Prolonged Low-Volume Resuscitation with HBOC-201 in a Large-Animal Survival Model of Controlled Hemorrhage

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Background: **Military guidelines call for two 500-mL boluses of Hextend for resuscitation in far-forward environments. This study compared a hemoglobin-based oxygen carrier (HBOC-201; Hemopure) to Hextend when used to treat hemorrhagic shock in situations of delayed definitive care military operations.**

Methods: **Yorkshire swine (55–65 kg) were hemorrhaged to a mean arterial blood pressure (MAP) of 30 mm Hg. Hypotension was maintained for 45 minutes followed by resuscitation with either Hextend (HEX) (n = 8) or HBOC-201 (HBOC) (n = 8). Over 8 hours, animals received up to 1,000 mL of either fluid in an effort to sustain an MAP of 60 mm Hg. At the end of 8 hours, HEX animals received 2 L of lactated Ringer's solution followed by shed blood. HBOC animals received 4 L of lactated Ringer's solution only. Animals were killed and necropsied on postprocedure day 5. Hemodynamic data were collected during shock and resuscitation. Complete blood counts, amylase, lactate, coagulation studies, and renal and liver function were measured throughout the experiment.**

Results: **Equivalent volumes were hemorrhaged from each group (HBOC, 44.3 ± 2.2 mL/kg; HEX, 47.4 ± 3.0 mL/kg). The HBOC group achieved the goal MAP (HBOC, 60.0 ± 2.3 mm Hg; HEX, 46.4 ± 2.3 mm Hg; $p < 0.01$) and required less volume during the initial 8 hours (HBOC, 12.4 ± 1.4 mL/kg; HEX, 17.3 ± 0.3 mL/kg; $p < 0.01$). The HBOC group had lower SvO_2 (HBOC, 46.3 ± 2.4%; HEX, 50.7 ± 2.5%; $p = 0.12$) and cardiac output (HBOC, 5.8 ± 0.4 L/min; HEX, 7.2 ± 0.6 L/min; $p = 0.05$), but higher systemic vascular resistance (HBOC, 821.4 ± 110.7 dynes · s · cm^{-5}; HEX, 489.6 ± 40.6 dynes · s · cm^{-5}; $p = 0.01$). Base excess, pH, lactate, and urine output did not differ between groups. HEX group survival was 50% (four of eight) versus 88% for the HBOC group (seven of eight). All animals survived the initial 8 hours. Animals surviving 5 days displayed no clinical or laboratory evidence of organ dysfunction in either group.**

Conclusion: **HBOC-201 more effectively restored and maintained perfusion pressures with lower volumes, and allowed for improved survival. These data suggest that hemoglobin-based oxygen carriers are superior to the current standard of care for resuscitation in far-forward military operations.**

Key Words: **Shock, resuscitation, hemoglobin-based oxygen carrier, HBOC-201, Hextend.**

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Soldiers injured in combat frequently require prompt medical attention; however, tactical combat environments can make provision of care a challenge. Injuries are typically sustained in austere settings with ongoing hostilities and limited medical resources. Furthermore, the time of evacuation to a higher echelons of care is variable, because of both geography and the nature of combat operations. Historically, up to 90% of deaths caused by injuries sustained during combat occur before reaching a medical treatment facility.[1,2]

Butler et al. outlined a basic casualty management plan for the battlefield, and divided the management of casualties into three phases. Tactical field care (TFC) is the stage of combat care that occurs once hostile fire has ceased but before evacuation of the injured combatant. This phase may be relatively brief or may last several hours. Resources for provision of care in this setting are typically limited to the contents of the medic's backpack.[3] In 2002, the U.S. Department of Defense Committee on Tactical Combat Casualty Care modified the treatment guidelines for TFC. This included changes to the algorithm for administration of fluid

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resuscitation, specifically, changes in the volume and type of fluid administered. These changes were largely based on previous combat experiences.[4] Current recommendations are for the combat medics to administer a 500-mL bolus of Hextend if the casualty is found to be in shock (as defined by absent peripheral pulses or altered mental status in the absence of brain injury), and to repeat this once at 30 minutes if the casualty remains in shock.[5]

In making these modifications, the committee recognized that the ideal fluid for field resuscitation remains elusive and called for further research. In addition, the committee suggested that ongoing studies should include scenarios to mimic delayed transport to definitive care. Hemoglobin-based oxygen carriers are currently being developed and may represent a better choice for field resuscitation fluids. Previous studies performed in our laboratory have evaluated the efficacy of HBOC-201 (Hemopure; Biopure Corp., Cambridge, MA) in the treatment of hemorrhagic shock.[6–8] These studies demonstrated that HBOC-201 effectively reverses anaerobic metabolism with no identified end-organ damage. In addition, HBOC-201 achieves these results with significantly smaller resuscitation volumes when compared with standard regimens and other low-volume resuscitative agents. The purpose of this study was to compare the effects of resuscitation with HBOC-201 to Hextend in the setting of simulated prolonged evacuation times.

MATERIALS AND METHODS

Animal Model

The study was approved by the Wilford Hall Medical Center Institutional Animal Care and Use Committee. All animals used in this study were cared for and handled according to the *Guide for the Care and Use of Laboratory Animals*.[9] This study used 16 adolescent female Yorkshire swine (*Sus scrofa*), weighing 55 to 65 kg each, obtained from a single provider. All animals were fasted after midnight with water available ad libitum. Animals were anesthetized with an intramuscular injection of ketamine (15–20 mg/kg) and atropine (0.04–0.4 mg/kg). An 18-gauge intravenous catheter was placed in an ear vein, and each animal received an initial bolus of 2 L of 0.9% sodium chloride solution. Each animal underwent endotracheal intubation and was maintained on mechanical ventilation with isoflurane for the duration of the experiment. End-tidal isoflurane levels were maintained at slightly above minimal alveolar concentration for swine (1.75%). Ventilation parameters included positive end-expiratory pressure of 3 mm Hg, Fio_2 of 40%, tidal volume of 7 to 10 mL/kg, and a respiratory rate of 10 to 12 breaths/min to maintain an end tidal CO_2 of 40 mm Hg. Electrocardiogram leads were placed to allow continuous monitoring. A bed warmer was used and room temperature manipulated to maintain the animal's core temperature at a minimum of 37°C.

Under sterile conditions, the right external jugular vein was percutaneously accessed and an 8.5-French introducer sheath was placed. A standard thermodilution Swan-Ganz catheter was positioned in the pulmonary artery. Bilateral groin incisions were made to expose the femoral vessels. The femoral arteries were cannulated with 16-gauge intravenous catheters. A lower midline laparotomy was then performed. A cystostomy tube was placed using a 16-French Foley catheter and was allowed to drain to gravity. All incisions were closed and covered with occlusive sterile dressings.

After instrumentation, animals were allowed to equilibrate for a period of 15 minutes, and baseline measurements were obtained. Animals then underwent a standardized controlled hemorrhage to a mean arterial pressure (MAP) of 30 ± 5 mmHg and were maintained at this level for a 45 minutes. Blood was collected in blood donation bags with citrate phosphate dextrose preservative and then placed at 4°C on an orbital shaker. Volumes were recorded during hemorrhage to determine the total volume of blood removed. After the shock period, the animals were resuscitated to a goal MAP of 60 ± 5 mmHg by one of two different resuscitation regimens: Hextend (6% hetastarch; BioTime, Inc., Berkeley, CA) (HEX) (n = 8) or HBOC-201 (n = 8). The animals in the HEX group received an initial 500-mL bolus of Hextend. This was repeated once if the goal MAP was not achieved. Animals in the HBOC group received up to 1,000 mL of HBOC-201 to achieve the goal MAP. Animals were maintained at this level for 8 hours. No additional fluids were given during this time.

At the end of 8 hours, animals in the HEX group were given 2 L of lactated Ringer's (LR) solution and their own shed blood. Animals in the HBOC group were given 4 L of lactated Ringer's solution. Under sterile conditions, the femoral artery catheters and cystostomy tube were removed and the incisions closed. The pulmonary artery catheter was removed and the external jugular vein introducer was exchanged for a tunneled indwelling venous catheter (Lifeport Port System; Horizon Medical Products, Manchester, GA). The animals were recovered from anesthesia and returned to their pens. Animals were killed with Euthanasia-5 solution on the morning of postoperative day 5. After the animals were killed, necropsies were performed.

Physiologic Data Collection

Heart rate (HR), arterial blood pressure, central venous pressure, and pulmonary artery pressure were monitored and recorded continuously. Thermodilution was used to determine cardiac output (CO). CO, systemic vascular resistance (SVR) and pulmonary vascular resistance were recorded at baseline (–60 minutes), completion hemorrhage (–45 minutes), 30 minutes of shock (–15 minutes), completion shock (0 minutes), 30 minutes of resuscitation (30 minutes), 60 minutes of resuscitation (60 minutes), 90 minutes of resuscitation (90 minutes), 2 hours of resuscitation (120 minutes), and then hourly until the end of the final resuscitation period. Arterial and mixed venous blood gas samples with lactate levels were also collected at these time points.

The oxygen extraction ratio (OER) was calculated by dividing the oxygen uptake by the oxygen delivery. Oxygen delivery was calculated as the product of the cardiac output and the arterial oxygen content, and oxygen uptake was calculated as the product of the cardiac output and the difference between arterial oxygen content and venous oxygen content.

Hematology and Chemistry Studies

Blood samples were drawn from the right femoral artery for hematology, chemistry, liver enzyme, and amylase determinations at baseline and hourly after the start of resuscitation. Samples were then collected from the Lifeport Port System in the morning and evening of each postoperative day. Samples for creatinine determination from HBOC animals were passed through Amicon Centrifree YM-30 filters (Millipore, Billerica, MA) to remove free hemoglobin from the solution to prevent interference with this colorimetric determination.

Oxidative Burst Assays

Blood samples were collected for oxidative burst determination at baseline; at completion shock; and at 60, 240, and 480 minutes after resuscitation. Samples were stained with 4 μL of 750 μmol/L dichlorofluorescein diacetate (Molecular Probes, Eugene, OR) for 10 minutes, in the dark, in a 37°C shaking water bath before stimulation. One-milliliter aliquots were placed into three separate tubes. One sample was stimulated with 5 μmol/L of phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co., St. Louis, MO), a second sample was stimulated with a standardized pansorbin solution (PAN; Calbiochem, San Diego, CA), and a third sample was left unstimulated. Samples were incubated for 30 minutes in a 37°C shaking water bath.

After a 30-minute incubation, the samples had red blood cells lysed and leukocytes fixed by the addition of BD FACS Lyse (BD Biosciences, San Jose, CA) for 10 minutes in the dark. Samples were then centrifuged to pellet ($300 \times g$) for 5 minutes and resuspended in phosphate-buffered saline (Sigma Chemical Co., St. Louis, MO). Cellular excitation was performed using the 488-nm line of a 15-mW argon laser and a band filter of 525 nm on a FACSCalibur Flow Cytometer (BD Biosciences). Forward- versus side-scatter plots were used to gate for neutrophils. Fluorescence intensity was determined on a log scale from 1 to 10^4, and at least 10,000 events were acquired for further analysis. To determine the effects of HBOC or HEX resuscitation on oxidative burst, the mean channel fluorescence of the baseline sample was determined for each parameter and study animal. The individualized baseline data were then used to calculate the percentage change in mean channel fluorescence for each parameter measured for each study animal. The percentage change data were then pooled and used in the remaining calculations and comparisons. Results were expressed as mean ± SEM values of channel fluorescence using CellQuest software (BD Biosciences).

Histology

A full gross necropsy was performed, and representative sections of stomach, duodenum (5 cm distal to the pylorus), ileum (5 cm proximal to the ileocecal valve), bilateral lower pole of the kidneys, right and left lateral lobes of liver, bilateral lower lobes of the lung, right and left ventricle of the heart, and frontal lobe of the brain were obtained. Tissue sections were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Histopathology on tissue sections was evaluated by a single veterinary pathologist blinded to study groups.

Statistical Analysis

A statistical power analysis, which was based on detecting differences in the serum chemistries and hematology values, indicated that the sample of eight animals per group would provide an 83% chance of detecting an interaction effect (group × time) with standardized group effect size of 0.42 (SD = 0.82), and an 88% chance of detecting a main effect size of 0.84, when testing at the 0.05 α level. The power for comparing the survival rates was limited because of the small sample size and the expectation that there would be comparable survival rates between the two groups.

Data are reported as mean ± SE. Statistical analysis was performed using Student's *t* test and repeated-measures analysis of variance with Tukey's post hoc analysis. The Statistical Package for the Social Sciences (SPSS version 11.5; SPSS, Inc., Chicago, IL) was used to perform calculations. Values of $p < 0.05$ were considered significant.

RESULTS

Survival

All animals survived the hemorrhage, shock, and initial resuscitation portions of the experiment. In the HEX group, two animals died during the administration of the definitive resuscitation, and two animals died during the postoperative recovery period. One animal in the HBOC group died as a result of an air embolus during insertion of the indwelling catheter. Overall survival was 50% in the HEX group and 88% in the HBOC group. This difference was not statistically significant.

Resuscitation Volumes

Study groups were equivalent for weight (HEX, 57.9 ± 1.0 kg; HBOC, 57.5 ± 1.1 kg; $p = 0.79$) and volume hemorrhaged per kilogram (HEX, 47.4 ± 2.2 mL/kg; HBOC, 44.3 ± 3.0 mL/kg; $p = 0.58$). The initial resuscitation volumes (HEX, 17.3 ± 0.3 mL/kg; HBOC, 12.4 ± 1.4 mL/kg; $p < 0.01$) and the total resuscitation volumes (HEX, 98.7 ± 2.7 mL/kg; HBOC, 80.8 ± 2.4 mL/kg, $p < 0.01$) were all significantly different (Fig. 1).

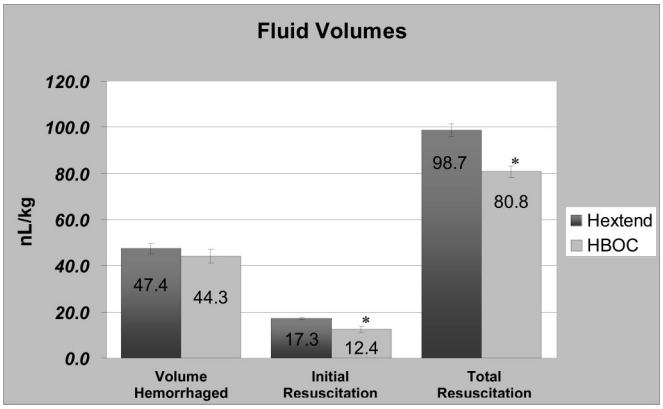


Fig. 1. *The volume hemorrhaged from each group as shown in milliliters per kilogram did not differ between study groups. However, the amount of resuscitation volume given in the HBOC group was significantly less with the initial resuscitation ($p < 0.01$) and total resuscitation ($p < 0.01$).*

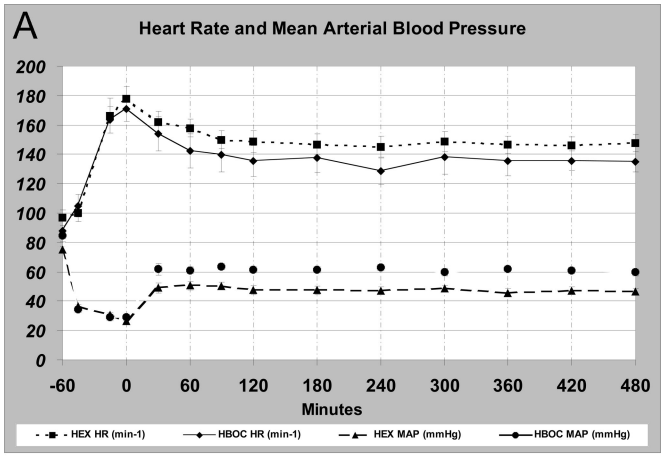
Hemodynamics

Hemodynamic parameters were equivalent for all groups at baseline. Both groups manifested similar hemodynamic responses to hemorrhage and shock: decreased MAP and cardiac output (CO), and increased HR and SVR. At the completion of the initial low-volume resuscitation period, the goal MAP of 60 mmHg was successfully achieved in the HBOC group; however, the MAP of the HEX animals remained depressed despite administration of the maximum allowed fluid volume in most instances (HEX, 46.4 ± 2.3 mm Hg; HBOC, 59.6 ± 2.3 mm Hg; $p < 0.01$). CO returned to baseline in the HEX group but remained depressed in the HBOC group (HEX, 7.2 ± 0.6 L/min; HBOC, 5.8 ± 0.4 L/min; $p = 0.05$). HR improved with resuscitation; however, it remained elevated above baseline in both groups (HEX, 148 ± 6 beats per minute; HBOC, 134 ± 7 beats per minute; $p = 0.21$). SVR showed a similar increase in both groups during the hemorrhage and shock periods; however, with resuscitation, the animals in the HEX group demonstrated a decrease below baseline, whereas the HBOC animals gradually returned to baseline values. (HEX, 489.6 ± 40.6 dynes · s · cm^{-5}; HBOC, 821.4 ± 110.7 dynes · s · cm^{-5}; $p < 0.01$) (Fig. 2).

Resuscitation Markers

Both groups manifested a metabolic response to shock with lactic acidosis, lower base excess, and decreased pH. The effect of the initial low-volume resuscitation was similar in both groups with regard to lactate (HEX, 1.6 ± 0.5 mmol/L; HBOC, 1.3 ± 0.1 mmol/L; $p = 0.59$), base excess (HEX, 4.3 ± 1.1 mmol/L; HBOC, 3.4 ± 0.9 mmol/L; $p = 0.40$), and pH (HEX, 7.47 ± 0.03; HBOC, 7.46 ± 0.01; $p = 0.33$).

Urine output decreased in both groups with hemorrhage and shock and remained depressed during the initial resuscitation (HEX, 0.2 ± 0.0 mL/kg/h; HBOC, 0.4 ± 0.1 mL/kg/h; $p = 0.90$) (Fig. 3). With administration of the definitive resuscitation, the urine output in the HEX group increased to 2.1 ± 0.7 mL/kg/h, whereas the HBOC group increased to 4.1 ± 0.7 mL/kg/h. Urine output was not measured during the 5-day survival period.



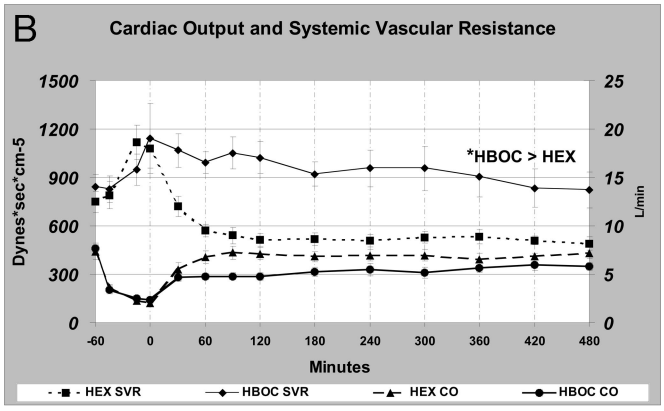


Fig. 2. *(A) The heart rate increased in both groups with hemorrhage and shock. There was improvement with resuscitation; however, neither group returned to baseline. Conversely, the MAP was decreased with hemorrhage and shock. The HBOC group successfully achieved the goal MAP with initial resuscitation. The HEX animals continued to have a persistently decreased MAP despite resuscitation ($p < 0.01$). (B) The SVR (left axis) was increased in both groups with hemorrhage and shock. The HBOC group gradually returned to baseline with resuscitation, whereas the HEX group had a depressed SVR with resuscitation ($p < 0.01$). CO (right axis) in both groups decreased with resuscitation. The HEX group had near normalization of CO with resuscitation, whereas the HBOC group had some improvement of CO but remained depressed ($p = 0.05$).*

Mixed venous oxygen saturation (SvO_2) values were similar for both groups. There was an initial decrease with hemorrhage and shock, with an incomplete return to baseline values with resuscitation (HEX, 50.7 ± 2.48%; HBOC, 46.3 ± 2.37%; $p = 0.12$). Similarly, the OER increased with hemorrhage and shock and remained elevated for the remainder of the resuscitation period (HEX, 49 ± 2%; HBOC, 51 ± 2%; $p = 0.38$) (Fig. 4).

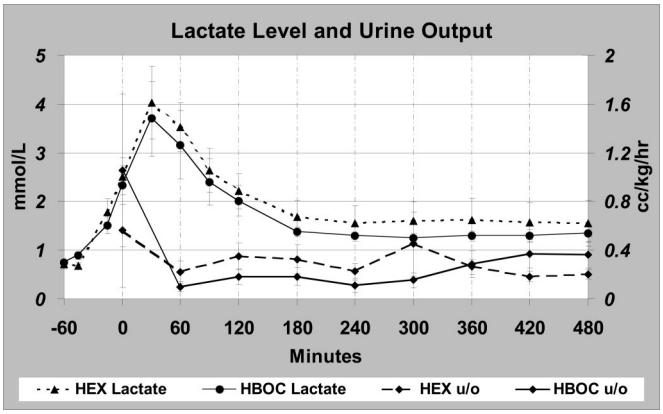


Fig. 3. *Metabolic markers demonstrated similar responses to hemorrhage, shock, and resuscitation in both groups. The lactate level (left axis) increased with hemorrhage and shock and improved with resuscitation, although there was a persistent slight elevation. Urine output (right axis) decreased in both groups with hemorrhage and shock and remained depressed.*

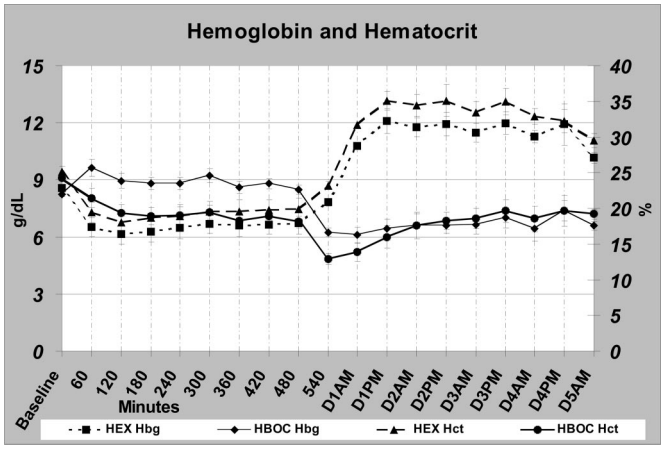


Fig. 5. *The hemoglobin level is significantly higher in the HBOC group during the initial resuscitation, whereas there is no difference between groups for hematocrit. This reflects the infusion of cell-free hemoglobin in the HBOC group. These trends are reversed with reinfusion of shed blood to the HEX animals with the final resuscitation.*

Hematology

Hemoglobin (Hb) and hematocrit (Hct) levels decreased in similar fashion with hemorrhage and shock. The hematocrit levels remained depressed in both groups during the initial resuscitation period (HEX, 19.8 ± 1.6%; HBOC, 18.1 ± 0.9%; $p = 0.92$). In contrast, the hemoglobin level in the HBOC group increased with initial resuscitation (HEX, 6.7 ± 0.6 g/dL; HBOC, 8.5 ± 0.2 g/dL; $p < 0.01$). These trends changed with the definitive resuscitation. The reinfusion of shed blood caused the HEX group to have significantly elevated hemoglobin and hematocrit values in the morning of the first postoperative day (Hb: HEX, 10.8 ± 0.2 g/dL; HBOC, 6.1 ± 0.4 g/dL; Hct: HEX, 31.7 ± 0.3%; HBOC, 13.9 ± 1.3%). This trend persisted over the 5-day survival period (Hb: HEX, 10.2 ± 0.3 g/dL; HBOC, 6.6 ± 0.2 g/dL; $p < 0.01$; Hct: HEX, 29.5 ± 1.0%; HBOC, 19.2 ± 0.71%; $p < 0.01$) (Fig. 5).

Prothrombin (PT) and fibrinogen levels did not change significantly with hemorrhage, shock, and initial resuscitation (PT: HEX, 15.8 ± 1.0 seconds; HBOC, 14.9 ± 0.5 seconds; $p = 0.25$; fibrinogen: HEX, 112.5 ± 9.6 mg/dL; HBOC, 127.5 ± 13.9 mg/dL; $p = 0.18$). Definitive resuscitation produced a slight decrease in PT and an increase in fibrinogen. These changes persisted over the 5-day survival period (PT: HEX, 13.3 ± 0.1 seconds; HBOC, 12.9 ± 0.2 seconds; $p = 0.65$; fibrinogen: HEX, 226.3 ± 33.8 mg/dL; HBOC, 266.1 ± 32.0 mg/dL; $p = 0.54$).

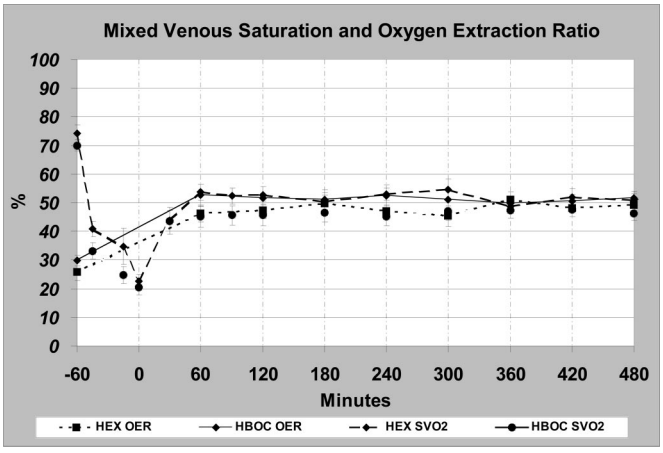


Fig. 4. *The mixed venous oxygen saturation (SvO_2) decreased in both groups with hemorrhage and shock. There was improvement with resuscitation; however, both remained depressed. In both groups, the OER increased with hemorrhage and shock and did not improve with resuscitation.*

Chemistry

Baseline potassium values were normal for both groups (HEX, 3.7 ± 0.2 mEq/L; HBOC, 3.5 ± 0.1 mEq/L). A similar increase in potassium levels was seen in both groups during the initial resuscitation (HEX, 5.7 ± 0.6 mEq/L; HBOC, 5.7 ± 0.4 mEq/L; $p = 0.76$). These values were normalized by the morning of the first postoperative day and remained normal during the survival period (HEX, 3.7 ± 0.1 mEq/L; HBOC, 4.1 ± 0.5 mEq/L; $p = 0.56$).

Creatinine levels in both groups increased from normal with hemorrhage and shock. Levels continued to gradually increase during the initial low-volume resuscitation period, with a slightly larger increase seen in the HEX group (HEX, 1.9 ± 0.3 mg/dL; HBOC, 1.5 ± 0.1 mg/dL; $p = 0.23$). The creatinine level in the HBOC group normalized by the morning of the first postoperative day. The level in the HEX group had a more gradual response, but was normalized by the completion of the survival period (HEX, 0.9 ± 0.1 mg/dL; HBOC, 1.0 ± 0.1 mg/dL; $p = 0.11$) (Fig. 6).

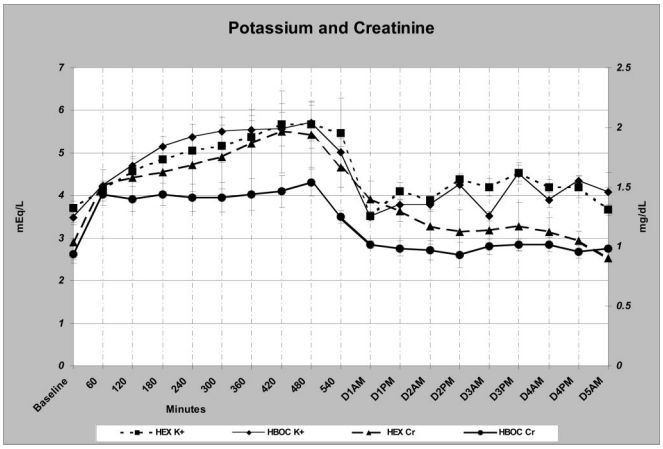


Fig. 6. *Both groups experienced an increase in potassium (K^+) (left axis) and creatinine (Cr) (right axis) levels during the initial resuscitation period. These values were normalized by completion of the study.*

Aspartate transaminase (AST) and alanine transaminase (ALT) levels did not vary with hemorrhage, shock, or initial resuscitation. However, levels were significantly increased in both groups by the morning of the first postoperative day. The HEX group had a larger increase in both AST and ALT than the HBOC group (AST: HEX, 2,046 ± 905 U/L; HBOC, 910 ± 250 U/L; ALT: HEX, 168 ± 53 IU/L; HBOC, 117 ± 23 IU/L). For both groups, AST levels were normalized by the end of the survival period and ALT levels were decreasing but had not yet reached baseline. There was no overall difference between groups (AST: HEX, 57 ± 23 U/L; HBOC, 83 ± 13 U/L; $p = 0.42$; ALT: HEX, 103 ± 44 IU/L; HBOC 103 ± 17 IU/L; $p = 0.19$).

Similarly, amylase levels did not change significantly in either group with hemorrhage, shock, or initial resuscitation (HEX, 2,043 ± 166 IU/L; HBOC, 1,575 ± 261 IU/L; $p = 0.85$). There was an increase seen on the morning of the first postoperative day (HEX, 3,365 ± 477 IU/L; HBOC, 2,324 ± 331 IU/L), but normalization was appreciated by the end of the survival period. No overall difference was noted between groups (HEX, 1,306 ± 709 IU/L; HBOC, 2,025 ± 185 IU/L; $p = 0.48$).

Oxidative Burst

There was essentially no increase in mean fluorescence of samples stimulated with PAN and PMA for animals resuscitated with HBOC (PAN, 1.8 ± 0.2%; PMA, 2.0 ± 0.6%). An increase in fluorescence values was seen in animals resuscitated with HEX (PAN, 5.9 ± 2.5%; PMA, 23.7 ± 20.3%). The between-group differences approached but did not achieve statistical significance for the PAN stimulation and were not significantly different for PMA stimulation (PAN, $p = 0.06$; PMA, $p = 0.29$).

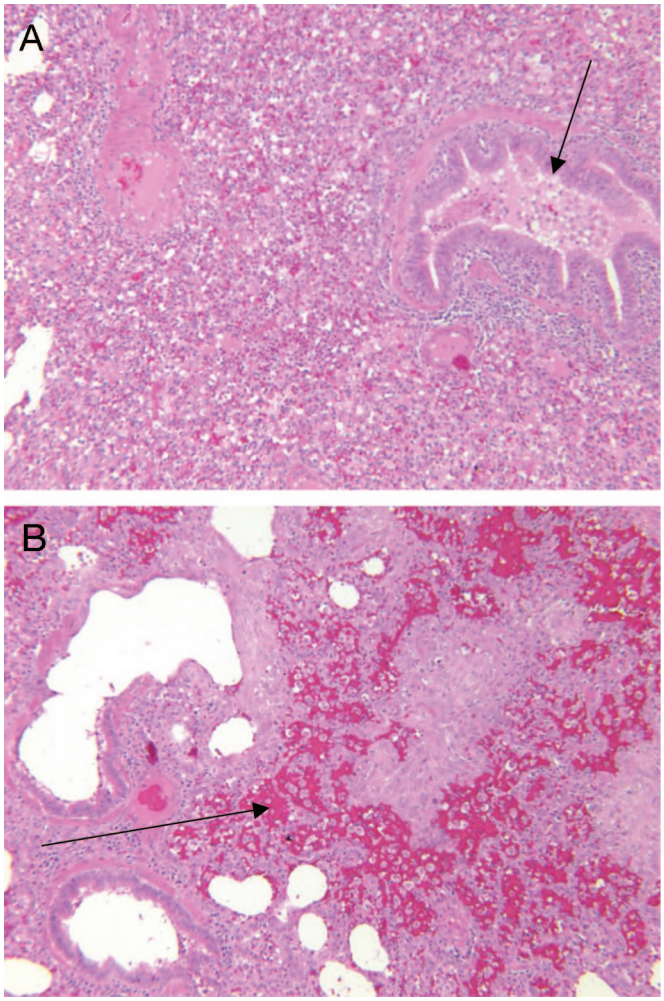


Fig. 7. *(A) This image depicts bronchopneumonia in lung tissue harvested from an HBOC animal. There is a dense inflammatory infiltrate and exudate obscuring the bronchioles (arrow). (B) Lung tissue taken from a HEX animal shows a similar inflammatory infiltrate and an area of hemorrhage (arrow).*

Histopathology

Histopathologic analysis of the animals that survived the entire experiment (HEX, n = 4; HBOC, n = 7) revealed lesions in the lungs, heart, liver, and kidney. Pneumonia, most frequently an acute bronchopneumonia, was noted in eight of the animals (HEX, n = 2; HBOC, n = 6). These lesions appeared to be acute and were most likely attributable to complications after prolonged anesthesia on the day of surgery (Fig. 7). Myocardial degeneration, with or without necrosis and regeneration, was noted in six animals (HEX, n = 3; HBOC, n = 3). This lesion was generally mild, most frequently involved the left ventricle, and was consistent with ischemia secondary to shock (Fig. 8). Liver changes were predominately centrilobular degeneration with or without necrosis and glycogen-type vacuolar change (HEX, n = 2; HBOC, n = 4). Centrilobular degeneration and necrosis are common findings secondary to hypoxia. The vacuolar change

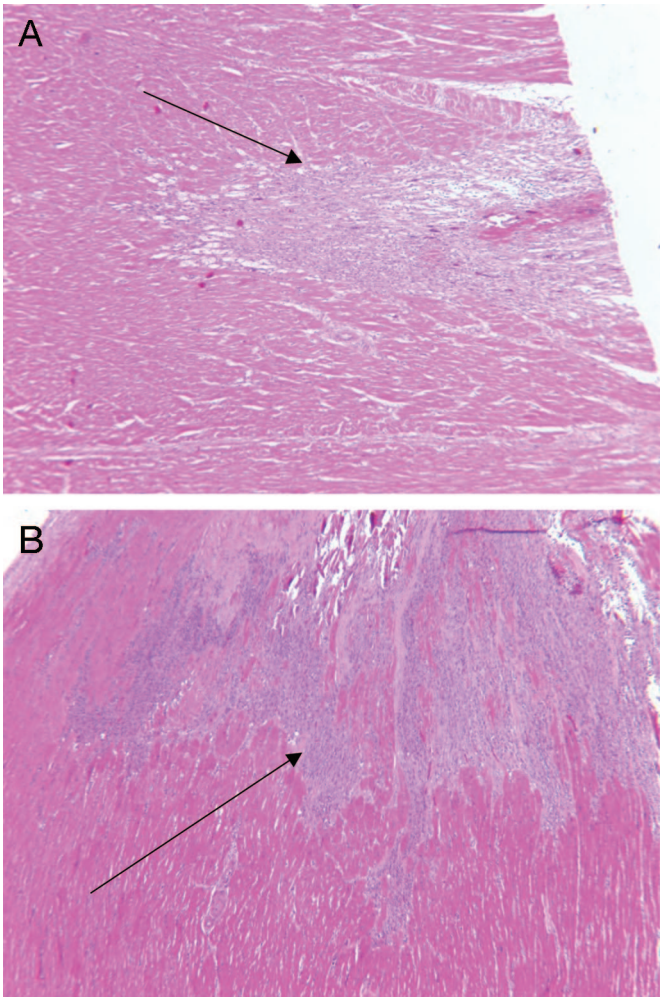


Fig. 8. *(A) Heart tissue harvested from an HBOC animals demonstrates a wedge-shaped area of infarction (arrow) with loss of cardiac myocytes. (B) The HEX heart tissue demonstrates a similar lesion (arrow).*

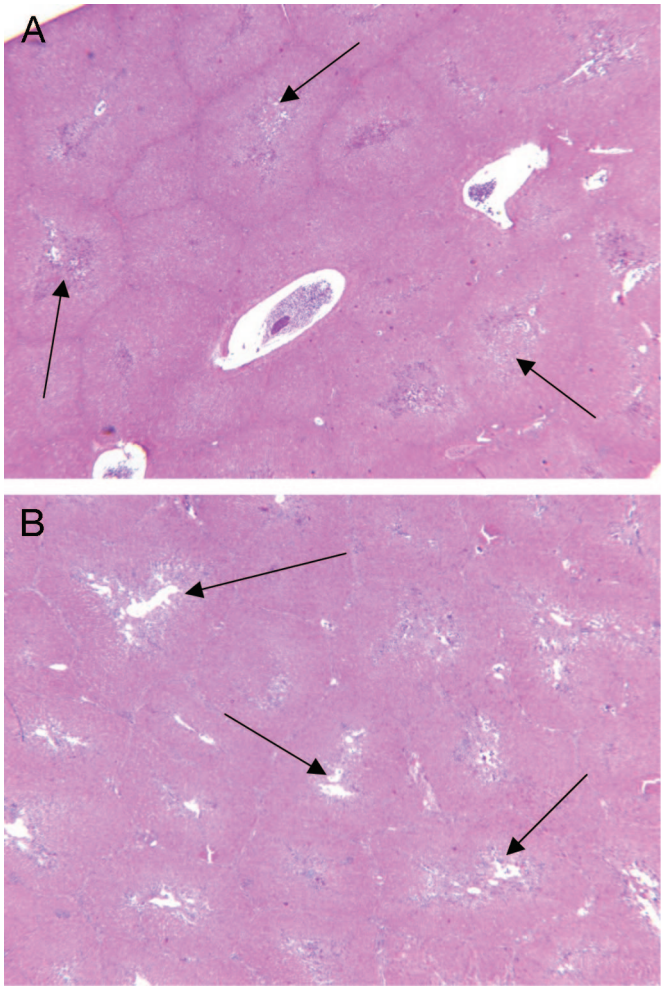


Fig. 9. *(A) Liver from an HBOC animal demonstrates centrilobular necrosis and loss of hepatocytes demonstrated by the loss of normal architecture in the centrilobular areas (arrows). (B) Similar lesions are seen in the HEX animals (arrows).*

is a nonspecific finding and was mild (Fig. 9). The predominant kidney lesion was multiple renal infarcts, a lesion consistent with shock (HEX, n = 2; HBOC, n = 1). (Fig. 10).

Histopathologic analysis of the four HEX animals that died prematurely revealed moderate to severe pulmonary edema (n = 3) or pulmonary hemorrhage (n = 1). One animal appeared to have preexisting bronchopneumonia, in addition to thrombus in the heart, degeneration in the liver and kidney, and congestion in the stomach, changes consistent with protocol-induced shock. Liver congestion was present in three animals, and hydronephrosis was seen in two animals.

DISCUSSION

This study compared the current standard of care for initial fluid resuscitation in the combat environment with a similarly designed resuscitation regimen using the hemoglobin-based oxygen carrier HBOC-201. The data demonstrate that HBOC-201 is at least as effective as the current standard, Hextend, in the treatment of hemorrhagic shock with prolonged low-volume resuscitation. Furthermore, there was no identified difference in long-term organ function, and HBOC-201 may provide a survival advantage.

The current choice of a colloid solution as the standard fluid for TCF resuscitation was based on its ability to maintain intravascular volume expansion for extended periods of time.[10] Hextend was specifically chosen for its relatively safe side-effect profile and its potential as an antioxidant.[11,12] Although no clinical evidence exists to support the use of Hextend as an initial resuscitation agent over other fluids, the logistical advantage imparted by the use of smaller volumes of Hextend to achieve improved volume expansion make it more advantageous for use in military operations. HBOC-201 has several unique properties of its own that make it appealing for use as a resuscitation fluid, particularly for military use: ability to transport oxygen, no need for refrigeration, universal compatibility, long shelf life, minimized risk of

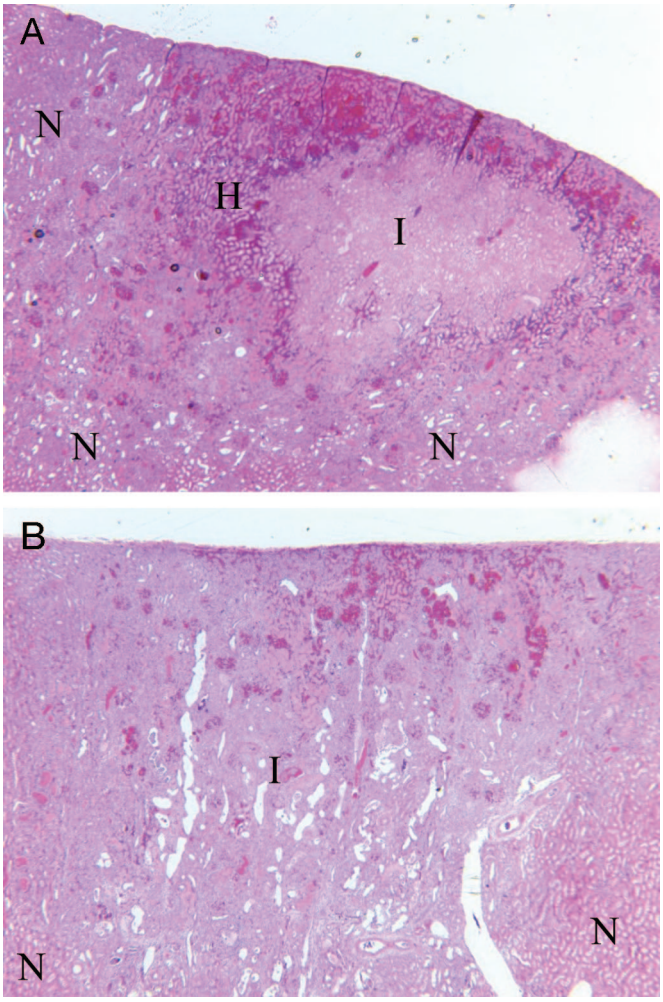


Fig. 10. *(A) Kidney tissue from an HBOC animal demonstrates an area of infarct (I), outlined by a zone of hemorrhage (H) and surrounded by normal (N) tissue. (B) Infarction (I) is also seen in HEX animal renal tissue; a minimal amount of normal (N) tissue is seen at the periphery of the image.*

disease transmission, and production from a renewable source. An additional logistical advantage of HBOC-201 is the requirement for less volume to achieve the same resuscitative goals provided by larger volumes of more traditional fluids. This was previously demonstrated by Sampson et al.[8] and was reproduced in the current study. The endpoint of resuscitation in this experimental protocol was a mean arterial blood pressure of 60 mmHg. All of the HEX animals required the entire volume of fluid permitted by the protocol (1,000 mL), and no animals in this group achieved the target blood pressure. In comparison, all of the HBOC animals achieved the target pressure, and many animals required less than the maximum allowed volume (average, approximately 700 mL).

Despite these differences in MAP, there were no statistically significant differences in several of the traditional measurements of adequate resuscitation. Lactate levels and base deficit increased in both groups with hemorrhage and shock and normalized with resuscitation. Conversely, pH showed a decrease with hemorrhage and shock and normalized with low-volume resuscitation. Urine output decreased in both groups and remained depressed during the initial low-volume resuscitation period. Decreased urine output in animals resuscitated with HBOC has been seen in previous studies using this animal model despite normalization of other resuscitation markers.[6–8] This has been attributed to the smaller resuscitation volumes needed with HBOC-201. However, survival studies showed no long-term effects on renal function despite the decreased urinary output.[7] Similarly, the low urinary output in the HEX group is most likely related to the low volume of initial resuscitation received by these animals. However, in the HEX group, this had a more significant impact on renal function than in the HBOC group, with the HEX group showing a larger increase in serum creatinine during the initial resuscitation, which took longer to normalize after completion of the experiment.

The similarity of SvO_2 and OER between the two study groups also suggests inadequate resuscitation in the HEX animals. The SvO_2 of HBOC animals is typically depressed when compared with animals receiving standard resuscitation fluids.[6–8] This is attributed to the higher P_{50} of the bovine hemoglobin molecule, resulting in greater oxygen off-loading in the capillary beds. In this study, however, the SvO_2 of both groups remains depressed. This is suggestive of a leftward shift in the oxygen-dissociation curve in the HEX group from an ongoing demand for increased oxygen delivery. Elevated serum chemistry markers suggesting worsened end-organ damage in the HEX animals compared with the HBOC animals lends support to this conclusion. Specifically, the serum amylase, creatinine, AST, and ALT values show a larger increase in the HEX animals compared with the HBOC animals in the early time period after definitive resuscitation.

However, in animals that survived the protocol, we did not identify any significant differences between study groups regarding long-term organ function. The combined results of the end-study serum chemistries and histopathology do not identify any significant differences between study groups. In the previous work of York et al., animals underwent a similar shock protocol and were maintained in a hypotensive state for 4 hours and survived for 3 days.[7] In that series, the only identified pathologic lesion was centrilobular necrosis of the liver in the animals receiving HBOC-201. There was concern this was the result of systemic elimination of HBOC, with subsequent loss of ability to deliver oxygen. The results of the current study, however, find that these pathologic lesions are more likely attributable to the severity of the shock protocol rather than the specific resuscitation fluid administered. Lesions were equally distributed between study groups and affected multiple organ systems.

Despite the similarities seen in both groups with regard to measurements of traditional markers of resuscitation, the animals in the HBOC group demonstrated a superior survival rate when compared with the HEX group (HBOC, 88%;

HEX, 50%). This result, however, was not statistically significant. In designing the experiment, a survival difference between groups was not expected; therefore, the study was not sufficiently powered to determine such a difference. Nonetheless, the difference seen is highly suggestive of a survival benefit with HBOC resuscitation. The single death in the HBOC group was felt to be attributable to an air embolism during Lifeport Port System insertion at the completion of the low-volume resuscitation segment of the experiment. In contrast, all of the deaths in the HEX group were protocol related, with two deaths occurring during definitive resuscitation and two occurring during the evening after surgery. Necropsy results from these animals showed acute pulmonary edema and evidence of liver congestion and hydronephrosis. Although the animals in the HEX group received a statistically greater amount of resuscitative fluid than the HBOC animals, these differences would not have been expected to lead to this result.

Evaluation of the oxidative burst data suggests a trend toward increased neutrophil activation in the HEX group. Results show an increase in oxidative burst in the HEX group during the initial low-volume resuscitation period, whereas there is relatively little change in the HBOC animals. However, these differences did not achieve statistical significance. In a previous study, Ortegon et al. specifically compared the oxidative burst of cells exposed to HBOC-201 with cells exposed to traditional resuscitation fluids.[13] This study demonstrated increased oxidative burst in cells exposed to HBOC-201 and in cells exposed to Hextend. These in vitro results do not appear to correlate with the current in vivo data.

The data from this study indicate that HBOC-201 is at least as effective as Hextend when used in a protocol that mimics the current care plan used by combat medics. HBOC-201 more effectively restored perfusion pressure with smaller volumes of fluid, and there were no significant differences in traditional markers of resuscitation between the HBOC and Hextend groups. No differences were detected in organ function over the course of the 5-day survival period; however, HBOC-201 appears to confer a survival advantage when compared with Hextend. Although the search for the ideal combat resuscitation fluid continues, this study suggests that hemoglobin-based oxygen carriers may be the fluid of choice in situations of delayed definitive care in far-forward and austere environments.

REFERENCES

1. Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty care research. *Mil Med.* 1984; 149:55–62.
2. Butler FK Jr, Hagmann JH, Richards DT. Tactical management of urban warfare casualties in special operations. *Mil Med.* 2000;165:1–48.
3. Butler FK Jr, Hagmann J, Butler EG. Tactical combat casualty care in special operations. *Mil Med.* 1996;161(suppl):3–16.
4. Holcomb JB. Fluid resuscitation in modern combat casualty care: lessons learned from Somalia. *J Trauma.* 2003;54:S46–S51.
5. Prehospital Trauma Life Support Committee of the National Association of Emergency Medical Technicians in cooperation with the Committee on Trauma of the American College of Surgeons. *PHTLS: Basic and Advanced Prehospital Trauma Life Support.* St. Louis, MO: Mosby; 2003.
6. McNeil JD, Smith DL, Jenkins DH, York GB, Josephs JJ. Hypotensive resuscitation using a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) leads to reversal of anaerobic metabolism. *J Trauma.* 2001;50:1063–1075.
7. York GB, Eggers JS, Smith DL, et al. Low-volume resuscitation with a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) provides adequate tissue oxygenation for survival in a porcine model of controlled hemorrhage. *J Trauma.* 2003;55:873–885.
8. Sampson JB, Davis MR, Mueller DL, Kashyap VS, Jenkins DH, Kerby JD. A comparison of the hemoglobin-based oxygen carrier HBOC-201 to other low-volume resuscitation fluids in a model of controlled hemorrhagic shock. *J Trauma.* 2003;55:747–754.
9. National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press; 1996.
10. Mortelmans Y, Merckx E, van Nerom C, et al. Effect of an equal volume replacement with 500 mL 6% hydroxyethyl starch on the blood and plasma volume of healthy volunteers. *Eur J Anaesthesiol.* 1995;12:259–264.
11. Gan TJ, Bennett-Guerrero E, Phillips-Bute B, et al. Hextend, a physiologically balanced plasma expander for large volume use in major surgery: a randomized phase III clinical trial–-Hextend Study Group. *Anesth Analg.* 1999;88:992–998.
12. Nielsen VG, Tan S, Brix AE, Baird MS, Parks DA. Hextend (hetastarch solution) decreases multiple organ injury and xanthine oxidase release after hepatoenteric ischemia-reperfusion in rabbits. *Crit Care Med.* 1997;25:1565–1574.
13. Ortegon DP, Dixon PS, Crow KK, Mueller DL, Kerby JD. The effect of the bovine hemoglobin oxygen therapeutic HBOC-201 on human neutrophil activation in vitro. *J Trauma.* 2003;55:755–760.

DISCUSSION

Dr. Christoph R. Kaufmann (Portland, Oregon): This study is important, thorough, and well written. The research question addressed relates to optimal care for the soldier, sailor, airman, or marine in the prehospital combat setting, specifically when there is unavoidable delay to definitive operative care. In essence, this is the Somalia, Black Hawk Down scenario simulating delayed evacuation of hypotensive soldiers from the battlefield. In this clinical scenario, the current Department of Defense Tactical Field Care policy is to give 500 mL of Hextend to a casualty in shock with a second 500 mL, if needed. This study was undertaken to compare the use of Hextend with the use of HBOC-201 in the setting of simulated prolonged evaluation times.

The study design involved the controlled hemorrhage in 16 swine to a mean arterial pressure of 30 for 45 minutes, then resuscitation with either 1,000 mL of Hextend—as all animals did receive both boluses—or up to 1,000 mL of HBOC-201 to a target mean pressure of 60, a pressure at which the animals were maintained for 8 hours before final

resuscitation with either 4 L of LR in the Hemopure group, or 2 L of LR plus shed blood in the Hextend group.

The authors conclude that the data demonstrate that the hemoglobin-based oxygen carrier, HBOC-201, is at least as effective as the current standard, Hextend, in the treatment of hemorrhage shock with prolonged low volume resuscitation. They further theorized that HBOC-201 may provide a survival advantage. I have some concerns and questions regarding both the methodology and the conclusions reached by the authors in the manuscript.

First, the methodologic concern. Realizing that a severe hemorrhage model used in this study was developed by your lab and others over time, I would like to ask a question regarding return of shed blood. The Hextend animals were permitted to remain hypotensive, below the target mean arterial pressure, for 8 hours, which certainly should have primed the neutrophils as was demonstrated by your oxidative burst analysis. These same animals were then given 9-hour old stored blood, which may have served as the second hit. My first question then is why infuse all of the shed blood only to the Hextend animals in this model, or for that matter, why re-infuse any at all? In a real military scenario, a soldier 8/9 or nine hours out from his injury may not need blood transfusion at all. Do you plan to study additional variations of this shock model, perhaps using additional Hextend beyond the two boluses to allow a soldier to be resuscitated to your target mean pressure of 60?

I have two questions regarding study conclusions. The manuscript states that HBOC-201 has several unique properties that make it appealing for use as a resuscitation fluid, including requiring less volume for achieving resuscitation goals as compared with traditional fluids. While cardiac output returned to baseline in the Hextend group after initial resuscitation, it remained depressed in the HBOC group following initial resuscitation. Systemic vascular resistance, which was elevated during shock as expected, rapidly returned below starting values in the Hextend resuscitation group, but took seven hours in the HBOC resuscitation group to return to normal. Does HBOC have a pressor effect that can explain these findings but is not mentioned in the manuscript? This would also be one explanation for the fact that all HBOC animals were able to be resuscitated to target mean arterial pressure with 1,000 mL of fluid, while none of the Hextend animals were. So my question is: Does a pressor effect exist for HBOC-201?

Finally, I quote, "HBOC-201 may provide a survival advantage." This statement is based on the fact that only a single HBOC animal died from an air embolism, and that four of eight Hextend animals died the first day of the study, either at the definitive resuscitation at 8 hours or later that same evening. Necropsy demonstrated that all four of these animals died from acute pulmonary edema. Rather than suggesting there is a survival advantage to HBOC resuscitation from unidentified causes, the authors should recognize the more likely reason for this apparent survival advantage. The total volume of resuscitation fluid given to each group was formulated by protocol, rather than based on physiologic endpoints, and not equal. This resulted in 99 ml L/kg total resuscitation for the Hextend group, versus 8L mL total resuscitation for the HBOC group. This statistically significant difference is nearly 20 mL/kg, and could well have caused the death from acute pulmonary edema in the Hextend group.

My final question is: Isn't over-resuscitation in the Hextend group the most likely explanation for these four acute pulmonary edema deaths? Perhaps stated otherwise, is there a combined effect on the lungs of shed blood transfusion with over-resuscitation? Military policy makers should carefully review this manuscript and others like it before making changes in the way our soldiers are resuscitated. In the vast majority of cases, evacuation for our soldiers, sailors, airmen, and marines is rapid, rather than prolonged as this model simulates. Should further investigation of HBOC-201 prove it to be a suitable resuscitation fluid for the military, that should not mean it needs to be used under all conditions as the first choice resuscitation fluid. Our medics must have a selection of resuscitative fluids, and the training to know when and how to use each one optimally. I encourage the authors to continue this important work.

Dr. Frederick A. Moore (Houston, Texas): I was impressed with the two groups at how much damage there was in the tissues. In last month's issue of *Shock*, George Kraemer was studying two different regimens of hypotensive resuscitation, and he happened to have a control group that was to put the pressure back to normal. What his study showed was that the animals resuscitated in the traditional approach—which is to normalize blood pressure—did much better, so I really would like to see a control group here just to see if you were to resuscitate the animals in the way we've done it in the past, just how much damage would be present.

Dr. Greg Beilman (Minneapolis, Minnesota): In a recent Shock Society meeting, Jill Sondeen presented results with Hextend and Polyheme in a very similar group of animals in a controlled hemorrhage shock model. In fact, she just demonstrated no survival difference to animals receiving Hextend, compared to Polyheme resuscitation. I was wondering if you had thoughts regarding the potential differences between Polyheme as an HBOC versus HBOC-201.

Dr. Lawrence P. Sue (San Jose, California): I don't know if there have been any studies done into this. What is the stability of HBOC-201 with regards to temperature? As you know, most combat medics don't carry their equipment in a temperature controlled environment. One hundred fifteen degrees in Saudi Arabia in the summer, or even worse—I haven't been there—what is the stability of this with temperature?

Dr. Colleen M. Fitzpatrick (Closing): With regard to the first question of using the shed blood to resuscitate only the Hextend animals; when we wrote the original protocol,

we initially intended to resuscitate both groups with crystalloid only. However, we found that when we infused the crystalloid into the Hextend animals, the animals died, essentially because we significantly diluted out the oxygen carrying capacity. So we opted to re-infuse the shed blood into those animals. I think certainly we could entertain looking at other study groups with additional Hextend infusion, though I don't know that would make a significant difference.

With regard to the possibility of a pressor affect, HBOC does appear to have a pressor affect. This has previously been attributed to nitric oxide scavenging; however, work in our lab has shown that it does not appear to be exclusively the result of nitric oxide scavenging, and other mechanisms may be in play. We have ongoing work in this area.

With regard to the question of over resuscitation, the animals in the Hextend group did receive a larger volume of fluid. If you look at the amount of volume they received in the final definitive resuscitation, the difference between the two groups was actually not clinically significant enough to produce the degree of pulmonary edema and hemorrhage that we see in the nonsurvival animals. So I think part of the difference in the survival advantage of the HBOC animals is that the Hextend animals were not able to achieve the same perfusion pressure and therefore, didn't have adequate oxygenation, or had sub-optimal oxygenation during the 8 hour period. When we draw the conclusion that HBOC may offer survival advantage, I suppose we ought to clarify that statement and say that it may offer survival advantage when compared to a similar protocol using Hextend.

The question of resuscitating back to baseline pressure and the possibility of this improving outcome is certainly an interesting question. We didn't resuscitate these animals back to baseline simply because we were trying to compare this directly with the current field care guidelines. But that is something that would certainly be interesting to explore.

No head-to-head comparisons between Polyheme and HBOC have been made yet. There are some differences in the formulations of the products, and I think that there is still a lot of work to be done with both products before any definitive comparisons can be made. It will be interesting to see how this plays out.

In terms of the temperature requirements of HBOC, it does not require refrigeration. This is certainly an advantage for use in the military, because it can be put in the backpack of a medic located somewhere in the desert without deleterious effects.