Blood volume and cardiac index in rats after exchange transfusion with hemoglobin-based oxygen carriers

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Migita, Russell, Armando Gonzales, Maria L. Gonzales, Kim D. Vandegriff, and Robert M. Winslow. Blood volume and cardiac index in rats after exchange transfusion with hemoglobin-based oxygen carriers. J. Appl. Physiol. 82(6): 1995–2002, 1997.—We have measured plasma volume and cardiac index in rats after 50% isovolumic exchange transfusion with human hemoglobin cross-linked between the α-chains with bis(3,5-dibromosalicyl) fumarate (αα-Hb) and with bovine hemoglobin modified with polyethylene glycol (PEGHb). αα-Hb and PEGHb differ in colloid osmotic pressure (23.4 and 118.0 Torr, respectively), oxygen affinity (oxygen half-saturation pressure of hemoglobin = 30.0 and 10.2 Torr, respectively), viscosity (1.00 and 3.39 cp, respectively), and molecular weight (64,400 and 105,000, respectively). Plasma volume was measured by Evans blue dye dilution modified for interference by plasma hemoglobin. Blood volumes in PEGHb-treated animals were significantly elevated (74.0 ± 3.5 ml/kg) compared with animals treated with αα-Hb (49.0 ± 1.2 ml/kg) or Ringer lactate (48.0 ± 2.0 ml/kg) or with controls (58.2 ± 1.9 ml/kg). Heart rate reduction after αα-Hb exchange is opposite to that expected with blood volume contraction, suggesting that αα-Hb may have a direct myocardial depressant action. The apparently slow elimination of PEGHb during the 2 h after its injection is a consequence of plasma volume expansion: when absolute hemoglobin (concentration × plasma volume) is compared for PEGHb and αα-Hb, no difference in their elimination rates is found. These studies emphasize the need to understand blood volume regulation when the effects of cell-free hemoglobin on hemodynamic measurements are evaluated.

αα-hemoglobin; cardiac output; polyethylene glycol-modified hemoglobin; blood substitutes; colloid osmotic pressure; plasma volume; Evans blue dye; bovine hemoglobin

HEMOGLOBIN-BASED OXYGEN carriers are not yet available for routine blood replacement, because the many biological consequences of high-concentration cell-free hemoglobin are not completely understood. Among these is a propensity to raise systemic blood pressure in humans and animals (28). Impurities were initially implicated as a cause (6, 23), but extensive purification has not eliminated this unwanted property. Several factors can potentially contribute to vasoconstriction; among them are vasoactivity due to NO scavenging (19), local autoregulation in the microcirculation (15, 26), and adrenergic stimulation. At least part of the cause for the vasoactivity is believed to be the high affinity of hemoglobin for NO (8), but to what extent the expansion of circulating blood volume due to oncotic effects of hemoglobin plays an additional role has not been established.

Oncotic pressure is only one of a number of properties of cell-free hemoglobin that will affect its physiological effects and clinical utility. For example, modified hemoglobins leave the plasma at different rates, presumably as a consequence of the specific type of modification and dose; polymerized and conjugated hemoglobins have the longest intravascular retention times, and intramolecular cross-linked hemoglobin appears to have the shortest plasma retention time (13). Hemoglobin solutions may also affect vascular permeability (2, 22). It is important to determine the most appropriate combination of oncotic pressure and vascular retention for a clinical application: higher oncotic pressure may be useful in shock, but in other situations, hyperoncotic hemoglobin solutions have been suggested to cause tissue damage in the heart and liver (4) or volume overload. Quantitative data to describe the effect of hemoglobin solutions on blood volume are not available in the literature. The purpose of the present study is to examine such effects with two representative solutions with markedly different oncotic properties.

Numerous methods have been used to determine plasma volume, usually by radiolabeled albumin (16, 21, 27) or by dye-dilution techniques (1, 9, 25). Evans blue dye (EBD, T-1824), an inert material used for >50 years for plasma volume determination (7), is attractive in blood substitute research, because measurement is simple, it is nonradioactive, and it has a long history of use in rats and other small animals. The use of EBD in the presence of cell-free plasma hemoglobin raises several special problems: 1) the optical spectra of EBD and hemoglobin overlap; 2) EBD binds to albumin and leaves the circulation at a rate that reflects the transudation of albumin through the vessel wall (7), and after a few hours EBD begins to reenter the bloodstream via the lymphatics; and 3) EBD, a diazo compound, could bind significantly to hemoglobin, as it does to albumin, and could alter the optical spectrum of hemoglobin and/or albumin. One objective of the present study is to validate the EBD method in the presence of cell-free hemoglobin.

METHODS

All experiments and procedures were approved by the San Diego Veterans Affairs Medical Center Animal Studies Subcommittee.

Characterization of hemoglobin solutions. The characteristics of the two hemoglobin solutions are given in Table 1. Human hemoglobin cross-linked between the α-chains with bis(3,5-dibromosalicyl) fumarate (αα-Hb) was prepared at the Letterman Army Institute of Research as previously described (29). It was formulated in Ringer lactate and supplied at ~15 or 7.9 g/dl. The latter solutions were used for the in vivo studies. Polyethylene glycol-modified hemoglobin (PEGHb) was a gift from Enzon (Piscataway, NJ) and was...
infused at a concentration of 5.5 g/dl. Human serum albumin (25 g/dl stock) was obtained from Baxter Health Care.

Hemoglobin concentration was measured using a spectrophotometer (Hemocue, Mission Viejo, CA). Viscosity measurements were made using a capillary viscometer, and colloid osmotic (oncotic) pressure (COP) was measured using a colloid osmometer (model 4420, Wescor, Logan, UT). Protein samples used for the oncotic pressure measurements were diluted using normal saline. Number-average molecular weight was determined by oncotic pressure measurements; values based on known structure are given in parentheses.

### Table 1. Characteristics of hemoglobin solutions

<table>
<thead>
<tr>
<th>Source</th>
<th>ααHb</th>
<th>PEGHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Hb], g/dl</td>
<td>7.9</td>
<td>5.5</td>
</tr>
<tr>
<td>P50, Torr</td>
<td>30.0</td>
<td>10.2</td>
</tr>
<tr>
<td>COP, mmHg</td>
<td>23.4</td>
<td>118.0</td>
</tr>
<tr>
<td>Viscosity, CP</td>
<td>1.0</td>
<td>3.39</td>
</tr>
<tr>
<td>Mol mass, Da</td>
<td>68,849 (64,400)</td>
<td>123,380 (105,000)</td>
</tr>
<tr>
<td>Excluded volume, nm³</td>
<td>1,034</td>
<td>93,273</td>
</tr>
</tbody>
</table>

ααHb, human hemoglobin cross-linked between α-chains with bis(3,5-dibromosalicyl)fumarate; PEGHb, polyethylene-modified hemoglobin; [Hb], hemoglobin concn; P50, oxygen half-saturation pressure of hemoglobin; COP, colloid osmotic pressure. Weight – average molecular weight was determined by oncotic pressure measurements; values based on known structure are given in parentheses.

replaced simultaneously via the femoral vein. Exchange transfusions were done at a rate of ~0.5 ml/min to a total volume of solution that equaled 50% of estimated total blood volume (60 ml/kg). In the case of Ringer lactate experiments, a 1:1 exchange led to hemodynamic instability. Thus the exchange ratio was 2:1, in which the volume of Ringer lactate returned to the animal was twice that removed. Several groups of animals were used for these studies. EBD cannot be given repeatedly to the same animal, so each animal represents a single time point after exchange transfusion. Five animals were used for each EBD blood volume determination. Serial cardiac output could be measured repeatedly in the same animal, however. Thus five rats each were used for ααHb and PEGHb and three for Ringer lactate to measure cardiac output after exchange transfusion.

### Plasma and blood volume determination.

After exchange transfusion, rats were allowed to rest in their restrainers for 0, 30, 60, or 120 min. Blood volume was measured in five ααHb- and PEGHb-transfused animals at each time point and in three animals transfused with Ringer lactate at each time point. A preinjection sample of 150 µl of blood was collected in three premeasurements, heparinized capillary tubes immediately before EBD injection. This preinjection sample was centrifuged in an IEC Micro MB centrifuge for 5 min to obtain the preinjection hematocrit. Each tube was then scored just above the red cell-plasma interface, and 50 µl of this plasma was pipetted into a 1-ml spectrophotometer cuvette (VWR Scientific) and diluted 20:1 in 0.1 M tris(hydroxymethyl)aminomethane-HCl buffer, pH 9.0 (this pH was chosen to minimize the effect of methemoglobin on the measured spectra). Plasma samples were also used to measure hemoglobin concentration using the Hemocue instrument. Optical spectra of the EBD-containing plasma samples were measured between 450 and 750 nm using a diode array spectrophotometer (model 3000, Milton Roy) and stored. All post-EBD injection samples were handled in a similar fashion. Immediately after collection of the preinjection sample, EBD (5 mg/ml solution; New World Trading, Debary, FL) was drawn into a Hamilton syringe to a volume that would deliver a dose of 0.05 mg/ml of predicted plasma volume. Blood samples were collected immediately before injection of EBD and then at 5-min intervals for a total of five samples. The absorbance at 620 nm (A620) was corrected first for the amount of cell-free hemoglobin. Repeat measurements showed a small discrepancy between measured and known blood volumes of only 0.4%.

### Plasma volume (PLV)

The femoral artery catheter was connected through a stopcock to a pressure transducer (model 1050, UFI, Morro, CA). Arterial pressure was recorded continuously using a data-collection system (model MP100WSW, BIOPAC Systems, Goleta, CA). The data were stored in digital form for subsequent analysis. Cardiac output was measured by injection of cold saline via the jugular vein. Mixing occurs in the pulmonary capillary beds, and cardiac output was measured by a thermocoupling catheter and cardiac output computer (Columbia Instruments, Columbus, OH). Systolic, diastolic, and mean pressures were also calculated for each heartbeat. Mean values were averaged for each minute of data collected at a rate of 100 Hz. Exchange transfusion. After anesthesia, rats were allowed to recover for 90 min and placed in Plexiglas restrainers. The arterial and venous cannulas were flushed with 200 and 100 µl, respectively, of heparinized saline (100 U/ml). Test solutions were filtered through a 0.22-µm filter immediately before infusion. The rats were connected to an infusion pump (model 4262000, Labconco, Kansas City, MO) so that blood was removed from the femoral artery and exchange fluid was

### Exchange transfusion

where Ci and Vi are the EBD concentration and volume injected and Cp is the extrapolated time 0 plasma EBD concentration. If the absorption spectrum in plasma is not altered by the presence of hemoglobin, then Cc and Cp can be replaced by the absorbances A0 and A0, respectively.

This method was validated by performing a series of experiments using EBD to determine the volume of a known amount of shed blood in vitro that contained a significant amount of cell-free hemoglobin. Repeat measurements showed an average discrepancy between measured and known blood volumes of only 0.4%.

A plasma trapping factor of 0.96 (7) and a total body-to-venous hematocrit ratio (Fv) of 0.74 (5) were obtained from
the literature, and total blood volume (TBV, ml/kg body wt) was calculated using the formula
\[
TBV = \frac{PLV}{1 - Hct \times \text{(trapping factor)} \times F_{cells}}
\]
where Hct is hematocrit.

Cardiac output, plasma hemoglobin, and blood pressure. In separate experiments, blood pressure, cardiac output, and plasma hemoglobin disappearance after a 50% exchange transfusion were measured. Hemodynamic measurements were made for each test material in five rats at 0, 30, 60, and 120 min after a 50% exchange transfusion. Plasma hemoglobin was measured in five rats at 0, 60, and 120 min after a 50% exchange transfusion to assess the apparent rate of disappearance of \(\alpha\alpha\)Hb and PEGHb.

EBD-hemoglobin binding. To determine the extent to which EBD binding to hemoglobin affects the measured optical spectrum, solutions of EBD, hemoglobin, and EBD + hemoglobin were compared. Test solutions consisted of rat plasma and hemoglobin mixtures. Rat plasma was collected in capillary tubes from cannulated arteries and was subsequently centrifuged and pooled. The concentration of EBD in the test mixtures was 2.5 µg/ml, and the hemoglobin concentration was 0.125 mM. Both of these concentrations approximate the in vivo conditions of subsequent experiments.

Statistical methods. Differences between all group means were evaluated using a one-way analysis of variance. Individual differences between groups were assessed using a post hoc Bonferroni multiple-comparisons test. Comparisons were performed only when \(P < 0.05\). \(P < 0.05\) was considered significant.

RESULTS

Characterization of test solutions. The hemoglobin solutions are compared in Table 1. The oxygen half-saturation pressure of \(\alpha\alpha\)Hb is taken from our previous studies (29). The value for PEGHb, 12.3 Torr, was obtained in our laboratory. COP is significantly higher for PEGHb than for \(\alpha\alpha\)Hb or albumin (Fig. 1). The molecular weights reported in Table 1 were calculated from the oncotic pressure measurements (3) and are therefore number-average values. The values in parentheses in Table 1 were calculated from the known structure of the molecules in the case of \(\alpha\alpha\)Hb and the value reported previously from PEGHb (20). The number-average molecular weight of PEGHb is significantly higher than its calculated molecular weight or the molecular weight of \(\alpha\alpha\)Hb and is consistent with the high excluded volume. Thus, although the protein mass of PEGHb is less than twice that of \(\alpha\alpha\)Hb, the molecule occupies \(\sim\)100 times the volume.

Hematocrit. Hematocrit was measured before EBD injection in the same animals used for measurements of plasma and blood volume (Fig. 2). Hematocrits were significantly lower in animals that received PEGHb than in those that received \(\alpha\alpha\)Hb or Ringer lactate at all time points (0, 30, 60, and 120 min). There was no significant difference between hematocrits at the various time points within the group that received PEGHb. Hematocrits in the PEGHb-transfused animals were 16.1% at 0 min, 18.1% at 30 min, 18.9% at 60 min, and 18.0% at 120 min.

When animals treated with \(\alpha\alpha\)Hb were compared with those treated with Ringer lactate alone, there were significant differences at only 30 and 60 min, with \(\alpha\alpha\)Hb-transfused animals having slightly lower hematocrits at both time points. Hematocrits in the \(\alpha\alpha\)Hb-transfused group were 21.3% at 0 min, 20.3% at 30 min, 21.8% at 60 min, and 24.4% at 120 min. Hematocrits in the group transfused with Ringer lactate were 22.6% at 0 min posttransfusion, 23.6% at 30 min, 25.0% at 60 min, and 24.6% at 120 min.

EBD-hemoglobin binding. Figure 3 shows the spectra of EBD, hemoglobin, and EBD + hemoglobin in plasma. Differences between the spectra of the mixture and the sum of both components measured independently were not significant. Figure 3 also demonstrates a small but significant overlap of the hemoglobin and
EBD spectra at 620 nm. Thus measurement of A_{620} in the plasma just before injection of EBD is essential to obtain the corrected A_{620} due to EBD alone in the plasma volume experiments (12).

EBD disappearance. Figure 4 shows the disappearance rates for EBD in the plasma in rats after injection. The rate of disappearance of EBD after exchange transfusion with αHb or hemodilution with Ringer lactate increases with time (more negative slope), but the rate in the PEGHb animals remains constant. The difference in rate between αHb- and PEGHb-transfused animals is significant (P < 0.05) at 60 and 120 min after exchange, indicating that EBD escapes at approximately twice the rate of PEGHb.

Plasma and blood volume. In control rats not subjected to hemodilution, we found a plasma volume of 39.6 ± 1.3 ml/kg and a blood volume of 58.2 ± 1.9 ml/kg. These values are in excellent agreement with those reported in the literature using 125I-labeled albumin (27), indocyanine green (65.7 ml/kg) (27), and EBD (60.8 ml/kg) (5). Total blood volume adjusted for body weight for the three solutions is shown in Fig. 5. Plasma and blood volumes were significantly higher in animals exchange transfused with PEGHb than in animals exchange transfused with αHb or Ringer lactate and were significantly higher than in controls at all time points. Average blood volumes were 50% greater in rats transfused with PEGHb than in controls immediately after completion of the exchange transfusion. Mean blood volume in PEGHb-transfused rats was 74 ± 3.5 ml/kg immediately after transfusion, 66 ± 11.5 ml/kg at 30 min, 62 ± 3.9 ml/kg at 60 min, and 61 ± 3.8 ml/kg at 120 min. There were no significant differences between blood volumes when PEGHb-treated groups were compared at different time points.

When animals treated with αHb were compared, total blood volumes were significantly lower than control only at 60 and 120 min. Whereas blood volumes were consistently higher in the αHb- than in the Ringer lactate-transfused group, there were no significant differences between the two groups when post hoc comparisons were made. Mean blood volumes in αHb-treated animals were 49 ± 1.2 ml/kg at 0 min, 48 ml/kg at 30 min, 45 ± 2.9 ml/kg at 60 min, and 42 ± 2.8 ml/kg at 120 min. When αHb-transfused animals were compared at different time points, animals had significantly lower blood volumes at 120 min than at 0 and 30 min posttransfusion.

In animals receiving only Ringer lactate solution, blood volumes were significantly lower than in controls at 0, 60, and 120 min after transfusion. Mean blood
volumes in these animals were 48 ± 2.0 ml/kg immediately after exchange transfusion, 47 ± 2.6 ml/kg at 30 min, 43 ± 0.2 ml/kg at 60 min, and 42 ± 5.8 ml/kg at 120 min. There were no significant differences in blood volume among animals receiving Ringer lactate at different time points.

Hemoglobin. The hemoglobin concentrations ([Hb]) are shown in Fig. 6. Figure 6A shows the measured plasma concentrations in grams per deciliter. The data were fit to the expression

\[
[Hb] = A(\exp^{-kt})
\]

where \(A\) is the y intercept, \(k\) is a constant, and \(t\) is time in minutes. By use of this expression, the half-life \(t_\text{\%}\) of the hemoglobin concentration is given by

\[
t_\text{\%} = \frac{\ln(2)}{k}
\]

The estimated parameters \(A\) and \(k\) and the \(t_\text{\%}\) values are given in Table 2. The \(t_\text{\%}\) values estimated from plasma hemoglobin concentration are very different for \(\alpha\)Hb and PEGHb: 3.53 and \(\geq 200\) h, respectively.

Expansion of the blood volume prevents an accurate picture of the actual clearance rate of the two hemoglobins, however, because of the dynamic changes in the blood volume (Fig. 5). The plasma hemoglobin concentration calculated on a gram per kilogram body weight basis (hemoglobin concentration × plasma volume) is shown in Fig. 6B. Analysis of these curves now yields identical \(t_\text{\%}\) values for the two hemoglobins: 2.96 h.

When \(t_\text{\%}\) values are calculated from plasma hemoglobin concentration over 10 h (Table 2), \(t_\text{\%}\) is 4.43 and 10.29 h for \(\alpha\)Hb and PEGHb, respectively. It is not possible to calculate absolute disappearance rates over 10 h, because we have blood volume data only for the first 2 h after exchange transfusion. However, Fig. 5 shows that there was little change in blood volume beyond 2 h and that blood volume has essentially returned to the control level in our model by that time. Thus, taken together, these data suggest that the absolute clearance rate for PEGHb may be biphasic: relative to \(\alpha\)Hb, it is initially removed more slowly. This could be a result of greater molecular size or, possibly, a reduced rate of metabolism.

Blood pressure and heart rate. In a different series of experiments, continuous mean arterial pressure readings were obtained from exchange-transfused rats. Five rats received PEGHb, six received \(\alpha\)Hb, and six received Ringer lactate (Fig. 7). The exchange transfusion was carried out from 30 to \(\sim 55\) min in these experiments. As shown in Fig. 7, \(\alpha\)Hb produces a significant \((P < 0.05)\) rise in mean arterial blood pressure compared with animals treated with Ringer lactate or PEGHb. Exchange with \(\alpha\)Hb also produces a significant decrease in heart rate compared with PEGHb or Ringer lactate \((P < 0.05)\). The heart rate rises in the animals treated with Ringer lactate compared with baseline or PEGHb-treated animals \((P < 0.05)\).

Cardiac output. Cardiac output was measured in a separate series of experiments at 0, 30, 60, and 120 min after 50% exchange transfusion. Results were corrected for body weight and are expressed as cardiac index in Fig. 8. In animals receiving PEGHb, cardiac outputs were markedly elevated over the 120 min after transfusion. There was little change in cardiac output in animals that received \(\alpha\)Hb, and cardiac output was decreased in animals receiving only Ringer lactate in a

Table 2. Plasma hemoglobin concentration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma concn, g/dl</th>
<th>Whole body Hb, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.694</td>
<td>1.579</td>
</tr>
<tr>
<td>k</td>
<td>3.260 × 10⁻³</td>
<td>4.930 × 10⁻³</td>
</tr>
<tr>
<td>(t_\text{%}), h</td>
<td>2 h</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td>10 h</td>
<td>4.43</td>
</tr>
</tbody>
</table>

\(A\), \(y\) intercept; \(k\), constant; \(t_\text{\%}\), half-life.

Fig. 6. Plasma hemoglobin disappearance after 50% exchange transfusion with PEGHb (○, solid line) and \(\alpha\)Hb (○, dashed line). A: plasma concentration. B: absolute hemoglobin. Absolute values were calculated using hemoglobin concentrations (A) and plasma volume of distribution (Fig. 5). Values are means ± SE; \(n = 5\) for each group.
50% exchange transfusion. Figure 9 shows that cardiac index is directly correlated to blood volume.

**DISCUSSION**

Red blood cells exert very little COP. However, cell-free hemoglobin, like other plasma proteins, does exert COP, and, in general, the magnitude of the COP is a colligative property, i.e., related to the concentration of molecules in the plasma. Thus traditional thinking has been to strive to develop hemoglobin solutions as red cell substitutes that minimize COP while maximizing oxygen capacity (10). In contrast, many non-oxygen-carrying plasma expanders, such as the starches, are effective precisely because they do exert COP and thereby maintain the vascular volume and cardiac output (24). Furthermore, considerable success has been achieved in the experimental resuscitation of animals in shock with hypertonic saline dextran (17), again because of its high COP. There has been little attention to the effects of various cell-free hemoglobin solutions on plasma and blood volume. This study compared two hemoglobin solutions with very different oncotic pressures with regard to their effects on blood volume and cardiac output using EBD dilution and thermodilution, respectively.

EBD has been used for many years to measure plasma and blood volumes. It is bound to albumin, and therefore the circulating albumin exchanges with interstitial albumin. The use of EBD to measure plasma volume in the presence of cell-free hemoglobin has not been described in the literature. Therefore, we were concerned 1) because of possible interference of the \(A_{620}\) measurement in plasma, 2) because of possible interaction of hemoglobin and EBD, and 3) because hemoglo-
bin could alter the disappearance volume of albumin from the plasma.

Spectral analysis of mixtures of EBD and hemoglobin solutions compared with spectra of each individually indicate that the EBD or hemoglobin spectra are not fundamentally altered at the wavelengths used in the calculation of plasma volume in our experiments. Furthermore, in preliminary experiments, we established that, by subtracting plasma A620 obtained after exchange transfusion and before EBD injection, we were able to accurately estimate premeasured volumes of blood and hemoglobin solutions. Our determination of normalized blood volume in normal rats agreed closely with published observations.

In regard to the disappearance of EBD from the plasma, we found that the rates were significantly different for PEGHb- and ααHb-treated animals, the latter being significantly faster after 60 min and approximately twice as fast 120 min after exchange transfusion. This unexpected result raises the interesting possibility that greater vascular leakiness is induced by ααHb than by PEGHb. In fact, the disappearance rate for the PEGHb animals did not change significantly over the 120 min of the experiments. This observation may provide a clue in understanding the interaction of cell-free hemoglobin with endothelium and is worthy of further experiments.

We found significantly higher blood volumes, lower hematocrits, and higher cardiac outputs in animals transfused with PEGHb than in those transfused with ααHb or Ringer lactate. These observations are all physiologically consistent with the oncotic pressures of these solutions. Influx of extravascular fluid in response to an oncotic gradient would increase blood volume and dilute the circulating red cell mass, causing a decreased hematocrit. Expanded blood volume should increase output, according to Starling's law.

The heart rate response to exchange transfusion is worthy of special comment. Tachycardia is expected when blood volume is depleted. Indeed, this is the case with exchange transfusion with Ringer lactate. However, ααHb and Ringer lactate produce the same contraction of the blood volume (Fig. 5), but ααHb depresses the heart rate (Fig. 7B). Thus it appears that the relative bradycardia produced by ααHb is not volume mediated, and our results suggest a direct depressive effect on the heart.

The lack of a significant change in blood pressure in PEGHb-treated animals is not easily understood. However, it does raise the question, Can the vasoactive properties of hemoglobin be generalized to all molecules? Moreover, until more detail is known about the ability of PEGHb to bind and release NO, whether this apparent difference between PEGHb and ααHb is due to other reactivities with NO or to some other property will remain unknown. There are at least two alternative explanations for the different vasoactivities. First, PEGHb is a much larger molecule (Table 1), and therefore its entry into the interstitial space might be restricted. Indeed, this is suggested by the difference in EBD disappearance slopes shown in Fig. 4. Second, the oxygen binding curves of the two solutions are markedly different (unpublished data): PEGHb displays a lower oxygen half-saturation pressure of hemoglobin and Hill coefficient, whereas these two properties of ααHb are closer to the values for blood.

ααHb-transfused animals show a slight but significant decrease in blood volume compared with controls but a large increase in blood pressure, in agreement with data reported by Hess and co-workers (14) in a dehydrated swine shock model. Thus, whereas ααHb may be less oncally active than PEGHb, it appears to be more vasoactive. In experiments carried out by Gulati and others (11), increases in cardiac output after infusion of commercially prepared ααHb (DCLHb) resulted in an increase, rather than a decrease, in cardiac output. It is not clear whether this discrepancy is due to some difference in the solutions or in the protocols studied. Resolution of the difference would be important as the solutions near clinical use. As expected, our animals that received Ringer lactate had lower blood volumes, higher hematocrits, and lower cardiac outputs than animals in the ααHb or the PEGHb group.

The oncotic effect on blood volume has important implications for the estimation of plasma retention times of modified hemoglobins. In our experiments, we were able to calculate the absolute plasma retention (hemoglobin concentration × plasma volume) in addition to hemoglobin concentration alone. This analysis (Fig. 6) led to a surprising result: whereas the plasma hemoglobin concentration t1/2 values for ααHb and PEGHb are markedly different, the absolute rate of disappearance from the circulation is approximately the same. This phenomenon should be taken into account when pharmacokinetic studies of any protein with oncotic activity are performed.

The model we have chosen for the present studies is not intended to be a clinical model. Rather, it was selected to permit study of fluid shifts after administration of hemoglobin solutions. In an elective surgical setting, hemodilution would probably be less aggressive, and the effects on blood volume and hemodynamics would not be as severe as we report here. In shock-resuscitation, where larger volumes might be used, oncotic effects could be much more significant. However, the importance of the model and of these experiments is to demonstrate that each modified hemoglobin may have different physiological effects and that, to use cell-free hemoglobin solutions wisely and safely in clinical settings, these effects must first be understood.

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