_2$ (SO$_2$), and regional distributions of blood flow. Neither KA nor UA rats showed arterial hypoxemia during hemorrhage. However, the arterial SO$_2$ increased in C rats as hemorrhage progressed but decreased after 30% hemorrhage in both anesthetized groups. This decrease could be related to an effect of low arterial pH on Hb saturation, leading to a shift in the oxyhemoglobin dissociation curve to the right (23). C, KA, and UA rats showed a similar quantitative evolution of main plasma ions, except for higher differences for [Lac$^-$] and [K$^+$]. Differences in [Lac$^-$] may reflect impaired tissue oxygenation but also a different [Lac$^-$] metabolism (19). On the other hand, hemorrhage is usually associated with hyperkalemia (1). It is well known that changes in acid-base balance affect K$^+$ homeostasis. Thus acidosis increases, whereas alkalosis decreases plasma [K$^+$] via a shift between intracellular and extracellular compartments (7). In the present study, only the anesthetized

Fig. 9. Davenport-van Slyke [HCO$_3^-$] vs. pH diagram for arterial plasma, showing changes in acid-base status during acute, graded hemorrhage (10, 20, and 30%) in C rats and in KA and UA groups; n = 7 rats each group. Data are means ± SE. Standard temperature was 37°C. Dashed lines depict the true plasma buffer line (β = 30.1 slykes; see Ref. 2). Solid lines depict isocapnic isopleths.
groups showed low pH values and higher plasma [K⁺]. Finally, the absence of significant differences in MAP between anesthetized groups suggests that ketamine could prevent the development of tissue hypoxia during hemorrhage by affecting peripheral cardiovascular mechanisms rather than central cardiovascular control (14, 26). A better peripheral vascular condition in KA rats would be reflected in the present study as an acid-base behavior intermediate between C and UA rats.

In summary, two factors were related to the appearance of a metabolic acid component in the arterial plasma of C rats during acute graded hemorrhage. During the first phase of hemorrhage, when MAP was maintained, an imbalance of strong inorganic ions was the main factor found to induce acidosis. In later phases, when MAP significantly decreased, significant increases in [Lac⁻] were found to contribute to the metabolic acid component. Both factors, strong inorganic ions and [Lac⁻], were responsible for a significant decrease in SID of arterial plasma that reflected the development of a metabolic acid-base disorder. Nevertheless, hemorrhaged C rats were able to maintain, through respiratory compensation, arterial pH at almost normal values at all levels of hemorrhage studied. On the other hand, care must be taken when extrapolating the acid-base response of anesthetized animals to that expected in other experimental conditions, such as conscious animals during hemorrhage. Rats anesthetized with ketamine or urethane showed a metabolic acidosis during acute, graded hemorrhage, which was also characterized by decreases in SID, due to an imbalance of strong inorganic ions in the initial phases and lactic acidosis in later phases. However, the development of primary metabolic acidosis and further respiratory compensation was dependent on the anesthetic employed. In both cases, anesthetized rats were not able to compensate for metabolic disturbances, their plasma pH having decreased to values far below normal.

The present study evaluated the mechanisms of acid-base regulation in the blood of the rat during acute hemorrhage. As ion imbalance played a significant role in the development of metabolic acidosis during early hemorrhagic phases, it will be of interest to consider in future experiments or, indeed, in clinical practice, the quantification of the main strong cations and anions (including lactate) during this pathological condition. SID measurement could be a useful and complementary tool in the evaluation of these acid-base disorders, together with the direct determination of blood pH and PCO₂ by electrodes. This complementary analysis may help to discriminate the relative contribution of the different components involved in the acid-base response to acute blood loss. Moreover, as the present results were obtained under non-steady-state conditions, it would be interesting to study the role of strong inorganic ions in the progress of compensatory changes leading to a new steady state after hemorrhage.

The authors are grateful to Jane Ferrier and Robin Rycroft for help with the English version of the paper.

This study was supported by Spanish National Programme of Scientific Research and Technological Development (Grant PB/93/0740).

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Received 15 June 1998; accepted in final form 21 January 1999.

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