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

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Background: **We have shown in a previous work that HBOC-201 is able to reverse anaerobic metabolism at low volumes in a porcine model of controlled hemorrhage. On the basis of these results, we hypothesize that low-volume resuscitation with HBOC-201 in a porcine model of controlled hemorrhage provides adequate tissue oxygenation to limit end-organ damage and allow for survival of the animal.**

Methods: **Twenty-four Yorkshire swine (55–65 kg) were rapidly hemorrhaged to a mean arterial pressure (MAP) of 30 mm Hg, maintained hypotensive for 45 minutes, and then divided into four groups. The first group, Shed Blood (BL), was resuscitated with shed blood to baseline MAP. A second group, Shed Blood (60), underwent resuscitation for four hours at an MAP of 60 mm Hg with shed blood. The third group, LR + Blood, was resuscitated with lactated Ringer's (maximum, 40 mL/kg) followed by shed blood to baseline MAP. The final group, HBOC (60), underwent resuscitation for 4 hours at an MAP of 60 mm Hg with HBOC-201. Hemodynamic variables, urine output, blood gas analyses, lactate levels, and jejunal oximetry were followed throughout the experiment. Animals were allowed to survive and underwent necropsy on postinjury day 3. Histologic comparisons were made. Data were analyzed using analysis of variance/Duncan's multiple range test.**

Results: **All animals survived the hemorrhage/resuscitation. One animal in the LR + Blood group died on postinjury day 1. Heart rate, MAP, and arterial pH were similar between groups. Cardiac output was significantly lower throughout resuscitation in the HBOC (60) group. Jejunal oximetry was similar throughout the experiment in all groups, revealing a decline in Po_2 during hemorrhage and return to baseline or near baseline during resuscitation. There was no evidence of renal dysfunction. Histologically, one animal in the LR + Blood group and four of six animals in the HBOC (60) group demonstrated mild hepatocellular damage. All other tissues examined were found to have no significant abnormalities. Elevations in serum aspartate aminotransferase levels were noted when comparing the HBOC (60) group to the Shed Blood (BL) and Shed Blood (60) groups on day 2. Significant decreases in hemoglobin levels were noted in the HBOC (60) group compared with all other groups beginning on day 2.**

Conclusion: **Low-volume resuscitation with HBOC-201 provides adequate tissue oxygenation for survival in a porcine model of controlled hemorrhagic shock with no long-term organ dysfunction identified. Although some animals did show mild hepatocellular damage with elevations of aspartate aminotransferase at day 2, these findings did not appear to have clinical relevance, and the enzyme elevations were trending toward normal by the third postoperative day. Decreases in hemoglobin levels at the later time points were expected, given the half-life of the product.**

Key Words: **Hemorrhage, Resuscitation, Hemoglobin-based oxygen-carrying (HBOC) solution, Blood substitute, Porcine.**

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Hemorrhage is a major cause of death in trauma. Initial therapy currently consists of bolus doses of isotonic crystalloid solutions followed by packed red blood cells, as necessary. This approach dates to animal research performed as early as the 1950s.[1–4] The ideal resuscitation solution would be abundant, universally compatible, inexpensive, an effective oxygen carrier, an intravascular volume expander, and free of negative side effects and infectious risk. The development of oxygen-carrying solutions based on acellular hemoglobin preparations has made significant advances in recent years.[5–8] The most promising are the polymerized hemoglobin-based oxygen-carrying (HBOC) solutions, with some of these products undergoing or having recently completed testing in clinical trials.[9–13]

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Table 1 Characteristics of HBOC-201

Characteristic	Value
Hemoglobin concentration (g/dL)	13
Size distribution	
< 68 kDa	<5%
128 kDa	17%
> 128 kDa	80%
P_{50} (mm Hg)	38
Colloid oncotic pressure (mm Hg)	25
Half-life ($t_{1/2}$) (h)	19
Osmolarity (mOsm/L)	300
Electrolytes (mmol/L)	
NaCl	113
KCl	4
$CaCl_2$	1.4
NaOH	10
Sodium lactate	27
N-acetyl-L-cysteine (mg/dL)	200
Free glutaraldehyde (μg/dL)	<3.5
Endotoxin (EU/mL)	<0.05

Data from York et al., 2002.[14]

HBOC-201 (Hemopure, Biopure Corporation, Cambridge, MA) is an ultrapure polymerized bovine hemoglobin-based oxygen carrier in a modified lactated Ringer's solution (Table 1).[14] The product can be stored at room temperature and has a shelf-life of 3 years. It requires no crossmatch and does not appear to elicit an immune response, allowing the same patient to receive additional doses of the product over time.[15] These logistic advantages make it ideal for far-forward military operations or rural trauma settings where transport times to higher echelons of care are long and where blood is not immediately available. This product has completed phase III clinical trials and a Biological License Application with the Food and Drug Administration has been filed for use in the treatment of surgical anemia.

We demonstrated recently, in a porcine model of hemorrhagic shock, that hypotensive resuscitation with HBOC-201 reversed anaerobic metabolism as compared with normotensive resuscitation with crystalloid solution.[16] We were also able to show that HBOC-201 achieved this effect at significantly lower volumes of resuscitation as compared with standard resuscitative fluids. Therefore, this product shows promise as an effective low-volume resuscitation fluid. However, this study was accomplished in a nonsurviving animal model and therefore end-organ effects and animal survival in the postinjury period were not fully evaluated. Of particular concern was the fact that the animals were oliguric for the period of the study. This is most likely because of the relative hypovolemic state given the low volumes of resuscitation required to reverse anaerobic metabolism. However, the long-term effects on renal function could not be evaluated. The purpose of this study was to extend these findings and evaluate the effects of low-volume resuscitation with HBOC-201 and potential sequelae in a surviving porcine model of hemorrhagic shock and resuscitation. We hypothesized that low-volume resuscitation with HBOC-201 would achieve tissue oxygenation similar to that of normotensive resuscitation with lactated Ringer's solution combined with blood. We also hypothesized that low-volume resuscitation with HBOC-201 would not increase the incidence of late organ dysfunction and subsequent survival in these animals.

MATERIALS AND METHODS

Animal Model

This 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*.[17] Twenty-four female Yorkshire swine (55–65 kg) obtained from a single provider source were used in this experiment. All animals were fasted 12 hours before surgery and only received water ad libitum. Each animal was sedated with an intramuscular injection of ketamine (15–20 mg/kg) and atropine (0.04–0.4 mg/kg). General anesthesia was induced with mask ventilation using isoflurane (3.5–5.0%) in 40% oxygen. An 18-gauge, 1.25-in, intravenous catheter was placed in an ear vein. Crystalloid intravenous fluid was administered at a rate of 2 to 4 mL/kg/h until initiation of the experimental protocol. Each animal then underwent endotracheal intubation followed by mechanical ventilation. End-tidal isoflurane levels were maintained at or slightly above the minimal alveolar concentration for swine (1.75%). Ventilation parameters included positive end-expiratory pressure of 3 mm Hg, F_{IO_2} of 40%, tidal volume of 7 to 10 mL/kg, and a respiratory rate of 10 breaths/min. A Foley catheter was inserted into the urinary bladder and electrocardiogram leads were placed. A bed warmer, room temperature adjustments, and blankets were used to maintain the animal's core temperature at 37°C.

All invasive procedures were accomplished using aseptic technique. An 8-Fr introducer sheath was placed percutaneously in the internal jugular vein followed by a standard thermodilution pulmonary artery catheter. On the left lower extremity, a small cutdown was accomplished to allow placement of a 5-Fr catheter into a small superficial artery. This catheter was advanced into the distal aorta, secured in position, and used for continuous arterial pressure monitoring. A second cutdown was accomplished on the right lower extremity to expose the common femoral artery. An 8-Fr introducer sheath was placed in the artery by means of the Seldinger technique. This sheath was used as the location of controlled hemorrhage and laboratory sampling. All lines were secured in place and incisions closed with skin staples.

A laparotomy was then performed. A 6-in section of jejunum, approximately 1 ft beyond the ligament of Treitz, was secured to the abdominal wall using 2-0 silk sutures. A 5-in, 18-gauge Angiocath was then introduced percutaneously through the abdominal wall into the lumen of the isolated jejunal segment and secured in place with a 2-0 silk suture. A calibrated Paratrend 7 sensor (Diametrics Medical,

St. Paul, MN) was then connected to the Angiocath and advanced slowly into the lumen of the jejunal segment. After verifying proper calibration and operation of the Paratrend 7 sensor, the laparotomy was closed with 1-0 polydioxanone suture and skin staples.

All animals were allowed to equilibrate for 15 minutes and then baseline measurements were recorded. After equilibration, each animal was rapidly hemorrhaged through the femoral artery introducer sheath over approximately 7 minutes until the mean arterial pressure (MAP) had decreased to 30 mm Hg. All animals were kept hypotensive at an MAP of 30 ± 5 mm Hg for 45 minutes by continued intermittent hemorrhage. The shed blood was collected in blood bags containing citrate phosphate dextrose and kept at room temperature until reinfusion. Net weight of the blood bags was used to calculate hemorrhage volume. Subjects were divided into four experimental groups. The Shed Blood (BL) (n = 6) group was resuscitated to baseline MAP with shed blood. The Shed Blood (60) (n = 6) group was resuscitated to an MAP of 60 mm Hg with shed blood. The LR + Blood (n = 6) group was resuscitated to baseline MAP with lactated Ringer's (LR) solution until a maximum of 40 mL/kg was infused, followed by shed blood. The HBOC (60) (n = 6) group was resuscitated to an MAP of 60 mm Hg with HBOC-201. All animals were observed for 4 hours after initiation of resuscitation. At the end of the 4-hour period, the Shed Blood (60) group was resuscitated to baseline MAP using remaining shed blood and the HBOC (60) group was resuscitated to baseline MAP using LR solution. All animals were then observed for an additional hour.

At the end of the fifth hour, the Paratrend 7 catheter was removed. The 5-Fr arterial catheter was removed and the small superficial artery ligated. The femoral introducer sheath was removed, the femoral artery repaired with 7-0 Prolene, and the skin closed with staples. On the side of the upper extremity introducer sheath, the pulmonary artery catheter was removed and the introducer was exchanged for an indwelling Port-a-Cath. This Port-a-Cath was used for daily laboratory blood sampling. Each animal was then awakened, extubated, and individually housed in a holding pen with food and water provided ad libitum.

Physiologic Data Collection

Hemodynamic variables (heart rate and arterial blood pressure) along with jejunal oximetry from the Paratrend 7 catheter were recorded continuously. Cardiac output (CO) was determined by the thermodilution technique and recorded at the times listed below. A mixed venous blood sample, arterial blood gas, and serum lactate level were all drawn at the following time points: baseline (t − 45 minutes); end hemorrhage (t − 30 minutes); end hypotension/begin resuscitation (t = 0); and then 30, 60, 90, 120, 150, 180, 210, 240, and 300 minutes into the resuscitation.

Pathologic Examination

End-organ damage was assessed both functionally with serum laboratory values and histologically by microscopic evaluation of selected tissues. Blood samples were taken through the previously paced Port-a-Cath. On the morning of postinjury day 3, each animal was sedated, intubated, and placed on mechanical ventilation as described above. A laparotomy was performed and representative samples were collected for histology from the stomach, duodenum, ileum, kidney, liver, and lung. The animal was killed with Euthanasia-5 solution immediately after tissue collection.

Tissue samples were fixed in 10% buffered formalin and processed to paraffin. Hematoxylin and eosin-stained, 5-μm sections were prepared for microscopic evaluation by a veterinary pathologist. All lesions were subjectively graded mild, moderate, marked, or severe using standard histopathologic nomenclature.

Blood Samples

Liver function tests, blood urea nitrogen, creatinine, and a complete blood count were drawn at baseline, t = 0, and hourly throughout the resuscitation. After the initial experiment, laboratory values were checked on postoperative days 1, 2, and 3. Before performing creatinine measurements, serum specimens were centrifuged through Amicon Centrifree filters (Millipore, Bedford, MA) to remove acellular hemoglobin from the sample. HBOC-201 does not interfere with any other serum measurements obtained in this study.

Statistical Analysis

Data reported are mean ± SD for each respective group and all animals as a whole. All statistical analyses were performed using a statistical software package for personal computers (SPSS for Windows version 6.0; SPSS, Inc., Chicago, IL). Statistical comparison of group demographic and hemodynamic data included a one-way analysis of variance with a post hoc test (Tukey's honest significant difference). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Group Characteristics

All groups were similar regarding weight, estimated blood volume, hemorrhage volume, and percentage of estimated blood volume hemorrhaged. Resuscitation volume was significantly higher in the LR + Blood group as compared with the other groups (Table 2). All animals survived the hemorrhage/resuscitation portion of the protocol. One animal in the LR + Blood group died on postinjury day 1.

Hemodynamics

MAP in all groups was equivalent at baseline and decreased in a similar pattern during hemorrhage. As the Shed Blood (60) and HBOC (60) groups experienced low-volume resuscitation, by design, each had a significantly lower MAP

Table 2 Group Characteristics

Group	Weight (kg)	Hemorrhage Volume (mL)	% of EBV Hemorrhaged	Resuscitation Volume (mL)
Shed Blood (BL)	61.8 ± 4.1	1,974 ± 566	45.2 ± 10.9	1,974 ± 566
Shed Blood (60)	58.6 ± 3.2	1,769 ± 243	43.1 ± 5.5	2,435 ± 294
LR + Blood	60.7 ± 1.9	1,744 ± 253	41.2 ± 6.9	4,227 ± 228
HBOC (60)	60.2 ± 1.6	1,754 ± 309	41.6 ± 6.8	2,392 ± 670
p Value (ANOVA)	0.314	0.663	0.804	<0.001

ANOVA, analysis of variance.

during resuscitation as compared with the other groups. By end resuscitation, MAP returned to baseline in all groups (Fig. 1). Heart rate in all groups was equivalent at baseline, rose in an expected fashion during hemorrhage, and returned to baseline by completion of resuscitation. CO for all groups was similar at baseline. During hemorrhage, CO in all groups declined in an expected fashion. Throughout resuscitation, the CO in the HBOC (60) group was significantly lower than both the LR + Blood and Shed Blood (BL) groups. By end resuscitation, all groups returned to baseline or near baseline CO (Fig. 2).

Metabolic Indicators

Metabolic values are shown in Table 3. Arterial pH in all groups was equivalent at baseline. All groups showed a decrease in pH that persisted until 30 minutes into resuscitation. During the last 2 hours of resuscitation, the pH in the HBOC (60) group was significantly lower than the Shed Blood (BL) group (7.45 ± 0.05 vs. 7.53 ± 0.03, $p < 0.05$); however, this finding is primarily because of an increase in arterial pH for the Shed Blood (BL) group, as the HBOC group values returned to baseline during resuscitation.

Lactate levels in all groups began to rise above normal during the hypotensive period and returned to baseline or near baseline by the end of the 4-hour period of initial resuscitation. However, after fluid resuscitation to baseline MAP in the two groups maintained at MAP 60 for the initial 4 hours, the lactate level in the HBOC (60) group rose to statistically significant levels when compared with the other groups (2.3 ± 0.9 mmol/L vs. 0.9 ± 0.1 mmol/L for the Shed Blood [BL]

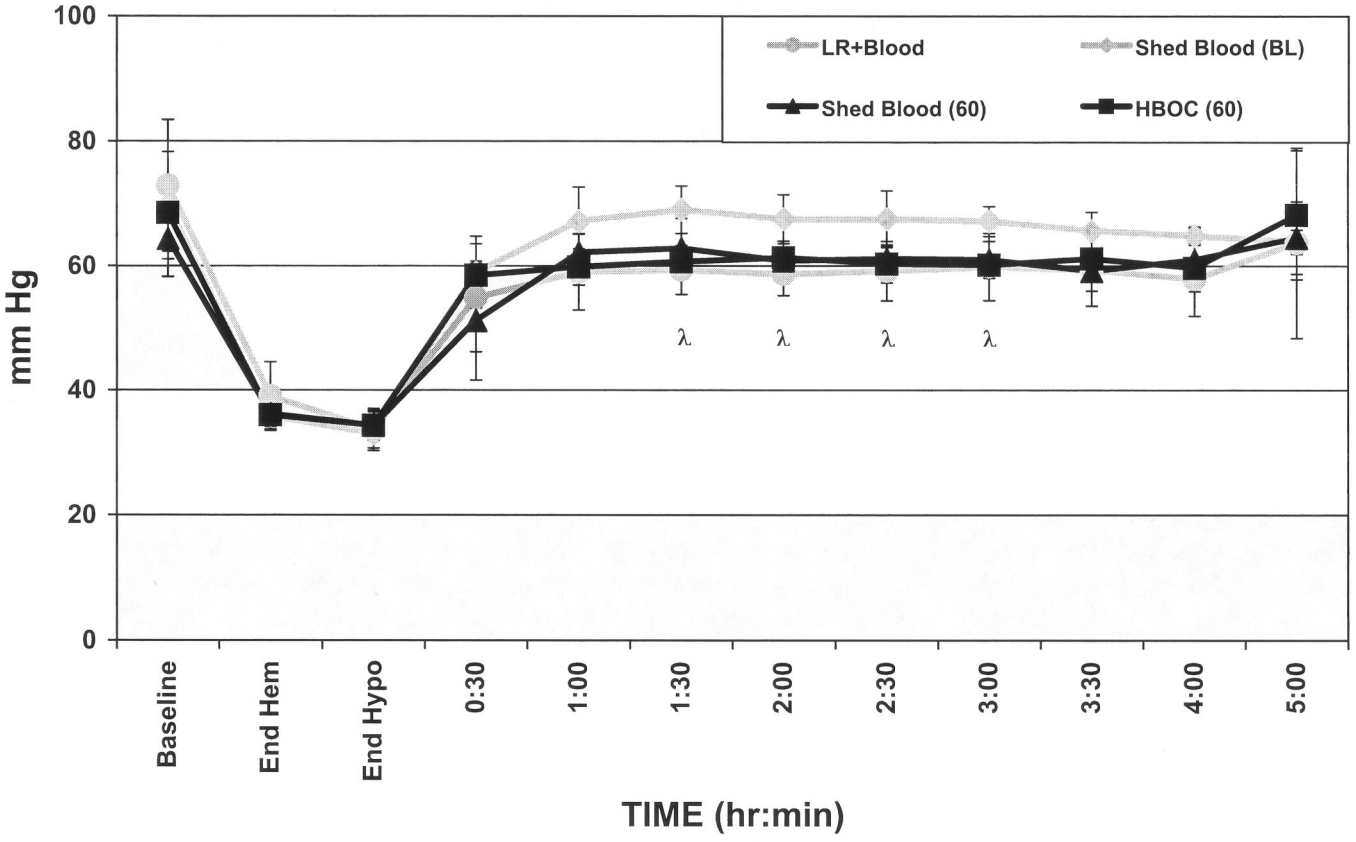

Fig. 1. *Mean arterial pressure.* λp < *0.05 HBOC (60) vs. Shed Blood (BL) group.*

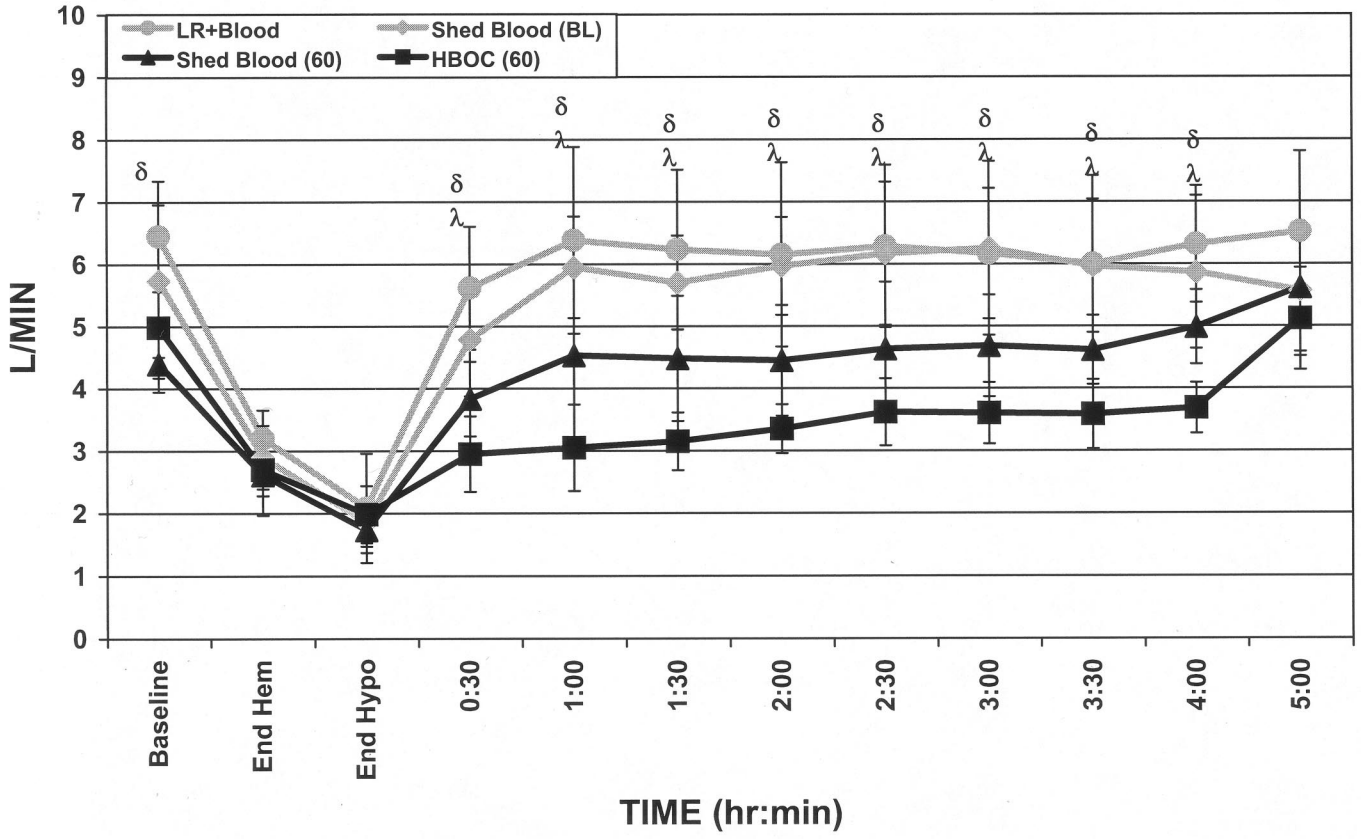

Fig. 2. *Cardiac output. λp < 0.05 HBOC (60) vs. Shed Blood (BL) group, δp < 0.05 HBOC (60) vs. LR + Blood group.*

group; 1.4 ± 0.1 mmol/L for the Shed Blood [60] group; and 1.3 ± 0.4 mmol/L for the LR + Blood group; all $p < 0.05$).

Hemoglobin Measurements

There were no significant differences in hemoglobin levels between groups during hemorrhagic shock and the 5-hour period of resuscitation (Fig. 3). Although hemoglobin levels tended to be lower for the LR + Blood group during this phase, this did not reach statistical significance. However, hemoglobin levels for the animals receiving HBOC-201 began to decline compared with the other groups beginning the evening of postoperative day 1 and were significantly decreased compared with the other groups beginning in the morning of day 2 and extending throughout the rest of the study period. Hemoglobin concentrations were lowest in the HBOC-201 group at the termination of the study on the morning of day 3 (7.67 ± 0.70 g/dL vs. 11.13 ± 0.99 g/dL for the Shed Blood [BL] group; 10.48 ± 1.13 g/dL for the Shed Blood [60] group; and 11.08 ± 1.99 g/dL for the LR + Blood group; all $p < 0.0001$).

End-Organ Function/Oxygenation

Urine output for the HBOC (60) group declined and remained lower than all other groups during resuscitation. These animals were oliguric during the 4-hour period of resuscitation and resumed normal urine output after being resuscitated back to baseline MAP between the fourth and fifth hours of resuscitation. All groups were producing adequate urine output by end resuscitation (Fig. 4). Creatinine in all groups rose above baseline during hemorrhage. Levels peaked at 4 hours postresuscitation and returned to baseline by postoperative day 1, remaining normal through the end of the study on postoperative day 3. Although creatinine levels in the HBOC (60) animals tended to be higher than other groups at the 4- and 5-hour postresuscitation time points, these differences did not reach statistical significance (Fig. 5).

Hepatic transaminase levels in all groups rose above baseline beginning on postoperative day 1. Levels were higher in the HBOC (60) and LR + Blood groups and were significantly different between HBOC (60) and both Shed Blood groups during day 2. Levels between treatment groups showed no statistical significance by day 3 (Fig. 6).

Jejunal P_{O_2} was equivalent in all groups at baseline and declined slightly during hemorrhage (Fig. 7). Jejunal P_{O_2} increased at initiation of resuscitation for all groups. At the 2-hour point, all groups had returned to baseline jejunal P_{O_2}, except for the HBOC (60) group, which remained higher than baseline through end resuscitation. Jejunal P_{CO_2} was equivalent in all groups at baseline and rose during hemorrhage. Within 1 hour of initiating resus-

Table 3 Metabolic Values

Parameter	Time (min)	Group: Shed Blood (BL)	Shed Blood (60)	LR + Blood	HBOC (60)
Arterial pH	Baseline (R–45)	7.47 ± 0.03	7.47 ± 0.02	7.48 ± 0.02	7.47 ± 0.06
	End Hem (R–30)	7.50 ± 0.04	7.49 ± 0.02	7.48 ± 0.05	7.46 ± 0.05
	End Hypo (R = 0)	7.43 ± 0.04	7.38 ± 0.03	7.39 ± 0.06	7.39 ± 0.05
	R + 30	7.41 ± 0.08	7.39 ± 0.07	7.36 ± 0.10	7.39 ± 0.06
	R + 60	7.46 ± 0.05	7.43 ± 0.04	7.39 ± 0.09	7.41 ± 0.07
	R + 90	7.48 ± 0.03	7.47 ± 0.03	7.41 ± 0.07	7.42 ± 0.07
	R + 120	7.50 ± 0.02	7.47 ± 0.02	7.46 ± 0.04	7.44 ± 0.07
	R + 150	7.49 ± 0.03	7.47 ± 0.05	7.46 ± 0.04	7.45 ± 0.05
	R + 180λ	7.52 ± 0.02	7.48 ± 0.03	7.47 ± 0.03	7.44 ± 0.05
	R + 210λ,ϕ	7.52 ± 0.02	7.49 ± 0.02	7.48 ± 0.03	7.43 ± 0.05
	R + 240λ	7.53 ± 0.02	7.48 ± 0.02	7.46 ± 0.04	7.44 ± 0.06
	R + 300λ	7.53 ± 0.03	7.48 ± 0.02	7.49 ± 0.03	7.45 ± 0.05
Serum lactate (mmol/L)	Baseline (R–45)	0.9 ± 0.3	1.2 ± 0.4	1.4 ± 0.6	1.2 ± 0.4
	End Hem (R–30)	1.0 ± 0.3	1.1 ± 0.3	1.4 ± 0.7	1.1 ± 0.3
	End Hypo (R = 0)	1.8 ± 0.6	1.9 ± 0.7	3.0 ± 2.0	1.7 ± 0.4
	R + 30	2.7 ± 1.4	2.6 ± 1.3	5.3 ± 3.6	2.7 ± 1.1
	R + 60	2.4 ± 1.4	2.3 ± 0.9	4.4 ± 3.6	2.6 ± 1.4
	R + 90	1.9 ± 0.9	1.8 ± 0.7	3.6 ± 3.2	2.5 ± 1.3
	R + 120	1.6 ± 0.7	1.5 ± 0.3	2.8 ± 2.4	2.1 ± 0.9
	R + 150	1.3 ± 0.4	1.4 ± 0.3	2.2 ± 1.6	2.0 ± 0.9
	R + 180	1.1 ± 0.3	1.3 ± 0.3	1.7 ± 1.0	1.9 ± 0.7
	R + 210λ	1.0 ± 0.1	1.2 ± 0.2	1.5 ± 0.7	1.9 ± 0.7
	R + 240	0.9 ± 0.1	1.3 ± 0.1	1.4 ± 0.5	2.0 ± 0.9
	R + 300	0.9 ± 0.1	1.4 ± 0.1	1.3 ± 0.4	2.3 ± 0.9

$^{\lambda}p < 0.05$ HBOC vs. Shed Blood (BL).
$^{\phi}p < 0.05$ HBOC vs. Shed Blood (60).
$^{\delta}p < 0.05$ HBOC vs. LR + Blood.

citation, jejunal P_{CO_2} returned to baseline or near baseline in all groups (Fig. 8).

Pathologic Analysis

Liver

Mild to moderate degeneration and/or necrosis of centrilobular to midzonal hepatocytes was observed in four of six animals in the HBOC (60) group (Fig. 9*A* and *B*) and one of six animals in the LR + Blood group. No pathologic changes were observed in the other groups.

Kidneys

Multifocal mild vacuolar change of the tubular epithelium was observed in several animals. This finding was independent of treatment group. No findings specific to the HBOC (60) group were identified (Fig. 9*C*).

Gastrointestinal Tract

Mild to moderate interstitial edema was noted in the lamina propria mucosa of the stomach and small intestines in many animals and was independent of treatment group. Histologic evidence of progressive ischemic change characterized by degeneration and loss of mucosal epithelium was not observed at the time points evaluated in this study (Fig. 9*D*).

Lung

All subjects showed signs of inflammation consistent with subclinical respiratory infection common in these animals. This precluded evaluation of more subtle pulmonary changes associated with the experimental treatment.

DISCUSSION

In a previous work, we compared hypotensive resuscitation with HBOC-201 to standard therapies in a nonsurviving porcine model of controlled hemorrhage. This model produces a severe physiologic insult to the animal and results in early mortality if the animals are not resuscitated. We were able to show that hypotensive resuscitation with HBOC-201 in this porcine model provided sufficient tissue oxygenation to reverse anaerobic metabolism.[16] However, although global markers of anaerobic metabolism were corrected, concerns persisted regarding the ultimate effect resuscitation with these agents would have on end-organ function. This study was undertaken to further evaluate end-organ function using a 3-day survival model after controlled hemorrhagic shock. Our primary interest is in studying these agents for potential use in military operations where blood products may not be immediately available. Therefore, our study design was based on immediate, primary resuscitation with HBOC-201 at the site of injury followed by a 4-hour period of resuscitation to mimic evacuation times to higher levels of care, after which

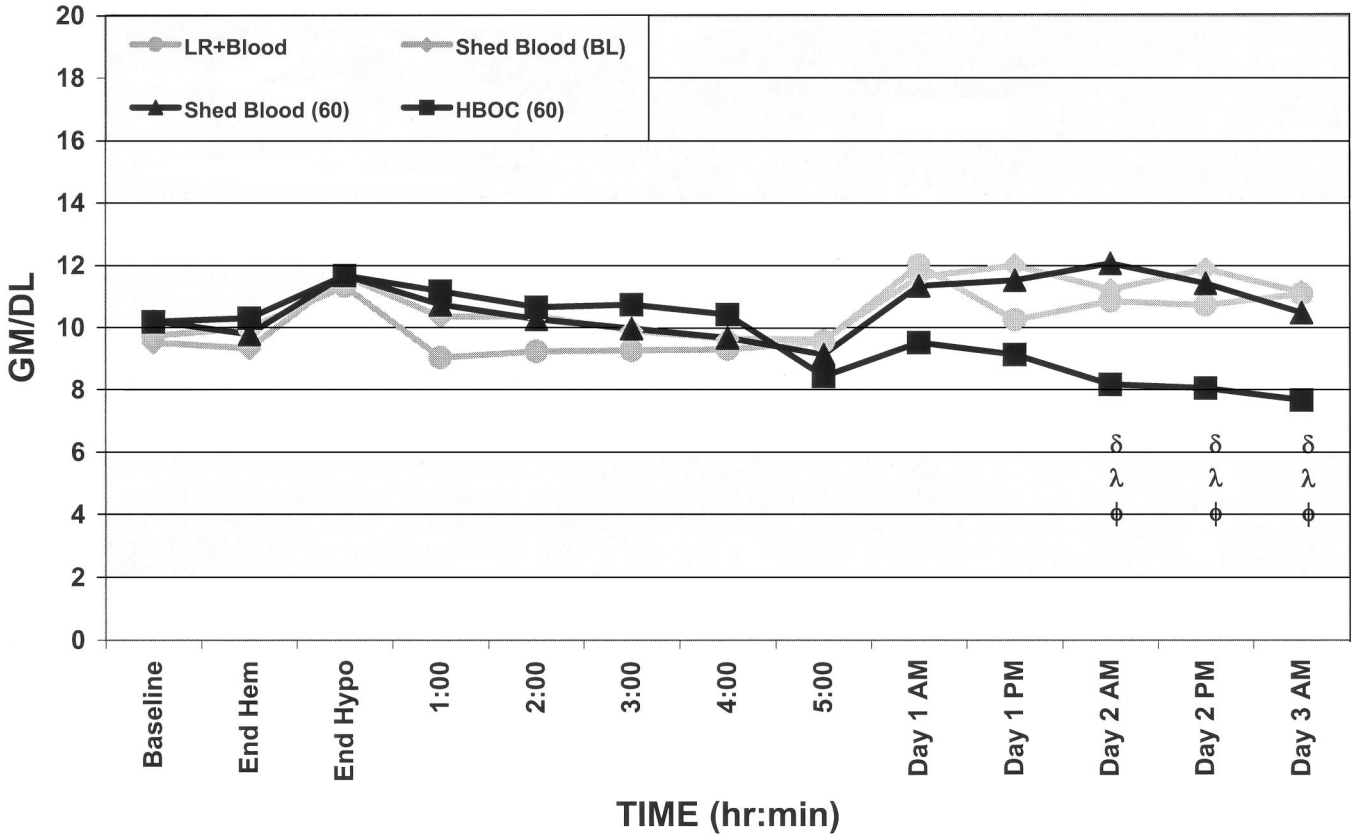

Fig. 3. *Hemoglobin.* $\lambda p < 0.0001$ *HBOC (60) vs. Shed Blood (BL) group,* $\phi p < 0.0001$ *HBOC (60) vs. Shed Blood (60) group,* $\delta p < 0.0001$ *HBOC (60) vs. LR + Blood group.*

the animals were volume resuscitated back to their baseline pressure. We hypothesized that low-volume resuscitation with HBOC-201 using this protocol in a porcine model of controlled hemorrhage would provide adequate tissue oxygenation and not result in clinically significant end-organ damage. We have previously shown that HBOC-201 reverses anaerobic metabolism in this model at significantly lower volumes of resuscitation when compared with traditional resuscitation fluids.[16] However, this survival study differs from the previous study in that the animals were resuscitated back to baseline MAP with LR solution at the end of the 4-hour resuscitation period. Even so, the total volume of resuscitation the animals received over the 5-hour experimental period was significantly less in the HBOC-201 group when compared with the LR + Blood group (2,392 ± 670 mL vs. 4,227 ± 228 mL; $p < 0.001$). The total amount of resuscitation was not significantly different between the HBOC-201 and Shed Blood groups. This shows a distinct advantage for HBOC-201 over crystalloid resuscitation as a primary resuscitative agent where blood is not immediately available. The implication is that potentially more casualties can be treated at the site of injury using HBOC-201 as the primary resuscitative fluid.

Despite relative hypovolemia, as indicated by low CO and oliguria, animals receiving HBOC-201 had normalization of arterial pH and serum lactate at the 4-hour time point after resuscitation. This result closely mimics findings from our previously published nonsurvival model.[16] Of note, there is an increase in the serum lactate at the 5-hour time point after resuscitation (2.3 ± 0.9 mmol/L vs. 2.0 ± 0.9 mmol/L) (Table 3). This increase, although statistically significant, may have occurred secondary to washout of tissue metabolites or may have just been the result of small sample size. Whether this effect would have been seen in animals resuscitated back to baseline MAP with HBOC-201 is unknown and was not studied. Regardless of the rise in serum lactate seen in the HBOC-201 animals after volume resuscitation to baseline MAP, all animals achieved the primary endpoint of survival to postinjury day 3 with normalization of serum lactate levels (Table 3).

It was unknown what effect potential vasoconstrictive properties of these agents would have on end-organ function. End-organ damage was assessed both clinically throughout the experiment and histologically at the end of the experiment on postinjury day 3. As expected, urine output was lower during the initial 4-hour resuscitation period in the group resuscitated with HBOC-201 when compared with the other groups. This reproduces data previously obtained in this model. The low urine output is most likely because of the relative hypovolemia seen with the low initial volumes of

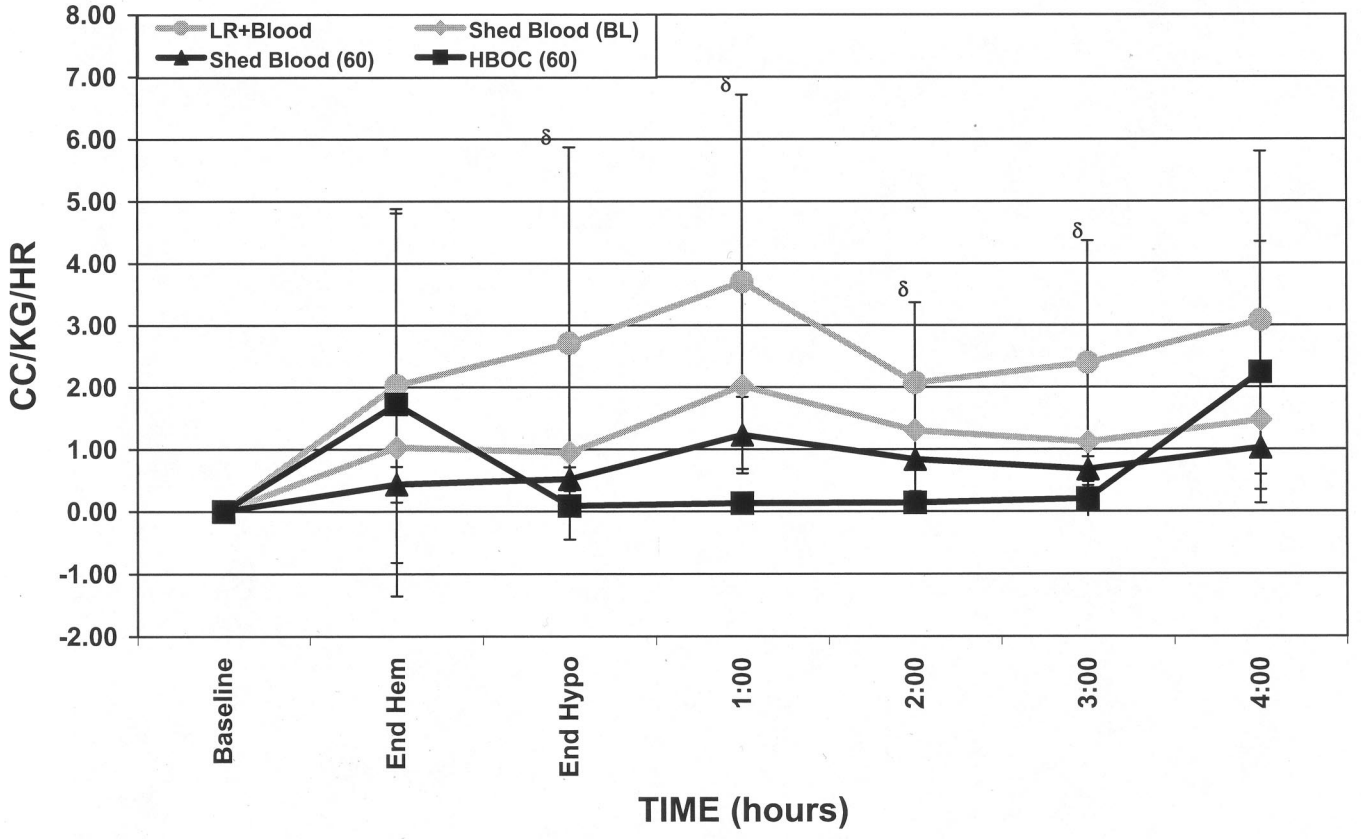

Fig. 4. *Urine output. δp < 0.05 HBOC (60) vs. LR + Blood group.*

HBOC-201 required. After volume resuscitation back to baseline MAP, urine output returned to normal levels in the HBOC-201 group. Serum creatinine in all groups rose above normal but returned to baseline by postinjury day 1, with no statistical differences noted between groups. Renal histology obtained after the animals were killed on postinjury day 3 revealed mild vacuolar change that was consistent among all groups. These findings suggest that despite low urine output, animals in the HBOC-201 group did not suffer an ischemic renal insult resulting in acute tubular necrosis or permanent renal impairment as indicated by serum chemistries and histology.

The most significant histologic finding was the multifocal mild to moderate hepatic degeneration/necrosis observed in four of the six animals in the HBOC (60) group and one of the five surviving LR + Blood animals. Centrilobular and midzonal hepatic necrosis is an unusual finding. It is not clear whether these lesions resulted from a direct toxic effect of the HBOC-201 or from decreased oxygen delivery and ischemia to the liver. However, because centrilobular hepatocytes are most sensitive to decreases in oxygen delivery, these lesions most likely represent ischemic change.[18] Similar changes have been noted in previous studies using stroma-free hemoglobin (SFH). Friedman et al. reported morphologic changes in the liver after 75% exchange transfusion in a small-animal model.[19] Centrilobular necrosis was seen in rats 12 and 24 hours after exchange transfusion but not at earlier time points of 1 and 5 hours. These changes were not attributable to direct toxicity of the native hemoglobin but rather to the relatively short half-life (3.5 hours) of SFH and the osmotic diuresis caused by the product as it was excreted by the kidneys. This ultimately led to a loss of oxygen-carrying capacity and intravascular volume depletion, thereby negating the early protective effect of SFH on the liver. Although the newer generation of polymerized hemoglobin preparations such as HBOC-201 are not excreted by the kidneys, the half-life of these products is still relatively short (Table 1). Therefore, an expected decrease in hemoglobin levels was seen over time in the animals that were resuscitated with HBOC-201 (Fig. 3). This loss of oxygen-carrying capacity over time may have contributed to the increase in the incidence of centrilobular necrosis similar to that described in the study by Friedman et al.[19] It is currently not known whether redosing with HBOC-201 on subsequent days to maintain higher hemoglobin levels in these animals would have prevented the histologic changes seen within the liver. This information is vitally important to fully understand how to use these products and will be investigated in future protocols.

It is also possible that the liver injury may have resulted from indiscriminate scavenging of nitric oxide. Except for the newer recombinant hemoglobins devoid of the nitric oxide-

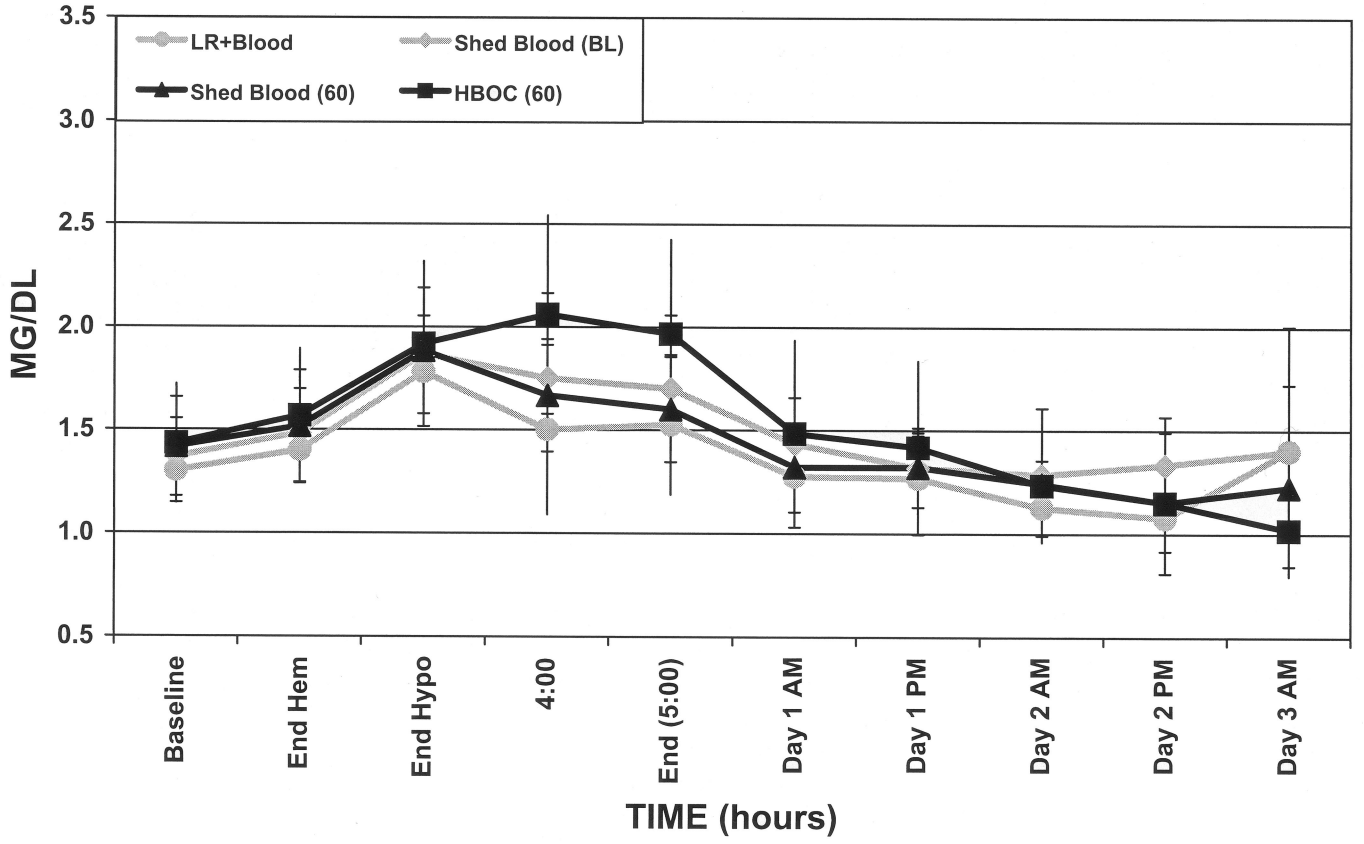

Fig. 5. *Serum creatinine.*

binding site, nitric oxide scavenging is a property inherent in all HBOC preparations.[20] The effect of nitric oxide on the modulation of hepatic injury after hemorrhagic shock has been previously studied.[21] This study found that inhibition of nitric oxide production with L-NAME, an L-arginine analog, in a rat model of hemorrhagic shock leads to increased hepatic injury, as determined by elevation of liver enzymes and changes in liver histology. These effects were reversed with the administration of L-arginine. Although it is difficult to prove nitric oxide scavenging as the cause of the histologic changes in the liver in this study, future studies investigating these mechanisms and the resultant effects on end-organ function would be warranted. In addition to histologic changes, measures of transaminase levels showed increases on postinjury day 1 in all groups, with steady return toward baseline after postinjury day 2. This finding is characteristic of liver function after resuscitation from hemorrhagic shock in other animal models with hemoglobin solutions or autologous blood.[22,23] In these studies, the changes in transaminase levels as a result of hepatocellular injury were felt to be attributable to the severity of the shock rather than to a particular resuscitation fluid. Although all groups in this study did show some degree of elevation in liver enzymes, there were statistically significant elevations noted in the HBOC (60) group compared with the Shed Blood groups on day 2. However, these differences disappeared by day 3. Most likely, the histologic findings noted in these animals are temporary and would not be observed in studies with longer recovery intervals. This is supported by studies that evaluated the HBOC product in other animal models and revealed very similar findings, with reported return of transaminase levels to normal by day 7 and no histologic evidence of injury.[24,25] Therefore, the true clinical significance of these findings is questionable.

Few regional indicators of tissue perfusion are known to be reliable. Gastric mucosal pH has been shown to have prognostic value in critically ill patients and may also be an important marker for adequate resuscitation.[26,27] Recent criticisms of gastric tonometry include instability of carbon dioxide (CO_2) in the tonometric solution, long equilibration times, and even regional tonometric differences within the stomach (J. McNeil et al., unpublished data).[28] Intestinal mucosal P_{O_2} and P_{CO_2} monitoring has risen as a promising new method for assessing inadequate tissue perfusion. Jejunal P_{O_2} and P_{CO_2} have recently been cited as promising indicators of perfusion at the tissue level.[29–32] Jejunal P_{O_2} has been shown to be superior to gastric tonometry as a regional indicator of tissue oxygenation in one study (H. Frankel et al., unpublished data). Our experience in this work reveals an expected decline in jejunal oxygen levels with hemorrhage followed by return to baseline or near baseline during resuscitation. Of note, approximately 2 hours into resuscitation,

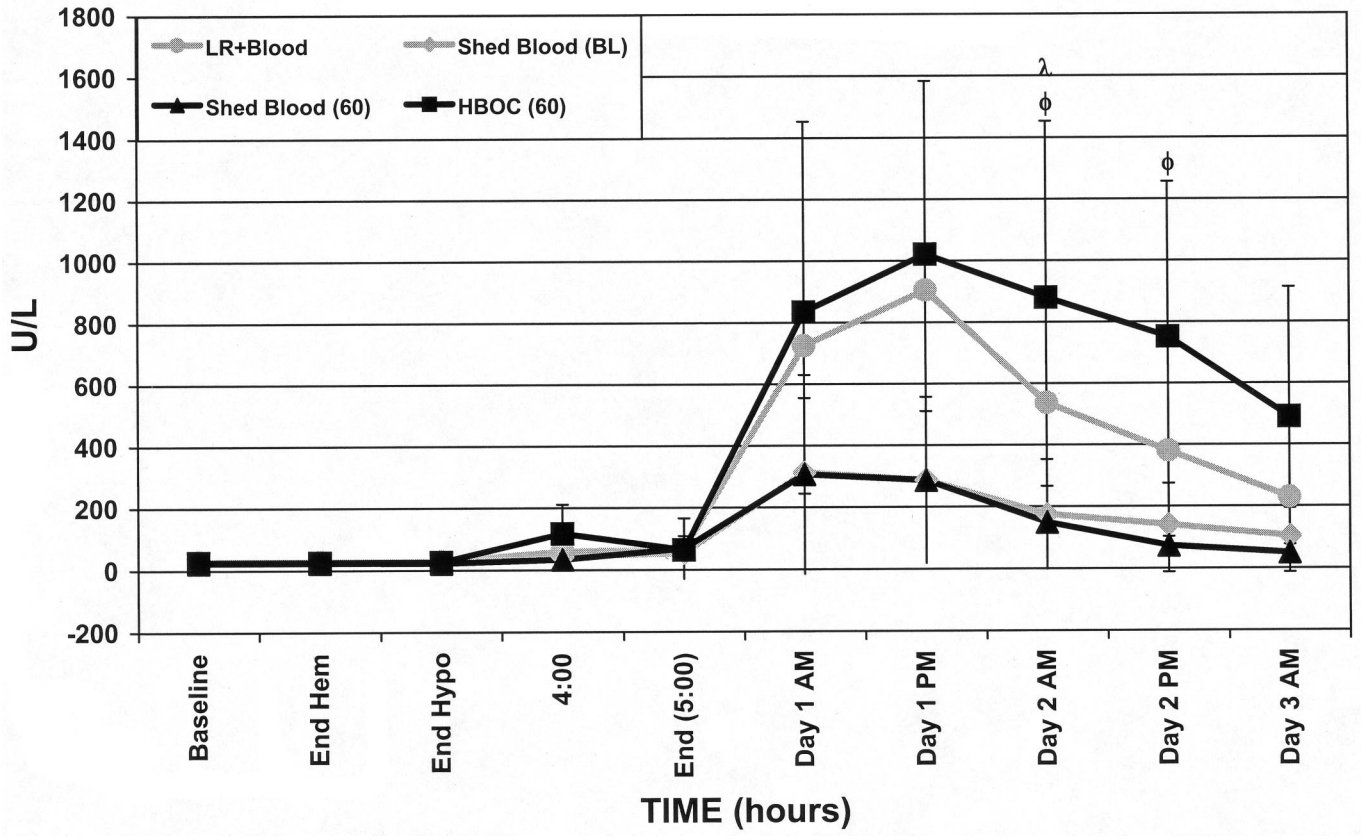

Fig. 6. *Serum aspartate aminotransferase (AST). λp < 0.05 HBOC (60) vs. Shed Blood (BL) group, ϕp < 0.05 HBOC (60) vs. Shed Blood (60) group.*

jejunal oxygen levels in the HBOC-201 group remained consistently higher, but not significantly, as compared with the other groups. Jejunal P_{CO_2} increased sharply with hemorrhage and soon declined with resuscitation. One explanation for the increased release of carbon dioxide would be an increase in anaerobic metabolism at the tissue level as a result of hemorrhage and hypovolemia. Jejunal CO_2 levels returned to baseline during resuscitation and subsequent resolving anaerobic metabolism. The finding of no histologic changes consistent with progressive ischemic change is consistent with the oximetry data showing normalization of jejunal oxygen levels after resuscitation. This is important given concerns over the vasoactivity of HBOC-201 and the effects this may have on regional and microcirculatory blood flow. Gastrointestinal symptoms thought to be related to this vasoactivity have been seen when these agents are given clinically. This could be exacerbated in situations of hemorrhage, where splanchnic blood flow is physiologically reduced to maintain central perfusion. However, our data show that jejunal oxygenation is returned to normal shortly after resuscitation with HBOC-201. This is similar to data recently published evaluating cerebral oxygenation in a swine model of hemorrhage and resuscitation. In this model, small-volume resuscitation with HBOC-201 restored brain tissue oxygen tension to levels 66% above baseline.[33] Therefore, it does not appear that the vasoactive effects of HBOC-201 limit its ability to deliver oxygen in regional circulatory beds during resuscitation in models of controlled hemorrhagic shock.

In conclusion, we were able to show that, in a swine model of controlled hemorrhage shock, animals were able to survive a 4-hour period of low-volume resuscitation with HBOC-201 without apparent clinically significant effects. Modest elevation in liver enzymes was noted and four animals showed areas of mild to moderate hepatocellular damage. These were not considered to be clinically significant, and liver enzymes were returning toward normal by postoperative day 3. Also significant is that no renal abnormality was noted even though urine output was minimal during the period of resuscitation. As demonstrated in earlier studies, anaerobic metabolism was reversed during the 4-hour resuscitation period with low volumes of HBOC-201 infusion. This suggests that the use of HBOC-201 in low-volume resuscitation may reduce mortality from hemorrhagic shock by maintaining adequate tissue oxygenation in the setting of decreased tissue perfusion. Further studies investigating the use of HBOC-201 for longer periods in both hypotensive and normotensive regimens of controlled and uncontrolled hemorrhagic shock will greatly enhance our understanding of these products and provide further insight into how best to apply these novel agents in field resuscitation protocols.

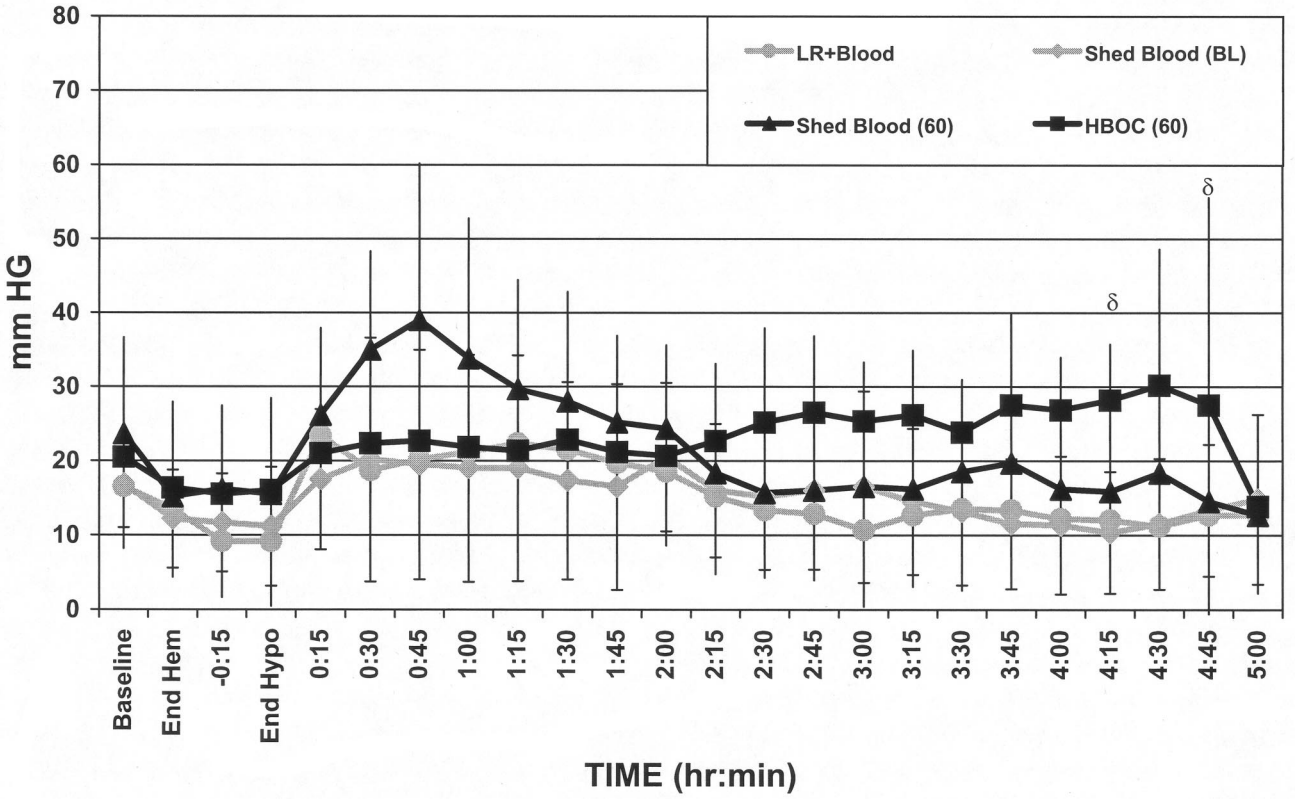

Fig. 7. *Jejunal oximetrics (Po_2). δp < 0.05 HBOC (60) vs. LR + Blood group.*

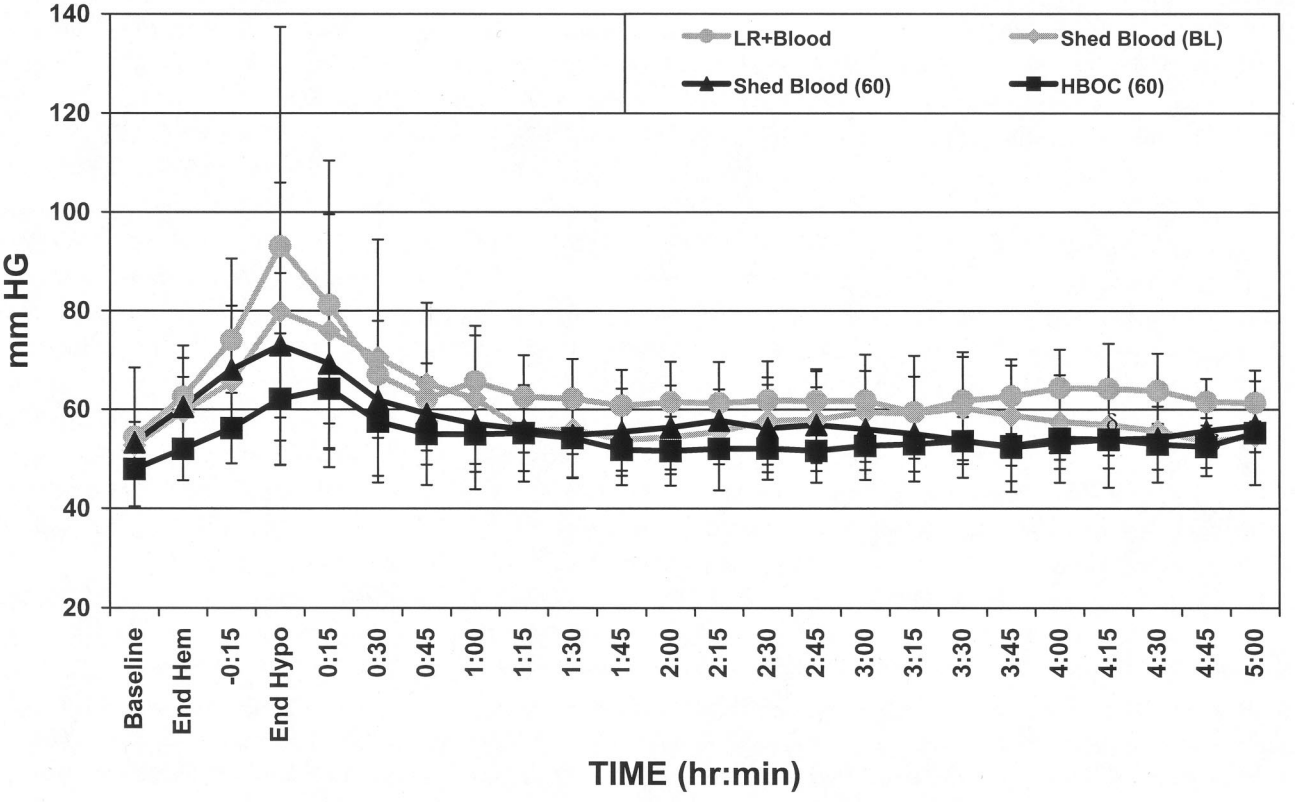

Fig. 8. *Jejunal oximetrics (pCO_2).*

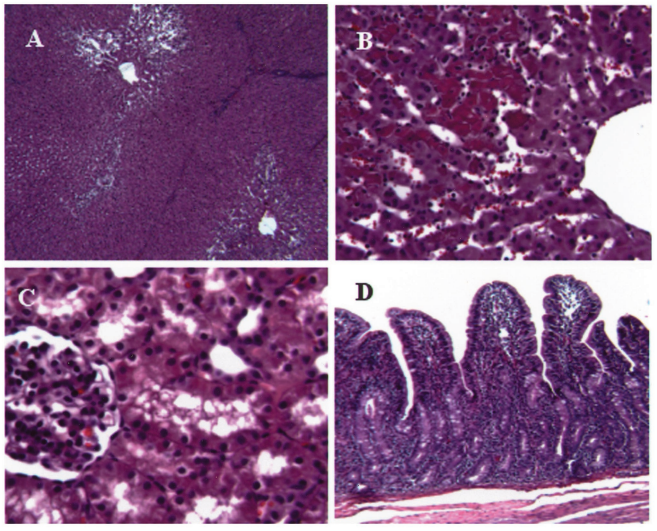

Fig. 9. *Tissue histology. (*A *and* B*) Liver, (*C*) kidney, and (*D*) jejunum.*

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