Systemic responses to prolonged hemorrhagic hypotension

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Hemorrhagic shock involves the loss of a substantial portion of the circulating blood volume. The loss of volume causes decreases in cardiac output (CO) and hence O2 delivery to the peripheral tissues. Hypoperfusion to many tissues may be exacerbated by neuroendocrine reflexes that cause vasoconstriction. Nearly one-half of all patients suffering from hypovolemia with hemorrhagic shock die within the first 24 h. If untreated, hemorrhagic shock can lead to acidosis, cellular hypoxia, microcirculatory damage, multiple organ failure, and ultimately death (12).

In marked hypotension and decreased tissue perfusion due to hypovolemic shock, correction of the initial problem may not correct the hypotension because peripheral vasodilation has supervened: vasodilatory shock can follow volume resuscitation in prolonged and severe hypotension due to hemorrhage (32). In addition, decreased systemic vascular resistance can be often found in less severe but prolonged hemorrhagic shock (19). Therefore, studies have focused on the vasodilatory aspect of shock decompensation and have suggested therapeutic procedures for restoring vasoreactivity to reverse the vasodilation (19).

An alternative approach is to focus on the oxygenation differences. It has been repeatedly demonstrated in clinical and experimental shock that survival from hemorrhage is related to the degree of developed O2 debt and that, given the same hemorrhage insult, survival cannot be predicted based on volume loss or the resulting central hemodynamic variables (9). The degree of O2 debt developed during hemorrhage is highly predictive of the severity of subsequent reperfusion injury and downstream immune and inflammatory events (23). The American College of Surgery defines shock as “an abnormality of the circulatory system that results in inadequate organ perfusion and tissue oxygenation” (1). Accordingly, many investigators consider that the measurement of perfusion-related variables such as O2 deficit and base excess (BE) are better indicators of the severity of hemodynamic decompensation (9, 27).

In contrast, monitoring of shock is done frequently by measuring mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), hematocrit, or urine flow. These traditionally monitored variables may not adequately reflect tissue oxygenation and the severity of the cellular injury (27). Therefore, careful selection of physiological parameters to be monitored is a critical step in the ability to distinguish between potential survivors and nonsurvivors to a prolonged hemorrhagic hypotension (HH) (9). This is decisive for triage in battlefield settings where delayed or prolonged transport times after traumatic shock are more likely. Similar scenarios are likely in civilian rural settings or with prolonged extrication in which resuscitation resources (crystalloids) are sparse and blood products may not be available (8). Prompt triage would allow a focus of resources.

Few studies provide a rigorous examination of the relevance of each monitored variable during a prolonged HH (7, 27). If a variable is unable to differentiate between survivors and nonsurvivors, it hardly qualifies as a useful clinical monitoring tool (27, 28). Therefore, this study was designed to provide a systematic investigation of physiological parameters that describe biochemical and O2 transport patterns in animals subjected to HH. Systemic parameters that could differentiate survivors from nonsurvivors were identified. An aortic flow probe was implanted in rats (n = 21) for continuous measurement of cardiac output. Experiments were performed 6–9 days after surgery. Rats were bled to a mean arterial pressure of 40 mmHg and kept at that level using Ringer-lactate solution. Arterial and venous blood pressures, arterial and venous blood pressures, gases, acid-base status, glucose, lactate, electrolytes, hemoglobin, O2 saturation, heart and respiratory rates, total peripheral resistance, and O2 delivery and consumption were measured before hemorrhage, soon after 40 mmHg was reached, and 0.5, 1, 2, 3, and 4 h later. Fifty-three percent of rats survived ≥3 h (survivors); others were considered nonsurvivors. Nonsurvivors showed a significantly greater degree of metabolic acidosis than survivors. Arterial P O2, respiratory rate, O2 saturation, O2 content, glucose, and pH were significantly higher in survivors. The rate of Ringer-lactate infusion, arterial K+, and P CO2 were lower in survivors. Arterial K+ and respiratory rate were the only parameters significantly different between survivors and nonsurvivors at all time points during HH. Arterial levels of K+ showed the clearest distinction between survivors and nonsurvivors and may explain the sudden death experienced by animals during HH. The data suggest that early respiratory and metabolic compensations are essential for survival of prolonged HH.
ability of some animals to survive to a model of prolonged HH. In this model, rats were hemorrhaged to a MAP of 40 mmHg and kept at that level using crystalloid solution. If the hypothesis is correct, then systemic parameters related to oxygenation (such as Po2 and pH) should be different between survivors and nonsurvivors and may be considered as predictors of mortality.

MATERIALS AND METHODS

Experimental Animals

This study was approved in advance by the Institutional Animal Care and Use Committee of Virginia Commonwealth University Health System and conforms to the Public Health Service Policy on Human Care and Use of Laboratory Animals (2002) and the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Twenty-one male Sprague-Dawley rats (Harlan; Indianapolis, IN) weighing 281 ± 9 g (mean ± SE) were used in the study. Rats were housed in plastic cages placed in a facility with a 12:12-h light-dark cycle and constant temperature (21°C) as hydration (48–50%) and maintained for an adaptation period of 1 wk. They were fed a commercial diet and had free access to water.

Aortic Flow Probe Implantation

Rats were initially anesthetized with isoflurane (3%, balance O2; Abbott Laboratories; North Chicago, IL), and an oral intubation was then performed to ventilate the rats mechanically with a rodent pressure-controlled ventilator (Kent Scientific; Torrington, CT). Isoflurane anesthesia was maintained, and, under aseptic conditions, a thoracotomy was performed. The ascending aorta was isolated by blunt dissection and retraction of the thymus. A transverse ultrasonic flow probe (model 2.5SB; Transonic Systems; Ithaca, NY) was then positioned around the ascending aorta, and the thorax was closed. The acute experiments were carried out after a 6- to 9-day recovery period after the surgical procedure.

Hemodynamic Measurements

The implanted animals were subjected to a tracheostomy and catheterizations under anesthesia with a mixture of ketamine (70 mg/kg ip, Fort Dodge Animal Health; Fort Dodge, IA) and acepromazine (3 mg/kg ip, Vedco; St. Joseph, MO), followed by a constant intravenous infusion (0.24–0.36 mg kg−1 h−1) of alfalfaxolone/alfadalone acetate (Saffian, Schering-Plough Animal Health; Welwyn Garden City, UK). The left femoral vein was cannulated with polyethylene (PE)-50 tubing for this purpose. The right carotid artery was cannulated with PE-50 tubing filled with a heparin-saline solution (10 U heparin/ml) and connected to a pressure transducer to continuously measure arterial blood pressure (AP). The right jugular vein was cannulated with PE-90 tubing advanced to the entrance of the right atrium. This line was used to collect central venous blood samples and to record CVP. The left femoral artery was cannulated and connected to a microprocessor-controlled infusion/withdrawal syringe pump (model PHD2000, Harvard Apparatus; Holliston, MA). The same artery was used to hemorrhage the animal (see below) and to collect blood samples. All catheters were flushed as needed with heparinized saline to inhibit formation of clots. The core temperature was monitored and maintained at 36.5–37.0°C using a thermostatically controlled heating blanket (Harvard Apparatus). Normal saline was administered at room temperature (23 ± 2°C) as hydration fluid before (5 ml/kg ip) and after (5 ml/kg ip) muscle extirpation.

The left splanchnicus muscle was prepared to allow intravital microscopy without impacting the systemic circulation. The muscle was exposed, covered with a plastic film (Saran Wrap, Dow Corning; Midland, MI), and placed on a heated platform. Finally, the platform holding the animal with the exteriorized muscle was placed on a microscope stage and kept there for the remainder of the experiment. Observations on the muscle microcirculation will be reported separately.

Blood Gas, Hematological, and Biochemical Measurements

Blood analyses were performed in paired arterial and venous samples (0.1 ml each) collected at various time points using heparinized glass capillary tubes (Clinitubes, D957G-70-100, Radiometer; Copenhagen, Denmark). All blood samples were immediately replaced by an equal volume of Ringer-lactate (RL) solution (Baxter; Deerfield, IL). Blood glucose, potassium, chloride, sodium, calcium, lactate, bicarbonate, BE, Po2, PCO2, and pH were measured with a blood gas analyzer (ABL 725, Radiometer). The total hemoglobin (Hb) concentration and HbO2 saturation were measured with a multia wavelength CO-oximeter adjusted for the HbO2 dissociation curve of the rat (OM3; Radiometer).

Inclusion Criteria

The following criteria had to be met by each animal before being enrolled in the experimental protocol: 1) full body weight recovery from the day of flow probe implantation, 2) baseline MAP above 85 mmHg, 3) starting bicarbonate between 18 and 30 mmol/l, 4) arterial O2 saturation (SaO2) above 90%, and 5) Hb concentration above 10 g/dl. This helped to minimize the effects of initial dehydration, excessive catheter flushing, and accidental food/water deprivation before surgery. However, some animals that were initially included in the protocol showed unusually poor responses to hemorrhage and were also excluded from analysis, as explained in RESULTS.

Protocol

Animals were heparinized (260 U/kg body wt), and, after at least a 20-min stabilization period, baseline measurements were begun by obtaining blood samples for the determination of arterial and venous blood gases and chemistry. During each blood collection, a time mark was placed in the computer recording of the systemic parameters to precisely match the data being collected. A second set of baseline measurements was performed 10 min later. Immediately after the sampling for baseline, hypotension was induced by the slow removal (0.3–0.5 ml/min) of blood over a 15- to 20-min period until MAP reached 40 mmHg. Small amounts of additional blood were withdrawn if needed to lower MAP to 40 mmHg. RL infusion was used to maintain MAP if it fell below 40 mmHg. The amount of RL infused was measured and did not exceed more than three times the shed blood volume. Systemic blood gases and metabolic parameters, along with systemic hemodynamics, were measured when the 40-mmHg target was reached [considered as time point (t) = 0] and at 0.5, 1, 2, 3, and 4 h later. All animals surviving the entire 4-h experiment, as well as those requiring early euthanization, received a pentobarbital overdose of 100 mg/kg iv. Control animals were subjected to all the surgical and experimental procedures described above except for the controlled hemorrhage.

Data Acquisition and Analysis

The amplified outputs from the pressure transducers (DA100C, BIOPAC Systems; Goleta, CA) and those from the aortic flowmeter were connected to a desktop computer for continuous on-line data acquisition at a rate of 500 Hz (Acqknowledge 3.7.2 + MP150 hardware and software, BIOPAC Systems). The inspiratory phase of each respiratory cycle generated negative pressure changes in the CVP tracings, easily distinguishable from right atrial pressure changes. The respiratory rate (RR) was calculated as the reciprocal of the interval between these successive negative pressure peaks in the CVP tracings. Systolic, diastolic, pulse, and mean arterial pressures were calculated from the original digitized traces of AP. HR was calculated from the aortic flow signal as the reciprocal of the interval
between successive flow peaks and recorded continuously. CO was estimated from the mean aortic flow. Mean stroke volume was calculated as CO/HR. Cardiac index (CI) and stroke index (SI) were computed by dividing the appropriate variables by body mass. Total peripheral resistance (TPR) was calculated as (MAP - CVP)/CI. All off-line calculations were based on 1-min segments of the digitized signals taken as close as possible to the blood collection time points. Global oxygen consumption ($V_{O_2}$) was calculated using the Fick principle as the product of CI and the difference between arterial ($CaO_2$) and venous $O_2$ contents ($CV_{O_2}$):

$$
CaO_2 = (Hb \times 1.34 \times SaO_2) + (0.003 \times PaO_2)
$$

$$
CV_{O_2} = (Hb \times 1.34 \times SvO_2) + (0.003 \times PvO_2)
$$

$V_{O_2} = CI \times (CaO_2 - CV_{O_2})$

where $SaO_2$ and $SvO_2$ are the arterial and venous $O_2$ saturations, respectively, and $PaO_2$ and $PvO_2$ are the arterial and venous $O_2$ tensions, respectively. Whole animal oxygen delivery ($D_{O_2}$) was computed as the product of $CaO_2$ and CI. The $O_2$ extraction ratio ($O_2ER$) was computed as $V_{O_2}/D_{O_2}$. $V_{O_2}$ deficit was calculated as the difference between $V_{O_2}$ during hypotension and the mean $V_{O_2}$ during baseline measurements. The net cumulative $O_2$ debt at each time point was calculated from the integrated area described by the time-$V_{O_2}$ deficit curve.

Statistics

Values are reported as means ± SE. Differences between groups were analyzed using two-way ANOVA with repeated measures. When a significant F-value was encountered, post hoc analyses were performed between groups with both the Student-Newman-Keuls test and with Bonferroni’s correction for multiple comparisons. A level of $P < 0.05$ was considered significant.

RESULTS

Control Animals

Animals subjected to all procedures except HH showed relatively stable physiological parameters throughout the experimental period of 5 h. The steady decrease in Hb over time (also shown in Fig. 1, right) was probably due to the small hemodilution consequent to successive blood sampling for gas/chemistry analyses. However, the animals maintained high $O_2$ saturation and $P_O_2$, with stable $D_O_2$ and $V_{O_2}$. Several parameters for the control animals are presented in conjunction with the description of the results from animals subjected to HH.

Hemorrhaged Animals

Some animals subjected to HH appeared to decompensate quite early and died <1 h after blood withdrawal. Detailed examination of the experimental records showed that, in two cases, MAP dropped below 30 mmHg due to poor handling during blood sampling and temperature probe placement. In two other cases, the necropsy showed that the rats had abnormal lungs, with several areas of edema and atelectasis. This could indicate that these animals had not fully recovered from the previous surgery (i.e., flow probe implantation). Therefore, these animals were not included in the present analysis. To examine the data, a cutting point was established before the execution of the experiments based on a mortality rate of 50%, as recommended previously (22). Nearly equal numbers of survivors and nonsurvivors were obtained using 3 h as the cut point. A 2-h cut point would produce only 6% mortality. With the use of 4 h as the dividing line for survivors versus nonsurvivors, a higher mortality (~78%) was seen. Therefore, a 3-h point was selected to divide survivors and nonsurvivors. To avoid any bias, this was done before the analysis of the physiological parameters was performed. Animals subjected to HH ($n = 17$) were divided into two groups: nine rats survived 3–4 h (survivors) and eight animals died <3 h after MAP reached 40 mmHg (nonsurvivors). Both groups of animals required similar bleed volumes to reach a $MAP$ of 40 mmHg: 33 ± 4 and 28 ± 2 ml/kg for nonsurvivors and survivors, respectively (Fig. 1, left). The mean hemorrhage time to reach the $MAP$ level of 40 mmHg was also similar: 17.4 ± 1.3 and 18.7 ± 1.0 min for nonsurvivors and survivors, respectively. The mean time from MAP = 40 mmHg to death was 129 ± 12 and 220 ± 12 min for nonsurvivors and survivors, respectively. All animals required crystalloid (RL) infusion to maintain MAP at 40 mmHg for 1 h or longer. Figure 1, left, shows that the rate and amount of infused RL solution at 120 min were significantly higher for nonsurvivors (0.43 ± 0.04 ml/kg·min−1 and 52 ± 6.4 ml/kg) than for survivors (0.27 ± 0.07 ml/min·kg−1 and 29 ± 4.3 ml/kg). Consequently, Hb concentration was lower for nonsurvivors at all points during hypotension, although differences did not reach statistical significance (Fig. 1, right). One of the possible causes for the higher demand for RL infusion among nonsurvivors could be the changes in TPR. Immediately after 40 mmHg was reached, survivors showed a larger increase in TPR (from baseline

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**Fig. 1.** Left: total volume of hemorrhaged blood followed by cumulative volume of Ringer-lactate (RL) solution infused to maintain mean arterial pressure (MAP) at 40 mmHg. Right: venous Hb concentration at various time points before and during hemorrhagic hypotension. Data points shown at baseline (BL) are measurements performed before hemorrhage. Time (t) = 0 min means the 40-mmHg target pressure was reached. Control animals, n = 4; animals surviving <3 h of hemorrhagic hypotension (nonsurvivors), n = 8; animals surviving 3–4 h (survivors), n = 9.

*Significantly different from survivors at the same time point; †significantly different from the baseline period; ‡significantly different from the control group at the same time point. All data are expressed as means ± SE.
values) than nonsurvivors (44.6 ± 11.3 vs. 21.7 ± 8.9%, respectively, P = 0.049). Although values only reached the significance level at t = 120 min, survivors showed higher TPR than nonsurvivors during the whole hypotensive period.

Respiratory, hemodynamic, and oxygenation responses. Hemodynamic and cardiac data from hemorrhaged animals are shown in Tables 1 and 2. Survivors showed a higher SI and lower HR than nonsurvivors at baseline, but differences did not reach statistical significance (0.1 > P > 0.05). APs and cardiac parameters were similar for both groups of animals during HH except at the last time point for nonsurvivors (120 min), where SI was higher in nonsurvivors, probably reflecting an increased venous return due to the larger volume of infused RL solution. A lower HR was also observed at t = 120 min for nonsurvivors. In contrast with the similar response of the cardiovascular parameters between survivors and nonsurvivors, the RR was systematically higher for survivors, from the start of the hypotensive period throughout the observation time (Fig. 2A).

Biochemical values of arterial blood reflected a stronger respiratory response from survivors: PO2, HbO2 saturation, and O2 content were higher in survivors, especially after the first hour of hypotension (Fig. 2, B–D). Similar values were found for venous blood.

The development of lactic acidosis with respiratory compensation is further characterized by the data shown in Figs. 3 and 4. Hemorrhaged animals exhibited decreased pH, PaCO2, BE, and HCO3− with a simultaneous increase in lactate. Changes in HCO3−, BE, and lactate were similar for nonsurvivors and survivors despite the larger volume received in nonsurvivors (Fig. 3). The survivor group showed better compensatory responses as expressed by higher levels of PaCO2 and pH (Fig. 4). Again, similar values were found for venous blood.

Table 3 presents data on whole body DO2, VO2, and O2ER. A clear region of DO2-dependent VO2 was present. The inflection in the VO2-DO2 relationship was similar for both groups of rats, and the calculated critical DO2 was 10.8 ml·min−1·kg−1 for survivors and 10.4 ml·min−1·kg−1 for nonsurvivors. Although similar values during hypotension were achieved by nonsurvivors and survivors, the data suggest that the responses were actually different because animals from the survivor group during baseline had lower O2ER (P = 0.06) than nonsurvivor rats. Therefore, during the first 2 h of HH, the survivors showed tendencies to a smaller average drop (from baseline) in VO2 (35 ± 2% vs. 42 ± 3%, P = 0.09). Because even small differences in VO2 may be significant over time, the O2 deficit was used to estimate the O2 debt and the cumulative O2 debt (Fig. 6). Although the variability of the data prevented the demonstration of statistical significance at all time points, the data shown in Fig. 6 are suggestive that the small difference in VO2 deficit between nonsurvivors and survivors translated into a larger O2 debt for nonsurvivor animals (0.1 > P > 0.05 for O2 debt and for cumulative O2 debt at t = 120 min).

Blood electrolytes. Transient hyperchloremia and progressive hypocalcemia (15% decrease from baseline, 0.1 > P > 0.05 at t = 120 min) were observed during HH, whereas blood Na+ levels did not vary significantly over time. All these changes followed similar patterns in nonsurvivors and survivors.

Despite the variability in oxygenation responses for nonsurvivor and survivor animals, the pattern of death was similar for all rats. Even though various levels of systemic parameters were found, all animals appeared to die in a similar and abrupt manner. Typically, an irreversible and rapid (completed in ~1 min) fall in AP, HR, and aortic flow were observed, suggestive of an acute cardiac event. A potential candidate for such an effect is the intravascular potassium level. In fact, changes in plasma K+ levels followed a very distinctive pattern, as illustrated in Fig. 7, A and B. At all time points during hypotension, nonsurvivors had a higher K+ level than that of survivors, the difference ranging from 1 to 3 mmol/l. However, the K+ levels at death were very high (above 9 mmol/l) and similar between survivors and nonsurvivors. Arterial glucose levels (Fig. 7C)

Table 1. Hemodynamic parameters during prolonged hemorrhagic hypotension

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
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<tr>
<td>Survivor</td>
<td>101.9±4.1</td>
<td>41.4±1.0†</td>
<td>37.7±1.1†</td>
<td>38.6±0.9†</td>
<td>39.8±0.3†</td>
<td>38.5±1.2†</td>
<td>36.1±3.1†</td>
</tr>
<tr>
<td>Nonsurvivor</td>
<td>110.8±4.9</td>
<td>41.4±0.7†</td>
<td>40.1±0.4‡</td>
<td>40.3±0.4†</td>
<td>40.3±0.7‡</td>
<td>38.6±1.3†</td>
<td>36.1±3.5†</td>
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<td>DP, mmHg</td>
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<td>Survivor</td>
<td>78.6±5.1</td>
<td>26.4±2.7†</td>
<td>24.1±1.7†</td>
<td>24.2±1.1†</td>
<td>23.6±1.2†</td>
<td>23.6±1.3†</td>
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<tr>
<td>Nonsurvivor</td>
<td>78.6±5.1</td>
<td>26.4±2.7†</td>
<td>24.1±1.7†</td>
<td>24.2±1.1†</td>
<td>23.6±1.2†</td>
<td>23.6±1.3†</td>
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<td>SP, mmHg</td>
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<tr>
<td>Survivor</td>
<td>128.7±4.9</td>
<td>71.8±6.6†</td>
<td>66.5±4.6†</td>
<td>71.2±8.5†</td>
<td>79.4±8.8†</td>
<td>86.7±7.6†</td>
<td>99.1±9.0</td>
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<tr>
<td>Nonsurvivor</td>
<td>130.5±4.5</td>
<td>59.6±6.2†</td>
<td>63.1±7.6†</td>
<td>69.3±7.9†</td>
<td>83.8±11.8†</td>
<td>86.7±7.6†</td>
<td>99.1±9.0</td>
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<td>PP, mmHg</td>
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<tr>
<td>Survivor</td>
<td>50.1±5.2</td>
<td>42.6±7.7</td>
<td>42.4±5.8</td>
<td>53.6±8.0</td>
<td>62.3±8.9</td>
<td>66.2±8.8</td>
<td>80.1±9.2</td>
</tr>
<tr>
<td>Nonsurvivor</td>
<td>39.6±2.9</td>
<td>30.8±6.4†</td>
<td>36.6±8.6</td>
<td>43.4±11.5</td>
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<tr>
<td>Survivor</td>
<td>0.7±0.3</td>
<td>−1.3±0.4</td>
<td>−0.3±0.6</td>
<td>−0.6±0.4</td>
<td>−0.1±0.3</td>
<td>0.3±0.3</td>
<td>1.6±0.5</td>
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<tr>
<td>Nonsurvivor</td>
<td>−0.3±0.5</td>
<td>2.2±0.3</td>
<td>−1.9±0.3‡</td>
<td>1.4±0.4‡</td>
<td>−0.2±0.5</td>
<td>0.3±0.3</td>
<td>1.6±0.5</td>
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<tr>
<td>CVP, mmHg · min−1</td>
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<td>0.67±0.04°</td>
<td>0.66±0.04°</td>
<td>0.55±0.04°</td>
<td>0.42±0.02°</td>
<td>0.39±0.03</td>
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<tr>
<td>Nonsurvivor</td>
<td>0.61±0.07</td>
<td>0.72±0.08</td>
<td>0.68±0.09</td>
<td>0.55±0.06</td>
<td>0.42±0.05</td>
<td>0.39±0.03</td>
<td>0.40±0.07</td>
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</table>

Values are means ± SE; n = 9 survivors and 8 nonsurvivors (NS). MAP, mean arterial pressure; DP, diastolic pressure; SP, systolic pressure; PP, pulse pressure; CVP, central venous pressure; TPR, total peripheral resistance. *Significantly different from baseline period; †Significantly different from the control group at the same time point.
were also significantly different between survivors and nonsurvivors, with survivors showing less pronounced hypoglycemia than nonsurvivors after the hyperglycemic response to acute blood withdrawal.

DISCUSSION

The main findings in this study were that 1) survivors showed higher PaO₂, arterial and venous HbO₂ saturation, and O₂ content than nonsurvivors; 2) better ventilation seems to explain these findings, because survivors showed a higher RR than nonsurvivors throughout the HH; 3) all animals died under high plasma K⁺ and lactate, together with low levels of glucose, Ca²⁺, BE, HCO₃⁻, and pH; and 4) the rate and amount of infused RL solution used to keep MAP at 40 mmHg was higher in nonsurvivors. Arterial K⁺ and ventilatory frequency were the only parameters significantly different between survivors and nonsurvivors at all time points during HH, suggesting that respiratory and metabolic compensations are essential for the survival to prolonged HH.

We developed a stable preparation that did not suffer deterioration in central hemodynamics during the 5-h experimental period. The mortality after hemorrhagic shock was ~50% within 2 h, producing a clinically relevant model as recommended previously (22), and sufficient number of surviving animals to allow statistical comparisons between survivors and nonsurvivors. Over 50 systemic parameters were investigated in hemorrhaged animals in an effort to identify the most discriminating ones between survivors and nonsurvivors. Sev-

Table 2. Cardiac parameters during prolonged hemorrhagic hypotension

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<tr>
<th></th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
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<tr>
<td>CI, ml·min⁻¹·kg⁻¹</td>
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<tr>
<td>Survivors</td>
<td>217.2±8.9</td>
<td>66.1±5.3†‡</td>
<td>60.5±6.3†‡</td>
<td>75.0±6.3†‡</td>
<td>96.1±4.3†‡</td>
<td>101.1±5.7†‡</td>
<td>94.8±19.7†‡</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>196.9±20.0</td>
<td>64.4±7.4†‡</td>
<td>67.2±7.7†‡</td>
<td>79.0±6.7†‡</td>
<td>100.5±11.4†‡</td>
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<tr>
<td>SI, µ/kg</td>
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<tr>
<td>Survivors</td>
<td>568.4±24.4</td>
<td>188.1±14.8†‡</td>
<td>164.1±14.2†‡</td>
<td>219.3±18.6†‡</td>
<td>293.0±21.9†‡</td>
<td>349.3±26.0†‡</td>
<td>379.6±61.9</td>
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<tr>
<td>Nonsurvivors</td>
<td>475.2±42.9</td>
<td>158.7±14.6†‡</td>
<td>179.3±20.9†‡</td>
<td>227.3±20.9†‡</td>
<td>398.6±43.2*</td>
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<tr>
<td>HR, beats/min</td>
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<td></td>
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<td></td>
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<tr>
<td>Survivors</td>
<td>383.3±9.6</td>
<td>354.9±15.1</td>
<td>366.1±16.1</td>
<td>346.1±19.4</td>
<td>335.4±17.2</td>
<td>297.5±20.7†‡</td>
<td>249.2±39.5†‡</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>411.4±8.6</td>
<td>400.9±11.1</td>
<td>376.9±14.6</td>
<td>351.0±17.0</td>
<td>264.2±31.6†‡</td>
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</tbody>
</table>

Values are means ± SE; n = 9 survivors and 8 nonsurvivors (NS). CI, cardiac index; SI, stroke index; HR, heart rate. *Significantly different from the survivor group; †significantly different from the baseline period; ‡significantly different from the control group at the same time point.
eral of these measurements were different between survivors and nonsurvivors and possibly of prognostic value in shock, especially because these measurements are clinically available. A protocol of prolonged HH was chosen because delayed interventions in hemorrhaged subjects may occur in both civilian and military scenarios, where resuscitation resources (crystalloids) are scarce and blood products are normally not available (8). However, there are relatively few studies examining the respiratory, cardiovascular, and metabolic consequences of prolonged HH (MAP of 40 mmHg). Such a time and severity combination is probably uniformly lethal in rats. Johnson et al. (15) found that the average time from initiation of hemorrhage to a MAP of 40 mmHg until death in a Wiggers’ model was under 3 h. Hemorrhage to 40 mmHg for only 1 h, followed by resuscitation was lethal in \( \frac{2}{3} \) of rats (41). We observed a mortality of 82% during 4 h of shock. However, some species are more tolerant of such an insult: 68% of conscious hamsters survived 4 h of shock at a MAP of 40 mmHg (17).

Although preheparinization (2,000 U/kg) has protective effects in hemorrhaged rats (37), it is unlikely that the single dose used in the present experiments (260 U/kg) affected the responses (40). In addition, the infusion of RL solution would have diluted even further the heparin given before baseline measurements. The anesthetic (Saffan) was chosen because it preserves both the interaction between injury and cardiovascular reflex activity and also the defense reactions (20). Injury-induced changes in baroreflex sensitivity under Saffan are similar to those seen in conscious humans (20). Saffan has been used in several studies on cardiovascular and respiratory functions in the rat (33), including studies involving determinations of \( \text{DO}_2 \) and \( \text{VO}_2 \) (10). Because CO cannot be accurately estimated from AP and HR (38), we continuously measured CO to calculate TPR, \( \text{VO}_2 \), and \( \text{DO}_2 \).

Vasoconstriction in the peripheral circulation is the normal immediate response to conditions in which the MAP is too low for adequate tissue perfusion, such as HH. Delayed hypotension (vasodilatory shock) may occur as a result of failure of the vascular smooth muscle to constrict. Our data are consistent with a vasodilatory shock after volume resuscitation during prolonged and severe HH (32). Hemorrhaged animals in this study showed a transient increase in TPR (more pronounced for survivors), and differences in TPR during HH between survivors and nonsurvivors corresponded to different rates of RL infusion. However, the different rates of RL infusion could also reflect differences in the venous capacitance between survivors and nonsurvivors.

Fig. 3. Arterial blood levels of base excess, \( \text{HCO}_3^- \), and lactate. Data are shown as in Fig. 1. †Significantly different from the baseline period; ‡significantly different from the control group at the same time point. All data are expressed as means ± SE.

Fig. 4. Arterial blood levels of pH and \( \text{PCO}_2 \). Data are shown as in Fig. 1. *Significantly different from survivors at the same time point; †significantly different from the baseline period; ‡significantly different from the control group at the same time point. All data are expressed as means ± SE.
Vasodilation found during the decompensatory phase of hemorrhagic shock, such as the one found in the present experiments, has been associated with K^+ channels (19) that can be opened by a decrease in intracellular ATP levels [ATP-sensitive K^-channels (K_{ATP}) channels] and intra- or extracellular acidosis. Hemorrhagic shock is associated with early derangements in the intracelluar metabolic status (5) and activation of K_{ATP} channels contribute to the vasodilation and early mortality. In rats hemorrhaged to a MAP of 35 mmHg, inhibition of K_{ATP} channels increased MAP and improved the survival rate (30). In addition, nitric oxide has been reported to increase in response to HH (29) and may modulate at least a portion of the observed changes in VO_2 (25).

Although vasodilation is an important component of irreversible shock, it may not have been the cause of death observed in the present experiments. Although hyperkalemia has not been directly associated with death after HH in rats, our data are suggestive that this may be the case. The sudden death of the rats seemed more characteristic of an acute toxic effect.

### Table 3. Oxygenation parameters during prolonged hemorrhagic hypotension

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do_2, ml-min^-1kg^-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Survivors</td>
<td>34.4±2.0</td>
<td>8.1±0.9†‡</td>
<td>7.2±0.6†‡</td>
<td>8.3±0.6†‡</td>
<td>9.8±0.5†‡</td>
<td>8.6±0.8†‡</td>
<td>8.1±1.7†‡</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>30.9±3.8</td>
<td>7.5±0.8†‡</td>
<td>7.5±0.8†‡</td>
<td>7.8±0.71†‡</td>
<td>7.9±1.3†‡</td>
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</tr>
<tr>
<td>Vo_2, ml-min^-1kg^-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>9.3±0.9</td>
<td>5.9±0.7</td>
<td>5.6±0.4†‡</td>
<td>5.6±0.5†‡</td>
<td>6.7±0.5</td>
<td>5.5±0.6†‡</td>
<td>4.5±0.6†‡</td>
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<tr>
<td>Nonsurvivors</td>
<td>10.7±1.1</td>
<td>6.2±0.8</td>
<td>6.1±0.8†‡</td>
<td>5.4±1.0†‡</td>
<td>6.4±1.2</td>
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<tr>
<td>O_2 ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>0.27±0.03†‡</td>
<td>0.74±0.03†‡</td>
<td>0.79±0.04†‡</td>
<td>0.69±0.06†‡</td>
<td>0.68±0.05†‡</td>
<td>0.64±0.05†‡</td>
<td>0.58±0.07</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>0.37±0.04†‡</td>
<td>0.82±0.03†‡</td>
<td>0.79±0.03†‡</td>
<td>0.78±0.04†‡</td>
<td>0.81±0.01†‡</td>
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</tbody>
</table>

Values are means ± SE; n = 9 survivors and 8 nonsurvivors. Do_2, systemic oxygen delivery; Vo_2, systemic oxygen consumption; O_2 ER, oxygen extraction ratio. *Significantly different from the survivor group; †significantly different from the baseline period; ‡significantly different from the control group at the same time point.
of high extracellular K\(^+\) than of a progressive hypoxia, acidosis, or hypoglycemia. In addition, the same maximum levels of K\(^+\) found in survivors and nonsurvivors in our study (8–10 mmol/l) have been previously found at death in rats (16, 21, 35). Ischemia-induced loss of hepatic K\(^+\) (16) could partially account for the increase in extracellular K\(^+\) because the liver during shock experiences the most severe reduction in \(V_O^2\) (2) and large decreases in blood flow (24), which are not reversed with RL resuscitation (36). Therefore, we suggest that acute cardiorespiratory failure leading to sudden death during prolonged HH is due to severe hyperkalemia. The decline in cardiac function during HH can be partially explained by additional findings in this study such as decreased Ca\(^{2+}\), glucose, pH, and RR (leading to low \(P_O^2\) and HbO\(_2\) saturation). Increased levels of TNF-\(\alpha\) (3) and lower ATP levels also contribute to cardiac depression during HH in rats (5, 11, 18).

The rate of rise in plasma K\(^+\) and its levels were remarkably different between survivors and nonsurvivors during HH. The finding of higher levels of K\(^+\) in nonsurvivors is particularly significant considering the major differences in the amount of infused RL solution. Nonsurvivors appeared to have higher release of intracellular K\(^+\) because the levels of plasma K\(^+\) were diluted by the infused RL solution. For instance, at \(t = 120\) min, the plasma concentration of K\(^+\) in nonsurvivors was 50% higher than in survivors despite a 67% higher amount of infused RL solution. Treatments (hypothermia) leading to decreasing levels of hyperkalemia during HH improve outcome (16, 39). In addition, our animals showed hypoglycemia during HH. Insulin and glucose have been suggested in the treatment of HH leading to increased hepatic ATP (4), but their beneficial hypoglycemic role should be counterbalanced with the hypokalemic effect.

Respiratory compensation may account for the survival or nonsurvival of the rats because the RR was significantly depressed in nonsurvivors, leading to hypoxia, more severe acidosis, and higher PaCO\(_2\) than in survivors. A similar response has been observed previously and reversed by central histamine injection (14). Taken together, the data suggest that RR and blood oxygenation parameters (such as \(P_A^2\), arterial and venous HbO\(_2\) saturation, and \(O_2\) content) may be important predictors of mortality.

The difference in blood oxygenation between survivors and nonsurvivors could favor the idea of \(O_2\) therapy in HH. However, \(O_2\) therapy to increase \(P_A^2\) should be used with caution because \(O_2\) breathing has improved the outcome in some studies with rats (6) but failed to do so in others (31). Differences in the severity of the HH, anesthetic agent, and \(P_A^2\) are probably involved in these discrepancies. In addition, beneficial actions of hyperoxia should be counterbalanced by its microvascular effects such as vasoconstriction and decreased functional capillary density (34). Nevertheless, our data support the concept of a “physiological” increase in blood oxygenation because S were breathing with a normal fraction of inspired \(O_2\) and achieved higher \(P_O^2\), HbO\(_2\) saturation, and \(O_2\) content by increased RR.

As in previous studies (24, 25), overall \(D_O^2\) was measured in the present study by the product of CO and arterial \(O_2\) content, whereas body metabolism was evaluated by \(V_O^2\) using the product of \(C_O\) and the arteriovenous \(O_2\) content difference. All hemorrhaged animals fell below critical \(D_O^2\) when the target MAP was reached, and a well-defined, delivery-dependent portion of the \(D_O^2\)-\(V_O^2\) relationship was found during HH. Although no significant differences were found between survivors and nonsurvivors in terms of \(D_O^2\), critical \(D_O^2\), and \(V_O^2\),
whole body D\textsubscript{O}\textsubscript{2} may be of limited value in evaluating the oxygenation in organs and tissues during HH. It has been shown that D\textsubscript{O}\textsubscript{2}, V\textsubscript{O}\textsubscript{2}, and O\textsubscript{2}ER in various organs are differentially altered after hemorrhagic shock in the rat (2). Therefore, although whole body D\textsubscript{O}\textsubscript{2} was similar in survivors and nonsurvivors, important differences might exist in regional oxygenation. The increased levels of K\textsuperscript{+} in nonsurvivors support the concept of a more severe liver hypoxia in these animals.

Tissue hypoxia were also estimated by calculating the O\textsubscript{2} debt from the difference between V\textsubscript{O}\textsubscript{2} before and during HH, as described previously (26). A pattern of higher O\textsubscript{2} debt was found in nonsurvivors during HH. The correlation between increased O\textsubscript{2} debt and outcome in HH has been shown for rats, pigs, dogs, and humans (9, 26, 28). Similar results were found in hamsters (17).

Traditional metabolic indexes of shock severity such as lactate, BE, and HCO\textsubscript{3} were not different between survivors and nonsurvivors, suggesting that these may not be considered as good predictors of mortality. This contrasts with reports in humans (9), but it is consistent with the view that lactate may be of limited value as an indicator of tissue hypoxia (13). In addition, CI was not significantly different between survivors and nonsurvivors. Similar findings have been reported in humans (27). However, during baseline, survivors had a tendency to show lower HR and higher SI than nonsurvivors. This finding may be significant because it suggests that better resting cardiac conditioning (traditionally expressed by low HR and high SI) may be an additional beneficial factor for the survival of prolonged HH.

In summary, the present study in hemorrhaged rats documents distinct patterns in survivors and nonsurvivors after the onset of HH. Animals that died in <3 h (nonsurvivors) showed greater metabolic acidosis than survivors, including higher Pco\textsubscript{2} and lower RR, P\textsubscript{O}\textsubscript{2}, HbO\textsubscript{2} saturation, O\textsubscript{2} content, and pH. Arterial K\textsuperscript{+} showed the clearest distinction between survivors and nonsurvivors, and the elevated levels of K\textsuperscript{+} may explain the sudden death experienced by animals during HH. The data suggest that respiratory and metabolic compensations are essential for the survival of prolonged HH.

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