Plasma volume expansion with solutions of hemoglobin, albumin, and Ringer lactate in sheep

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Fischer, Stefanie R., Michael Burnet, Daniel L. Traber, Donald S. Prough, and George C. Kramer. Plasma volume expansion with solutions of hemoglobin, albumin, and Ringer lactate in sheep. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H2194–H2203, 1999.—We have measured plasma volume expansion (Evans blue and hematocrit changes) and hemodynamic responses in conscious hemorrhaged and normovolemic splenectomized sheep after a 30-min infusion of either 20 ml/kg of diaspirin cross-linked hemoglobin (DCLHb), 20 ml/kg of human albumin (Alb), or 60 ml/kg of a solution of Ringer lactate (RL). All regimens expanded blood volume and increased blood pressure and cardiac output after hemorrhage. However, only 15 ± 3% of the infused volume of RL was evident as intravascular expansion 10-min postinfusion, compared with 67 ± 16% and 139 ± 139% for Alb and DCLHb, respectively. DCLHb infusions were associated with higher blood pressures and lower cardiac outputs compared with RL and Alb infusions, but the increased oxygen content of blood with DCLHb resulted in systemic delivery of oxygen similar to that of the other infusions. These differences in hemodynamics and vascular volume continued for 6 h, and at 24 h vascular volume and all hemodynamics were similar in all three groups. The better volume expansion with DCLHb may be due to greater mobilization of endogenous interstitial protein or reduced transcapillary loss as total intravascular endogenous plasma protein increased after infusion of DCLHb, whereas there was an apparent loss of endogenous intravascular protein after infusions of Alb and RL. Vasodilatation by DCLHb is one mechanism that could lower blood-to-tissue transport of fluid and protein. In addition to its oxygen-carrying capacity and vasoactivity, DCLHb is associated with volume expansion properties out of proportion to its colloid osmotic pressure. Oxygen delivery; hemorrhage; resuscitation; shock

As defined by Advanced Trauma Life Support guidelines, the suggested treatment of hemorrhagic hypotension is to stop bleeding and infuse Ringer lactate solution (RL) to restore vascular volume and increase venous return, cardiac output, and tissue oxygen delivery (2). Two limitations of RL for emergency resuscitation are its inefficiencies as a volume expander and its dilution of red blood cell (RBC) concentration and thus oxygen-carrying capacity. An ideal resuscitation fluid would provide an adequate intravascular volume and intravascular retention time; it would increase oxygen-carrying capacity and should be without toxicity or risk of transfusion-related infections. For practicality, particularly for prehospital or field resuscitation, administration of small volumes would be desirable.

Free hemoglobin solutions appear to be logical candidates for the ideal resuscitation fluid; however, the use of unmodified free hemoglobin is associated with an unsuitably high oxygen affinity, short intravascular half-life, and nephrotoxicity (12). These undesirable effects can be diminished through chemical modification of hemoglobin (7, 8). The use of new hemoglobin-based, oxygen-carrying solutions that are undergoing advanced development may provide a particularly efficient means to treat hemorrhage (8). Free hemoglobin solutions are colloids and should be efficient volume expanders; also, plasma hemoglobin may load oxygen from the lungs and unload oxygen to tissues more efficiently than RBC-carried hemoglobin (31, 32). Many varieties of hemoglobin-based, oxygen-carrying solutions are under development, and such solutions have been extensively studied with respect to their oxygen-carrying capacity and their effects on blood pressure and blood flow in normovolemic and hypovolemic humans and animals (9, 12, 27, 33). Surprisingly, there has been little research into the volume expansion properties of blood substitutes.

One of the more extensively studied free hemoglobin solutions is diaspirin cross-linked hemoglobin (DCLHb), which is manufactured from modified human blood (3, 29). Infusion of DCLHb into hemorrhaged animals increases arterial blood pressure, restores plasma lactate concentrations to prehemorrhage baseline levels, and increases oxygen-carrying capacity (4, 6, 19, 23). Greater resuscitation effectiveness in animal studies has been reported for DCLHb compared with that for RL, human serum albumin (Alb), or hypertonic saline (4, 24). The increase in blood pressure with DCLHb resuscitation is caused in part by the binding of nitric oxide and hemoglobin (1) and possibly through the interaction of hemoglobin with endothelin (13). Little is known about the effects of DCLHb on intravascular volume compared with the effects of other volume expanders in hemorrhagic shock. In concentrations of 10 g/dl, DCLHb is hyperoncotic (oncotic pressure 30–43 mmHg) and, therefore, in theory, expands vascular volume by a volume exceeding the infused volume. On the other hand, hemoglobin is a slightly smaller molecule than Alb, is less negatively charged, and leaves the vasculature more rapidly than Alb (5). Thus the in vivo volume expansion properties of DCLHb could be different from those of Alb.

The “control” solution that has most often been used to compare the resuscitative effects of 10% DCLHb is...
Animals and Surgical Preparation

Nine adult female sheep (33–50 kg, mean 40 ± 2 kg) were anesthetized with halothane and instrumented for chronic study. Catheters were inserted in the right and left femoral artery and vein, and a pulmonary arterial catheter was placed into the common jugular vein and positioned in the pulmonary artery such that wedge pressure could be obtained when the balloon on the catheter tip was inflated. A left lateral subcostal incision was made, and a splenectomy was performed to prevent splenic erythrocyte sequestration and release to allow the use of hematocrit (Hct) changes as a measure of plasma volume changes. The animals were awakened after surgery and allowed 1 wk to recover from the operative procedure. They were maintained in metabolic cages with free access to food and water. On the day after surgery, catheters were connected to pressure transducers with continuous flushing devices (heparin-saline solution 3 U/ml). An infusion of RL (Baxter Healthcare, Deerfield, IL) was started at 2 ml·kg⁻¹·h⁻¹. Twenty-four hours before the experiment, infusion and access to drinking water were discontinued and a urinary catheter (14-Fr, Sherwood Medical, St. Louis, MO) was placed.

Experimental Protocol

Six animals were assigned to fixed-pressure hemorrhage experiments. Three animals did not undergo hemorrhage but served as controls for the study of effects of the treatments during normovolemia. In each animal, three different treatment experiments were performed in random order, with a minimum recovery time of 1 wk between the experiments as described previously (2–4).

Hemorrhage experiments. Baseline hemodynamic values and blood samples were collected twice during a 1-h monitoring period. The sheep were then bled via the large-bore femoral arterial catheter to reach and then maintain a mean arterial blood pressure of 50 mmHg. The blood was collected in sterile 450-ml-capacity blood bags (Teruflex blood bag system, Terumo, Tokyo, Japan) containing 63 ml of citric acid and then stored at 4°C. The bags were weighed before and after being filled with blood. Two hours after the beginning of the hemorrhage, the sheep were resuscitated over a period of 120 min with one of three treatments: 60 ml/kg RL (RL group), 20 ml/kg 10% DCLHb (DCLHb group), or 20 ml/kg 8% Alb (Alb group). Six hours after the end of resuscitation, the sheep were given free access to water and the infusion with RL was restarted at 2 ml·kg⁻¹·h⁻¹. The experiments were completed after the last data collection at 24 h after resuscitation, and then each sheep was retransfused with the collected blood.

Normovolemic control experiments. In the three control sheep, the resuscitation fluids were infused immediately after the baseline measurements were taken. Otherwise, the protocol was performed identically to that for the hemorrhaged sheep.

Measurements and Data Collection

Hemodynamic data, including heart rate and mean arterial, mean pulmonary arterial, and central venous pressures were continuously monitored. These variables plus cardiac output and pulmonary wedge pressure were recorded twice during the baseline period before hemorrhage (or at a comparable interval in the normovolemic sheep), every 30 min during hemorrhage, and 10, 20, 30, 60, 90, and 120 min and 4, 6, and 24 h after resuscitation (or after infusion in the normovolemic sheep). Arterial, central venous, and pulmonary arterial pressures were measured with transducers (P × 3.3 Disposable Transducers, Baxter Edwards Critical Care) and recorded on a hemodynamic monitor (model 78304, Hewlett-Packard, Santa Clara, CA). A horizontal plane 12 cm above the sternum was taken as a zero reference point for vascular pressures. Cardiac output was determined in triplicate by the thermodilution technique with 10 ml of ice-cold 5% dextrose solution as the indicator on a cardiac output computer (model 9530, Baxter Edwards Critical Care). Cardiac index (CI) was calculated using the following formula for body surface area of the sheep: body surface area (m²) = body weight (kg)⁰⁸³ × 0.087. Heart rate was determined from the arterial tracing.

At the same time points, samples from arterial and mixed venous blood were drawn and analyzed for gas tension and pH (System 1302, Instrumentation Laboratory, Lexington, MA). The data were corrected for core body temperature by the apparatus. Total hemoglobin (sheep RBC hemoglobin and DCLHb was measured using an oximeter (CO-Oximeter 482, Instrumentation Laboratory). Evans blue dye did not interfere with the measurements of total hemoglobin as determined by dilutions of blood performed in normal saline and in saline with Evans blue. Hct was determined by capillary tube centrifugation. In groups infused with DCLHb, the plasma hemoglobin after resuscitation (DCLHbₚ) was calculated as follows: DCLHbₚ = total Hbₚ – Hct divided by a correction factor, where Hbₚ is total measured hemoglobin. The correction factor for each time point was determined as the mean of the ratio of the measured Hct divided by total Hbₚ for each individual animal before the infusion of DCLHb. Correction factors were between 2.9 and 3.15 for different animals and were consistent for different samples from an individual animal. Intravascular hemoglobin was determined as the product of plasma hemoglobin and plasma volume.

Plasma volume was measured by the indicator dilution technique using Evans blue dye as the indicator (11, 20). In the hemorrhaged animals, 1.5, 2, 6, 12, and 4 ml of Evans blue dye were rapidly infused intravenously at baseline, at the end of hemorrhage (120 min), and at 10 min and 2 and 24 h after

METHODS

Animals and Surgical Preparation

In this study, we investigated the effects of DCLHb on plasma and blood volume expansion. We compared DCLHb with an isooncotically matched equal volume of human Alb solution and a threefold larger volume of RL. The experiments were performed in conscious splenectomized sheep after controlled hemorrhage/hypotension and also in normovolemic sheep that did not undergo hemorrhage.

VOLUME EXPANSION EFFECTS OF DCLHb

H2195

~8% Alb, a concentration prepared to have a colloid osmotic pressure matched (isooncotic) with that of 10% DCLHb (24). The expectation is that, if the volumes and oncotic pressures of DCLHb and Alb were matched, initial volume expansion would be identical. On the other hand, a more clinically relevant control solution would be a larger volume of crystalloid such as RL, which is the solution most often used clinically to treat hemorrhagic shock and that exerts no oncotic pressure (4, 24). Recent data from an interim analysis of the US phase III trauma trial of DCLHb has shown that patients treated with DCLHb had an increased mortality compared with those in the control group. Clearly, more information on the resuscitative effects of DCLHb is needed either to define the proper clinical use of DCLHb or to contribute to the design of a better second-generation hemoglobin-based oxygen carrier.

The experiments were performed in conscious splenectomized sheep after controlled hemorrhage/hypotension and also in normovolemic sheep that did not undergo hemorrhage.
resuscitation, respectively. In the normovolemic animals, 1.5,
4, 8, and 4 ml were rapidly infused intravenously at baseline
and at 10 min and 2 and 24 h after infusion, respectively.
Arterial blood samples were collected before and 1, 2, 4,
and 6 min after each Evans blue injection. The plasma sample
taken just before dye injection served to correct for residual
Evans blue and free hemoglobin. Evans blue concentration
was measured in the plasma of these samples with a spectro-
photometer (model 1001, Spectronic, Milton Ray, Rochester,
NY) at 620 nm. The values were fit to a logarithmic decay
curve of plasma dye concentration over time using linear
regression to extrapolate to a calculated dye concentration at
the time of injection. This value is representative of the
plasma concentration if instantaneous mixing is assumed to
have occurred at the time of injection. Standard curves were
made using the plasma of each animal, which was collected
before the beginning of each experiment. Serial dilutions of
DCLHb were made and photometrically analyzed. DCLHb
and Evans blue absorbed light at different wavelengths and
exhibited only a small overlap in spectra.

Estimated blood volume (BV) was calculated from the
measured plasma volume (PV) and Hct as

\[ BV = \frac{PV}{(1 - Hct)} \]

where Hct is expressed as a fraction. Independent measures of
volume expansion were provided by Evans blue-measured
plasma volume and blood volume changes calculated from the
preresuscitation blood volume (BV_o) and changes in Hct
before infusion (Hct_o) and at specific times (t) after infusion
(Hct_t) as

\[ BV_t = BV_o \times \frac{(Hct_o - Hct_t)}{Hct_t} \]

where BV_t is the blood volume change at t.

We did not correct our calculations for F-cell ratio. The
F-cell ratio is the ratio of whole body Hct compared with
large-vessel Hct. For most animals, including sheep, the
F-cell ratio is <1 (~0.9). The addition of a correction for F-cell
ratio to our blood volume calculations would have resulted in
an ~10% increase in all calculations of blood volume.

The total protein content of plasma including endogenous
plasma protein and infused albumin and hemoglobin was
measured with a refractometer. Oncotic pressure was mea-
sured in plasma (4100 Colloid Osmometer fitted with an
AM-030 membrane, Wescor, Logan, UT). Intravascular pro-
tein content of the plasma was calculated as the product of
total protein content and plasma volume. Lactate was mea-
sured in arterial blood using a Lactate Analyzer (YSI, Yellow
Springs, OH).

Fluid intake, including fluids for measuring cardiac output
and flushing lines, and fluid output, including urinary output
and the amount of blood withdrawn during hemorrhage and
for blood sampling, were recorded at each time point. For
calculation of the postresuscitation fluid balances, the time
point immediately before resuscitation (or infusion in control
animals) was taken as reference.

Systemic oxygen delivery (DO2) was calculated from CI,
arterial saturation of oxygen (SaO2), arterial partial pressure
of oxygen (PaO2), and measured total blood hemoglobin (Hb)
as

\[ DO2 = CI \times (0.136 \times Hb \times SaO2 + 0.03 \times PaO2) \]

Arterial oxygen saturation was estimated from a human
hemoglobin-oxygen dissociation curve because Evans blue
dye interferes with oximetric analysis of hemoglobin satu-
rations. Mean PaO2 was in excess of 95 mmHg, and calculated
saturation were in excess of 97% for all groups at all time
points. Thus subtle differences between sheep hemoglobin
and DCLHb on the upper end of the oxygen dissociation curve
were of little consequence.

Preparation of Test Solutions

DCLHb as a 10% solution and ~8% human Alb, prepared to
have an oncotic pressure identical to that of the DCLHb, were
provided by Baxter Healthcare, (Deerfield, IL) (29). These
solutions were isoosmotic (colloid osmotic pressure 31–32
mmHg) as measured in our laboratory using an oncometer
using a 30-kDa cutoff membrane. They had a colloid osmotic
pressure of 43 mmHg when measured by the manufacturer with a 10-kDa cutoff
membrane. The chemical characteristics of DCLHb have been
described in previous publications (29). Evans blue (Sigma
Chemical, St. Louis, MO) was dissolved in saline to yield a
conection of 4.52 mg/ml. We used commercially available RL (Baxter Healthcare).

Statistical Analysis

Data from the experiments with hemorrhage were ana-
yzed using analysis of variance for a two-factor experiment
with repeated measures for time. The two factors were group
and time. Only a limited number of comparisons were ana-
yzed for statistical significance differences by a priori deci-
sion. For all groups, the end-of-hemorrhage value was com-
pared with the baseline value, and the 10-min, 2-h, and
24-h postresuscitation values were compared with the value at
the end of hemorrhage. Group differences were analyzed at 10
min, 2 h, and 24 h after infusion. If there was a group
difference at a specific time point, then a post hoc analysis
was done using Fisher’s least significant difference procedure
with Bonferroni correction for the number of comparisons.
Statistical significance was set at a P value <0.05. Data are
presented as means ± SE. Because of the small number of
animals in the normovolemia experiments, these data were
analyzed for summary statistics only and not for comparative
statistics.

RESULTS

All animals tolerated the hemorrhage and the infu-
sions well.

Hemorrhaged Sheep

During hemorrhage mean arterial pressure was main-
tained near 50 mmHg in all groups (Fig. 1). This level of
hypotension was accompanied by a significant decrease
in CI to <50% of baseline (Fig. 1). Ten minutes after
resuscitation, mean arterial pressure had returned to
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rise in CI above baseline levels but within 20 min achieved a level similar to baseline. In the DCLHb-treated animals, CI rose close to baseline levels but was significantly lower than in the Alb-treated animals for 2 h after resuscitation. Systemic vascular resistance index (SVRI) tended to increase during hemorrhage (albumin group: from 1,761 ± 155 to 1,857 ± 218 dyn·s·cm⁻²·m⁻²; RL group: from 1,444 ± 130 to 1,781 ± 158 dyn·s·cm⁻²·m⁻²; DCLHb group: from 1,356 ± 71 to 1,837 ± 186 dyn·s·cm⁻²·m⁻²) and fell 10 min after infusion of either Alb or RL.

Pulmonary arterial pressure and vascular resistance index (PVRI). During hemorrhage, pulmonary arterial pressure decreased and returned to baseline after Alb and RL infusion. After DCLHb, pulmonary arterial pressure rose transiently above baseline levels. PVRI increased during hemorrhage and stayed increased after DCLHb for ~90 min but decreased immediately after infusion of either Alb or RL.

Hct and plasma volume decreased equally in all three groups during hemorrhage (Table 1). After resuscitation, Hct decreased further in all groups. Figure 3 shows Evans blue-measured plasma volume at the end of hemorrhage and 10 min after resuscitation for each individual experiment and for the mean of each group. Plasma volume increased after resuscitation, significantly more so in the DCLHb group than in the RL and Alb groups. Figure 4 summarizes the mean blood loss, the mean resuscitation volume, and the mean volume expansion (in ml/kg) of all three groups. The bled volume was equal in all three groups. In the RL and Alb groups, plasma volume expansion was lower than the infused volume with ratios of plasma volume expansion to infused volume equal to 0.15 ± 0.03 for RL and 0.67 ± 0.16 for Alb. In contrast, animals resuscitated with DCLHb had plasma volume expansion that exceeded the amount of infused volume (ratio 1.39 ± 0.25).

The greater volume expansion with DCLHb versus Alb was a surprising finding considering that the dose (20 ml/kg) and oncotic pressure of the Alb and DCLHb solutions were perfectly matched. An independent measure of the volume expansion can be calculated from the change in Hct in these splenectomized animals. Figure 5 shows percent blood volume expansion calculated from the change in Hct, expressed over time for the three groups, confirming progressively greater volume expansion for 20 ml/kg DCLHb versus 20 ml/kg Alb.
versus 60 ml/kg RL. Table 2 compares volume expansion measurements expressed in milliliters per kilogram at 10 min postinfusion with those at the end of hemorrhage measured with Evans blue and with Hct. With both methods blood volume expansion was comparable, with the exception of the RL group, which showed a larger expansion when calculated from Hct alone. Total blood hemoglobin also decreased similarly in all three groups during hemorrhage and decreased further after resuscitation in the Alb and DCLHb groups (Fig. 6). After DCLHb infusion, the total hemoglobin was equal to the prehemorrhage level, with −30% or 3.03 ± 0.17 g/dl being free hemoglobin from DCLHb. Total intravascular content of free hemoglobin in the DCLHb group was calculated as plasma volume times free hemoglobin concentration and was 1.83 ± 0.18 g at 10 min after resuscitation and 1.80 ± 0.17 g at 2 h. Because the infused dose of DCLHb was 2 g/kg, this suggests that −10% of the infused DCLHb left the circulation soon after infusion.

Oncotic pressure decreased during hemorrhage and decreased further after resuscitation with RL (Fig. 7). Alb and DCLHb caused a rise in oncotic pressure, and there was no statistical difference between these two groups. Table 3 shows the time course of lactate, bicarbonate, and plasma protein. The increase in lactate and the decrease in bicarbonate levels during hemorrhage were reversible within 2 h of resuscitation irrespective of the resuscitation fluid response. The plasma protein was measured using a refractometer, which measures all plasma solutes including endogenous protein, as well as the plasma DCLHb and human albumin infused. Total intravascular plasma protein concentration (Fig. 7) and content (Fig. 8) decreased equally in all three groups during hemorrhage. Each increased after resuscitation with albumin and DCLHb, with higher values in the DCLHb-treated animals; however, this difference did not reach statistical significance. The total fluid balance [all fluids in − urinary output + blood sampling)] at 10 min and 2 h after resuscitation was 18.5 ± 0.3 and 19.7 ± 1.5 ml/kg (Alb), 18.6 ± 0.3 and 15.5 ± 1.8 ml/kg (DCLHb), and 58.5 ± 0.3 and 60.6 ± 1.0 ml/kg (RL), respectively. All groups received −3 ml/kg additional fluid in the 2 h postresuscitation associated with the measurement of cardiac output and flushing of lines. These data show that most of the resuscitative fluid over the first 2-h time period was retained in the body for all three groups, despite the significant differences in vascular expansion.

### Normovolemic Sheep

Infusion of RL and Alb caused a transient and small rise in mean arterial blood pressure and CI in normovolemic animals (Table 4). DCLHb led to a significant increase in mean arterial blood pressure for 4 h before returning to preinfusion baseline levels. The increased pressure was associated with a decreased CI. Hct decreased in all three groups after fluid administration. The initial blood volume expansion based on changes in Hct tended to be greatest in the DCLHb group. However, the magnitude of the expansion for all three solutions and the differences among groups were much smaller compared with those for the hemorrhaged animals and were not statistically significant. The plasma volume measurements with Evans blue dye showed considerable variations, and two experiments were excluded due to a technical error during sample collection. Therefore, the Evans blue data are not given. Two hours after infusion there was no apparent blood volume expansion, and blood volume was virtually identical in the three groups. Two hours after fluid infusion, the net fluid balance was higher in the animals that received RL (RL, 26.8 ± 8.5 ml/kg; Alb, 7.5 ± 1.2 ml/kg; DCLHb, 1.2 ± 10.0 ml/kg), and this pattern persisted for the remainder of the observation period. Plasma hemoglobin concentration was 2.6 ± 0.2 g/dl 10 min after infusion of DCLHb, compared with 3.0 ± 0.2 g/dl after the same dose in the hemorrhaged animals. A lower concentration would be expected in normovolemic animals due to the distribution of the DCLHb in their larger plasma volume.

### DISCUSSION

Our study investigated the effects of three different resuscitation fluids on plasma and blood volume in a model of controlled hemorrhage as well as in normovolemic animals. This model is well established to produce...
a clinically relevant level of circulatory shock and has been previously used in several studies from our laboratory (21, 28, 34). The study resulted in cardiac output values of less than one-half the baseline values and a lactic acidosis, indicating tissue hypoxia and induced anaerobic metabolism. The hemorrhage volume was ~60% of the total baseline blood volume. Approximately one-half of this deficit is made up by transcapillary refill during the 2 h of hypotension (28). Other investigators have reported similar results in other animal models by withdrawing a fixed amount of blood (4) or withdrawing blood until a defined arterial blood pressure or base deficit was reached (15, 22, 27).

We determined plasma volume with the dye dilution method using Evans blue dye as the indicator. This

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Fig. 3. Plasma volume expansion in 6 individual experiments, measured for each test solution at 10 min after a 30-min infusion of 60 ml/kg RL (A), 20 ml/kg DCLHb (B), or 20 ml/kg Alb (C) compared with plasma volume at end of hemorrhage period. Means ± SE are also plotted for each period. †P < 0.001, 10 min after resuscitation vs. hemorrhage for each treatment group. Increase in plasma volume after DCLHb infusion was significantly greater (P < 0.05) than increases with either Alb or RL.

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Fig. 4. Mean bled volume, infused volume, and blood volume expansion in RL, Alb, or DCLHb groups. All data are expressed as means ± SE in ml/kg. †P < 0.05, DCLHb vs. Alb and RL.

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Fig. 5. Time course of blood volume expansion calculated from changes in measured Hct. †P < 0.05, RL vs. Alb and DCLHb; ‡P < 0.05, DCLHb vs. Alb and RL.
method is well established (11, 20). Its advantages are that the technique is easy to use, the dye is inert, and the method does not involve any radioactively labeled materials. Evans blue dye is rapidly bound to serum albumin and leaves the circulation together with albumin extravasation into the interstitium. The rate of disappearance of Evans blue-bound albumin is measured and corrected for by taking several samples after injection. The possible interference between the light spectra of Evans blue and hemoglobin has recently been addressed by Migita et al. (20), who measured plasma volume in rats after isovolemic exchange transfusion. They found that combining both solutions allowed accurate plasma volume determination in the presence of free hemoglobin and did not significantly alter the spectra for Evans blue dye and hemoglobin. Similarly, we also found that both solutions absorb light at distinctively different wavelengths but that there is a small overlap of the hemoglobin spectra at the wavelength used to measure Evans blue dye (620 nm). This is easily corrected by taking a sample of plasma before dye injection to correct for the presence of any plasma hemoglobin or previously injected Evans blue dye.

RL was an extremely poor volume expander, with only 15% of the infused dose remaining in the circulation 10 min after infusion. Whereas clinical textbooks often suggest that about one-third of infused crystalloid should remain in the circulation, actual measured volume expansion in patients and volunteers consistently shows that <20% of infused crystalloid remains in the vasculature shortly after infusion (18, 30). This poor volume expansion is due to the distribution of the crystalloid through the entire extracellular space, which is four- to fivefold larger than the plasma volume, and to diuresis. Additionally, RL is slightly hypotonic compared with normal extracellular fluid, and water will partially distribute in the intracellular space to balance intra- and extracellular osmotic pressure. Increasing the dose of RL may have increased volume expansion but would likely have precipitated significant edema formation in soft tissues.

In the Alb-treated animals, plasma volume did not increase as much as the volume of the infusion. This finding was a surprise in that the Alb solution was hyperoncotic compared with normal plasma. In contrast, the plasma expansion after DCLHb infusion was significantly greater than the infused volume. The difference in the volume expansion of DCLHb and Alb is at first difficult to explain, because both solutions were matched with respect to oncotic pressure. The hemoglobin molecule is only slightly smaller than Alb, and hemoglobin has a more positive charge and is more rapidly cleared from the circulation than Alb (5). This would suggest that the DCLHb would be a short-lived and inefficient volume expander. Because we found better volume retention with DCLHb, there may have been a greater early vascular retention of infused hemoglobin than for infused Alb. In support of this view, both total protein content of the plasma and the
measured plasma colloid osmotic pressures tended to be greater in the DCLHb group after infusion. A greater plasma protein concentration could be partly due to the slightly greater (0.4 g/kg) infused protein dose with 20 ml/kg infusions of 10 g/100 ml of DCLHb versus 8 g/100 ml of Alb. Figure 8 shows that the calculated vascular content of protein in the plasma increased 2.3 g/kg after an infusion of 2 g/kg of DCLHb, whereas the measured total vascular content plasma hemoglobin was only 1.8 g/kg, suggesting that some additional mobilized protein (~0.5 g/kg) entered the circulation. In contrast, after a 1.6 g/kg infusion of human Alb, the calculated vascular content of protein increased only 1.1 g/kg, and after infusion of RL, the vascular content of plasma protein decreased by 0.35 g/kg. These data suggest that DCLHb recruited extravascular plasma protein, whereas infusion of Alb and RL was associated with an increased loss of plasma protein. After 24 h, the total vascular content of plasma protein for all three groups reached similar levels. Fluid infusions and volume expansion have previously been shown to cause a net loss of plasma proteins from the circulation (25, 36).

The explanation for the DCLHb-induced net gain of plasma volume and plasma protein content may be due to the vasoactivity of DCLHb. Vasconstriction would tend to lower capillary pressure and augment transcapillary fluid reabsorption, whereas traditional volume expanders result in reduced peripheral resistance, increased capillary surface area and pressure, and increased fluid filtration. Increased fluid filtration could also cause increased protein extravasation due to solute drag. If the vasoconstriction of DCLHb occurs preferentially in skin or muscle, as some studies suggest (14), then this may offer an explanation for better volume expansion, because these organs comprise about two-thirds of body weight and provide a significant source of interstitial fluid.

Alternately or additionally, DCLHb could be a stimulus for increased lymphatic pumping, which could be a mechanism for mobilizing interstitial protein. However, the record of the effects of free hemoglobin on lymphatic function suggests varied responses (1, 5). An infusion of 20 ml/kg of DCLHb in conscious, normovolemic sheep increased lung lymph flow to two to three times the baseline levels for up to 4 h, whereas prefemo-

| Table 3. Blood lactate, bicarbonate, and plasma protein measured in conscious sheep |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Baseline        | Hemorrhage      | After Resuscitation |
| **Lactate, mmol/l**            |                 |                 |                  |
| Alb                             | 0.7 ± 0.1       | 6.5 ± 1.3*      | 5.7 ± 1.3        |
| DCLHb                           | 0.8 ± 0.1       | 10.5 ± 2.3*     | 10.2 ± 2.5       |
| RL                              | 0.9 ± 0.2       | 9.0 ± 1.5*      | 8.7 ± 1.6        |
| **HCO3-, meq/l**                |                 |                 |                  |
| Alb                             | 24.1 ± 1.3      | 20.0 ± 1.6      | 21.0 ± 1.4       |
| DCLHb                           | 26.7 ± 0.6      | 18.8 ± 1.1*     | 19.0 ± 1.3       |
| RL                             | 25.9 ± 1.3      | 19.6 ± 1.4*     | 18.5 ± 1.8       |
| **Plasma protein, g/100 ml**   |                 |                 |                  |
| Alb                             | 6.3 ± 0.4       | 4.5 ± 0.4*      | 5.6 ± 0.3†       |
| DCLHb                           | 6.6 ± 0.4       | 4.2 ± 0.3*      | 5.9 ± 0.3†       |
| RL                             | 6.3 ± 0.4       | 4.3 ± 0.4*      | 2.7 ± 0.3†       |

Values are means ± SE. Sheep were hemorrhaged for 2 h and then resuscitated with a 30-min infusion of 20 ml/kg Alb, 20 ml/kg DCLHb, or 60 ml/kg RL solution. *P < 0.05, end of hemorrhage vs. baseline; †P < 0.05, time postinfusion vs. end of hemorrhage.
Table 4. Hemodynamic changes in mean arterial pressure, cardiac index, and volume expansion measured in normovolemic conscious sheep

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10 min</th>
<th>2 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb</td>
<td>90 ± 4</td>
<td>102 ± 4</td>
<td>89 ± 5</td>
<td>93 ± 7</td>
</tr>
<tr>
<td>DCLHb</td>
<td>92 ± 4</td>
<td>134 ± 10</td>
<td>132 ± 5</td>
<td>103 ± 3</td>
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<tr>
<td>RL</td>
<td>93 ± 5</td>
<td>106 ± 5</td>
<td>91 ± 1</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>CI, l·min⁻¹·m⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alb</td>
<td>4.9 ± 0.5</td>
<td>6.1 ± 0.6</td>
<td>5.7 ± 0.6</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>DCLHb</td>
<td>5.4 ± 0.9</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.6</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>RL</td>
<td>5.2 ± 0.9</td>
<td>6.3 ± 1.2</td>
<td>4.9 ± 0.8</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>Hct, %</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Alb</td>
<td>24.7 ± 1.3</td>
<td>20.3 ± 2.0</td>
<td>21.7 ± 2.6</td>
<td>23.3 ± 2.0</td>
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<tr>
<td>DCLHb</td>
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<td>24.3 ± 2.7</td>
<td>27.7 ± 3.8</td>
<td>30.0 ± 0.5</td>
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<tr>
<td>RL</td>
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<td>27.3 ± 0.9</td>
<td>27.3 ± 0.3</td>
</tr>
<tr>
<td>Blood volume expansion, %</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb</td>
<td>17.8 ± 5.1</td>
<td>16.2 ± 8.6</td>
<td>5.6 ± 5.0</td>
<td>5.6 ± 5.0</td>
</tr>
<tr>
<td>DCLHb</td>
<td>26.6 ± 2.7</td>
<td>15.3 ± 6.5</td>
<td>2.9 ± 2.9</td>
<td>2.9 ± 2.9</td>
</tr>
<tr>
<td>RL</td>
<td>3.1 ± 8.6</td>
<td>16.3 ± 7.9</td>
<td>15.9 ± 5.5</td>
<td>15.9 ± 5.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sheep were administered a 30-min infusion of 20 ml/kg Alb, 20 ml/kg DCLHb, or 60 ml/kg RL solution. MAP, mean arterial pressure; CI, cardiac index.

Hemodynamic changes in normovolemic conscious sheep. Table 4. Hemodynamic changes in mean arterial pressure, cardiac index, and volume expansion measured in normovolemic conscious sheep.

VOLUME EXPANSION EFFECTS OF DCLHb

In general, lymphatic flow from skin and muscle increased only 60% for ~0.5 h and then fell below baseline (5). An oncotically matched infusion of albumin increased lymphatic flows similarly in those studies (5). On the other hand, hemolysate, which contains free hemoglobin and other cellular debris, appeared to inhibit mesenteric lymphatic pumping (10, 35).

Our data are consistent with the better volume-expansion effects of DCLHb, which are attributable to a higher vascular content of endogenous plasma protein due to a reduced extravasation rate of fluid and endogenous plasma protein or an increased lymphatic return of protein into the circulation. In support of this view, the mean plasma colloid osmotic pressure was higher after infusion of DCLHb than after Alb, despite both infusions being equal in volume and colloid osmotic pressure, although this difference was not statistically significant.

Another possible explanation of the better volume expansion of DCLHb is that the free hemoglobin could interact with normal plasma protein differently than Alb to generate a higher colloid osmotic pressure for a mixture of DCLHb and sheep plasma protein compared with that for a mixture of human Alb and sheep plasma protein. To test this possibility, we performed serial dilutions of the DCLHb and Alb solutions in normal saline and in sheep plasma. We found identical relationships between the measured colloid osmotic pressure and the serial dilutions of DCLHb and Alb using either saline or plasma dilutions. Thus the most likely mechanism for the better volume expansion remains better vascular retention of fluid and protein or interstitial mobilization of fluid and endogenous protein.

Other studies with DCLHb resuscitation of hemorrhage (3, 4, 5, 9) have not reported enhanced volume expansion compared with matched isotonic saline or albumin infusions. However, in these studies, plasma volume was not measured and animals were not splenic tomized. If splenic contraction were to occur after DCLHb infusion, then mobilization of RBCs could prevent a lower Hct after infusion despite volume expansion after DCLHb.

In the normovolemic animals the three solutions did not result in dramatic changes or differences among treatments. Although DCLHb did appear to cause the greatest initial volume expansion, the response was short lived. Overall, the normovolemic animals exhibited an initial expansion that was much less than that in the hemorrhaged animals. Several physiological mechanisms (increased glomerular filtration rate, lowered antidiuretic hormone secretion, reduced aldosterone levels, and altered microvascular Starling forces) tend to rapidly normalize vascular volume when vascular expansion above normal occurs. While in a preexisting hypovolemic state, these mechanisms are adjusted in the opposite direction to maintain or increase blood volume, and thus more infused fluid remains intravascular. The hemorrhaged animals of all groups are in a state of net reabsorption and low capillary pressure before infusion of the test solutions, whereas normovolemic animals are in a state of net filtration and normal capillary pressure. This is the most likely explanation for the reduced volume expansion with all treatments in the normovolemic sheep versus the hemorrhaged sheep.

In the hemorrhaged sheep, infusion with any one of the three regimes was effective in restoring cardiovascular function. Blood pressure was increased with all solutions, with DCLHb causing the largest increase. Blood pressure after DCLHb exceeded the prehemorrhage baseline levels and was substantially greater than in the other two groups. Cardiac output increased in all three groups, but the increase was significantly less after DCLHb. Similar changes have been reported by other investigators studying DCLHb and other hemoglobin-based, oxygen-carrying solutions (9, 16). The elevated pressures with DCLHb are most likely due to hemoglobin causing a generalized vasoconstriction due to binding of nitric oxide, which also causes increased pulmonary arterial pressure and resistance (17, 22, 26).

Because total blood hemoglobin was 30–40% greater in the DCLHb groups versus the RL and Alb groups and because CI was ~10–15% less, Do₂ was comparable or slightly greater with DCLHb resuscitation.

All three fluid regimens were effective in decreasing lactate levels and ameliorating acidosis. Even though the lactate levels of the DCLHb group were highest before treatment, the rate of decrease was similar in all groups. Other investigators have reported that DCLHb caused a quicker resolution of acidosis compared with Alb, RL, and hypertonic saline (4, 24).

In summary, the present study suggests that, in addition to their well-described effects on oxygen-carrying capacity and vasoactivity, free hemoglobin solutions may have another important characteristic: enhanced volume expansion. Possible interactions between the vasoactivity and the volume effects of hemoglobin-based, oxygen-carrying solutions merit further study.
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