Systemic and microcirculatory effects of autologous whole blood resuscitation in severe hemorrhagic shock

Heinz Kerger, Klaus F. Waschke, Klaus V. Ackern, Amy G. Tsai, and Marcos Intaglietta. Systemic and microcirculatory effects of autologous whole blood resuscitation in severe hemorrhagic shock. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H2035–H2043, 1999.—Systemic and microcirculatory effects of autologous whole blood resuscitation after 4-h hemorrhagic shock with a mean arterial pressure (MAP) level of 40 mmHg were investigated in 63 conscious Syrian golden hamsters. Microcirculation of skeletal skin muscle and subcutaneous connective tissue was visualized in a dorsal skinfold. Shed blood was retransfused within 30 min after 4 h. Animals were grouped into survivors in good (SG) and poor condition (SP) and nonsurvivors (NS) according to 24-h outcome after resuscitation and studied before shock, during shock (60, 120, and 240 min), and 30 min and 24 h after resuscitation. Microvascular and interstitial PO2 values were determined by phosphorescence decay. Shock caused a significant increase of arterial PO2 and decrease of PCO2, pH, and base excess. In the microcirculation, there was a significant decrease in blood flow (Qb), functional capillary density (FCD; capillaries with red blood cell flow), and interstitial PO2 [1.8 ± 0.8 mmHg (SG), 1.3 ± 1.3 mmHg (SP), and 0.9 ± 1.1 mmHg (NS) vs. 23.0 ± 6.1 mmHg at control]. Blood resuscitation caused immediate MAP recompensation in all animals, whereas metabolic acidosis, hyperventilation, and a significant interstitial PO2 decrease (40–60% of control) persisted. In NS (44.4% of the animals), systemic and microcirculatory alterations were significantly more severe both in shock and after resuscitation than in survivors. Whereas in SG (31.8% of the animals) there was only a slight (15–30%) but still significant impairment of microscopic tissue perfusion (Qb, FCD) and oxygenation at 24 h, SP (23.8% of the animals) showed severe metabolic acidosis and substantial decreases (≥50%) of FCD and interstitial PO2, FCD, interstitial PO2, and metabolic state were the main determinants of shock outcome.

Current therapy of severe hemorrhagic shock focuses on the avoidance of homologous (whole) blood or blood products transfusion because of side effects and drawbacks such as transmission of viral diseases (e.g., hepatitis and acquired immunodeficiency syndrome), immunosuppression, cancer recurrence, allergic reactions, and limited availability (26, 33). Although primary volume replacement is usually made by infusion of crystalloids and/or colloids (8, 26), thus engaging the therapeutic advantage of induced hemodilution (20, 21, 26), shock survival is directly dependent on the restitution of adequate blood oxygen carrying capacity once hematocrit falls below a critical range of 20–25% (20, 21). This, however, can currently be accomplished only by transfusion of autologous or homologous blood, since artificial oxygen carriers such as cell-free hemoglobin solutions are not yet routinely available (26, 33).

Previous investigations have shown that the severity of hemorrhagic shock and the success of volume therapy with oxygen and/or nonoxygen carriers may be predicted by the extent of metabolic and respiratory perturbations both during and after the shock period (16, 17). Arterial base excess has been identified as an accurate indicator of persisting volume deficit and whole body oxygen debt in shock (6, 7, 10) and as a valuable and reliable predictor of shock outcome (10, 25), a finding also supported by results from our laboratory using a severe 2- and 4-h hemorrhagic shock model (40 mmHg), respectively (16, 17). These studies also revealed that at the level of microscopic tissue perfusion, nonsurvivable shock was directly related to a significant decrease of functional capillary density, with consequent impairment of tissue oxygenation and poor removal of (toxic) metabolites causing local and systemic blood acidosis (6, 10, 16, 17).

This study investigates systemic and microcirculatory effects of (isovolemic) autologous whole blood resuscitation after severe (40 mmHg) 4-h hemorrhagic shock and determines whether shock outcome after shed blood retransfusion can be predicted by alterations in systemic and/or microcirculatory conditions during the shock period and/or after volume resuscitation. Investigations were conducted in conscious Syrian golden hamsters fitted with a dorsal skinfold preparation (11), a chronic microcirculatory model without the effects of acute surgery and anesthesia (15). Microvascular and interstitial PO2 measurements in skeletal skin muscle and subcutaneous connective tissue were accomplished by the recently developed phosphorescence decay methodology (29, 30, 32).

Materials and Methods

Animal model. Systemic and microcirculatory effects of 4-h hemorrhagic shock and after fluid resuscitation with autologous whole blood were studied in male Syrian golden hamsters (45–70 g body wt) fitted with a dorsal skinfold chamber window (11). This model provides microscopic access to the vasculature of skeletal skin muscle and subcutaneous connective tissue, and limited availability (26, 33). Although primary volume replacement is usually made by infusion of crystalloids and/or colloids (8, 26), thus engaging the therapeutic advantage of induced hemodilution (20, 21, 26), shock survival is directly dependent on the restitution of adequate blood oxygen carrying capacity once hematocrit falls below a critical range of 20–25% (20, 21). This, however, can currently be accomplished only by transfusion of autologous or homologous blood, since artificial oxygen carriers such as cell-free hemoglobin solutions are not yet routinely available (26, 33).
tive tissue and allows investigation of the microcirculation in conscious animals for a prolonged experimental period. Pentobarbital sodium (50 mg/kg body wt ip, Nembutal, Abbott Laboratories, North Chicago, IL) was used as an anesthetic for chamber window implantation and catheterization of the carotid artery and jugular vein. All animals were allowed a recovery period of 2 days after completion of surgical procedures. Experimentation was performed only in animals with healthy appearance and absence of inflammation, bleeding, edema, or low flow states in the skinfold preparation.

Shock model. Shock was induced by stepwise hemorrhage of 50% of the animals’ total blood volume, which was estimated to be 7% of the body weight (18). The shock level was defined by a mean arterial pressure (MAP) of 40 mmHg maintained over a period of 4 h. Within 30 min, blood was taken in four identical quantities from the carotid artery by means of heparinized syringes (heparin sodium, 1,000 U/ml, Elkins-Sinn, Cherry Hill, NJ). Additional small aliquots of blood (0.1–0.3 ml) were withdrawn if the 40-mmHg blood pressure level was not achieved or exceeded 45 mmHg during the following shock period. With the exception of small quantities of saline (0.05 ml) necessary for catheter flushing after blood withdrawal, animals did not receive any fluid or blood while in shock.

Autologous whole blood resuscitation. All animals (n = 63) surviving the 4-h shock period were fluid resuscitated with autologous whole blood where all shed blood collected during induction and maintenance of shock was retransfused. Heparinized shed blood (heparin sodium, 1,000 U/ml, Elkins-Sinn) was stored in a refrigerator at 4°C after withdrawal and heated to room temperature (25°C) in a water bath (37°C) before retransfusion. Transfusion was performed in four identical quantities within 30 min.

Evaluation of systemic parameters. Blood samples for measurement of hemoglobin concentration (B-Hemoglobin Photometer Hemocue, Angelholm, Sweden), hematocrit (Readacrit Centrifuge, Clay Adams, Division of Becton-Dickinson, Parsippany, NJ), arterial blood gases, pH, and base excess (Ciba Corning 238 pH/blood gas analyzer, Ciba Corning Diagnostics, Pleasanton, CA) were collected in heparinized microtubes (microhematocrit capillary tubes, Scien-Photometer Hemocue, Angelholm, Sweden), hematocrit (Readacrit Centrifuge, Clay Adams, Division of Becton-Dickinson, Parsippany, NJ), arterial blood gases, pH, and base excess (Ciba Corning 238 pH/blood gas analyzer, Ciba Corning Diagnostics, Pleasanton, CA) were collected in heparinized microtubes (microhematocrit capillary tubes, Scientific Products, Division of Travenol Laboratories, McGraw Park, IL). All blood samples were analyzed immediately after withdrawal, and each animal’s prevailing body temperature was taken into account for determination of arterial blood gas and acid-base state. Central body temperature was measured rectally, while dorsal skin temperature was assessed epidermally in three different locations outside the skinfold preparation area by means of a microthermometer (Physitemp, model BAT-12, Physitemp Instruments, Clifton, NJ). Room temperature was kept constant at 25°C during experimentation. An analog recording system (Beckman R611, Beckman Instruments, Schiller Park, IL) was used for continuous monitoring of MAP and heart rate.

Analysis of microhemodynamic parameters. Microvessels in skeletal skin muscle and subcutaneous connective tissue were observed with an inverted microscope (IMT-2, Olympus, New Hyde Park, NY) using a ×40 water-immersion objective (Olympus WP1an, numerical aperture = 0.7) and transillumination technique. Microscopic images were recorded by a video camera (Cohu 4815–2000) and transferred to a television-videocassette recorder (Sony Trinitron PVM-1271Q monitor and Panasonic AG-7355 video recorder). Microvessels were classified according to their exact up- or downstream position within the microvascular network (15–17). Arteriolar microvessels were grouped by diameter into large feeding arterioles (A1, 79.0–88.1 µm), small arcading arterioles (A2, 24.3–33.3 µm), transverse arterioles (A3, 8.7–10.3 µm), and terminal arterioles (A4, 6.4–6.8 µm). Venules were classified by diameter as small collecting venules (23.2–33.0 µm) and large venules (98.0–112.4 µm). Capillary segments and interstitial tissue locations (interstitium) were supplied and drained by the selected arteriolar and venular tree, thus forming a functional unit with these microvessels.

Microvascular diameter and red blood cell (RBC) velocity (vRBC) were analyzed on-line in arterioles and venules. Vessel diameter (d) was measured with an image-shearing system (13) (digital video image shearing monitor, model 908, IPM, San Diego, CA), while vRBC was analyzed by photodiodes (fiber-optic photo diode pickup system, IPM) and cross-correlation technique (velocity tracker mod-102 B, IPM) (12). Blood flow rates (Qb) were calculated from evaluated vessel diameters and RBC velocities (14) according to the formula

\[ Q_b = \alpha \times v_{RBC} \times \pi \times d^2 / 4 \]

where \( \alpha \) represents a correction factor for different-sized microvessels. According to the literature (14), a factor of 2.0 was used for vessels >60 µm (A1 arterioles and large venules), whereas 1.6 was used for vessels between 20 and 60 µm (A2 arterioles and small collecting venules). The factor 1.3 was used for the smaller vessels (A3 and A4 arterioles). The factor initially selected for each individual blood vessel was also used for flow calculation over time.

Functional capillary density (FCD), defined as the number (percentage) of capillary segments that have RBC transit of at least one RBC in a 30-s period, was analyzed from four to six different video-recorded microscopic fields exhibiting four to six different capillaries each. We selected a total of 15–20 different capillaries per animal that were supplied from different terminal (A4) arterioles branching off the selected transverse (A3) arteriole. Only capillaries with straight segments of an average length of 600 µm (400–800 µm) were considered for calculation of FCD. The initially selected fields were followed throughout the experimental protocol. This procedure was chosen to relate functionality or nonfunctional-ity of capillaries directly to the microhemodynamic conditions in the supplying arteriolar microvessels (15–17).

Determination of microvascular and interstitial PO2. Subcutaneous microvascular and interstitial (tissue) PO2 values were determined with the previously described phosphorescence decay technique (29, 30, 32). The method is based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complexes after pulsed light excitation and allows noninvasive assessment of intravas-ular as well as interstitial PO2, since intravascularly injected porphyrin complexes extravasate into the interstitium over time (15–17, 30). The relationship between phosphorescence lifetime (τ) and oxygen tension is given by the Stern-Volmer equation

\[ \tau_0 / \tau = 1 + k_q \times \tau_0 \times PO_2 \]

where \( \tau_0 \) and \( \tau \) are the phosphorescence lifetimes in the absence of molecular oxygen and at a given PO2, respectively, and \( k_q \) is the quenching constant, with both factors being pH and temperature dependent.

Palladium-meso-tetra(4-carboxyphenyl)porphyrin (Porphyрин Products, Logan, UT) bound to serum albumin and diluted in saline (0.9% sodium chloride, Elkins-Sinn) to a final concentration of 15 mg/ml was used as a phosphorescent dye (\( \tau_0 = 600 \mu s, k_q = 325 \text{Torr}^{-1} \cdot s^{-1} \cdot \text{at pH 7.4 and 37°C} \)) and intravenously injected (30 mg/kg body wt).

Phosphorescence was excited by light pulses (30 Hz) generated by a 45-W xenon strobe arc (EGG&E Electro Optics,
Salem, MA). The pulsed light illuminated a round area of ~140 µm diameter, while $P_O_2$ measuring sites were microscopically vignetted by an adjustable slit. For microvascular $P_O_2$ measurements, the slit was longitudinally fitted within the vessel, whereas for analysis of interstitial $P_O_2$, it vignetted intercapillary spaces avoiding interference with blood vessels. Filters of 420 and 630 nm were used for porphyrin excitation and phosphorescence emission, respectively. Phosphorescence signals were captured by a photomultiplier (EMI, 9855B, Knut Elektronik, Munich, Germany). One hundred twenty-eight decay curves were averaged, visualized, and saved by a digital oscilloscope (Hitachi Oscilloscope V-1065, 100 MHz, Hitachi Denshi). Decay time constants were determined by a computer fitting the averaged decay curves to a single exponential (29), using the Stern-Volmer equation. The probe constants $t_0$ and $k_p$ were corrected to reflect the prevailing temperature (~28–30°C) in the skinfold preparation. Both factors were also corrected for pH, using the actual blood value. This was done also for interstitial $P_O_2$ measurements, since we could not measure tissue pH in this preparation. Because it may not be excluded that interstitial pH is slightly lower than systemic blood pH, interstitial $P_O_2$ values may be slightly overestimated (32), since $t_0$ decreases and $k_p$ increases on the acidic side of pH 7.2 (17 and 8%, respectively). This discrepancy, however, should be small, as shown by the agreement between phosphorescence decay and microelectrode measurements of tissue $P_O_2$ made in this preparation (3).

Experimental design and statistics. Sixty-three animals were subjected to hemorrhage and resuscitated with autologous whole blood after the 4-h shock period. There were 35 survivors and 28 nonsurvivors (NS) at 24 h after resuscitation. Surviving animals were further classified according to their general health appearance on the basis of consciousness, motor activity, and sleeping and feeding habits. Results were grouped according to the different outcome into animals being in good condition at that time point (SG) and animals with poor appearance (SP). Animals succumbing already during the shock period were not included in the data.

Systemic and microhemodynamic parameters were analyzed in all (63) animals at baseline, during shock (60, 120, and 240 min), and 30 min and 24 h after autologous whole blood resuscitation.

Microvascular and interstitial $P_O_2$ values were determined in groups of seven animals for each time point of shock as well as 30 min (surviving and nonsurviving animals) and 24 h after shed blood retransfusion (surviving animals). To avoid microhemodynamic side effects due to excessive light exposure and porphyrin excitation, repeated $P_O_2$ measurements in the same animals were avoided, and $P_O_2$ control values were assembled in an independent group of seven animals, the systemic and microhemodynamic conditions of which were not significantly different from those undergoing hemorrhagic shock and autologous whole blood resuscitation.

All data are shown as means ± SD. The minimum level of significance considered was 5% ($P < 0.05$) for all statistical tests. For repeated measurements, time-related changes were assessed by ANOVA. Differences among groups (SG, SP, NS) at the various time points were also evaluated by ANOVA.

RESULTS

Blood shed volume for induction and maintenance of shock was not significantly different between surviving and nonsurviving animals [in ml/100 g body wt: 3.7 ± 0.5 and 5.3 ± 0.3 (SG), 3.8 ± 0.4 and 5.2 ± 0.3 (SP), and 3.9 ± 0.5 and 5.5 ± 0.5 (NS), respectively]. The decrease in hemoglobin and hematocrit levels at the beginning and the end of the shock period was also not significantly different between groups (Table 1). Hemoglobin concentrations and hematocrit levels remained significantly suppressed in all animals on the order of 11.6–12.6 g/dl and 34.3–37.9%, respectively, 30 min and 24 h after autologous whole blood transfusion; differences between groups, however, did not reach significance (Table 1).

Transfusion of autologous whole blood caused recompensation of MAP in all animals, whereas animals surviving in poor condition exhibited a significantly decreased blood pressure 24 h after resuscitation (87.1 ± 10.6 vs. 104.0 ± 10.9 mmHg at baseline) (Fig. 1). Survivors had a nonsignificant decrease in heart rate during shock and normal heart rates after shed blood retransfusion, whereas nonsurvivors developed significant bradycardia during the shock period that persisted after resuscitation (Fig. 1).

The 4-h hemorrhagic shock period caused metabolic acidosis and compensatory hyperventilation in all animals as evidenced by a significant decrease in arterial pH, base excess, and $PCO_2$ and increased $P_O_2$ levels (Fig. 2). Alterations in arterial blood gas and acid-base state were significantly more severe in nonsurvivors at various time points of the shock period when compared with surviving animals, with $P_O_2$ and $PCO_2$ levels reaching 117.1 ± 9.8 and 30.8 ± 5.9 mmHg, respectively, at the end of the shock period when compared with 65.8 ± 9.3 and 46.2 ± 7.3 mmHg at baseline (Fig. 1). In nonsurvivors, arterial pH and base excess decreased already in an early shock stage from 7.36 ±

Table 1. Hemoglobin concentrations and hematocrit before and during shock as well as after autologous whole blood resuscitation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Before</th>
<th>Shock 60 Min</th>
<th>Shock 120 Min</th>
<th>Shock 240 Min</th>
<th>Resuscitation 30 Min</th>
<th>Resuscitation 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dl</td>
<td>SG</td>
<td>15.6 ± 0.5</td>
<td>8.8 ± 1.3*</td>
<td>8.2 ± 0.6*</td>
<td>7.9 ± 0.9*</td>
<td>12.0 ± 0.8</td>
<td>12.6 ± 1.1*</td>
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<tr>
<td></td>
<td>SP</td>
<td>16.2 ± 0.5</td>
<td>8.7 ± 0.9*</td>
<td>8.3 ± 1.3*</td>
<td>8.0 ± 0.2*</td>
<td>11.7 ± 1.3</td>
<td>12.0 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>15.0 ± 1.3</td>
<td>8.1 ± 0.9*</td>
<td>8.2 ± 0.9*</td>
<td>7.5 ± 0.8*</td>
<td>11.6 ± 1.0</td>
<td>35.1 ± 4.4*</td>
</tr>
<tr>
<td>Hct, %</td>
<td>SG</td>
<td>48.4 ± 2.3</td>
<td>26.9 ± 3.8*</td>
<td>23.8 ± 3.3*</td>
<td>20.3 ± 4.3*</td>
<td>36.3 ± 5.2</td>
<td>35.9 ± 7.0*</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>47.1 ± 3.5</td>
<td>25.1 ± 3.5*</td>
<td>23.1 ± 4.5*</td>
<td>22.0 ± 3.5*</td>
<td>34.3 ± 4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>46.0 ± 3.8</td>
<td>24.8 ± 2.1*</td>
<td>23.9 ± 3.7*</td>
<td>21.3 ± 3.6*</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SD. Hb, hemoglobin; Hct, hematocrit; SG, survivors in good condition (n = 20 animals); SP, survivors in poor condition (n = 15); NS, nonsurvivors (n = 28 animals). All statistical comparisons were made against baseline in each group and between groups (ANOVA). *Significantly different from baseline ($P < 0.05$).
0.04 and 2.0 ± 3.1 mM (baseline) to 7.22 ± 0.06 and −9.1 ± 3.5 mM at shock end. In surviving animals, corresponding values were 7.31 ± 0.05 and −4.3 ± 2.1 mM (SG) and 7.30 ± 0.02 and −5.1 ± 2.0 mM (SP), respectively (Fig. 2).

Thirty minutes after autologous whole blood transfusion, surviving animals showed a decrease in PO2 and an increase in PCO2, pH, and base excess levels relative to the shock period, being indicative of mitigated metabolic acidosis and compensatory hyperventilation (Fig. 2). However, except for arterial pH, all parameters remained significantly different from baseline conditions. Although animals surviving in good condition (n = 20) had normal pH, only slightly decreased base excess levels, and a tendency of hyperventilation 24 h after resuscitation, survivors in poor condition (n = 15) exhibited severe metabolic acidosis and hyperventilation at that time point (Fig. 2). Corresponding pH and base excess values were 7.29 ± 0.04 and −6.0 ± 1.3 mM, respectively, whereas PO2 and PCO2 levels reached 97.4 ± 10.9 and 38.3 ± 4.6 mmHg, respectively (Fig. 2).

In contrast, nonsurviving animals showed persistence of severe metabolic acidosis 30 min after autologous whole blood transfusion when compared with the shock period, where pH and base excess levels were 7.23 ± 0.05 and −8.1 ± 3.2 mM, respectively (Fig. 2). Resulting alterations in arterial PO2 (112.8 ± 15.0 mmHg) and PCO2 (33.0 ± 5.1 mmHg) levels were also significantly more pronounced than in surviving animals (Fig. 2).

In all animals, 4-h hemorrhagic shock caused a significant reduction of arteriolar, venular, and interstitial PO2 levels (Fig. 3) in the skinfold preparation. Oxygen tension in the A1, A2, A3, and A4 arterioles (53.7 ± 7.4, 45.1 ± 6.5, 40.3 ± 8.1, and 35.1 ± 8.5 mmHg at control, respectively) decreased to an extent of (in mmHg) 37.8 ± 16.9 (A1), 25.3 ± 10.1 (A2), 16.3 ± 6.9 (A3), and 7.0 ± 3.6 (A4) at the end of the shock period, where relatively lower values in nonsurvivors differed in some instances significantly from PO2 values in surviving animals. Resulting interstitial PO2 levels were (in mmHg) 1.8 ± 0.8 (SG), 1.3 ± 1.3 (SP), and
0.9 ± 1.1 (NS) vs. 23.0 ± 6.1 at control (Fig. 3). Interstitial PO2 values also differed in some instances significantly between surviving and nonsurviving animals (Fig. 3), whereas no significant difference could be found for slightly higher venular PO2 levels.

Autologous whole blood resuscitation caused a marked increase of arteriolar, venular, and interstitial PO2 (Fig. 3) in all animals when compared with the shock period, although values still remained significantly lower (20–60%) than under control conditions. Microvascular PO2 levels were higher in surviving animals, although these values did not differ in all microvessel types significantly from those in nonsurviving animals. Thirty minutes after resuscitation, surviving animals exhibited A1 and A4 arteriolar PO2 values of 44.8 ± 9.7 and 24.0 ± 6.2 mmHg (SG) and 44.0 ± 9.3 and 21.0 ± 3.6 mmHg (SP), respectively, whereas in nonsurvivors, corresponding values were 36.7 ± 9.3 and 16.6 ± 6.9 mmHg, respectively. Resulting interstitial PO2 levels [15.8 ± 6.1 mmHg (SG), 14.3 ± 4.5 mmHg (SP), and 9.4 ± 4.1 mmHg (NS)] were significantly different between surviving and nonsurviving animals (Fig. 3).

Although animals surviving in poor condition showed a substantial decrease in arteriolar, interstitial, and venular PO2 levels 24 h after resuscitation relative to control, survivors in good condition exhibited only a slight PO2 decrease, with differences between animal groups reaching significance. A1, A4 arteriolar, and interstitial PO2 values were 37.0 ± 2.8, 19.7 ± 9.1, and 12.7 ± 5.2 mmHg in the first group (SP), whereas they reached 47.8 ± 6.1, 27.5 ± 5.7, and 18.8 ± 4.1 mmHg in the second group (SG) (Fig. 3).

Hemorrhagic shock also caused a significant constriction of A1 arteriole diameters in all animals, which was in some instances significantly more pronounced in nonsurviving animals (Fig. 4). Although all animals exhibited no significant changes of A2 vessel diameters during the shock period, A3 arteriole diameters significantly decreased in nonsurvivors. All animals also showed significant vasodilation of A4 arterioles and a decline in vessel diameters of large venules (Fig. 4), whereas no significant changes could be observed in small collecting venules during shock.

After shock resuscitation, a significant constriction of A1 arteriole diameters persisted in nonsurvivors (NS) and at 24 h in animals surviving in poor condition (SP) (Fig. 4). Diameters of A1 arterioles in animals surviving in good condition (SG) were also slightly decreased; these changes, however, did not reach significance (Fig. 4). There was also a significant decline in vessel diameters of large venules in all animals 30 min after shed blood retransfusion (Fig. 4), whereas no significant changes could be found for the other microvessel types.
The 4-h hemorrhagic shock period was associated with a significant and parallel decrease of microvascular \( v_{RBC} \) and \( Q_B \) in all animals (Fig. 4). Arteriolar \( v_{RBC} \) and \( Q_B \) levels were higher in surviving animals during shock, but only in some instances was there a significant difference between survivors and nonsurvivors (Fig. 4). Relatively higher \( v_{RBC} \) and \( Q_B \) levels of large venules in nonsurviving animals (50–60% of baseline) were also not significantly different from values in survivors (30–40% of baseline) (Fig. 4).

Autologous whole blood resuscitation caused a marked and comparable increase in microvascular \( v_{RBC} \) and \( Q_B \) relative to the shock period, although resulting values were still significantly lower than under baseline conditions (Fig. 4). Both parameters differed only in some microvessel types significantly between surviving and nonsurviving animals. Persisting deficits were up to 50% in surviving animals, whereas they reached 75% in nonsurvivors (Fig. 4). Normal microvascular \( v_{RBC} \) and \( Q_B \) rates could not be observed in surviving animals even 24 h after resuscitation (Fig. 4). However, although animals surviving in good condition (SG) showed only a slight reduction of 20–30% compared with baseline, \( v_{RBC} \) and \( Q_B \) decreases were >60% in survivors with poor appearance (SP). These differences between animals reached significance in various vessel types (Fig. 4).

Hemorrhagic shock also caused a significant reduction of FCD in all animals, with values being lowered to 15–25% of baseline at shock end (Fig. 5). FCD levels of surviving animals exceeded values of nonsurvivors, with differences reaching significance at various time points of the shock period (Fig. 5).

Although autologous whole blood transfusion increased FCD in all animals relative to the shock period, normal values could not be reestablished (Fig. 5). Thirty minutes after resuscitation, FCD levels were 75.6 ± 17.7% (SG), 59.7 ± 32.2% (SP), and 37.4 ± 20.6% (NS) relative to baseline, respectively, with differences between survivors and nonsurvivors reaching significance (Fig. 5). Whereas survivors in good condition (SG) showed only a slight FCD decrease (87.2 ± 14.3% of baseline) 24 h after resuscitation, a substantial reduction to ~50% of baseline could be observed in survivors with poor appearance (SP) (Fig. 5).

Overall, 24-h survival rate of hemorrhagic shock and after fluid resuscitation with autologous whole blood was 55.6% (35 of 63 animals). However, only 20 of 35 surviving animals (31.8%) were in good physical condition at that time point as judged by the normalcy of consciousness, motor activity, and sleeping and feeding habits, whereas the remainder of animals (23.8%) showed a poor physical appearance.

**DISCUSSION**

The current study investigated systemic and microcirculatory effects of autologous whole blood resuscitation after severe 4-h hemorrhagic shock (MAP, 40 mmHg) in a conscious animal model to relate differences in shock outcome to the various degree of systemic and microcirculatory alterations both during shock and after fluid resuscitation. A principal finding was that the prolonged hemorrhagic shock period and after shed blood transfusion were survivable for a period of 24 h only by 55.6% of the animals. Even though 57.1% of these survivors appeared to be in good physical condition at that time point and showed normal arterial pressure and metabolic conditions, microscopic tissue perfusion (\( Q_B \), FCD) and interstitial oxygenation in the skinfold remained slightly but significantly impaired, and \( Q_B \), FCD, and interstitial Po2 were 15–30% lower than normal. In the remainder of survivors, poor health appearance correlated with macrohemodynamic and metabolic instability and a substantial (~50–60%) decrease in microscopic tissue perfusion and interstitial oxygenation relative to preshock conditions. In contrast, nonsurvivors showed significantly more severe systemic and microcirculatory perturbations already during the shock period, which could not be overcome by fluid resuscitation. Differences in outcome appeared to be directly related to the degree of microscopic tissue perfusion (\( Q_B \), FCD) and interstitial oxygenation impairment, factors crucially determining systemic metabolic conditions.

Return to normal MAP in all animals already during autologous whole blood transfusion is in accordance with the findings of numerous previous investigations (4, 23, 28) and can be attributed to the increase in circulating intravascular volume and rise in cardiac output that may be reduced by >80% during severe hemorrhagic shock (5, 28). Persistence of significant bradycardia in nonsurviving animals 30 min after resuscitation, however, may be primarily due to reflex activity and has been observed also in other shock models of comparable severity (28, 31). This, however, did not affect arterial blood pressure levels, since there was not a significant difference between surviving and nonsurviving animals both in shock and after resuscitation.

Therefore, it may be possible that normalization of MAP in nonsurviving animals after shed blood transfusion was in part related to a persisting increase in sympathetic tone causing increased vascular resistance and centralization of the circulation by stimula-
the PO₂ decrease within the arteriolar system is a consequence of the precapillary "loss of oxygen," since the blood vessel wall is only a partial barrier to the extravascular oxygen diffusion gradient (9, 16, 22, 27, 30). This arteriolar oxygen exit is accentuated during shock because a lower blood flow rate increases residence time of blood in the vessels, and the prevailing low interstitial PO₂ levels determine a steeper intra-/extravascular oxygen diffusion gradient (9, 16, 22, 27, 30). Although the arteriolar oxygen exit ultimately becomes a part of the oxygen supply of the tissue and, therefore, contributes to its oxygenation, some of this oxygen is shunted into the venous return as evidenced by the fact that large venules had relatively higher PO₂ levels than the tissue and small collecting venules (data not shown).

The marked decrease in FCD was a critical difference in microcirculatory parameters between survivors and nonsurvivors. In a previous study, we found that survival during a prolonged shock protocol was directly related to the maintenance of FCD (16). Substantially decreased FCD was also related to lower interstitial PO₂ levels in nonsurvivors both during the shock period and after resuscitation, whereas the difference in interstitial tissue oxygenation impairment between surviving and nonsurviving animals was mirrored by corresponding alterations in arterial blood gas and acid-base state.

Lowered FCD in nonsurvivors corroborates results of different animal models where FCD decreased by 70% after shock resuscitation with autologous whole blood (1, 2). This reduction was in part related to endothelial swelling that was maintained after shed blood transfusion (19). Capillary perfusion impairment may also be due to capillary plugging due to trapped leukocytes (1, 2). A contributing factor for capillary perfusion deficits may also be the increase in blood viscosity induced by autologous blood transfusion, leading to increased hydraulic resistance within the capillaries (20, 26).

Shock studies in rats have shown complete return to normal metabolic conditions after shock resuscitation with autologous whole blood (23, 24), a finding that corresponded to restoration of normal tissue oxygenation (23). In our study, all animals showed significant metabolic acidosis as evidenced by decreased base excess levels at 30 min after shed blood retransfusion. Recompensation of pH levels in surviving animals may in part be the result of compensatory hyperventilation (16, 17). Persisting metabolic derangements are the expression of a significant global oxygen deficit (6, 10), which agrees with the findings in our microcirculatory model, where perfusion and interstitial oxygenation remained significantly impaired in all groups after resuscitation. Persisting metabolic instability in this current study may in part be due to the long (4-h) duration of the hemorrhagic shock period when compared with previous investigations (23, 24).

Metabolic and respiratory alterations were significantly more pronounced in nonsurviving animals during the 4-h shock period (16) and after autologous whole blood resuscitation (17). This may be indicative of a significantly more severe oxygen supply deficit and oxygen debt already during the shock period (6, 10, 16), which could not be overcome by fluid resuscitation (17). These clear-cut differences in metabolic conditions between surviving and nonsurviving animals were correlated with the degree of FCD reduction and tissue oxygenation impairment. It should also be noted that global metabolic disorders are also dependent on oxygen supply and consumption in all other organs and tissues not investigated in our study.

Differences in blood gas and acid-base state between survivors and nonsurvivors and differences in outcome after blood retransfusion could not be related to the level of hemorrhage, since blood-shed volumes did not differ significantly between animals. Given our and previous findings (6, 10, 16, 17, 34), it would appear that the oxygen supply deficit and oxygen debt during hemorrhagic shock as indicated by systemic metabolic and respiratory perturbations significantly determine long-term outcome and survival rates after fluid resuscitation with blood. Survival appears to be directly related to the generation and management of metabolic by-products causing systemic blood acidosis, which, in turn, should be the ultimate consequence of capillary perfusion and tissue oxygenation impairment observed in this microcirculatory environment.

Animals surviving for a period of 24 h after shed blood retransfusion could readily be grouped into animals with good physical appearance and into those with poor health conditions as judged by the parameters consciousness, motor activity, and sleeping and...
feeding habits. The general health conditions of animals thereby correlated well with findings at the systemic and microcirculatory levels. The 24-h survivors in poor condition exhibited significantly decreased arterial blood pressure, severe metabolic acidosis, and compensatory hyperventilation, changes that were paralleled by substantial decreases (≥50%) in microvascular blood flow, FCD, and interstitial oxygenation in this microcirculatory environment. Lowered arterial blood pressure, significant vasoconstriction of feeding (A1) arterioles, and particularly the reduction of FCD, in turn, appeared to be the main causes of tissue perfusion and oxygenation impairment (1, 2, 4, 16, 17, 34). On the other hand, survivors with good physical appearance showed stable macrohemodynamic conditions, only minor metabolic and respiratory alterations, and a 15–30% reduction in microscopic tissue perfusion and interstitial oxygenation. The persisting microcirculatory perturbations, however, may account for the slight respiratory and metabolic disorders observed in these animals.

Because of the mild character of these changes, it may be assumed that this small fraction of animals, i.e., 31.8%, would have eventually fully recovered and survived for a longer period of time. On the other hand, persisting alterations at the systemic and microcirculatory levels in these animals are indicative of the severity and long duration of the hemorrhagic shock period (16).

Differences between 24-h survivors in good and bad conditions can, however, also be attributed to a different grade of hemorrhage, since blood-shed volumes did not differ significantly between both groups. Interestingly, also no significant differences at the systemic and microcirculatory levels could be observed during the 4-h hemorrhagic shock period and 30 min after autologous whole blood resuscitation. However, systemic and microcirculatory conditions tended to be slightly more compromised already at these time points in 24-h survivors with poor appearance. A crucial factor for the different outcome of survivors also appeared to be FCD, which critically determines (interstitial) tissue oxygenation, removal of metabolites, and, therefore, systemic metabolic and respiratory conditions (1, 2, 10, 16, 17). In 24-h survivors with poor health appearance, FCD was lowered by ~50%, which may represent a threshold detrimental to survival. This is corroborated by findings in shock nonsurvivors, which also exhibited a FCD reduction of ≥50% at 30 min after fluid resuscitation. Further studies, however, are required to determine the minimal FCD level times compatible with long-term survival.

Findings of this study may be relevant for clinical medicine, suggesting that volume replacement and restitution of adequate oxygen-carrying capacity per se may not be sufficient to restore normal microcirculatory perfusion, (interstitial) tissue oxygenation, and metabolic conditions in severe hemorrhagic shock (17). Although results cannot be translated directly to other (central) organs and microcirculatory environments, they imply that (improved) long-term survival after severe hemorrhagic shock may depend on further therapeutic interventions, particularly on those aimed at the maintenance of adequate FCD and perfusion.

In conclusion, isovolemic autologous whole blood resuscitation after 4-h hemorrhagic shock was associated with physical recovery and compensation of systemic parameters in only a small fraction (31.8%) of animals, even though slight deficits (15–30%) in microscopic tissue perfusion and interstitial oxygenation persisted. The 24-h survivors in poor condition (23.8%) showed severe metabolic instability, coupled with substantial decreases (≥50%) in microvascular blood flow, FCD, and interstitial Po2 values. In nonsurvivors (44.4%), deficits on the systemic and microcirculatory levels were significantly more severe already during the 4-h shock period, alterations that were sustained despite of fluid resuscitation. Impairment of FCD beyond a threshold of 50% appeared to be a crucial factor for the differences in outcome, determining (interstitial) tissue oxygenation, removal of metabolites, and systemic metabolic conditions compatible or not compatible with life, respectively.

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