Oxygen Transport Dynamics After Resuscitation With a Conjugated Hemoglobin Solution

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**Hypothesis:** Pyridoxalated hemoglobin polyoxyethylene conjugate (PHP), a hemoglobin-based oxygen carrier, is effective in restoring hemodynamic balance and oxygen delivery after moderate hemorrhage but may be less effective in off-loading oxygen at the tissue level.

**Design:** Before-after trial.

**Setting:** Animal research laboratory of an academic institution.

**Participants:** Ten female Yorkshire swine.

**Interventions:** Anesthetized swine underwent a 25% controlled hemorrhage followed by resuscitation with crystalloid plus either shed blood or PHP. Hemodynamic parameters, including heart rate, mean arterial pressure, mean pulmonary arterial pressure, and cardiac index were continuously monitored. Arterial and mixed venous blood samples were collected at baseline, after hemorrhage, after resuscitation, and every 15 minutes for 90 minutes after resuscitation. Oxygen delivery and consumption, oxygen extraction ratios, and percentage of contribution to oxygen delivery and consumption were determined in whole blood, red blood cells, and plasma by using a compartmentalized approach.

**Main Outcome Measures:** Intergroup and intra-group comparisons were performed for hemodynamic parameters and oxygen transport dynamics.

**Results:** Heart rate returned closer to baseline levels in the PHP group \( (P < .05) \) and mean pulmonary arterial pressure was transiently elevated after infusion of PHP \( (P = .028) \), but otherwise no significant differences in hemodynamic balance were observed. The extraction ratio from the red blood cells in the PHP group more than doubled, whereas the extraction ratio from plasma remained constant. The percentage of contribution of plasma, including PHP, to oxygen delivery exceeded 20\% \( (P < .05) \), but the relative contribution to oxygen consumption did not markedly change from baseline.

**Conclusions:** Pyridoxalated hemoglobin polyoxyethylene conjugate is at least as effective as shed blood in restoring hemodynamic balance and oxygen delivery after moderate hemorrhage. There is a disproportionately low contribution from plasma to oxygen consumption, which suggests that PHP may act as an oxygen sink in moderate anemia.

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**EMOGLOBIN-BASED OXYGEN carriers are being developed and tested as a desirable therapeutic option in acute anemic states resulting from trauma or surgery associated with substantial blood loss. Purported advantages of hemoglobin (Hb)-based oxygen carriers over allogeneic red blood cell (RBC) transfusions include ready availability, a long shelf life, universal compatibility, and reduction or elimination of disease transmission. For trauma, Hb-based oxygen carriers would be useful when allogeneic blood is not readily available, such as in the prehospital setting, or to minimize or altogether avoid allogeneic transfusions. Clinical use of Hb-based oxygen carriers in trauma and urgent surgery is limited, but there is accumulating evidence that the infusion of an acellular Hb solution after hemorrhage can restore and maintain total Hb (RBC Hb plus plasma [PL] Hb) concentration ([Hb]), reduce the number of allogeneic transfusions, and sustain life at critically low hematocrit levels.**

Previous work in our laboratory has characterized the hemodynamic changes associated with resuscitation with various Hb solutions after hemorrhage and the oxygen transport dynamics of acellular Hb solutions in an isovolemic hemodilution model. Results of the isovolemic hemodilution study demonstrated that pyridoxalated Hb polyoxyethylene conjugate (PHP) contributed significantly to oxygen delivery \( (\Delta O_2) \) but less so to oxygen consumption \( (\Delta V_2) \) until a critically low RBC mass (hematocrit level of 8%) was reached. Therefore, PHP might be most effective, at least in terms of off-loading oxygen at the tissue level, in states of severe anemia.

Herein is an extension of our previous work that seeks to delineate the oxygen transport dynamics of PHP in a resuscitation model after moderate hemorrhage.

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Our analysis hinges on the notion that oxygen carried by acellular Hb solution resides in PL and thus can be quantitatively differentiated from oxygen carried by the RBCs. We hypothesized that, after moderate hemorrhage, resuscitation with PHP is an effective solution to restore hemodynamic balance but might act as an oxygen sink because of a relatively lower contribution to $V_\text{O}_2$ than to $D_\text{O}_2$.

**METHODS**

**SURGICAL PREPARATION**

The institutional animal care and use committee approved the methods we described. Ten female Yorkshire swine (Looper Farms, Granite Falls, NC), each weighing 8.5 to 12.3 kg, were randomized between 2 experimental arms. They received 5 mg of tiletamine-zolazepam intramuscularly per kilogram of body weight after 12-hour food restriction. A 100-µg bolus of fentanyl citrate was then administered though a standard 22-gauge peripheral vascular catheter (Quik-Cath; Baxter Healthcare Corp, Deerfield, Ill) and was followed by a continuous intravenous fentanyl infusion at 60 µg/kg per hour for the duration of the experiment. The swine received 2 mg of pancuronium bromide intravenously initially with the tiletamine-zolazepam, and additional pancuronium was administered as needed. The experiment did not begin until normocarbia and normoxia had been achieved, after which ventilator settings were not adjusted.

The right common femoral artery and vein were cannulated with over-the-needle 16-gauge intravascular catheters (Quik-Cath, Baxter Healthcare Corp) by means of a cutdown technique. The right external jugular vein was exposed and cannulated with a 6F catheter. Median sternotomy was then performed to access the pulmonary artery. After mobilization of the pulmonary artery, a flow probe (model 10A; Transonic Systems Inc, Ithaca, NY) was placed around it to measure cardiac output. An 18-gauge intravascular catheter (Quik-Cath) was placed over a needle into the pulmonary artery via the right ventricle and secured with a 4-0 polypropylene suture to collect mixed venous samples. A flexible 3.5F transducer catheter (Mikro-Tip SPR-524; Millar Instruments Inc, Houston, Tex) was placed in the pulmonary artery via the right ventricle and secured with a 4-0 polypropylene suture. Animals received a 100-mL bolus of lactated Ringer solution for 30 minutes plus maintenance intravenous fluids at 25 mL/kg per hour during surgical preparation. Animals were heparinized initially with 300 U/kg, with an additional 150 U/kg administered every hour after the first dose for the duration of the experiment.

Baseline (BL) hemodynamic data were collected, including mean arterial pressure, mean pulmonary arterial pressure, cardiac output, and heart rate. These hemodynamic parameters were continuously monitored (M176A monitor; Hewlett-Packard Co, Palo Alto, Calif). Limb-lead electrocardiograms were also monitored throughout the experiment.

**ACELLULAR HB SOLUTION**

Pyridoxalated Hb polyoxyethylene conjugate is an acellular Hb solution produced from the lysate of outdated human RBCs (Curacyte Inc, Durham, NC). Details of the chemical modifications of Hb leading to the production of PHP have been previously described. The PHP solution used in this experiment was sterile and passed the general safety test for biologics. The endotoxin concentration was 0.04 equivalent units per milliliter, the osmolality was 263 mmol/kg of water, the [Hb] was 8 g/dL, and the partial pressure of oxygen corresponding to 50% oxygen saturation of Hb ($P_{O_2}$) was 23.6 mm Hg. Methemoglobin concentration was 1.9%. Pyridoxalated Hb polyoxyethylene conjugate is in phase 3 clinical trials and is not currently approved for use in humans.

**HEMORRHAGE AND RESUSCITATION**

The animals underwent controlled, exponentially decreasing hemorrhage for 1 hour while maintenance intravenous fluids were withheld. An estimated 23% of the intravascular blood volume was removed from the jugular vein, which corresponds to a 17.5 mL/kg reduction in blood volume, assuming an estimated blood volume of 70.0 mL/kg. A withdrawal pump was used to remove 7.0 mL/kg of estimated blood volume for the first 15 minutes and then 5.0, 3.5, and 2.0 mL/kg during the 3 subsequent 15-minute intervals. This graded-hemorrhage swine model was described by Hess et al and used in our prior studies.5,7

After the controlled hemorrhage, all animals were initially resuscitated with a 200-mL bolus of lactated Ringer solution for 30 minutes. For another 30 minutes, the animals in the control group received shed blood, and the experimental group received PHP. Animals resuscitated with PHP received an amount of PHP equal to shed blood, and those resuscitated with shed blood were given back the blood volume that had been withdrawn. After resuscitation, maintenance intravenous fluids were resumed at 25 mL/kg per hour for the duration of the experiment.

**SAMPLE ANALYSIS**

The following blood samples were withdrawn at BL, after hemorrhage, and after resuscitation: a 3-mL sample of mixed venous blood from the pulmonary artery catheter and a 3-mL sample of arterial blood from the right common femoral artery catheter. Additionally, 3 mL of mixed venous and arterial blood samples were collected and analyzed in 15-minute increments for 90 minutes after resuscitation with PHP or shed blood. Whole blood (WB) samples were collected in 3-mL heparinized syringes and analyzed with a standard blood gas analyzer (IL 1610; Instrumentation Laboratory Co, Lexington, Mass) and co-oximeter (IL 482; Instrumentation Laboratory Co) to measure arterial oxygen partial pressure ($P_{O_2}$), mixed venous oxygen partial pressure, [Hb], arterial oxygen saturation ($S_aO_2$), and mixed venous oxygen saturation ($S_vO_2$).

The WB was then transferred from the 3-mL syringe to a 3-mL heparinized vacuum tube. The 3-mL vacuum tube was centrifuged at 5000 revolutions per minute for 5 minutes with a standard centrifuge to separate RBCs and PL. The PL containing the acellular Hb was anaerobically removed by using a 19-gauge needle. The PL was then similarly analyzed with blood gas analysis and co-oximetry as described in the previous paragraph. This protocol was based on that of Rosen et al who established the basis and reproducibility of this compartmentalized approach to analysis. At the conclusion of the experiment, the animals were killed with potassium chloride intracardiac injection.

To calculate [Hb] in RBCs, a compartmentalized approach was used whereby $[\text{Hb}]_{\text{RBC}} = [\text{Hb}]_{\text{WB}} - [\text{Hb}]_{\text{PL}}$. The measured PL [Hb] is in units of grams per deciliter of PL and must be multiplied by (1 − hematocrit) to convert to grams per deciliter of WB. Thus, in the following equations $[\text{Hb}]_{\text{RBC}} = [\text{Hb}]_{\text{WB}} - [\text{Hb}]_{\text{PL}}$.

The following formulas were used to calculate arterial oxygen concentration ($C_{aO_2}$) for RBCs and PL, which are expressed as volume percentage:

$C_{aO_2} = [\text{Hb}]_{\text{WB}} \times 1.36 \times S_aO_2 + 0.003 \times P_{O_2}$

$C_{aO_2} = [\text{Hb}]_{\text{PL}} \times 1.36 \times S_vO_2 + 0.003 \times P_{O_2}$

$C_{aO_2} = C_{aO_2} - (C_{aO_2})_{\text{PL}}$
The same equations were used to calculate mixed venous oxygen content (CvO₂), with mixed venous oxygen saturation and mixed venous oxygen partial pressure values instead of SaO₂ and PaO₂. Similarly, the oxygen content of RBCs in mixed venous blood is as follows:

\[(\text{CvO}_2)_{\text{RBC}} = (\text{CvO}_2)_{\text{WB}} - (\text{CvO}_2)_{\text{PL}}\]

The Do₂ is expressed as milliliters of oxygen per minute per kilogram and was calculated as follows:

\[(\text{Do}_2)_{\text{WB}} = (\text{CaO}_2)_{\text{WB}} \times \text{CI} \times 10\]
\[(\text{Do}_2)_{\text{PL}} = (\text{CaO}_2)_{\text{PL}} \times \text{CI} \times 10\]
\[(\text{Do}_2)_{\text{RBC}} = (\text{CaO}_2)_{\text{RBC}} \times \text{CI} \times 10\]

In these equations, CI is the cardiac index in liters per minute per kilogram, which was calculated as the ratio of the cardiac output to the weight in kilograms. The Vo₂ was calculated as follows:

\[(\text{Vo}_2)_{\text{WB}} = \left(\frac{(\text{CaO}_2)_{\text{WB}} - (\text{CvO}_2)_{\text{WB}}}{\text{CI} \times 10}\right)\]
\[(\text{Vo}_2)_{\text{PL}} = \left(\frac{(\text{CaO}_2)_{\text{PL}} - (\text{CvO}_2)_{\text{PL}}}{\text{CI} \times 10}\right)\]
\[(\text{Vo}_2)_{\text{RBC}} = \left(\frac{(\text{CaO}_2)_{\text{RBC}} - (\text{CvO}_2)_{\text{RBC}}}{\text{CI} \times 10}\right)\]

STATISTICAL ANALYSIS

Data are presented as mean ± SD. Statistical analysis was performed by using analysis of variance. A P value of less than .05 was considered to indicate statistical significance.

RESULTS

The calculations described in the previous section were performed for each animal’s data set. These calculated values were then averaged, and these are the data presented. The mean ± SD weight of the swine was 10.2 ± 1.4 kg in the control group and 10.8 ± 1.0 kg in the PHP group.

HEMODYNAMIC BALANCE

The same equations were used to calculate mixed venous oxygen content (CvO₂), with mixed venous oxygen saturation and mixed venous oxygen partial pressure values instead of SaO₂ and PaO₂. Similarly, the oxygen content of RBCs in mixed venous blood is as follows:

\[(\text{CvO}_2)_{\text{RBC}} = (\text{CvO}_2)_{\text{WB}} - (\text{CvO}_2)_{\text{PL}}\]

The Do₂ is expressed as milliliters of oxygen per minute per kilogram and was calculated as follows:

\[(\text{Do}_2)_{\text{WB}} = (\text{CaO}_2)_{\text{WB}} \times \text{CI} \times 10\]
\[(\text{Do}_2)_{\text{PL}} = (\text{CaO}_2)_{\text{PL}} \times \text{CI} \times 10\]
\[(\text{Do}_2)_{\text{RBC}} = (\text{CaO}_2)_{\text{RBC}} \times \text{CI} \times 10\]

In these equations, CI is the cardiac index in liters per minute per kilogram, which was calculated as the ratio of the cardiac output to the weight in kilograms. The Vo₂ was calculated as follows:

\[(\text{Vo}_2)_{\text{WB}} = \left(\frac{(\text{CaO}_2)_{\text{WB}} - (\text{CvO}_2)_{\text{WB}}}{\text{CI} \times 10}\right)\]
\[(\text{Vo}_2)_{\text{PL}} = \left(\frac{(\text{CaO}_2)_{\text{PL}} - (\text{CvO}_2)_{\text{PL}}}{\text{CI} \times 10}\right)\]
\[(\text{Vo}_2)_{\text{RBC}} = \left(\frac{(\text{CaO}_2)_{\text{RBC}} - (\text{CvO}_2)_{\text{RBC}}}{\text{CI} \times 10}\right)\]

STATISTICAL ANALYSIS

Data are presented as mean ± SD. Statistical analysis was performed by using analysis of variance. A P value of less than .05 was considered to indicate statistical significance.

OXYGEN TRANSPORT DYNAMICS

Figure 1 shows Do₂ in WB across time in the control and PHP groups. The Do₂ decreased significantly in the PHP group (P = .030) and the control group (P = .016) after hemorrhage and increased after resuscitation. The Do₂ did not return to BL values at any time after resuscitation, but the differences were not statistically significant.

Figure 2 shows Vo₂ in WB across time in the control and PHP groups. The Vo₂ remained at or above BL levels after resuscitation in the PHP group (P = .028) but did not significantly change in the control group. Cardiac index significantly decreased by about 50% in both groups after hemorrhage (P = .013 vs BL for the PHP group and P = .012 vs BL for the control group) and recovered after resuscitation.

Figure 3 shows [Hb] in WB, RBCs, and PL across time for the PHP group. After infusion of PHP, the [Hb] in PL in arterial blood samples from animals with PHP infusion was between 0.8 and 1.0 g/dL of WB. Total [Hb] in WB was 6.8 ± 3.0 g/dL at BL and decreased to 5.6 ± 3.4 g/dL at 15 minutes vs at BL. After infusion, [Hb] in WB decreased significantly from 124 ± 56 to 182 ± 74 beats per minute in PHP animals and from 130 ± 32 to 187 ± 52 beats per minute in control animals. Heart rate was significantly higher in the control group at the 60-minute, 75-minute, and 90-minute points, as compared with BL values (P < .05). Arterial pressure decreased from 93 ± 13 to 44 ± 10 mm Hg in the PHP animals (P = .003) and from 80 ± 23 to 50 ± 25 mm Hg in the control animals after hemorrhage. Mean arterial pressure recovered to near BL levels after resuscitation with shed blood or PHP. Mean arterial pressure in both groups decreased gradually from the after-resuscitation point to the 90-minute point but was not significantly different from the BL level in either group. Pulmonary arterial pressure increased to 31 ± 6 mm Hg after resuscitation in the PHP group (P = .028) but did not significantly change in the control group. Cardiac index significantly decreased by about 50% in both groups after hemorrhage (P = .013 vs BL for the PHP group and P = .012 vs BL for the control group) and recovered after resuscitation.

Table 1. Changes in Hemodynamic Parameters Across Time*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After Hemorrhage</th>
<th>After Resuscitation</th>
<th>Minutes After Resuscitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats per minute</td>
<td>124 ± 56</td>
<td>182 ± 74</td>
<td>132 ± 44</td>
<td>143 ± 58</td>
</tr>
<tr>
<td>PHP group</td>
<td>143 ± 48</td>
<td>139 ± 62</td>
<td>146 ± 66</td>
<td>157 ± 67</td>
</tr>
<tr>
<td>Control group</td>
<td>130 ± 32</td>
<td>159 ± 32</td>
<td>170 ± 42</td>
<td>207 ± 36†</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>93 ± 13</td>
<td>44 ± 10†</td>
<td>88 ± 8</td>
<td>79 ± 13</td>
</tr>
<tr>
<td>PHP group</td>
<td>66 ± 25</td>
<td>71 ± 13</td>
<td>74 ± 15</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>Control group</td>
<td>80 ± 23</td>
<td>89 ± 19</td>
<td>83 ± 37</td>
<td>73 ± 35</td>
</tr>
<tr>
<td>Mean pulmonary arterial pressure, mm Hg</td>
<td>50 ± 25</td>
<td>50 ± 25</td>
<td>56 ± 32</td>
<td>58 ± 39</td>
</tr>
<tr>
<td>PHP group</td>
<td>22 ± 7</td>
<td>31 ± 6†</td>
<td>27 ± 6</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Control group</td>
<td>23 ± 12</td>
<td>21 ± 12</td>
<td>18 ± 9</td>
<td>20 ± 12</td>
</tr>
<tr>
<td>Cardiac index, L/min per kilogram</td>
<td>0.087 ± 0.035</td>
<td>0.091 ± 0.022</td>
<td>0.080 ± 0.020</td>
<td>0.064 ± 0.024</td>
</tr>
<tr>
<td>PHP group</td>
<td>0.066 ± 0.029</td>
<td>0.072 ± 0.015</td>
<td>0.064 ± 0.026</td>
<td>0.062 ± 0.011</td>
</tr>
<tr>
<td>Control group</td>
<td>0.088 ± 0.018</td>
<td>0.086 ± 0.013</td>
<td>0.066 ± 0.019†</td>
<td>0.057 ± 0.014</td>
</tr>
</tbody>
</table>

Abbreviation: PHP, pyridoxalated hemoglobin polyoxyethylene conjugate.

*Data are shown as mean ± SD.
†P < .05 vs baseline.
percentage of contribution to D˙O2 reached a maximum level vs BL) and remained at BL levels in the control group (P < .05 for the pyridoxalated hemoglobin polyoxyethylene conjugate (PHP) group). The remaining times are given in minutes. Asterisk indicates P < .05 vs BL.

Figure 1. Oxygen delivery in whole blood across time. BL indicates baseline; AH, after hemorrhage; AR, after resuscitation; and PHP, pyridoxalated hemoglobin polyoxyethylene conjugate. The remaining times are given in minutes. Asterisk indicates P < .05 vs BL.

Figure 2. Oxygen consumption in whole blood across time. BL indicates baseline; AH, after hemorrhage; and AR, after resuscitation. The remaining times are given in minutes. Asterisk indicates P < .05 vs BL; dagger, P < .05 from BL), despite the decrease in RBC [Hb] after hemorrhage. Figure 3B shows that the total [Hb] in the control animals remained nearly constant after resuscitation (P > .05 from BL).

Table 2 summarizes oxygen extraction ratios in RBC, PL, and WB in the PHP group and WB extraction ratios for the control group. The extraction ratio for WB is (V˙O2)WB / (D˙O2)WB, with similar calculations for PL and RBC. Notably, in the PHP group the PL extraction ratio did not significantly change from BL, whereas the RBC extraction ratio increased from a BL level of 0.42 to a maximum of 0.93 at 90 minutes (P < .001).

Figure 4 depicts the percentage of contribution to D˙O2 from PL across time. The percent contribution to D˙O2 is calculated as the ratio of D˙O2 from PL to D˙O2 from WB: (D˙O2)PL / (D˙O2)WB. The values at BL were not significantly different between the 2 groups: 5.8%±2.3% for the PHP group and 7.4%±4.1% for the control group. After resuscitation, the percentage of contribution to D˙O2 increased significantly at all time points in the PHP group (P < .03 vs BL) and remained at BL levels in the control group (P ≤ .02 for the PHP group vs the control group). The percentage of contribution to D˙O2 reached a maximum level of 34.2% in the PHP group at 30 minutes.

Table 2

<table>
<thead>
<tr>
<th>Time</th>
<th>PHP Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>2.3±1.2</td>
<td>3.4±2.1</td>
</tr>
<tr>
<td>20 min</td>
<td>3.5±1.8</td>
<td>4.6±2.4</td>
</tr>
<tr>
<td>30 min</td>
<td>4.7±2.1</td>
<td>5.8±2.7</td>
</tr>
<tr>
<td>45 min</td>
<td>5.9±2.4</td>
<td>7.0±2.9</td>
</tr>
<tr>
<td>60 min</td>
<td>7.1±2.8</td>
<td>8.2±3.1</td>
</tr>
<tr>
<td>90 min</td>
<td>8.3±3.1</td>
<td>9.4±3.5</td>
</tr>
</tbody>
</table>

Figure 5 depicts the percentage of contribution to VO2 from PL across time. The percent contribution to VO2 is calculated as the ratio of VO2 from PL to VO2 from WB: (V˙O2)PL / (V˙O2)WB. The values at BL were 7.0%±5.0% for the PHP group and 8.1%±4.0% for the control group (P > .05). After infusion of PHP, the percentage of contribution to VO2 increased to a maximum of 20.4% at 30 minutes (P < .003 vs BL and P < .001 vs the control group) and then decreased to BL levels.

COMMENT

In this swine model of moderate hemorrhage and resuscitation, we studied the hemodynamic effects and oxygen transport properties of PHP, an acellular Hb solution. The observed hemodynamic response to hemorrhage and resuscitation was generally consistent with results from our previous studies. The animals in the control group, however, demonstrated a greater degree of tachycardia and systemic hypotension than did the animals in the PHP group. This finding could represent incomplete resuscitation in the control animals. Alternatively, these data suggest that PHP is effective as a resuscitation fluid.

As previously described, some acellular Hb solutions, including PHP, are associated with the transient development of pulmonary hypertension. In the present study, mean pulmonary arterial pressure increased from 17 to a peak of 31 mm Hg immediately after resuscitation in the PHP group. This effect is thought to be due to the scavenging of nitric oxide by the Hb molecule. The pul-
The intravascular half-life of PHP is less than 12 hours, and in clinical trials may elicit different degrees of vascular activity, or no measurable vasoconstrictive effect at all, so any effects of PHP would be expected to be transient. Nonetheless, the use of Hb solutions for the treatment of acute hemorrhage may be associated with a reduction in the allogeneic RBC transfusion requirement, as well as the ability to sustain life at critically low hematocrit levels when blood is not readily available.

The ultimate measure of the effectiveness of an Hb solution is how well it delivers and off-loads oxygen. To address this issue, we used a compartmentalized approach, whereby the contribution to DO2 and VO2 by RBCs and by PL, including PHP, could be quantified. This information cannot be obtained by simply calculating D˙O2 or off-loading from the RBCs, or both. Hence, to determine the relative contribution to DO2 and VO2, we used the compartmentalized approach.

The oxygen extraction ratio remained relatively constant in PL in the PHP group. On the other hand, the extraction ratio from RBCs increased progressively from 0.42 to 0.93. Thus, the increase in extraction ratio from the RBCs could be due to a decrease in DO2, an increase in VO2 (or off-loading from the RBCs), or both.

### Table 2. Oxygen Extraction Ratios in Whole Blood, Plasma, and Red Blood Cells*

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>After Hemorrhage</th>
<th>After Resuscitation</th>
<th>Minutes After Resuscitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>PHP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>0.42 ± 0.14</td>
<td>0.66 ± 0.13†</td>
<td>0.57 ± 0.20</td>
<td>0.70 ± 0.13†</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.42 ± 0.28</td>
<td>0.43 ± 0.29</td>
<td>0.33 ± 0.14</td>
<td>0.39 ± 0.13</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0.42 ± 0.16</td>
<td>0.69 ± 0.15†</td>
<td>0.63 ± 0.23</td>
<td>0.77 ± 0.13†</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>0.42 ± 0.09</td>
<td>0.65 ± 0.14†</td>
<td>0.42 ± 0.06</td>
<td>0.47 ± 0.09†</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.47 ± 0.07</td>
<td>0.43 ± 0.26</td>
<td>0.42 ± 0.27</td>
<td>0.45 ± 0.27</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0.42 ± 0.10</td>
<td>0.67 ± 0.14†</td>
<td>0.42 ± 0.08</td>
<td>0.48 ± 0.10†</td>
</tr>
</tbody>
</table>

Abbreviation: PHP, pyridoxalated hemoglobin polyoxyethylene conjugate.

*Data are shown as mean ± SD.
†P < .05 vs baseline.
‡P < .05 PHP group vs control group.

### Figure 4. Percentage of contribution of plasma to oxygen delivery across time. BL indicates baseline; AH, after hemorrhage; and AR, after resuscitation. The remaining times are given in minutes. Asterisk indicates P < .05 vs BL and for the pyridoxalated hemoglobin polyoxyethylene conjugate (PHP) group vs the control group.

### Figure 5. Percentage of contribution of plasma to oxygen consumption across time. BL indicates baseline; AH, after hemorrhage; and AR, after resuscitation. The remaining times are given in minutes. Asterisk indicates P < .05 vs BL and for the pyridoxalated hemoglobin polyoxyethylene conjugate (PHP) group vs the control group.
This issue can be explored further by looking at Figures 4 and 5. The percent contributions of PL to $\dot{D}O_2$ and $\dot{V}O_2$ are perhaps better measures than is the oxygen extraction ratio to determine the delivery and off-loading characteristics of PHP because delivery and consumption are treated independently rather than as a ratio. From Figure 4 it is clear that PL, including PHP, contributes significantly to $\dot{D}O_2$. After resuscitation with PHP, the percentage of contribution to $\dot{D}O_2$ from PL increased from about 5% to more than 20%, reaching a peak of 34% at 30 minutes. The PHP concentration in WB was approximately 1 g/dL, and the total [Hb] after the hemorrhage was around 6 g/dL, so PHP represented approximately 16.6% of total [Hb]. The $\dot{D}O_2$ of 20% or higher from PL, including dissolved oxygen and oxygen bound to PHP, is therefore consistent with the expected contribution to delivery from PL on the basis of the relative amount of PHP to total [Hb].

Figure 5 shows that dissolved oxygen in PL accounts for about 7% of the $\dot{V}O_2$ prior to PHP infusion. After resuscitation with PHP, the percentage of contribution of PL to $\dot{V}O_2$ increased to a maximum of 20% at 30 minutes and thereafter was no different than BL levels. These results suggest that even if PHP is able to effectively deliver oxygen to the tissues, the tissues preferentially consume the delivered oxygen from RBCs, not from PL. Because PHP has a lower $P_{50}$ than does normal RBC Hb, the relative preference for off-loading oxygen from RBCs vs PHP Hb may result in relatively tighter binding of oxygen to PHP. Evidence against this mechanism, however, is included in prior study results that showed that PHP did not have a significantly greater percentage of contribution to $\dot{V}O_2$ than did a hemoglobin solution with a low $P_{50}$ of 12.7 mm Hg.

The percentage of contribution to $\dot{V}O_2$ from PL is highest immediately after resuscitation. In the period immediately after hemorrhagic shock, the peripheral tissues are the most ischemic and acidic. Acidosis shifts the oxygen dissociation curve to the right, which allows for more oxygen off-loading from Hb. The PHP curve may shift more than does the RBC Hb curve and result in a greater percentage of contribution to $\dot{V}O_2$ from PL throughout the first 30 minutes after resuscitation. Another possibility is that $\dot{V}O_2$ was highest shortly after resuscitation, and the erythrocytes were unable to provide enough oxygen for consumption, necessitating increased $\dot{V}O_2$ from PHP. A final possibility is that, at least in moderate anemic states, PHP acts as a sink for oxygen released from RBC Hb. This latter possibility, that is, part of the oxygen off-loaded by the RBCs may be taken up by PHP, is consistent with PHP’s lower $P_{50}$ than that of RBC Hb and PHP’s lack of a cell membrane, which would increase the resistance to oxygen diffusion. This finding also corroborates the results of a previous study that demonstrated a disproportionately low percentage of contribution to $\dot{V}O_2$ by PHP at moderately low hematocrit levels in an isovolemic hemodilution model.

If PHP acts as a sink for oxygen off-loaded from RBC Hb, this effect would be most pronounced in states of mild to moderate anemia, as in the present study. As long as there is sufficient RBC mass for $\dot{D}O_2$, the net effect on $\dot{V}O_2$ is a greater percentage of contribution from RBCs, as opposed to PL. In severe anemia, on the other hand, or in cases of RBC dysfunction that impairs oxygen off-loading, an increasing contribution to $\dot{V}O_2$ from PL would be expected.

This finding was demonstrated in our previous study, in which the total $\dot{V}O_2$ during isovolemic hemodilution was no different at a hematocrit level of 8% than it was at 30%, yet the percentage of contribution to $\dot{D}O_2$ from PL increased to 41% at the lowest hematocrit level. At hematocrit levels lower than 8%, the contribution to $\dot{D}O_2$ from PL would be expected to increase even further. Therefore, the most beneficial effect of PHP, in terms of $\dot{D}O_2$ and $\dot{V}O_2$, may be in states of severe, not mild or moderate, anemia. This behavior of PHP cannot be extrapolated to other Hb-based oxygen carriers, which would likely demonstrate different oxygen transport dynamics because of different chemical properties, $P_{50}$, and so on. It would be useful to characterize other acellular Hb solutions in development with regard to their oxygen transport properties.

To summarize, these data support the conclusion that PHP may be a useful resuscitation solution after hemorrhage. Hemodynamic balance is restored and the relative contribution to $\dot{D}O_2$ is significant. However, in this model of moderate hemorrhage, there may be preferential off-loading of oxygen from RBC Hb rather than from PHP, as evidenced by the high extraction ratio from RBC Hb and the relatively low percentage of contribution from PL to $\dot{V}O_2$. This preferential $\dot{V}O_2$ from RBC Hb may relate to the behavior of PHP as an oxygen sink in moderate anemia. Future research in this area might be useful to further delineate the oxygen transport properties of PHP and other Hb-based oxygen carriers.

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