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Hemodilution with Oxyhemoglobin

Mechanism of Oxygen Delivery and Its Superaugmentation with a Nitric Oxide Donor (Sodium Nitroprusside)

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Background: **Hemodilution (HD) with oxyhemoglobin colloid (oxyHb) provides a greater arterial oxygen content (Ca_{O_2}) than HD with conventional colloids; however, oxygen delivery ($\dot{D}O_2$) is essentially the same, because, in contrast to conventional HD, cardiac output (CO) is not augmented. This study seeks to elucidate the mechanism that limits CO during oxyHb-HD and to test whether infusion of a nitric oxide (NO) donor would augment $\dot{D}O_2$, because oxyHb is known to inactivate *in vitro* endothelial-derived NO.**

Methods: **Anesthetized dogs were isovolemically hemodiluted with 10% oxyHb, 8% albumin, or 10% methemoglobin (weak NO inactivator) to 20% hematocrit. After HD, sodium nitroprusside (SNP) was titrated intravenously until decreases (>10 mmHg) in mean aortic pressure (P_{ao}) indicated the presence of exogenous NO. Systemic hemodynamics and regional blood flows (microsphere method) were measured.**

Results: **Albumin-HD and metHb-HD produced typical HD-mediated responses: increased CO (63–65%), slight decreases (13–15%) in $\dot{D}O_2$, decreases in systemic vascular resistance (SVR) proportional to the decreases (49–52%) in blood viscosity of all three groups, and increased regional blood flows (RBF). Responses to oxyHb-HD were atypical: CO and its determinants were not changed, $\dot{D}O_2$ decreased (23%) proportional to CaO_2, and SVR and most RBF were not changed except for a net redistribution of CO to myocardium and skeletal muscle. In albumin-HD or metHb-HD, SNP (2–5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) induced comparable decreases in mean P_{ao} (29–37%) and SVR (39–41%); however, CO, RBF, and $\dot{D}O_2$ were not affected. In oxyHb-HD, exceptionally large doses of SNP (54 ± 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) decreased mean P_{ao} only 19 ± 1%; however, CO increased 78 ± 5% and decreases (61 ± 3%) in SVR were slightly greater than viscosity reductions. Other determinants of CO were not affected. Most RBF increased proportional to CO; there was, however, preferential distribution to myocardium and skeletal muscle. Consequently, the augmented CO, and CaO_2 of oxyHb-HD, produced large increases in $\dot{D}O_2$, 77 ± 5% from HD alone and 43 ± 3% from prehemodilution values.**

Conclusions: **This study indicates that the limited CO and $\dot{D}O_2$ of oxyHb-HD resulted from opposing changes in two determinants of flow, *i.e.*, reduced blood viscosity and increased arterial resistance (vasoconstriction). The vasoconstriction was not evident with metHb-HD and was reversed by the SNP infusion, indicating that oxyHb inactivated *in vivo* endothelial-derived NO. The ability of the NO donor (SNP) to facilitate large viscosity-mediated increases in $\dot{D}O_2$ during oxyHb-HD is an important finding that could potentially render oxyHb colloids more useful than conventional colloids, particularly for the individual with a compromised circulation who would benefit from an increased oxygen supply. (Key words: Blood, hemodilution: albumin; hematocrit; methemoglobin; oxyhemoglobin; viscosity. Hemodynamics, heart: cardiac output; oxygen delivery. Hemodynamics, peripheral: regional blood flows; resistance. Pharmacology: nitric oxide; sodium nitroprusside.)**

OXYHEMOGLOBIN variants derived from recombinant,[1] transgenic,[2] or other sources[3] have stimulated a resurgence in the commercial formulation of oxygen-transporting colloids. Typically, the oxyhemoglobin colloids have a di-α or di-β crosslink to prevent tetramer dissociation and to enhance oxygen dissociation (P_{50}) that is superior to banked erythrocytes.[1,3,4] However, the hemodynamic response to hemodilution with oxyhemoglobin colloids is not completely similar to that using conventional colloids. Despite a similar reduction in blood viscosity,[5] hemodilution with oxyhemoglobin produces no change in cardiac output,[5–11] although hemodilution with conventional colloids will cause a significant increase.[5,11–15] The nonaugmented cardiac output with oxyhemoglobin cannot be simply correlated with such factors as heart rate,[5,7,8,9,11] blood pressure,[5–11] blood volume,[6] or myocardial contractility.[5,6,16] The unchanged cardiac output is not caused

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by oxygen availability;[10,17] in fact, oxygen delivery is significantly decreased from prehemodilution values despite the additional plasma oxygen provided by oxyhemoglobin.[7-9]

A growing body of *in vitro* evidence indicates that oxyhemoglobin, but not methemoglobin, produces or enhances vasoconstriction,[17-22] and inhibits vasodilation by endothelium-derived nitric oxide (NO) or related compounds.[18,19,20] Whether, and how, oxyhemoglobin mediates this "vasoconstrictor" property in the systemic circulation, and the role of endothelial-derived NO, are unclear. For example, oxyhemoglobin-mediated changes in venous or arteriolar tone could affect stroke volume, or a selective redistribution of regional flows to capacitance beds could effect venous return.[23,24]

The purpose of the current study was to examine the mechanism(s) of the nonaugmented cardiac output and decreased oxygen delivery of hemodilution with oxyhemoglobin, including the role of endothelial-derived NO. Systemic and regional circulations were evaluated in dogs hemodiluted with oxyhemoglobin, methemoglobin (weak NO inactivator), and albumin, in the absence and presence of an infusion of an exogenous NO donor (sodium nitroprusside).

Materials and Methods

This study was approved by Loyola University Animal Care and Use Committee and performed in accordance with the National Research Council's Guide for the Use of Laboratory Animals. Twenty-nine conditioned, heartworm-free male mongrel dogs (18–27 kg) were anesthetized with sodium pentobarbital (30 mg/kg intravenously) followed by intravenous maintenance dose of 4 $mg \cdot kg^{-1} \cdot h^{-1}$. After the dogs' tracheas were intubated, their lungs were mechanically ventilated (Siemens-Elema 900D Servo Ventilator, Solna, Sweden) with 100% oxygen at a rate sufficient to achieve normocarbia.

Surgical Preparation

The dog was placed supine and polyethylene catheters were inserted for monitoring arterial pressure, collection of reference microspheres, isovolemic exchange transfusion, administration of intravenous fluids, and collection of blood samples. A 5-French thermodilution catheter was inserted into the pulmonary artery for measurement of cardiac output and right atrial pressure, and for monitoring blood temperature, which was maintained at 39° C with water-circulated heating pads. A Foley catheter was inserted into the bladder for urine collection. Under fluoroscopy, a 5-French volume-conductance catheter (Mansfield Webster, Baldwin Park, CA) was inserted, *via* the left carotid artery, across the aortic valve to the apex of the left ventricle to measure instantaneous volume change. An 8/10-French Fogarty venous thrombectomy catheter was placed, *via* the left femoral vein, into the inferior vena cava just above the diaphragm to produce occlusive unloading of the left ventricle.

The animal was then placed on its right side and paralyzed with pancuronium bromide (0.15 mg/kg). A left thoracotomy was performed in the fourth intercostal space. The exposed lung was retracted and 5 cm H_2O positive end-expiratory pressure was instituted to prevent atelectasis. A small incision was made in the pericardium near the left atrial appendage. The appendage was protracted and a PE 90 catheter was inserted for microsphere injection. A 3-French micromanometer-tipped pressure catheter (Millar, Houston, TX) was then inserted, *via* the appendage, into the left ventricle for pressure recording. The exposed thoracic surface was covered with plastic film to prevent evaporation.

Measurements and Calculations

Heart rate, aortic pressure (P_{ao}), left ventricular pressure and volume, and right atrial pressure (P_{ra}) were recorded continuously on an analog thermal array recorder (Gould Model TA4000, Cleveland, OH). Cardiac output (CO) was measured in triplicate using a Spectramed Hemopro 1 (Oxnard, CA) thermodilution computer. Systemic vascular resistance (SVR), in $dyne \cdot s \cdot cm^{-5}$, was calculated from ([mean P_{ao} − mean P_{ra} in mmHg] ÷ CO) × 80 $dyne \cdot s \cdot l \cdot cm^{-5} \cdot mmHg^{-1} \cdot min^{-1}$. Systemic vascular hindrance (SVH) was calculated from SVR ÷ η,[15] where η is the apparent viscosity of whole blood in centipoise (cps).

Arterial blood pH, P_{CO_2}, P_{O_2}, and electrolytes were measured with a Nova Stat Profile 1 analyzer (Waltham, MA). Plasma colloid osmotic pressure (COP) was determined with a Wescor 4400 Colloid Osmometer (Logan, UH). Blood viscosity was measured with a Brookfield Cone/Plate DV-II viscometer (Stoughton, MA) at shear rates ($\approx$ 225 s^{-1}) assumed in the aorta.[15] Hematocrit was determined volumetrically. Oxyhemoglobin (gm%), methemoglobin (%), and percent oxygen saturation were measured with a cooximeter (Model 482; Instrumentation Laboratories, Lexington,

MA). Oxygen content was measured with the cooximeter and added to the dissolved oxygen ($0.003 \times P_{O_2}$) to give total blood oxygen content (vol%). Whole-body oxygen delivery ($\dot{D}O_2$) in ml/min was calculated from CO × arterial blood O_2 content. Blood cyanide levels (mg/l) were measured spectrophotometrically by SmithKline Beecham Clinical Laboratories (Schaumburg, IL). The lower limit of cyanide detection with this method is 0.1 mg/l and the covariance is 12.0%.

Total blood volume was computed from plasma volume (indicator dilution of iodinated I^{125}-albumin; Mallinckrodt Medical, St. Louis, MO) and whole body hematocrit.§ According to Gold and Murray,[12] this method may underestimate total blood volume during hemodilution approximately 6% because of paradoxical sequestration of red cells in the spleen.

Colloid Preparation

Dog hemoglobin (10%) was prepared by a modification of methods from Fairbanks *et al.*[25] and Rooney *et al.*[26] Red cells, stored in CPDA-1, were separated from plasma and washed three times with 5 mmol sodium phosphate-buffered saline (pH 7.4). One volume of red cells was hypotonically lysed with two volumes of 5 mmol sodium phosphate (pH 8.0). The hemolysate-red cell suspension (≈ 12 gm% hemoglobin) was then centrifuged at least $0.7 \times 10^7 g_{max} \cdot min$ at 4° C to remove partially formed red cell ghosts.[26]¶ The hemoglobin supernatant was adjusted to isotonicity with physiologic levels of NaCl, KCl, and $CaCl_2$, and then centrifuged ($0.6 \times 10^6 g_{max} \cdot min$ at 4° C) to separate residual annealed red cell ghosts. The final supernatant (10 gm%) was then passed (flow rate ≈ 250 ml/min) through a series of 1.0-, 0.45-, and 0.25-μm filters (Whatman Polycap, Maidstone, England). Methemoglobin (metHb) was prepared by titration of oxyhemoglobin (oxyHb) with potassium ferricyanide until the solution was 100% metHb as measured with the cooximeter. The metHb solution was dialyzed against 40 volumes of physiologic electrolytes at room temperature. After 8 h, the bath was changed and the dialysis repeated. Human albumin, in normal saline, was purchased as Albuminar-25 (25 gm%) from Armour Pharmaceutical Company (Kankakee, IL) and diluted with saline to 8 gm%. Physical and chemical properties of the colloids are shown in table 1.

Table 1. Physical and Chemical Properties of the Oxyhemoglobin and Albumin Solutions

Property	10% Oxyhemoglobin	8% Albumin
Methemoglobin (%)	0.6 ± 0.2	—
P_{50}* (*p*H 7.4) (mmHg)	23.2 ± 0.6	—
*p*H (U)	7.0 ± 0.3	6.9 ± 0.2
$[Na^+]$ (mM)	150 ± 3	150 ± 3
$[K^+]$ (mM)	2.5 ± 0.2	0.1 ± 0.01
$[Ca^{2+}]$ (mM)	1.2 ± 0.1	0.06 ± 0.01
COP (mmHg)	41 ± 1.1	39 ± 1.0
Viscosity (cps)	1.7 ± 0.2	1.6 ± 0.2
Total lipid† (mg/L)	<2§	91 ± 2
Endotoxin‡ (EU/ml)	<0.13§	<0.13§

Values are mean ± SE; n = 12.

COP = colloid osmotic pressure.

* Determined by methods of Rooney MW, Joseph NJ, Crystal GJ, Salem MR: Ionic and newer nonionic contrast media adversely affect oxyhemoglobin dissociation. ANESTHESIOLOGY 69:A828, 1988.

† Determined with USEPA method 413.1, Springfield, Illinois, 1978.

‡ Determined with limulus amebocyte lysate assay (LAL), BioWhittaker, Inc., Walkersville, MD, according to Cooper JF: Resolving LAL test interferences. J Parenter Sci Technol 44:13–15, 1990.

§ Below lower limit of detection.

Regional Blood Flows and Distribution of Cardiac Output

Regional blood flows ($ml \cdot min^{-1} \cdot 100\ g^{-1}$ tissue) were measured with the reference isotope technique using 15-μ microspheres, as previously described.[27] To maintain isovolemic conditions during baseline sampling of reference microspheres, 8% albumin (≈30 ml) was infused simultaneously. After hemodilution, either oxyhemoglobin or albumin was infused simultaneously. These infusions did not significantly affect hematocrit. After the final injection of microspheres, the heart was stopped by intravenous injection of potassium chloride. Skin and bone (rib) were sampled from a shaved area distal to the thoracotomy. Skeletal muscle samples were taken from the hindlimb, back, forelimb, and head. These and all other organs were weighed. Organ blood flows ($ml \cdot min^{-1} \cdot 100\ g^{-1}$) and organ weights (grams) were used to calculate total organ flow. Organ blood flows (BF_{organ}) were summed to yield total systemic flow (ΣBF_{organ}) from which the fractional distribution of cardiac output to each organ was computed from $BF_{organ} \div \Sigma BF_{organ} \times 100$. Values of CO determined with indicator thermodilution, and ΣBF_{organ} determined with microspheres, were not significantly different, and the latter was used to determine

§ International Committee for Standardization in Haematology: Recommended methods for measurement of red-cell and plasma volume. J Nucl Med 21:793–800, 1980.

¶ Rooney MW: Sickling of liposomes containing hemoglobin S. Master's Thesis, Bioengineering, University of Illinois, 1980.

the fractional distribution (%) to each organ tissue. Skeletal muscle, skin, and bone weights were assumed to be 40, 9, and 8% of body weight, respectively.[28]

Left Ventricular End-Systolic Elastance (E_{lves})

Left ventricular contractility was determined from end-systolic elastance (Ees) using pressure-volume relationships according to methods of Kass *et al.*[29] Briefly, a volume-conductance catheter was used to measure left ventricular volumes simultaneous with pressure during a 15–20-s inflation/deflation of the inferior vena caval occluder. The acquired data were used to construct a set of pressure-volume loops. A linear regression line was fitted to the end-systolic pressure-volume points of the acquired loops. The slope of the regression line, called the end-systolic elastance (E_{lvcs}), is a load-independent measure of global left ventricular contractility. Increases and decreases in E_{lvcs} correspond to increases and decreases in contractility, respectively. Changes in blood conductivity (inverse of resistivity) that occurred with hemodilution were measured by withdrawing whole blood into a syringe-like cuvette equipped with two ring-shaped stainless-steel excitation electrodes. A 25-kHz AC current was applied, and in-phase voltage reflects the resistivity of the blood. Because only relative volume changes were considered in this study, parallel conductance (offset volume) of structures surrounding the left ventricle was measured only at the start of the study to bring absolute volume signals into the physiologic range. A potential limitation of this technique is that the parallel conductance may change from cardiac cycle to cardiac cycle during an occlusion, thereby skewing the ESPVR in one direction or another. However, studies by Lankford *et al.*[30] have shown that the conductance-offset volume does not change within a cardiac cycle.

Experimental Protocol

On arrival at the laboratory, typically, all dogs had hematocrits of 45 vol% or greater and filling pressures (P_{lved}) less than 5 mmHg. After cannulation, all animals received 8 gm% albumin to increase P_{lved} and to bring hematocrit to near 40 vol%. After 30 min of hemodynamic stability, baseline measurements were obtained. Hemodilution (HD) was produced by simultaneous isovolemic exchange of blood for colloid at a rate of 20 ml/min (≈45 ml/kg) to reduce hematocrit to 50% of baseline (≈20 vol%). Twelve dogs were hemodiluted with 10 gm% oxyhemoglobin (oxyHb-HD), 12 were hemodiluted with 8 gm% albumin (Ab-HD), and 5 were hemodiluted with 10% methemoglobin (metHb-HD). After the exchange transfusions, measurements and samples were obtained within 30 min. To administer a NO donor after HD, sodium nitroprusside (SNP) was infused intravenously until mean P_{ao} decreased > 10 mmHg, indicating the presence of exogenous NO. All 12 dogs in the oxyHb-HD group, 5 of 12 randomly selected dogs in the Ab-HD group, and all 5 dogs in the metHb-HD group received SNP. Standard SNP concentrations (100 μg/ml) were used in the Ab-HD and metHb-HD groups. However, in the oxyHb-HD group, excess volumes of the standard SNP solution produced < 10-mmHg decreases in mean P_{ao} in the first two dogs, as well as unacceptable decreases in hematocrit. In the remaining ten dogs, the SNP dose was increased, by adjusting concentration and the rate of intravenous infusion, in a step-wise manner until mean P_{ao} decreased > 10 mmHg to indicate the presence of NO. The SNP solution was prepared, from 50-mg vials (Elkins-Sinn, Cherry Hill, NJ), so that not more than 50 ml of solution was infused per animal. Blood volume measurements were repeated after the SNP infusion was discontinued.

Statistical Analysis

The paired Student's *t* test was used to compare control and hemodilution data within groups. Analysis of variance (ANOVA) was used in all other cases. Because different SNP concentrations were used in the groups, between-group analyses of the SNP results were not made. When significance with ANOVA was found, a modified Student–Newman–Keuls test (Bonferroni method) was used to make comparisons. Results are expressed as mean ± SE. In all cases, a $P < 0.05$ was considered indicative of a statistically significant difference.

Results

Effects of Each Colloid

Physical and chemical properties of the oxyhemoglobin and albumin solutions are shown in table 1. The diluted commercial albumin solution had lower potassium and calcium concentrations and higher total lipid content compared with the oxyhemoglobin solution. The pH, sodium concentration, colloid osmotic pressure, viscosity, and endotoxin level were not different for the two solutions. Total lipid in the oxyhemoglobin solution was less than the detection limits of the method. Properties of the methemoglobin and oxyhemoglobin solutions were the same, except oxy-

Table 2. Arterial Blood Gases, Electrolytes, and Cyanide Levels during Control, Hemodilution (HD), and HD plus Sodium Nitroprusside (SNP)

	pH (U)	P_{CO_2} (mmHg)	P_{O_2} (mmHg)	Hct (%)	Hb (g/dl)	MetHb (%)	$[Na^+]$ (mM)	$[K^+]$ (mM)	$[Ca^+]$ (mM)	$[CN^-]$ (mg/L)
Oxyhemoglobin (n = 12)										
Control	7.39 ± 0.01	35 ± 1	384 ± 21	42 ± 1	13.9 ± 0.3	0.7 ± 0.1	152 ± 1	3.5 ± 0.1	1.18 ± 0.02	<0.10
HD	7.38 ± 0.01	34 ± 2	411 ± 31	21 ± 1§	11.1 ± 0.5§	0.8 ± 0.1	152 ± 1	3.7 ± 0.2	1.15 ± 0.05	0.13 ± 0.03§
SNP*	7.36 ± 0.02	35 ± 2	424 ± 33	20 ± 1§	10.5 ± 0.2§	1.0 ± 0.1	152 ± 1	3.6 ± 0.1	1.12 ± 0.06	0.46 ± 0.04§¶
Albumin (n = 12)										
Control	7.38 ± 0.02	34 ± 2	371 ± 30	41 ± 1	13.8 ± 0.3	0.8 ± 0.1	154 ± 1	3.4 ± 0.1	1.22 ± 0.02	<0.10
HD	7.36 ± 0.01	36 ± 1	393 ± 32	20 ± 1§	7.0 ± 0.2§	0.8 ± 0.1	152 ± 2	3.2 ± 0.3	1.11 ± 0.03§	<0.10
SNP†	7.33 ± 0.02§¶	35 ± 2	312 ± 42	21 ± 1§	6.9 ± 0.2§	0.5 ± 0.1	151 ± 2	3.2 ± 0.2	1.10 ± 0.02§	<0.10
Methemoglobin (n = 5)										
Control	7.40 ± 0.02	35 ± 2	347 ± 35	39 ± 1	12.8 ± 0.3	0.7 ± 0.2	151 ± 1	3.4 ± 0.1	1.20 ± 0.02	<0.10
HD	7.37 ± 0.03	36 ± 3	311 ± 37	19 ± 1§	10.3 ± 0.5§	34.6 ± 1.1§	150 ± 2	3.7 ± 0.2	1.21 ± 0.04	0.16 ± 0.02§
SNP‡	7.32 ± 0.02§¶	36 ± 2	364 ± 47	18 ± 2§	9.9 ± 0.6§	26.2 ± 1.3§¶	150 ± 1	3.5 ± 0.2	1.23 ± 0.04	0.20 ± 0.03§

Values are mean ± SE.

P_{CO_2} = arterial carbon dioxide tension; P_{O_2} = arterial oxygen tension; Hct = hematocrit; Hb = hemoglobin; metHb = methemoglobin.

* 54.2 ± 4.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 10.

† 2.3 ± 0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 5.

‡ 5.4 ± 0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$.

§ $P < 0.05$ *versus* Control.

¶ $P < 0.05$ *versus* HD.

gen dissociation (P_{50}), which is not relevant to the former. The effects of hemodilution with each colloid on blood parameters are shown in table 2. Arterial pH and blood gases were not different from baseline levels and, except for a small decrease in serum ionized calcium during Ab-HD, electrolyte concentrations did not change from respective controls. Hematocrit was intentionally reduced to about 50% of baseline; however, total hemoglobin did not decrease by this amount during oxyHb-HD or metHb-HD because of the presence of these colloids in the plasma. Whole blood cyanide levels were below the limit of detection during each of the baselines and Ab-HD; however, during oxyHb-HD and metHb-HD, cyanide increased slightly above the lower limit of detection.

Hemodilution with oxyhemoglobin (oxyHb-HD) had no affect on cardiac output, but hemodilution with albumin (Ab-HD) or methemoglobin (metHb-HD) increased cardiac output 65 ± 2% and 63 ± 4% ($P < 0.05$), respectively (table 3). Mean aortic pressure and heart rate were not changed by oxyHb-HD or Ab-HD; however, these parameters were significantly decreased with metHb-HD. Right and left ventricular filling pressures (P_{ra} and P_{lved}) were not changed with oxyHb-HD or Ab-HD, but they decreased about 64% ($P < 0.05$) with metHb-HD. Plasma colloid osmotic pressure increased about 4 mmHg after hemodilution with each of the three colloids. Left ventricular end-systolic elastance (E_{lves}), an index of contractility, and total blood volume were not altered by hemodilution with the colloids.

Figure 1 shows oxygenation parameters of oxyHb-HD, Ab-HD, and metHb-HD. Despite an approximately 50% greater arterial O_2 content in oxyHb-HD compared with Ab-HD or metHb-HD, O_2 delivery was not significantly different ($P < 0.05$) among the three. The compensating effect of increased cardiac output in Ab-HD and metHb-HD permitted only 13–15% decreases in O_2 delivery from prehemodilution control values, although, in oxyHb-HD, the nonaugmented cardiac output caused O_2 delivery to fall proportional to arterial O_2 content. Values for systemic vascular resistance, blood viscosity, and systemic vascular hindrance are given in figure 2. These graphs demonstrate that, if vascular resistance is normalized for changes in viscosity (resistance ÷ viscosity), the actual state of vessel diameter (termed "hindrance" here) is apparent. In the case of Ab-HD, the decreased vascular resistance was caused by, and paralleled, the decreased blood viscosity and, therefore, no actual change in vessel diameter oc-

Table 3. Hemodynamic Parameters during Control, Hemodilution (HD), and HD plus Sodium Nitroprusside (SNP)

	CO (L/min)	P_{ao} (mmHg)	P_{ra} (mmHg)	P_{lved} (mmHg)	P_{pco} (mmHg)	E_{lves} (mmHg/ml)	HR (beats/min)	BV (ml)
Oxyhemoglobin (n = 12)								
Control	2.1 ± 0.1	131 ± 5	2.5 ± 0.4	11.1 ± 1.3	18.6 ± 0.4	4.5 ± 0.4	158 ± 6	1874 ± 69
HD	2.3 ± 0.2	136 ± 6	3.8 ± 0.4	14.1 ± 1.5	22.8 ± 0.8§	4.9 ± 0.6	153 ± 5	1892 ± 125
SNP*	4.1 ± 0.3§¶	110 ± 6§¶	3.8 ± 0.9	10.1 ± 1.7	20.9 ± 0.6§	4.3 ± 0.6	153 ± 11	1805 ± 118
Albumin (n = 12)								
Control	2.3 ± 0.1	136 ± 5	3.4 ± 0.3	10.6 ± 1.2	18.6 ± 0.5	5.3 ± 0.3	160 ± 6	1953 ± 121
HD	3.8 ± 0.2§	126 ± 4	3.2 ± 0.3	8.5 ± 1.2	22.5 ± 0.7§	5.3 ± 0.4	158 ± 6	1856 ± 102
SNP†	3.9 ± 0.2§	80 ± 5§¶	1.4 ± 0.4§¶	2.4 ± 1.7§¶	24.2 ± 1.0§	5.0 ± 0.7	155 ± 9	1901 ± 111
Methemoglobin (n = 5)								
Control	2.6 ± 0.2	132 ± 8	2.8 ± 0.4	9.1 ± 1.1	17.9 ± 0.9	4.6 ± 0.2	170 ± 12	1729 ± 112
HD	4.4 ± 0.3§	98 ± 6§	1.0 ± 0.1§	3.3 ± 0.9§	21.6 ± 0.7§	4.4 ± 0.3	153 ± 11§	1875 ± 59
SNP‡	5.0 ± 0.4§	70 ± 8§¶	1.2 ± 0.8§	5.2 ± 0.9§	21.0 ± 1.0§	4.1 ± 0.6	147 ± 13§	1872 ± 69

Values are mean ± SE.

CO = cardiac output; P_{ao} = mean aortic pressure; P_{ra} = mean right atrial pressure; P_{lved} = mean left ventricular end-diastolic pressure; P_{pco} = plasma colloid osmotic pressure; E_{lves} = left ventricular end-systolic elastance; HR = heart rate; BV = blood volume.

* 54.2 ± 4.76 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 10.

† 2.3 ± 0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 5.

‡ 5.4 ± 0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$.

§ $P < 0.05$ *versus* Control.

¶ $P < 0.05$ *versus* HD.

curred. During metHb-HD, however, some vasodilation was evident. In the case of oxyHb-HD, although calculated systemic resistance was not changed, blood viscosity decreased 51 ± 4% ($P < 0.05$) and systemic vascular hindrance increased 102 ± 6% ($P < 0.05$). Thus, with Ab-HD or metHb-HD, cardiac output increased as viscosity decreased, thereby averting substantial reductions in O_2 delivery. With oxyHb-HD, the flow-increasing effects of reduced viscosity were masked by systemic vasoconstriction, and O_2 delivery decreased with arterial O_2 content.

As shown in table 4, the nonaugmented cardiac output (and decreased O_2 delivery) of oxyHb-HD did not involve a redistribution of regional organ blood flows to beds with slow transit times (*e.g.*, splanchnic). In fact, flows were selectively redistributed from other beds to skeletal muscle and myocardium (fast transit-time beds), but with no net affect on cardiac output. Although regional hindrances were not determined, the data in table 4 indicate that the vasoconstrictor effects of oxyHb-HD were less evident in skeletal muscle, myocardium, and brain. In Ab-HD and metHb-HD, regional flows increased in approximate proportion to their respective cardiac outputs (tables 5 and 6) and there was no evidence of a redistribution of flows to beds with fast transit times (*i.e.*, extrasplanchnic).

Effect of SNP on Each Colloid

Standard hypotensive doses of SNP were not effective in oxyHb-HD, and large doses (54.2 ± 4.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$) decreased mean P_{ao} only 19 ± 1% (table 3). However, the large SNP doses markedly increased CO, 78 ± 5% ($P < 0.05$), from oxyHb-HD alone. Right and left ventricular filling pressures (P_{ra} and P_{lved}) were not significantly affected by the large SNP doses, indicating that, during oxyHb-HD, the NO-dilating properties of SNP were selectively effective only in resistance vessels, and not in capacitance beds. Neither left ventricular contractility (E_{lves}) nor heart rate were affected by SNP. In Ab-HD and metHb-HD, much smaller SNP doses (2.3 ± 0.4 and 5.4 ± 0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively) produced a 37 ± 1% and 29 ± 2% decrease ($P < 0.05$) in mean P_{ao}, respectively (table 3). In Ab-HD, the SNP infusion significantly decreased P_{ra} and P_{lved} (56 ± 4% and 72 ± 9%, respectively; $P < 0.05$) indicating NO-dilating effects in both resistance and capacitance vessels. Except for modest decreases in arterial pH in Ab-HD and metHb-HD, the

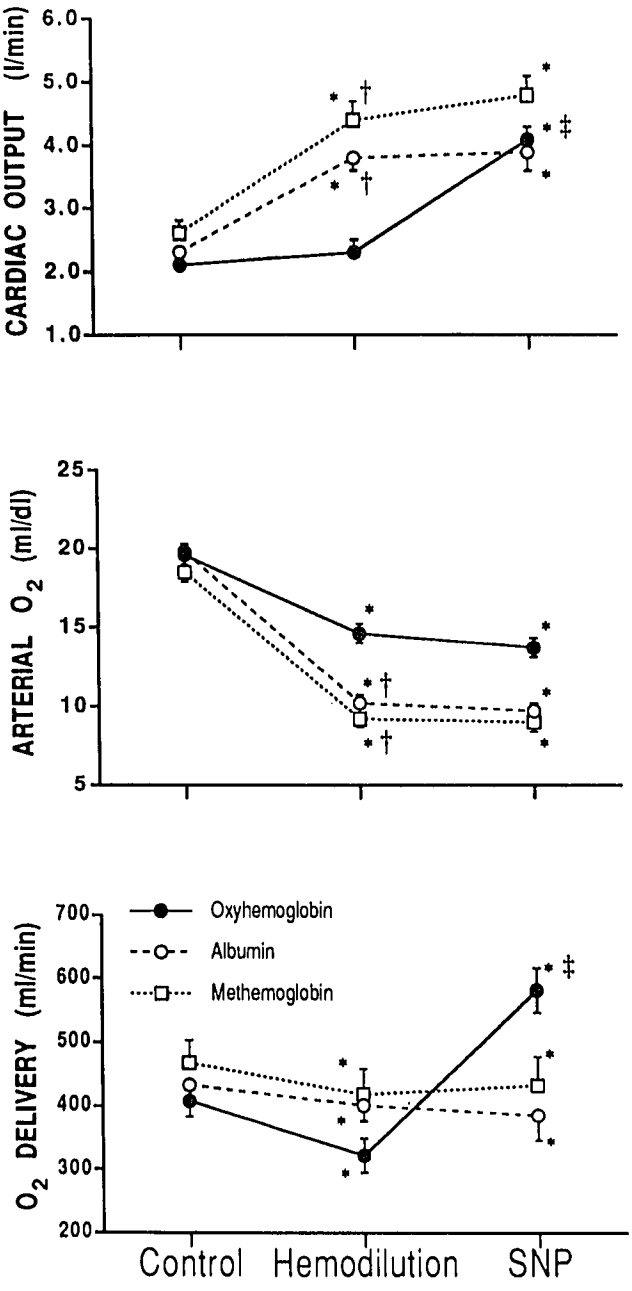

Fig. 1. Oxygenation parameters during control, hemodilution with each colloid, and hemodilution plus intravenous infusion of sodium nitroprusside (SNP). Details provided in legend of table 2 or 3. *$P < 0.05$ from control; †from oxyhemoglobin-hemodilution; ‡from within-group hemodilution.

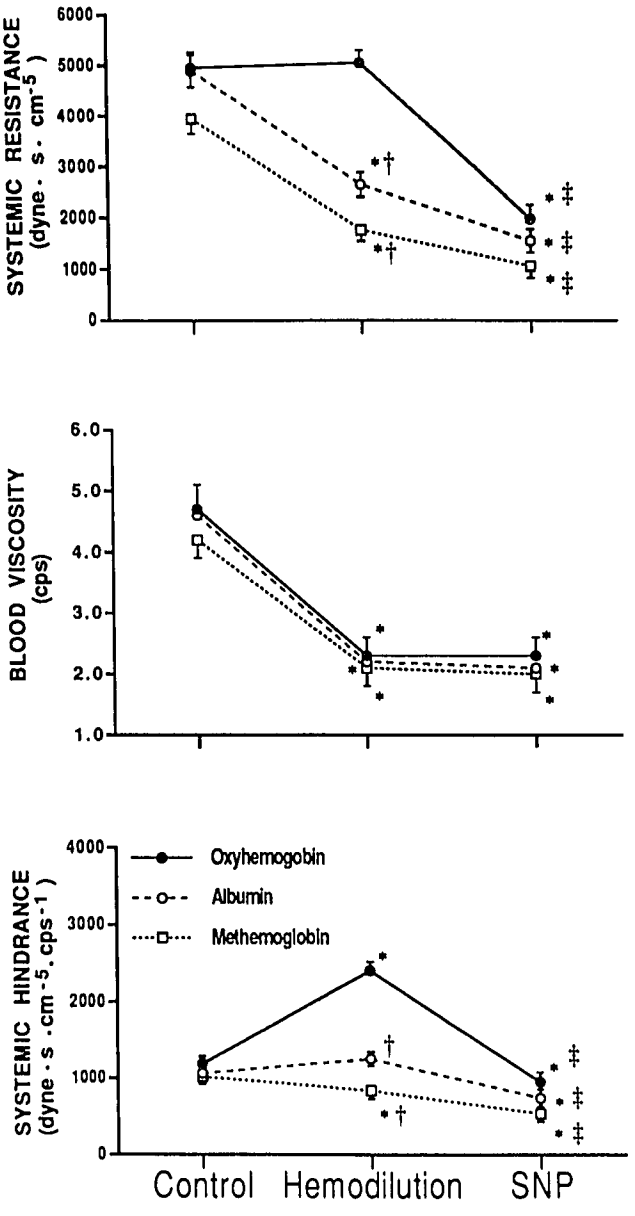

Fig. 2. Systemic vascular resistance, blood viscosity, and systemic vascular hindrance during control, hemodilution with each colloid, and hemodilution plus intravenous infusion of sodium nitroprusside (SNP). Details provided in legend of table 2 or 3. *$P < 0.05$ from control; †from oxyhemoglobin-hemodilution; ‡from within-group hemodilution.

SNP infusions produced no significant change in blood gases or electrolytes (table 2). At the time of SNP infusion in metHb-HD, endogenous conversion of methemoglobin to oxyhemoglobin was indicated by the significant decrease in total methemoglobin levels from HD alone. The effects of methemoglobin conversion on the dilating potency of SNP were not studied in a concentration-dependent manner; however, about twice the SNP dose used in Ab-HD was needed in metHb-HD to achieve comparable decreases in mean

Table 4. Regional Blood Flows (ml · min^{-1} · 100 g^{-1}) and Fraction of Cardiac Output (% CO) during Control, Hemodilution with Oxyhemoglobin (OxyHbHD), and OxyHbHD plus 54.2 ± 4.6 μg · kg^{-1} · min^{-1} Sodium Nitroprusside (OxyHbHD + SNP)

Organ	Control		OxyHbHD		OxyHbHD + SNP (n = 10)	
	Blood Flow	% CO	Blood Flow	% CO	Blood Flow	% CO
Kidney	577 ± 38	32.9 ± 1.8	492 ± 45	27.5 ± 2.3	564 ± 87	11.5 ± 1.2*†
GI tract	34 ± 3	11.7 ± 0.7	37 ± 3	12.9 ± 0.7	105 ± 10*†	17.7 ± 2.2
Spleen	162 ± 19	5.2 ± 0.6	152 ± 34	5.1 ± 0.8	155 ± 43	2.3 ± 0.5*†
Pancreas	24 ± 4	0.6 ± 0.1	27 ± 3	0.6 ± 0.1	55 ± 9*†	0.8 ± 0.3
Hepatic	35 ± 7	8.3 ± 1.1	27 ± 5	6.8 ± 1.1	86 ± 19*†	8.0 ± 1.8
Bronchial	59 ± 10	7.0 ± 1.4	39 ± 6*	3.4 ± 0.7*	144 ± 64*†	5.7 ± 2.7
Muscle	3.1 ± 0.4	13.5 ± 1.1	5.3 ± 0.7*	21.1 ± 1.5*	9.6 ± 1.4*†	18.7 ± 2.5*
Skin	2.5 ± 0.5	2.2 ± 0.2	3.3 ± 0.4	2.8 ± 0.2	9.1 ± 0.6*†	4.2 ± 0.9
Bone	13.9 ± 1.5	10.5 ± 1.2	11.4 ± 1.8	8.4 ± 1.0	16.5 ± 2.7	6.9 ± 1.1*
R Vent	52 ± 4	0.9 ± 0.1	99 ± 9*	1.4 ± 0.1*	413 ± 76*†	3.3 ± 0.5*†
L Vent	92 ± 7	3.7 ± 0.4	173 ± 16*	6.3 ± 0.5*	564 ± 86*†	12.0 ± 1.8*†
Septum	80 ± 7	1.2 ± 0.1	174 ± 20*	2.1 ± 0.2*	563 ± 85*†	4.3 ± 0.9*†
Brain	27 ± 1	1.3 ± 0.1	42 ± 3*	1.7 ± 0.2	93 ± 14*†	1.8 ± 0.3

Values are mean ± SEM; n = 12.

GI tract = stomach, small and large intestines; Hepatic = arterial flow; Bronchial = arterial flow; R Vent = right myocardium; L Vent = left myocardium; Brain = cerebrum and cerebellum.

* $P < 0.05$ *versus* Control

† $P < 0.05$ *versus* OxyHb-HD.

P_{ao} (table 3). In oxyHb-HD, although arterial pH was not affected, the large SNP infusions resulted in small but significant increases in blood cyanide levels from oxyHb-HD alone. In metHb-HD, the SNP infusions did not change blood cyanide levels from metHb-HD alone.

The SNP-mediated increases of CO during oxyHb-HD had profound effects on O_2 delivery (fig. 1). Although cardiac output increased to levels observed in Ab-HD (≈4 l/min), the greater arterial O_2 of oxyHb-HD caused O_2 delivery to increase 77 ± 5% ($P < 0.05$) from oxyHb-HD alone and 43 ± 3% from prehemodilution control values. Because the SNP infusions in Ab-HD

Table 5. Regional Blood Flows (ml · min^{-1} · 100 g^{-1}) and Fraction of Cardiac Output (% CO) during Control and Hemodilution with Albumin (AbHD)

Organ	Control		AbHD	
	Blood Flow	% CO	Blood Flow	% CO
Kidney	634 ± 34	31.1 ± 1.1	997 ± 72*	28.0 ± 0.9
GI tract	37 ± 3	12.7 ± 0.8	82 ± 5*	15.3 ± 0.7
Spleen	163 ± 18	5.8 ± 0.5	186 ± 27	3.5 ± 0.3*
Pancreas	21 ± 2	0.5 ± 0.1	52 ± 5*	0.6 ± 0.1
Hepatic	33 ± 3	9.4 ± 0.7	70 ± 5*	9.5 ± 0.6
Bronchial	64 ± 12	9.2 ± 2.2	177 ± 50*	9.0 ± 2.1
Muscle	2.9 ± 0.2	13.5 ± 1.2	7.1 ± 0.3*	14.2 ± 0.7
Skin	2.5 ± 0.2	2.4 ± 0.2	6.6 ± 0.7*	2.7 ± 0.3
Bone	11.5 ± 1.1	9.4 ± 0.9	22.0 ± 1.8*	9.4 ± 0.6
R Vent	56 ± 3	0.9 ± 0.1	178 ± 17*	1.6 ± 0.2*
L Vent	93 ± 4	3.4 ± 0.2	283 ± 25*	5.4 ± 0.4*
Septum	86 ± 5	1.1 ± 0.1	270 ± 22*	2.7 ± 0.2*
Brain	32 ± 2	1.4 ± 0.2	82 ± 6*	1.5 ± 0.1

Values are mean ± SEM; n = 12.

GI tract = stomach, small and large intestines; Hepatic = arterial flow; Bronchial = arterial flow; R Vent = right myocardium; L Vent = left myocardium; Brain = cerebrum and cerebellum.

* $P < 0.05$ *versus* Control.

Table 6. Regional Blood Flows (ml · min^{-1} · 100 g^{-1}) and Fraction of Cardiac Output (% CO) during Control and Hemodilution with Methemoglobin (metHbHD)

	Control		metHbHD	
Organ	Blood Flow	% CO	Blood Flow	% CO
Kidney	488 ± 43	24.1 ± 2.5	877 ± 94*	23.5 ± 2.9
GI tract	57 ± 2	17.4 ± 2.8	115 ± 10*	16.3 ± 0.8
Spleen	187 ± 27	6.1 ± 1.1	196 ± 25	2.6 ± 0.7*
Pancreas	38 ± 3	0.7 ± 0.1	48 ± 4	0.4 ± 0.1*
Hepatic	41 ± 12	11.5 ± 3.5	118 ± 23*	13.1 ± 2.5
Bronchial	54 ± 3	6.0 ± 0.7	102 ± 37*	6.2 ± 1.2
Muscle	4.3 ± 0.1	13.9 ± 3.4	8.9 ± 0.2*	14.5 ± 1.3
Skin	3.2 ± 0.2	2.5 ± 0.6	7.9 ± 0.8*	3.5 ± 1.0
Bone	20.1 ± 1.2	11.4 ± 0.8	21.6 ± 1.3	6.5 ± 0.8*
R Vent	88 ± 8	0.7 ± 0.1	295 ± 34*	1.4 ± 0.1*
L Vent	137 ± 11	3.6 ± 0.3	487 ± 56*	7.5 ± 1.1*
Septum	124 ± 8	1.1 ± 0.1	528 ± 40*	2.6 ± 0.2*
Brain	35 ± 4	1.0 ± 0.1	94 ± 9*	1.2 ± 0.2

Values are mean ± SEM; n = 5.

GI tract = stomach, small and large intestines; Hepatic = arterial flow; Bronchial = arterial flow; R Vent = right myocardium; L Vent = left myocardium; Brain = cerebrum and cerebellum.

* $P < 0.05$ *versus* Control.

and metHb-HD had no significant affect on cardiac output or regional blood flows (data not shown), O_2 delivery also was not changed (fig. 1). Statistical comparisons of the effects of SNP were not made between oxyHb-HD, Ab-HD, and metHb-HD because of the different SNP doses used in each group. However, the SNP-induced increase in O_2 delivery during oxyHb-HD is assumed to be significant from all three baselines and hemodilution groups. In figure 2, the dilating effects of SNP were minor in Ab-HD and metHb-HD, but were markedly obvious in oxyHb-HD. The SNP-induced decrease (61 ± 3%) in systemic vascular resistance during oxyHb-HD was primarily caused by the reduction in blood viscosity that was made apparent by the parallel decrease in systemic hindrance. As seen in the regional blood flows shown in table 4, the decreased hindrance in oxyHb-HD unmasked the flow-increasing effects of reduced viscosity. Renal and splenic blood flow, however, were not increased from control levels, demonstrating that, in the presence of oxyHb-HD, SNP was not effective in these vascular beds. In contrast, blood flow to skeletal muscle and myocardium more than tripled from control values, again reflecting selectively less "vasoconstrictor" effects of oxyHb and greater SNP effectiveness in these vascular beds. In general, the SNP infusion during oxyHb-HD produced increases in regional blood flows that were very similar to those observed during Ab-HD and metHb-HD (tables 4–6).

Discussion

Oxygen delivery during hemodilution with oxyhemoglobin colloid (oxyHb) is comparatively the same as hemodilution with conventional colloids, despite the additional oxygen provided by plasma oxyHb. Basically, this response is caused by a lack of increased cardiac output that, otherwise, would have resulted from the reduced blood viscosity. An examination of the determinants of cardiac output revealed that, in dogs hemodiluted with oxyHb, there was an increase in the geometric (vasoconstrictive) component of afterload that opposed and countered the reduced viscosity component. When the vasoconstrictive component of afterload was attenuated with a vasodilator (sodium nitroprusside), the large (viscosity-mediated) increases in cardiac output were observed. It is of particular significance that the hemodynamic consequences of this pharmacologic intervention resulted in a superaugmentation of oxygen delivery above prehemodilution levels, a finding that reveals a potential method and advantage of using oxyhemoglobin colloids over conventional hemodiluents.

The attenuated $\dot{D}O_2$ and unchanged CO with oxyHb-HD is not a new finding. Early studies by Sunder-Plassmann *et al.*[6] and Moss *et al.*[7] showed that isovolemic exchanges of blood with oxyhemoglobin did not produce a hyperdynamic state characteristic of anemia,

i.e., increased cardiac output and stroke volume. Conventional hemodiluents, such as albumin, increase systemic flow, primarily by reducing the viscosity component of resistance.[13] At the level of the heart, stroke volume, rather than rate, increases because the viscosity component of left ventricular afterload is reduced.[14] Changes in preload, contractility, and arteriolar tone may be contributing factors, but are not essential.[13] In the case of hemodilution with oxyhemoglobin, blood viscosity is reduced to the same extent, or even lower, than hemodilution with conventional colloids,[5] and, in the immediate stages, the unchanged cardiac output is not caused by alterations in blood pressure,[5-11] blood volume,[6] heart rate,[5,7-9,11] contractility,[5,6,16] or physical state of the oxyhemoglobin (*i.e.*, intermolecularly crosslinked).[8,9] Other studies have shown that oxygen availability is not a factor, because oxyhemoglobins with a wide range of oxygen affinity (P_{50}) produce the same response.[10,17] In fact, oxygen delivery is significantly decreased from prehemodilution levels, despite additional plasma oxygen provided by oxyhemoglobin.[7-9]

The above studies indicate that, indeed, the unchanged cardiac output with oxyhemoglobin-hemodilution appears to involve peripheral hemodynamics and the resultant effects on ventricular-vascular coupling. If oxyhemoglobin reduced venomotor tone in capacitance vessels, the pressure gradient for venous return would be decreased and, ultimately, so would ventricular preload.[13] Or, as is indicated by *in vitro* evidence,[17-22] if resistance vessels are affected, there may be a problem with afterload. However, if oxyhemoglobin causes a redistribution of regional blood flow to vascular beds with long transit times (*i.e.*, splanchnic), ultimately, venous return would be decreased.[23,24] In the current study, oxyHb-HD did not cause a redistribution of regional blood flows to splanchnic beds. In fact, blood flows were redistributed from other organs to skeletal muscle and myocardium (fast transit-time beds),[31] indicating selective regional effects of oxyHb-HD. Net systemic flow, however, was unchanged. During albumin-hemodilution (Ab-HD), the increased cardiac output was not associated with a redistribution of regional flows to organ beds with faster transit times. Right atrial and left ventricular end-diastolic pressures were not significantly affected in either group, indicating that the respective cardiac output responses were not caused by changes in filling pressures or preload.

For analysis of the arterial side, we used the relationship[15]

$$R = \eta \cdot Z,$$

to quantify vasoconstriction, where R = systemic vascular resistance (mean $P_{ao} - P_{ra} \div CO$); η = whole blood viscosity; and Z = vascular geometric hindrance. Increased hindrance implies vasoconstriction.[15] With normal hematocrit values and constant blood viscosity, changes in resistance usually reflect alterations in vascular hindrance. In the current study, hemodilution decreased hematocrits to about 20%, causing blood viscosity (η) to fall to one-half of baseline levels. With oxyHb-HD, resistance (R) was constant and, thus, hindrance (Z) was approximately doubled. With Ab-HD, both resistance and viscosity decreased about 50% and, therefore, no change in hindrance was evident, indicating that increased cardiac output was caused entirely by the flow-increasing effects of reduced viscosity. With oxyHb-HD, it appears that increased hindrance, caused by vasoconstriction of resistance vessels, countered the flow-increasing effects of reduced viscosity and, therefore, there was no net change in cardiac output. This dominant effect of vasoconstriction over viscosity becomes obvious if one considers Poiseuille's Law, which states that:

$$Q = \frac{dP \cdot \pi \cdot r^4}{8 \cdot \eta \cdot L},$$

where Q = flow; dP = pressure gradient; r = radius of the vessel; η = viscosity of the fluid; and L = length of the vessel. Because flow varies directly with the fourth power of vessel radius and inversely with the first power of fluid viscosity, changes in vessel radius (vasoconstriction) may have considerably more influence than changes in fluid viscosity. This fact may explain the lack of increased cardiac output in experiments that conducted extreme hemodilution with oxyhemoglobin.[8-10]

The use of 100% oxygen to ventilate the animals' lungs (normobaric hyperoxia) may be a vasoconstrictor influence.[32,33] Hyperoxia can generate such toxic oxygen metabolites[34] as superoxide anion (O_2^-), a known inactivator of NO,[35] and hydroxyl radical ($\cdot OH$), a membrane destabilizer.[22] Dedichen *et al.*[32] reported that the usual increase in cardiac output with dextran-hemodilution was not affected, whether ventilation was

under normobaric hyperoxia or under hyperbaric hyperoxia. However, cardiac output was attenuated after hemodilution if the initial condition of normobaric hyperoxia was switched to hyperbaric hyperoxia. In a related study, Chapler *et al.*[33] found that switching from normobaric normoxia to normobaric hyperoxia, after dextran-hemodilution, caused significant attenuation of the augmented cardiac output. Because the animals in our groups were ventilated in the same way at all times, and with no switching of oxygenation parameters, hyperoxia should not have been a factor. Furthermore, in other studies,[5-11] the nonaugmented cardiac output during oxyHb-HD is consistent, whether the animals were ventilated with room air or 100% oxygen. Another potential oxygen-related vasoconstrictor influence is the generation of O_2^- or $\cdot OH$ during the oxidation of oxyhemoglobin to methemoglobin.[22] Methemoglobin levels, in our study, were not increased during oxyHb-HD, indicating that, in this case, an "oxygen-driven" mechanism is an unlikely cause of vasoconstriction.

In vitro studies[18,19] have shown that oxyhemoglobin inhibits the vasodilation by endothelial-derived NO (EDNO) and related compounds. In contrast, methemoglobin has a markedly reduced EDNO inhibition[18,19] as well as weak and unstable binding to NO.[36] In the current study, animals hemodiluted with methemoglobin showed no evidence of vasoconstriction, and cardiac output and all regional blood flows were increased in a manner similar to hemodilution with albumin. The absence of increased systemic hindrance by methemoglobin indicates that the nonaugmented cardiac output and systemic vasoconstriction in oxyHb-hemodiluted dogs may be caused by *in vivo* inactivation of EDNO by oxyHb. Although direct binding by oxyHb, with inactivation of EDNO, can be invoked from *in vitro* studies,[18,19,36] we cannot rule out the possibility that oxyHb, but not metHb, caused release of a local vasoconstrictor, such as endothelin.

Sodium nitroprusside (SNP) was infused after oxyHb-HD to test whether a NO donor would override the vasoconstriction that, as indicated by *in vitro* evidence,[18-21] could be caused by inactivation of EDNO. The advantage of using SNP as a NO donor, unlike other nitrovasodilators, are its equieffectiveness in the venous and arterial circulations** with no apparent need of cofactors or oxidative processes.[37,38] The disadvantage of using SNP is the cyanide byproduct generated at some point during its decomposition.[39,40] In the current study, cyanide levels increased during SNP infusion, but never reached toxic or hemodynamic-altering levels.[41-43]

To decrease mean P_{ao} in oxyHb-HD, the dose of SNP had to be increased to 20 times the amount used in Ab-HD. This indicates that the NO potency, *i.e.*, vasodilation, of SNP in oxyHb-HD was attenuated, either from an endogenously released vasoconstrictor or, as indicated by *in vitro* studies,[18,19] *via* direct inactivation of SNP NO by oxyHb. However, when mean P_{ao} was decreased by SNP in oxyHb-HD, simultaneous increases in cardiac output were observed. Furthermore, the combination of augmented cardiac output and arterial oxygen provided by oxyHb resulted in an 80% increase in whole-body $\dot{D}O_2$ from oxyHb-HD alone, and a 45% increase in $\dot{D}O_2$ from prehemodilution levels. The SNP administration during oxyHb-HD did not decrease right atrial and left ventricular end-diastolic pressures as it did in Ab-HD. Because there were no changes in blood volume, cardiac contractility, heart rate, or pleural pressure during oxyHb-HD, and because afterload (mean P_{ao} component) was decreased, the unchanged filling pressures indicated a greater antagonism of SNP effectiveness in the venous circulation compared with the arterial circulation. Although SNP decreased the vasoconstrictive component of afterload (mean P_{ao}), the reduction in the viscosity component was now made apparent by the large increases in cardiac output. In regional circulations, the SNP-induced dilation of resistance vessels resulted in large increases in blood flow that were primarily caused by the presence of low viscosity. Therefore, because of the peculiar effectiveness of the nitrovasodilator to decrease only arteriolar tone and unmask the resistance-lowering effects of reduced blood viscosity, $\dot{D}O_2$ was superaugmented during oxyHb-hemodilution as a result of the increased cardiac output and greater arterial O_2 content (50% more than albumin-hemodilution).

The selective resistance to SNP in systemic veins may be related to findings that SNP relaxes smooth muscle in canine veins by a "hyperpolarizing" mechanism, rather than by a direct binding of NO to the ferrous heme moiety on soluble guanylate cyclase.[44,18,19] This "hyperpolarizing" mechanism, or factor, is neither inactivated by oxyhemoglobin nor potentiated by super-

** Van Zwieten PA: Vasodilator drugs with direct action on smooth muscle. Handbook of Hypertension: Pharmacology of Antihypertensive Drugs 3:308–330, 1984.

oxide dismutase plus catalase.[44] In the current study, a selective antagonism of SNP by oxyHb was also observed in regional resistance beds—in particular, the renal circulation, where flow never increased as it did in the albumin group. In general, the SNP infusion increased blood flow in most of the organ beds in a manner similar to albumin-hemodilution; however, the relative effectiveness of SNP in vascular beds was as follows: skeletal muscle = myocardium > most arterial beds > systemic veins = renal arteries.

In summary, the mechanism of nonaugmented CO and decreased $\dot{D}O_2$ during oxyHb-hemodilution appears to involve an increased vasoconstrictive component of afterload that counters the reduced viscosity component. Thus, stroke volume and cardiac output were not augmented, and $\dot{D}O_2$ was attenuated by the hemodilution-mediated decreases in arterial O_2 content. Infusion of a nitrovasodilator (SNP) reversed the vasoconstriction and unmasked viscosity-induced increases in CO and regional blood flows. However, the dilating effectiveness of SNP was significantly affected by oxyHb-hemodilution. Unusually large doses of SNP were needed to decrease mean P_{ao}, and there were no changes indicative of venodilation. Furthermore, SNP caused selective distribution of the increased CO to myocardium and skeletal muscle. As a result of the augmented CO and plasma oxyHb O_2, $\dot{D}O_2$ was increased markedly from prehemodilution levels. The attenuated effectiveness of SNP in oxyHb-hemodilution, and the absence of vasoconstriction with methemoglobin (weak NO inactivator), indicates that the molecular mechanism of the oxyHb-mediated vasoconstriction may involve *in vivo* inactivation of endothelial-derived NO.

This study raises new questions about the evolving pharmacology of oxyHb colloids and their interaction with nitrovasodilators. Because many nitrovasodilators are activated locally in vascular smooth muscle, and would be less vulnerable to inactivation by plasma oxyHb, would these nitrovasodilators be more suitable? Would nonnitro vasodilators be as effective? Regarding the "vasoconstrictor" mechanism, would the same hemodynamic effects, *i.e.*, no increase in cardiac output, be evident in the albumin group if L-NMMA, a competitive inhibitor of NO synthase,[18] was administered before hemodilution? Does oxyHb deplete L-arginine, the substrate for NO synthesis?[18] In any event, these studies may have bearing on the design of the O_2-delivering properties of oxyhemoglobin colloids. Moreover, the ability to combine oxyHb-hemodilution and suitable pharmacologic management to superaugment $\dot{D}O_2$, at essentially the same metabolic costs as albumin-hemodilution, is a finding that, potentially, could render oxyHb colloids more clinically useful than conventional colloids, particularly for individuals with a compromised circulation who would benefit from an increased oxygen supply.

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References

1. Looker D, Abbott-Brown D, Cozart P, Durfee S, Hoffman S, Mathews AJ, Miller-Roehrich J, Shoemaker S, Trimble S, Fermi G, Komiyama NH, Nagai K, Stetler GL: A human recombinant haemoglobin designed for use as a blood substitute. Nature 356:258–260, 1992

2. Behringer RR, Ryan TM, Reilly MP, Asakura T, Palmiter RD, Brinster RL, Townes TM: Synthesis of functional human hemoglobin in transgenic mice. Science 245:971–973, 1989

3. Vandegriff KD, Medina F, Marini MA, Winslow RM: Equilibrium oxygen binding to human hemoglobin cross-linked between the α chains by bis (3,5-dibromosalicyl) fumarate. J Biol Chem 264:17824–17833, 1989

4. Keipert PE, Aderiran AJ, Kwong S, Benesch RE: Functional properties of a new cross-linked hemoglobin designed for use as a red cell substitute. Transfusion 29:768–773, 1989

5. Biro GP: Comparison of acute cardiovascular effects and oxygen-supply following haemodilution with dextran, stroma-free haemoglobin solution and fluorocarbon suspension. Cardiovasc Res 16:194–204, 1982

6. Sunder-Plassmann L, Dieterle R, Seifert J, Jesch F, Messmer K: Stromafree haemoglobin solution as a blood replacement fluid. Actual state and problems. Eur J Int Care Med 1:37–42, 1975

7. Moss GS, DeWoskin R, Rosen AL, Levine H, Palani CK: Transport of oxygen and carbon dioxide by hemoglobin-saline solution in the red cell-free primate. Surg Gynecol Obstet 142:357–362, 1976

8. Hobbhahn J, Vogel H, Kothe N, Brendel W, Peter K, Jesch F: Hemodynamics and oxygen transport after partial and total blood exchange with pyridoxalated polyhemoglobin in dogs. Acta Anaesthesiol Scand 29:537–543, 1985

9. Vlahakes GJ, Lee R, Jacobs EE Jr, LaRaia PJ, Austen WG: Hemodynamic effects and oxygen transport properties of a new blood substitute in a model of massive blood replacement. J Thorac Cardiovasc Surg 100:379–388, 1990

10. Rosen AL, Gould S, Sehgal LR, Noud G, Sehgal HL, Rice CL, Moss GS: Cardiac output response to extreme hemodilution with hemoglobin solutions of various P_{50} values. Crit Care Med 7:380–383, 1979

11. Rooney MW, Crystal GJ, Salem MR: Regional blood flows during hemodilution with dextran or hemoglobin solution (abstract). FASEB J 3:A433, 1989

12. Gold PM, Murray JF: Changes in red cell distribution, hemodynamics, and blood volume in acute anemia. J Appl Physiol 26: 589–593, 1969

13. Chapler CK, Cain SM: The physiologic reserve in oxygen carrying capacity: Studies in experimental hemodilution. Can J Physiol Pharmacol 64:7–12, 1986

14. Murray JF, Escobar E, Rapaport E: Effects of blood viscosity on hemodynamic responses in acute normovolemic anemia. Am J Physiol 216:638–642, 1969

15. Fan F-C, Chen RYZ, Schuessler GB, Chien S: Effects of hematocrit variations on regional hemodynamics and oxygen transport in the dog. Am J Physiol 238:H545–H552, 1980

16. Yamakawa T, Miyauchi Y, Nishi K: Effects of pyridoxalated hemoglobin polyoxyethylene conjugate and stroma-free hemoglobin on cardiac function in isolated rat heart. Artif Organs 14:208–217, 1990

17. Vogel WM, Dennis RC, Cassidy G, Apstein CS, Valeri CR: Coronary constrictor effect of stroma-free hemoglobin solutions. Am J Physiol 251:H413–H420, 1986

18. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142, 1991

19. Ignarro LJ, Byrns RE, Buga GM, Wood KS: Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ Res 61:866–879, 1987

20. Hongo K, Kassell NF, Nakagomi T, Sasaki T, Tsukahara T, Ogawa H, Vollmer DG, Lehman RM: Subarachnoid hemorrhage inhibition of endothelium-derived relaxing factor in rabbit basilar artery. J Neurosurg 69:247–253, 1988

21. Tanishima T: Cerebral vasospasm: Contractile activity of hemoglobin in isolated canine basilar arteries. J Neurosurg 53:787–793, 1980

22. Steele JA, Stockbridge N, Maljkovic G, Weir B: Free radicals mediate actions of oxyhemoglobin on cerebrovascular smooth muscle cells. Circ Res 68:416–423, 1991

23. Greenway CV, Lautt WW: Blood volume, the venous system, preload, and cardiac output. Can J Physiol Pharmacol 64:383–387, 1986

24. Hoka S, Siker D, Bosnjak ZJ, Kampine JP: Alteration of blood flow distribution and vascular capacitance during induced hypotension in deafferented dogs. ANESTHESIOLOGY 66:647–652, 1987

25. Fairbanks G, Steck TL, Wallach DFH: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606–2617, 1971

26. Rooney MW, Lange Y, Kauffman JW: Acyl chain organization and protein secondary structure in cholesterol-modified erythrocyte membranes. J Biol Chem 259:8281–8285, 1984

27. Rooney MW, Crystal GJ, Salem MR: Influence of nifedipine on systemic and regional hemodynamics during adenosine-induced hypotension in dogs. Anesth Analg 68:261–269, 1989

28. Quillen EW, Reid IA: Effect of intravertebral angiotensin II on cardiac output and its distribution in conscious dogs. Circ Res 63:702–711, 1988

29. Kass DA, Beyar R, Lankford E, Heard M, Maughan WL, Sagawa K: Influence of contractile state on curvilinearity of in situ end-systolic pressure-volume relations. Circulation 79:167–178, 1989

30. Lankford EB, Kass DA, Maughan WL, Shoukas AA: Does volume catheter parallel conductance vary during a cardiac cycle? Am J Physiol 258:H1933–H1942, 1990

31. Caldini P, Permutt S, Waddell JA, Riley RL: Effect of epinephrine on pressure, flow, and volume relationships in the systemic circulation of dogs. Circ Res 34:606–623, 1974

32. Dedichen H, Race D, Schenk WG: Hemodilution and concomitant hyperbaric oxygenation: Effects on cardiovascular function. J Thorac Cardiovasc Surg 53:341–348, 1967

33. Chapler CK, Cain SM, Stainsby WN: The effects of hyperoxia on oxygen uptake during acute anemia. Can J Physiol Pharmacol 62: 809–814, 1984

34. Zweier JL, Duke SS, Kuppusamy P, Sylvester JT, Gabrielson EW: Electron paramagnetic evidence that cellular oxygen toxicity is caused by the generation of superoxide and hydroxyl free radicals. FEBS Lett 252:12–16, 1989

35. Rubanyi GM, Vanhoutte PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. Am J Physiol 250: H822–H827, 1986

36. Sharma VS, Traylor TG, Gardiner R, Mizukami H: Reaction of nitric oxide with heme proteins and model compounds of hemoglobin. Biochemistry 26:3837–3843, 1987

37. Bassenge E, Mulsch A: Anti-ischemic actions of molsidomine by venous and large coronary dilatation in combination with antiplatelet effects. J Cardiovasc Pharmacol 14:S23–S28, 1989

38. Feelisch M: The biochemical pathways of nitric oxide formation from nitrovasodilators: Appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. J Cardiovasc Pharmacol 17:S25–S33, 1991

39. Butler AR, Glidewell C: Recent chemical studies of sodium nitroprusside relevant to its hypotensive action. Chem Soc Rev 16: 361–380, 1987

40. Vesey CJ, Sweeney B, Cole PV: Decay of nitroprusside II: In vivo. Br J Anaesth 64:704–709, 1990

41. Vesey CJ, Krapez JR, Varley JG, Cole PV: The antidotal action of thiosulfate following acute nitroprusside infusion in dogs. ANESTHESIOLOGY 62:415–421, 1985

42. Michenfelder JD: Cyanide release from sodium nitroprusside in the dog. ANESTHESIOLOGY 46:196–201, 1977

43. Krasney JA: Cardiovascular responses to cyanide in awake sinoaortic denervated dogs. Am J Physiol 220:1361–1366, 1971

44. Miller VM, Vanhoutte PM: Is nitric oxide the only endothelium-derived relaxing factor in canine femoral veins? Am J Physiol 257: H1910–H1916, 1989