

Amy G. Tsai, Barbara Friesenecker, Michael McCarthy, Hiromi Sakai and Marcos Intaglietta

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P. Cabrales, B. Y. S. Vazquez, A. G. Tsai and M. Intaglietta

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P. Cabrales and A. G. Tsai

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Blood viscosity maintains microvascular conditions during normovolemic anemia independent of blood oxygen-carrying capacity

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Am J Physiol Heart Circ Physiol, August 1, 2006; 291 (2): H581-H590.

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Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skinfold model

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Tsai, Amy G., Barbara Friesenecker, Michael McCarthy, Hiromi Sakai, and Marcos Intaglietta. Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skinfold model. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H2170–H2180, 1998.—Effect of increasing blood viscosity during extreme hemodilution on capillary perfusion and tissue oxygenation was investigated in the awake hamster skinfold model. Two isovolemic hemodilution steps were performed with 6% Dextran 70 [molecular weight (MW) = 70,000] until systemic hematocrit (Hct) was reduced by 65%. A third step reduced Hct by 75% and was performed with the same solution [low viscosity (LV)] or a high-molecular-weight 6% Dextran 500 solution [MW = 500,000, high viscosity (HV)]. Final plasma viscosities were 1.4 and 2.2 cP (baseline of 1.2 cP). Hct was reduced to $11.2 \pm 1.1\%$ from $46.2 \pm 1.5\%$ for LV and to $11.9 \pm 0.7\%$ from $47.3 \pm 2.1\%$ for HV. HV produced a greater mean arterial blood pressure than LV. Functional capillary density (FCD) was substantially higher after HV ($85 \pm 12\%$) vs. LV ($38 \pm 30\%$) vs. baseline (100%). PO_2 levels measured with Pd-porphyrin phosphorescence microscopy were not statistically changed from baseline until after the third hemodilution step. Wall shear rate (WSR) decreased in arterioles and venules after LV and only in arterioles after HV. Wall shear stress (WSR \times plasma viscosity) was substantially higher after HV vs. LV. Increased mean arterial pressure and shear stress-dependent release of endothelium-derived relaxing factor are possible mechanisms that improved arteriolar and venular blood flow and FCD after HV vs. LV exchange protocols.

functional capillary density; shear rate; shear stress; dextran; tissue oxygenation

HEMODILUTION CHANGES the physical properties of blood by reducing the number of circulating red blood cells (RBCs) thus decreasing the oxygen-carrying capacity of blood and blood viscosity. During moderate levels of hemodilution, reduction of the systemic hematocrit (Hct) up to 50% is compensated for with a decrease in blood viscosity leading to an increased blood flow velocity and decreased diffusional oxygen exit from arterioles, resulting in augmented or maintained oxygen delivery to tissue (28, 30). In addition, reduction of systemic Hct during intentional hemodilution is not mirrored at the microcirculatory level, since capillary Hct is sustained near control levels (26, 29, 37, 38) thus maintaining tissue oxygenation.

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When systemic Hct is reduced by 60% or more (Hct that is 40% of normal or less) there is a significant decrease in blood viscosity and a lowering of blood hemoglobin concentration to levels where oxygen-carrying capacity is marginal and tissue oxygenation may be impaired. Under these conditions, oxygen delivery to tissue hinges on increasing oxygen extraction and maintaining the surface area for exchange, which is determined by the functional capillary density (FCD).

Blood viscosity is mainly determined by the concentration of RBCs, whereas the shear rate at the wall is a function of the plasma viscosity (15). The viscous properties of the plasma and the plasma layer in setting vessel wall shear stress are particularly significant in the microcirculation due to the migration of RBCs to the centerline of the vessel (1). Viscosity and flow velocity of blood define shear stress at the blood vessel wall, which stimulates vascular endothelium to produce vasoactive substances such as prostacyclin (12) and nitric oxide [NO (4)]. In moderate hemodilution, the reduction in viscosity is compensated by the increased flow; thus the shear stress sensed by the endothelium does not change significantly.

We hypothesize that, during extreme hemodilution, the cardiac output can no longer be sustained, and consequently blood flow will not develop the sufficient shear stress required for shear stress-dependent release of mediators, resulting in a reduced capillary perfusion. This is not the situation found in moderate hemodilution where the reduction in Hct and thus blood viscosity is compensated for by an increased blood velocity. The shear stress sensed by the endothelium in this situation is actually increased due to the increased flow and an essentially unchanged plasma viscosity. To determine if viscosity is a factor involved in regulating FCD and microvascular flow during extreme hemodilution, we designed a study in which we lower oxygen-carrying capacity but augment plasma viscosity by exchanging blood with a high-viscosity (HV) ultrahigh-molecular-weight dextran solution. Increased plasma viscosity at extreme hemodilution levels was analyzed in terms of microvascular tone, blood flow, FCD, and oxygen distribution to determine the functional state of the microvasculature under these conditions.

MATERIALS AND METHODS

Animal Preparation

Investigations were performed on 55- to 65-g golden Syrian hamsters (Simonsen, Gilroy, CA). Animal handling and care were provided following the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (National Re-

search Council, 1996). The study was approved by the local Animal Subjects Committee. The hamster chamber window model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere (8, 11). Briefly, the animal was prepared for chamber implantation with a 5 mg/kg ip injection of pentobarbital sodium anesthesia. After hair removal, sutures were used to lift the dorsal skin away from the animal, and one frame of the chamber was positioned on the animal's back. A chamber consisted of two identical titanium frames with a 15-mm circular window. With the aid of backlighting and a stereomicroscope, one side of the skinfold was removed following the outline of the window until only a thin monolayer of retractor muscle and the intact subcutaneous skin of the opposing side remained. Saline and then a cover glass were placed on the exposed skin held in place by the other frame of the chamber. The intact skin of the other side was exposed to the ambient environment. The animal was allowed at least 2 days for recovery; then its chamber was assessed under the microscope ($\times 650$) for any signs of edema, bleeding, or unusual neovascularization. Barring these complications, the animal was anesthetized again with pentobarbital sodium. Arterial and venous catheters were implanted in the carotid artery (PE-50) and jugular vein (PE-10), respectively. The catheters were filled with a heparinized saline solution (30 IU/ml) to ensure their patency at the time of experiment. Catheters were tunneled under the skin and exteriorized at the dorsal side of the neck where they were attached to the chamber frame with tape. The experiment was performed after at least 24 h but within 48 h after catheter implantation.

Inclusion criteria. Animals were suitable for the experiments if 1) systemic parameters were within normal range, namely, heart rate (HR) >320 beats/min, mean arterial blood pressure (MAP) >80 mmHg, systemic Hct $>45\%$, and arterial $PO_2 >50$ mmHg; and 2) microscopic examination of the tissue observed under $\times 650$ magnification did not reveal signs of edema or bleeding.

Systemic Parameters

MAP was measured continuously over the entire experimental period, except during the actual blood exchange, by attaching the arterial catheter to a pressure transducer (Beckman Recorder; Spectramed Pressure Transducer). HR was determined from the pressure trace. Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge; Clay Adams, Division of Becton-Dickinson, Parsippany, NJ).

Blood Chemistry and Rheology

Arterial blood sampled in two heparinized capillary tubes from the carotid artery catheter was immediately analyzed for arterial PO_2 , PCO_2 , and pH at $37^\circ C$ using a pH/blood gas analyzer (model 248; Chiron Diagnostics, Norwood, MA). Hemoglobin content of blood was determined from a drop of blood using a handheld photometric device (B-Hemoglobin Photometer Hemocue). Blood samples for viscosity and colloid osmotic pressure measurements were quickly withdrawn from the animal with a heparinized 3-ml syringe at the end of the experiment for immediate analysis or refrigerated for next-day analysis. The small animal size allowed for only ~ 2 ml of blood to be withdrawn from the animal after an experiment, which was not sufficient volume to measure colloid osmotic pressure, blood viscosity, and plasma viscosity. Because of the small sample volume, only one or two of these parameters could be measured in each animal. Measurements were then combined and taken to be representative of

the entire group. Blood samples were centrifuged, and colloid osmotic pressure in the plasma was measured using a colloid osmometer (model 420; Wescor, Logan, UT). Calibration of the osmometer was made with a 5% albumin solution using a 30,000 molecular weight cut off membrane (Amicon, Danvers, MA) (44). The viscosity of plasma and whole blood was determined at a shear rate of 160 s^{-1} at $37^\circ C$ in a 500- μm -diameter capillary viscometer (33).

Microhemodynamic Parameters

Detailed mappings were made of the chamber vasculature so that the same vessels studied in control could be followed throughout the experiment. Functional capillaries, defined as those capillary segments that have RBC transit of at least a single RBC in a 30-s period, were assessed in 10 successive microscopic fields, totaling a region of $\sim 0.46 \text{ mm}^2$. Observation of the fields was done systematically by displacing the microscopic field of view by a field width in 10 successive steps in the lateral direction (relative to the observer). Each step was viewed on the video monitor and was 240 μm long when referred to the tissue. The first field was chosen by a distinctive anatomic landmark (i.e., large vascular bifurcation) to easily and quickly reestablish the same fields and vessels at each observation time point. Each field had between two and five capillary segments with RBC flow. FCD (cm^{-1}), i.e., total length of RBC perfused capillaries divided by the area of the microscopic field of view, was evaluated by measuring and adding the length of capillaries that had RBC transit in the field of view. The relative change in FCD from baseline levels after each intervention is indicative of the extent of capillary perfusion.

Arteriolar and venular blood flow velocity were measured on-line using the photodiode/cross-correlator system of Intaglietta et al. (19; Fiber Optic Photo Diode Pickup and Velocity Tracker model 102B; Vista Electronics, San Diego, CA). The measured centerline velocity was corrected according to vessel size to obtain the mean RBC velocity (V ; see Ref. 27). The video image shearing technique was used to measure vessel diameter (D) on-line (Digital Video Image Shearing Monitor model 908; Vista Electronics). Blood flow rate (\dot{Q}) is equal to the mean RBC velocity times the cross-sectional area of the vessel and was calculated according to the expression $\dot{Q} = V \cdot \pi(D/2)^2$. Wall shear stress (WSS) is defined by the formula

$$\text{WSS} = \text{WSR} \cdot \eta \quad (1)$$

where WSR is the wall shear rate expressed as $8 \cdot VD$, and η is the plasma viscosity.

Intravascular and Extravascular PO_2 Measurements

Oxygen tension measurements were made using palladium-porphyrin phosphorescence quenching microscopy (39, 45), which is based on the relationship between the decay rate of excited palladium-mesotetra-(4-carboxyphenyl)porphyrin (Porphyrin Products, Logan, UT) bound to albumin and the partial pressure of oxygen according to the Stern-Volmer equation (45). Animals received a slow intravenous injection of 15 mg/kg body wt at a concentration of 10.1 mg/ml of the phosphorescence dye ~ 10 min before PO_2 measurements. Albumin exchange between plasma and tissue allows sufficient concentrations of albumin-bound dye within the interstitium to achieve an adequate signal-to-noise ratio for interstitial PO_2 measurements (41). Simultaneous tissue PO_2 measurements using this system and the classic microelectrode technique have found nearly identical values (3).

In our system described in detail elsewhere (39), intravascular measurements are made by placing an optical rectangular window within the vessel of interest, and the longest side of the rectangle is positioned parallel to the vessel wall. Intravascular PO_2 measurements were made in large feeding arterioles, smaller arcading arterioles, large venules, and smaller collecting venules. Tissue PO_2 measurements were made in interstitial regions devoid of large vessels with a $10\ \mu\text{m}$ by $10\ \mu\text{m}$ optical window to obtain an estimate of the lowest oxygen level within the chamber (21). The decay curves are analyzed off-line, using a standard single exponential least squares numerical fitting technique. Resultant time constants are applied to the Stern-Volmer equation to calculate PO_2 , where the quenching constant and the phosphorescence lifetime in the absence of oxygen measured are $325\ \text{mmHg}^{-1}\cdot\text{s}^{-1}$ and $600\ \mu\text{s}$, respectively, in this system. The phosphorescence decay due to quenching at a specific PO_2 yields a single decay constant (42), and in vitro calibration has been demonstrated to be valid for in vivo measurements.

Acute Isovolemic Hemodilution

Progressive hemodilution to a final systemic Hct level of 25% of baseline was accomplished with three isovolemic exchange steps. The volume of each exchange was a percentage of the animal's total blood volume, estimated at 7% of the body weight. The *level 1* exchange of 40% of the blood volume was followed by *level 2* and *level 3* exchanges, where 35% of the blood volume was exchanged at each step. *Level 1* exchange results in a moderate hemodilution. *Level 2* and *level 3* exchanges result in an extreme hemodilution where systemic Hct falls below 40% of normal. Using an infusion pump, we first passed the exchange solution through an in-line $0.22\text{-}\mu\text{m}$ syringe filter and then into the animal via the jugular vein catheter at a rate of $100\ \mu\text{l}/\text{min}$. Blood was simultaneously withdrawn by hand from the carotid artery at the same rate, a method found to be more accurate than using the automated syringe pump where the detection of catheter obstruction usually due to blood clotting is delayed. Because of the size of the animals, a slow rate of exchange was chosen to ensure a stable blood pressure during the exchange period. The animal was given a 5-min recovery period before data acquisition.

Experimental Design

The unanesthetized animal was placed in a restraining tube where it had access to wet feed during the entire experimental period. The animal was given 30 min to adjust to the tube environment before the control systemic parameters (MAP, HR, blood gases, and Hct) were measured. The conscious animal in the tube was then affixed to the microscopic stage of a transillumination intravital microscope (Leitz Ortholux II). The tissue image was projected onto a charge-coupled device camera (COHU 4815-2000) connected to a videocassette recorder (AG-7355; Panasonic) and viewed on a monitor (PVM-1271Q; Sony). The baseline FCD was assessed using a $\times 25$ (numerical aperture = 0.7, Leitz) salt water objective. For easier detection of RBC passage, the contrast between RBCs and tissue was enhanced with a BG12 (420 nm) bandpass filter. The animal, still situated in the restraining tube, was then repositioned on an inverted microscope (IMT-2 Olympus, New Hyde Park, NY) equipped with a $\times 20$ dry objective (numerical aperture = 0.46; Olympus), where arterioles and venules chosen for study (4-7 of each type) were characterized by their blood flow velocity and caliber. Microscopic images were televised on another identical video system. The animal was repositioned on each microscope after each exchange to follow the changes of the

microvascular parameters. Fields of observations and vessels were chosen for study at locations in the tissue where the vessels were in sharp focus. The same fields and vessels were investigated throughout the experiment so that comparisons are related directly to baseline levels.

Animals were randomly divided into the following five experimental groups: 1) control (no hemodilution); 2) *level 1* [hemodilution with Dextran 70 (6% wt/vol mean molecular weight of 70,000; Pharmacia) to Hct of 60% of baseline]; 3) *level 2* (hemodilution with Dextran 70 to Hct of 40% of baseline); 4) *level 3* low viscosity (LV; hemodilution with Dextran 70 to Hct of 25% of baseline); 5) *level 3* HV [hemodilution with Dextran 70 to Hct of 35% of baseline followed by hemodilution with 6% wt/vol Dextran 500 (mean molecular weight: 500,000; Pharmacia) in 0.9% normal saline to Hct of 25% of baseline].

Immediately after baseline systemic measurements, animals in the control group received a bolus injection of palladium-porphyrin dye, and measurements of oxygen distribution and microvascular hemodynamics were performed. In the experimental exchange groups, successive hemodilution was performed after baseline systemic, microvascular, and hemodynamic characterization. After each exchange and the ensuing stabilization period, measurements were performed following the schedule shown in Fig. 1, where exchanges begin every hour, i.e., the second exchange commences exactly 1 h after the first exchange. Repeated PO_2 measurements over time were not made to reduce possible complications from light and dye overexposure, which may affect the microhemodynamic response of the tissue. Blood samples were withdrawn from *level 3* exchange animals at the end of the experiment for subsequent analysis of viscosity and colloid osmotic pressure. The duration of the experiments was between 1 and 5 h depending on the degree of hemodilution.

The concentration of the high-molecular-weight Dextran 500 solution and the exchange protocol were determined from pilot studies where the animals were hemodiluted to the exchange *level 3* with a 25% wt/vol solution, and their venular microvasculature was observed for signs of RBC aggregation, since rheological disturbances appear in vessels with low shear (15). Pilot studies found that animals could withstand an entire third exchange with 6% Dextran 500 solution without visible in vivo RBC aggregation. To avoid this critical concentration of Dextran 500, the *level 3* exchange protocol began with 10% of the total blood volume exchange using 6%

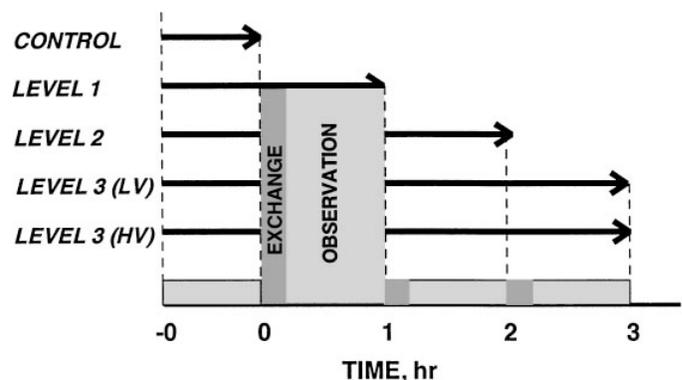


Fig. 1. Time course of experimental protocol. Control/baseline characterization was performed before the exchange protocol. Progressive hemodilution was performed at the beginning of each hour and was followed by an observation period. Three levels of hemodilution were performed. *Levels 1, 2, and 3* low viscosity (LV) were performed with Dextran 70. *Level 3* high viscosity (HV) was performed with a combination of Dextran 70 and high-molecular-weight Dextran 500.

Table 1. *Intrinsic physical characteristics of the dextran solutions*

	Average Molecular Mass, Da	Viscosity,* cP	COP, mmHg
Dextran 70 (6%)	70,000	2.8	49.9
Dextran 500 (6%)	500,000	6.4	31.6

* Shear rate of 160 s⁻¹ at 37°C; COP, colloid osmotic pressure.

Dextran 70 solution followed by 25% of the total blood volume exchange with the 6% Dextran 500. This procedure ensured an increased blood viscosity compared with a *level 3* exchange made solely with Dextran 70 and an absence of rheological complications. Pertinent physical properties of the exchange solutions used in this study are listed in Table 1.

Data Analysis

Results are presented as means \pm SD unless otherwise noted. Data are presented relative to levels at baseline. All measurements were compared with their levels at baseline before the blood exchange except for the PO₂ measurements in which oxygen levels were compared between groups of animals. For repeated measurements, time-related changes were assessed by analysis of variance. Comparison between different groups of animals was performed with the Student's *t*-test and the Mann-Whitney rank sum test. Multiple comparisons were made with the Student-Newman-Keuls test (SigmaStat Windows 95/v2.0; Jandel Scientific). Changes were considered statistically significant at $P < 0.05$.

RESULTS

Thirty-nine animals were entered into this study and assigned randomly to the following protocol exchange groups: control ($n = 9$); *level 1* ($n = 7$); *level 2* ($n = 6$); *level 3* LV ($n = 9$); *level 3* HV ($n = 8$). All animals tolerated the entire hemodilution protocol without visible signs of discomfort except two out of nine animals in the *level 3* LV exchange group who experienced a decline of MAP below 50 mmHg and a complete shut-down in flow to the subcutaneous vascular bed under study.

Systemic and Blood Gas Parameters

Changes in the systemic and blood gas parameters for each experimental group are presented in Table 2. There were no statistically significant differences between experimental groups before the exchange protocol. Systemic Hct after *level 1* and *2* exchange was 0.56 ± 0.04 and 0.40 ± 0.04 of baseline ($P < 0.05$ relative to baseline), respectively. *Level 3* LV and HV exchange reduced the systemic Hct to 0.24 ± 0.02 and 0.25 ± 0.02 of baseline, respectively, which were statistically reduced from baseline ($P < 0.05$) but not statistically different from each other.

MAP was not changed from baseline in the *level 1* exchange group, but upon further hemodilution with Dextran 70 MAP fell to 0.92 ± 0.05 and 0.62 ± 0.10 of baseline in *level 2* and *level 3* LV groups, respectively ($P < 0.05$). In the *level 3* HV group, MAP fell to 0.90 ± 0.09 of baseline ($P < 0.05$), a statistically higher level than the MAP after *level 3* LV exchange. HR was not affected by any of the hemodilution protocols.

Systemic arterial blood gas analysis showed a statistically significant rise in PO₂ from baseline and a fall in PCO₂ with increasing degree of hemodilution in all groups. There were no statistical differences in arterial blood gases between the *level 3* exchange groups. Blood pH was not statistically changed from baseline among all the experimental groups.

Physical Properties of Blood

Comparison of rheological properties and colloid osmotic pressure of the blood after *level 3* LV and HV exchange is presented in Table 3. Changes in blood and plasma viscosity were statistically different from baseline and between HV and LV groups ($P < 0.05$). Blood viscosity was reduced to 0.47 and 0.63 of baseline, and plasma viscosity was increased to 1.15 and 1.83 of baseline levels for *level 3* exchange LV and HV groups, respectively. Colloid osmotic pressure was statistically unchanged during the progressive hemodilution.

Table 2. *Macrohemodynamic parameters before and after blood exchange*

	<i>Level 1</i>		<i>Level 2</i>		<i>Level 3</i> LV		<i>Level 3</i> HV	
	Before	After exchange	Before	After exchange	Before	After exchange	Before	After exchange
Hct, %	49.5 \pm 1.4	0.56 \pm 0.04*	48.8 \pm 1.2	0.40 \pm 0.04*	46.2 \pm 1.5	0.24 \pm 0.02*	47.3 \pm 2.0	0.25 \pm 0.02*
MAP, mmHg	100.9 \pm 5.6	0.99 \pm 0.05	97.5 \pm 5.5	0.92 \pm 0.05*	92.7 \pm 9.6	0.62 \pm 0.10*†	96.9 \pm 9.1	0.90 \pm 0.09*†
HR, beats/min	434.3 \pm 51.3	0.97 \pm 0.07	393.3 \pm 18.9	1.04 \pm 0.06	410.0 \pm 46.6	0.95 \pm 0.15	422.5 \pm 26.1	1.01 \pm 0.07
[Hb], g/dl	14.4 \pm 0.8	0.61 \pm 0.38*	14.2 \pm 0.9	0.39 \pm 0.25*	13.9 \pm 1.1	0.25 \pm 0.02*	14.3 \pm 0.9	0.27 \pm 0.02*
PaO ₂ , mmHg	60.2 \pm 7.7	1.19 \pm 0.15*	65.9 \pm 5.1	1.25 \pm 0.11*	59.7 \pm 7.2	1.76 \pm 0.16*	56.1 \pm 3.5	1.82 \pm 0.12*
PaCO ₂ , mmHg	55.4 \pm 3.7	1.03 \pm 0.06	57.3 \pm 3.7	0.82 \pm 0.14*	62.1 \pm 7.5	0.76 \pm 0.13*	65.8 \pm 4.8	0.72 \pm 0.08*
Arterial pH	7.33 \pm 0.2	1.00 \pm 0.00	7.35 \pm 0.03	1.00 \pm 0.01	7.36 \pm 0.04	0.99 \pm 0.01	7.36 \pm 0.03	1.00 \pm 0.01

Values are means \pm SD. Before values are absolute values obtained at baseline conditions. After exchange values are presented as a fraction of the Before values and serve as an indicator of the relative change from baseline. No significant differences were detected between the baseline values of each group or between the values after the second exchange before the exchange with the high-viscosity solution Dextran 500. *Level 1*, hemodilution with Dextran 70 (6% wt/vol mean mol wt of 70,000); *level 2*, hemodilution with Dextran 70 to hematocrit of 40% of baseline; *level 3* low viscosity (LV), hemodilution with Dextran 70 to hematocrit of 25% of baseline; *level 3* high viscosity (HV), hemodilution with Dextran 70 to hematocrit of 35% of baseline followed by hemodilution with 6% wt/vol Dextran 500 (mean mol wt 500,000) in 0.9% normal saline to hematocrit of 25% of baseline; Hct, systemic hematocrit; MAP, mean arterial blood pressure; HR, heart rate; [Hb], hemoglobin content of blood; PaO₂, arterial partial O₂ pressure; PaCO₂, arterial partial pressure of CO₂. * $P < 0.05$ vs. baseline; † $P < 0.05$, *level 3* LV vs. *level 3* HV.

Table 3. *Rheological properties and colloid osmotic pressure*

	Blood Viscosity, cP		Plasma Viscosity, cP		COP, mmHg	
	Mean	n	Mean	n	Mean	n
Level 3 LV	2.12 ± 0.35*†	3	1.38 ± 0.14*†	3	16.7 ± 1.3	3
Level 3 HV	2.80 ± 0.22*†	5	2.19 ± 0.08*†	3	15.6 ± 0.9	5
Whole blood	4.47 ± 0.50	8	1.20 ± 0.04	4	17.5 ± 1.7	6

Values are means ± SD; n, no. of animals studied. * $P < 0.05$ vs. whole blood; † $P < 0.05$ between groups, low and high viscosity.

Microhemodynamics

Vascular tone. The changes in the diameter of large feeding and small arcading arterioles (range, 23–95 μm) and small collecting venules and large venular vessels (range, 24–203 μm) were measured after each hemodilution step. Figure 2A shows that arteriolar diameter was unchanged after *level 1* exchange. Upon further blood exchange to *level 2*, arterioles dilated to 1.15 ± 0.28 ($n = 46$, $P < 0.001$) of baseline. This trend reversed after *level 3* LV exchange, resulting in a slight arteriolar vasoconstriction to 0.94 ± 0.15 ($n = 49$, $P < 0.05$) of baseline. After the *level 3* HV exchange, arterio-

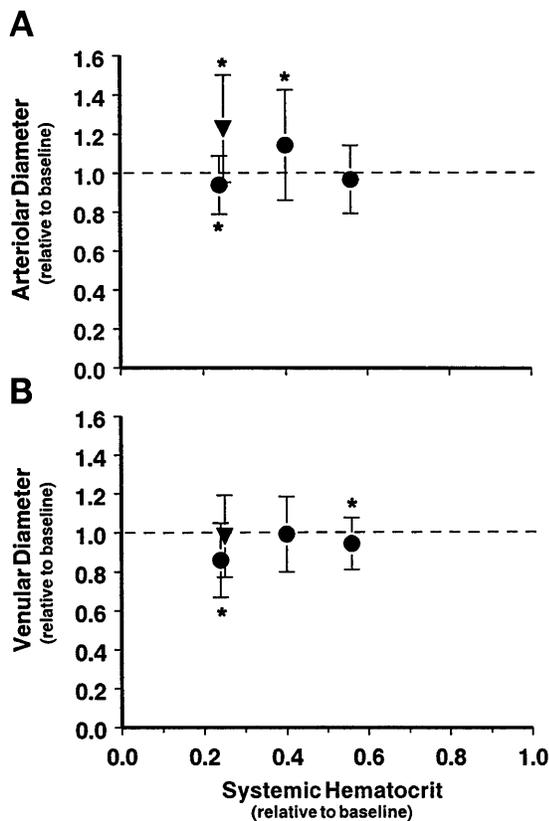


Fig. 2. Vascular tone vs. systemic hematocrit. Data are presented as means ± SD. ●, Dextran 70 exchange; ▼, Dextran 500 exchange. Broken line represents baseline level. * $P < 0.05$. Baseline diameters (μm) in each animal group were as follows: *level 1* [arterioles (A): 62.4 ± 18.2 , $n = 44$, venules (V): 68.8 ± 37.5 , $n = 42$]; *level 2* (A: 57.9 ± 17.8 , $n = 46$, V: 70.9 ± 39.2 , $n = 36$); *level 3* LV (A: 58.6 ± 12.3 , $n = 49$, V: 78.5 ± 23.9 , $n = 37$); *level 3* HV (A: 57.4 ± 15.3 , $n = 47$, V: 68.9 ± 32.7 , $n = 38$). n, No. of vessels studied.

lar diameter remained dilated at 1.22 ± 0.28 ($n = 47$, $P < 0.001$) of baseline.

Venular changes due to the hemodilution protocol are shown in Fig. 2B as a function of the Hct. Venules responded to *level 1* exchange by constricting to 0.95 ± 0.13 of baseline ($n = 42$, $P < 0.05$) and then returning to baseline levels after *level 2* exchange ($n = 36$). When the exchange protocol was continued to the *level 3* LV protocol, the venules constricted to 0.85 ± 0.20 ($n = 37$, $P < 0.001$) of baseline. *Level 3* HV protocol did not change venular diameters from baseline levels ($n = 38$).

RBC velocity and blood flow. Figure 3 shows the change in RBC velocity in arterioles (A) and venules (B) as a function of the Hct. An increase in both arteriolar and venular RBC velocity was detected after *level 1* exchange to 1.67 ± 1.03 ($P < 0.05$) and 1.55 ± 1.23 ($P < 0.05$) of baseline, respectively. After *level 2* exchange, arteriolar RBC velocity remained increased from baseline (1.39 ± 0.93 , $P < 0.05$), whereas venular RBC velocity returned to baseline levels. *Level 3* LV exchange reduced both arteriolar and venular RBC velocity to 0.67 ± 0.58 and 0.70 ± 0.76 of baseline, respec-

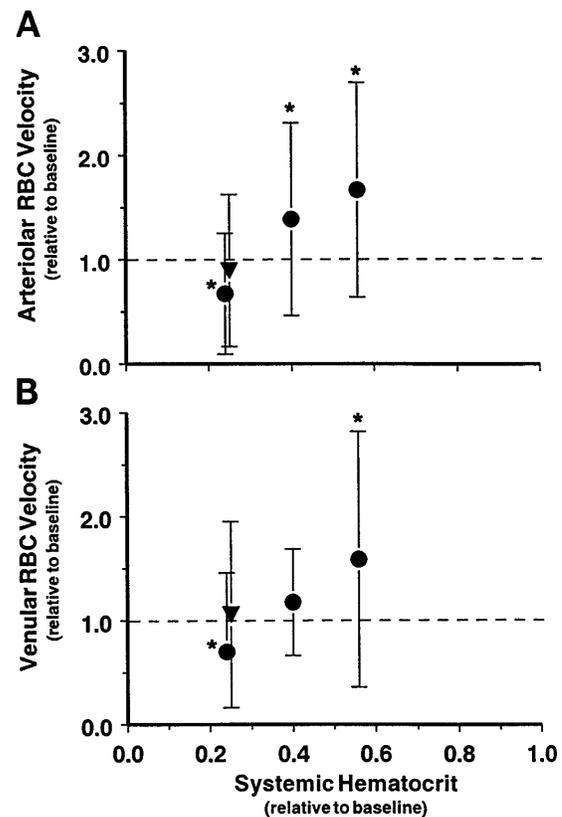


Fig. 3. Arteriolar and venular red blood cell (RBC) velocity vs. systemic hematocrit. Initial increase in arteriolar RBC velocity was followed by a return to baseline with HV protocol, whereas LV protocol led to a reduced RBC velocity. Similar pattern was observed in venular RBC velocity except the return to baseline levels was earlier, occurring after the second exchange. Data are presented as means ± SD. ●, Dextran 70 exchange; ▼, Dextran 500 exchange. Broken line represents baseline level. * $P < 0.05$. Baseline RBC velocities (mm/s) in each animal group were as follows: control (A: 4.9 ± 3.8 , V: 1.0 ± 0.7); *level 1* (A: 4.3 ± 2.4 , V: 1.2 ± 0.8); *level 2* (A: 4.5 ± 2.5 , V: 1.2 ± 1.4); *level 3* LV (A: 4.0 ± 2.3 , V: 1.0 ± 0.8); *level 3* HV (A: 4.1 ± 2.7 , V: 1.1 ± 0.9).

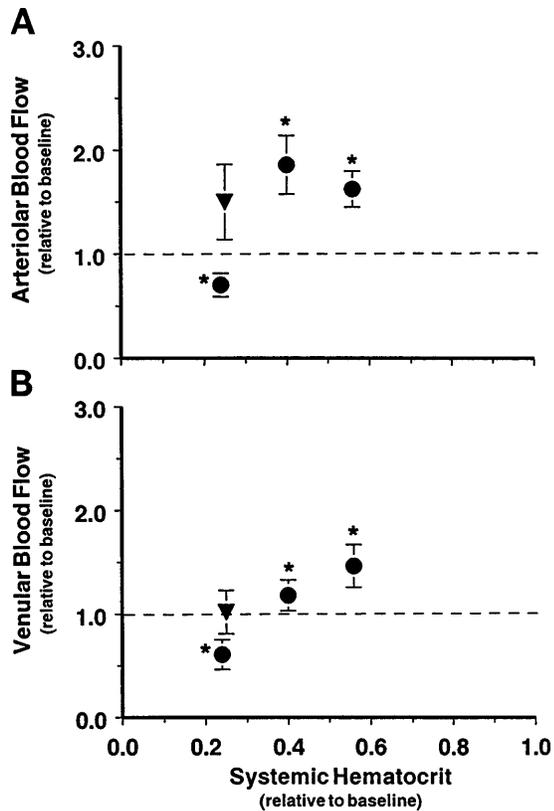


Fig. 4. Arteriolar and venular blood flow vs. systemic hematocrit. Hemodilution led to initial increases in blood flow in both vessel types. At the *level 3* exchange, HV protocol was able to maintain blood flow at baseline levels, whereas LV protocol resulted in reduction. Data are presented as means \pm SE relative to baseline levels. ●, Dextran 70 exchange; ▼, Dextran 500 exchange. Broken line represents baseline level. * $P < 0.05$.

tively ($P < 0.05$). *Level 3* HV exchange did not alter RBC velocity from baseline levels in either vessel type.

The relationships between arteriolar and venular blood flow after the hemodilution protocols and Hct are presented in Fig. 4. The results are given as means \pm SE to show the trend of this parameter calculated from vessel diameter and RBC velocity. Both arteriolar and venular blood flow were statistically increased from baseline after *level 1* and *level 2* exchange. Upon further hemodilution with Dextran 70 these increased levels could not be sustained, and blood flow was statistically reduced from baseline levels in both arterioles and venules. However, *level 3* HV exchange caused a return of the arteriolar and venular blood flow to baseline levels.

Wall Shear Rate

Wall shear rate (s^{-1}) is calculated using the diameter and mean velocity of each vessel studied after *level 3* LV and HV exchanges. The calculated values of wall shear rate are presented as means \pm SE to show the trend rather than the distribution of the parameter. Before the exchange protocol, there were no statistically significant differences in wall shear rate (s^{-1}) among the arterioles (LV: 614.5 ± 35.0 ; HV: 574.5 ± 34.4) and venules (LV: 107.6 ± 18.0 ; HV: 146.7 ± 17.9) in each

group. After the *level 3* LV exchange, arteriolar and venular wall shear rate (s^{-1}) were reduced to 0.68 ± 0.09 ($P < 0.001$) and 0.65 ± 0.15 ($P < 0.001$) of baseline. *Level 3* HV exchange decreased arteriolar wall shear rate to 0.82 ± 0.10 ($P < 0.01$) of baseline. In the venules, wall shear rate was 1.36 ± 0.21 of baseline and not statistically different from baseline. Statistical comparison between the LV and HV groups after the exchange protocol found no statistical difference in arteriolar wall shear rate. However, *level 3* HV exchange did significantly increase wall shear rate relative to the LV exchange protocol in venules ($P < 0.001$).

Functional Capillary Density

Figure 5 shows the effect of hemodilution on the length of RBC perfused capillaries per unit area. FCD after *level 1* exchange was 0.93 ± 0.05 of baseline and not statistically different from baseline. *Level 2* exchange reduced FCD to 0.84 ± 0.04 of baseline ($P < 0.05$). FCD was further reduced after *level 3* LV and HV to 0.38 ± 0.38 and 0.85 ± 0.12 of baseline, respectively ($P < 0.05$). A greater increase in capillary perfusion was obtained after *level 3* exchange with the HV than with the LV protocol ($P < 0.05$). This preparation under normal conditions and during the course of the experimental procedures shows a decrease of FCD of 13% over a period of 6 h. Therefore, the loss in FCD found after *level 2* and *level 3* HV is near the expected change of this parameter.

Microvascular Oxygen Distribution

Oxygen distribution was measured in the animals that had blood flow to the tissue under study. The effect of the hemodilution with Dextran 70 (*level 1*, *2*, and *3* LV) and with augmented plasma viscosity using high-molecular-weight Dextran 500 (*level 3* HV) for arterioles, venules, and tissue is presented in the histograms of Fig. 6. The median and quartiles of each distribution are listed in Table 4. *Level 1* and *2* exchange groups did

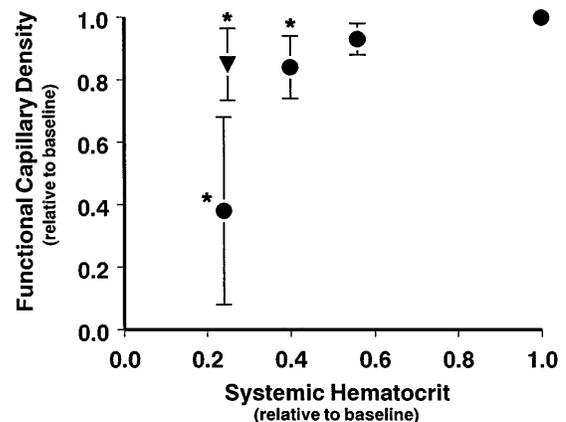
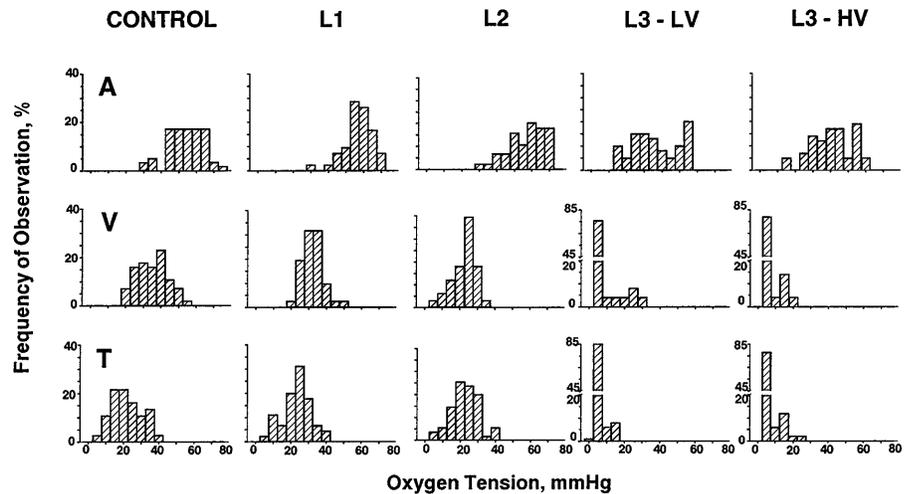


Fig. 5. Effect of hemodilution on capillary perfusion. Functional capillary density (FCD) was unchanged after *level 1* exchange. Drop in FCD was greater after *level 3* LV than *level 3* HV exchange. Data points are means \pm SD relative to baseline. ●, Dextran 70 exchange; ▼, Dextran 500 exchange. * $P < 0.05$. Baseline FCD (cm^{-1}) in each experimental group was as follows: *level 1* (105.8 ± 22.1); *level 2* (121.2 ± 20.9); *level 3* LV (109.2 ± 22.2); *level 3* HV (107.6 ± 22.3).

Fig. 6. Distribution of microvascular PO_2 vs. hemodilution level. A, arterioles; V, venules; T, tissue. Shift in arteriolar PO_2 to the right and venular PO_2 shift to the left after *level 1* and *level 2* exchange maintained tissue oxygenation at baseline levels. *Level 3*, extreme hemodilution, resulted in significant reduction across all categories. Both *level 3* LV and *level 3* HV caused a significant reduction in PO_2 in all categories. PO_2 measurements could only be made in vessels that had blood flow; thus the histograms for *level 3* LV do not include data from 2 animals that did not have blood flow in the tissue under study. Control group vessel diameters (means \pm SD, μm) were A: 57.0 ± 18.5 ($n = 58$), V: 69.9 ± 35.3 ($n = 56$), and RBC velocities (mm/s) were A: 4.9 ± 3.8 ($n = 58$), V: 1.0 ± 0.7 ($n = 56$). n , No. of vessels studied.



not show statistical changes in arteriolar, venular, and tissue PO_2 histograms from the control group. However, the shape of the three distributions narrowed relative to the control group after *level 1* exchange, and the greater separation between the mean arteriolar and venular PO_2 suggests an increased oxygen extraction. After *level 2* exchange, there is a shift in the arteriolar PO_2 toward higher values; however, the changes were not statistically different from the control group. *Level 3* exchange with both LV and HV fluids caused a widening of the arteriolar PO_2 histogram as evidenced by the 25 and 75% quartiles, resulting in a decreased median PO_2 . Both venular and tissue histograms for *level 3* exchange were shifted completely to the left. The PO_2 histograms obtained after *level 3* LV and HV exchange were significantly different from control but were statistically unchanged from each other.

DISCUSSION

Our principal finding is that normal levels of tissue perfusion in terms of arteriolar and venular blood flow and the FCD could be obtained during extreme hemodilution when plasma viscosity is increased by the addition of a high-molecular-weight Dextran 500 solution in the blood volume replacement fluid, a scenario that could not be achieved with a lower-viscosity solution. These results suggest that an increase in plasma viscosity, thus returning whole blood viscosity toward baseline conditions, elicits a regulatory mechanism that becomes inactivated during extreme hemodilution with a lower-viscosity fluid.

Oxygen delivery or systemic oxygen transport capacity during moderate intentional hemodilution is maintained because the decrease in the number of circulating RBCs reduces blood viscosity thereby enhancing cardiac output. Given the premise that higher blood viscosity should decrease blood flow and reduce tissue perfusion, an increase in blood viscosity, particularly with dextrans, which has been shown to cause aggregation by macromolecular bridging (6), could be potentially detrimental (14, 23). In our study, we found that increasing plasma viscosity was beneficial to tissue perfusion. This concept is supported by previous investigators who showed that organ blood flow and oxygen delivery were not impaired by plasma hyperviscosity. Comparison of tissue oxygenation achieved during moderate hemodilution with 3 and 6% Dextran 60, a 33% difference in plasma viscosity, showed similar elevations in PO_2 levels on the surface of liver and skeletal muscle (2). Investigators found that a higher-viscosity replacement fluid was able to achieve increased organ blood flow not observed with a lower-viscosity fluid. Krieter et al. (22) progressively hemodiluted by small infusions of a similar high-molecular-weight dextran used in the present study and found that an increase in plasma viscosity to 3 mPa·s did not compromise perfusion or oxygenation of vital organs. They proposed that the concomitant decrease in Hct from the hemodilution was completely offset by the elevated plasma viscosity, resulting in normal tissue oxygenation and organ perfusion. Use of high-molecular-weight Dextran 500 solutions (molecular weight = 500,000) has also been

Table 4. *Microvascular oxygen tension distribution*

	Control	<i>n</i>	<i>Level 1</i>	<i>n</i>	<i>Level 2</i>	<i>n</i>	<i>Level 3 LV</i>	<i>n</i>	<i>Level 3 HV</i>	<i>n</i>
Arteriolar	51.8 (45.0, 58.8)	58	55.2 (51.2, 59.7)	44	57.3 (46.3, 62.2)	46	32.8*†‡ (23.5, 48.0)	46	39.0*†‡ (29.9, 49.5)	46
Venular	32.7 (25.5, 38.8)	56	29.9 (26.0, 32.2)	42	29.2 (24.7, 37.9)	36	0.5*†‡ (0.3, 5.1)	32	1.4*†‡ (0.5, 4.4)	34
Tissue	17.6 (12.8, 25.8)	37	21.0 (17.3, 27.6)	45	20.4 (15.3, 25.9)	48	0.4*†‡ (0.3, 1.5)	71	0.5*†‡ (0.3, 5.0)	50

Data are presented as medians followed by the 25 and 75% quartiles in parentheses; *n*, no. of vessels studied. Units are mmHg. * $P < 0.05$ vs. control group; † $P < 0.05$ vs. *level 1*; ‡ $P < 0.05$ vs. *level 2*.

shown to better lower mortality and limit the severity of pancreatitis (34) over the levels achieved with lower-weight dextran solutions. In further support of our finding, Waschke et al. (43) found that cerebral perfusion was not compromised when they altered the viscosity of plasma while keeping the oxygen content of blood constant. They achieved this by performing a complete blood exchange with a fluid consisting of cell-free hemoglobin and 2% polyvinylpyrrolidone (molecular weight = 400,000). Chen et al. (5) exchanged whole blood with a 20% wt/vol solution of Dextran 500 in blood, elevating plasma viscosity fourfold (4 mPa·s), and observed compensatory vasodilation reflected by a reduction of vascular hindrance in several vital organs, which served to maintain blood flow and nutrient transport despite the induced hyperviscosity. These investigations on the effects of induced hyperviscosity in different animals and tissues support the concept that induced plasma hyperviscosity is not detrimental to tissue perfusion and in some instances improves perfusion over lower-viscosity fluids, as shown in our study.

Our findings show that the reduction in FCD observed after *level 3* LV exchange can be prevented by hemodilution using high-molecular-weight Dextran 500 to augment plasma viscosity. A mechanism directly related to an increase in plasma viscosity is the enhancement of shear stress via an increase in the viscous drag on the vessel wall. Release of vasodilators from the vascular endothelium such as NO (4) and prostacyclin (12) by endothelial cells has been shown to be shear stress induced. Increase in vessel wall shear stress by elevation of plasma viscosity using Dextran 500 has been shown to induce sustained NO-mediated dilation in the hamster cremaster muscle in vivo (9); increasing the plasma viscosity by 64.3% led to a 24.3% dilation in large feeding arterioles. In our study, increasing the plasma viscosity by 58.7% during the last exchange with Dextran 500 led to a 28.8% arteriolar vasodilation over the levels achieved with Dextran 70 as the sole hemodilutant. Systemic vasodilation, implying a reduction in total peripheral resistance during moderate hemodilution, has also been shown to be a result of endogenous NO release (10). Theoretical analysis applied to artificial blood replacement fluids suggests that lowered blood viscosity during blood substitution without proportionate increases in blood flow to maintain a constant vessel wall shear stress would lead to diminished mechanotransduction of the viscous drag by blood flow to the endothelium, resulting in vasoconstriction (18). Wall shear stress is also directly a function of fluid flow, and increases in blood flow have also been found to induce vasodilation (35). In the present investigation, the increase in arteriolar and venular blood flow achieved with the HV protocol beyond that of the LV protocol suggests that the difference in wall shear stress, increase in viscosity and blood flow, led to the observed higher levels of tissue perfusion during extreme hemodilution.

The relative viscosity of the two RBC suspensions obtained after HV and LV protocols being different even

though the concentration of RBCs is similar suggests the presence of interactions among the blood components. Dextran is known to interact with RBCs as a function of molecular size (15), and the dissimilarity between the relative viscosities of the two RBC suspensions may be indicative of either differences in the extent of RBC aggregation/adhesion and/or RBC deformability in the two suspensions. RBC aggregation could cause RBC sedimentation in horizontal venules, leading to venular occlusion, which was not observed. Moreover, because FCD was not affected by the HV protocol, it is likely that RBC aggregation is not the predominant mechanism, as this would lead to capillary occlusion by RBC aggregates and result in a decrease in FCD. Changes in RBC deformability could affect RBC distribution at vessel bifurcations; however, in the present study, capillaries without RBC flux are usually characterized by the presence of stationary RBCs. Therefore, RBC deformability does not appear to be a major factor affecting our FCD measurements. The average shear rate of 160 s^{-1} used in these viscosity measurements is one at which whole blood is somewhat non-Newtonian. To test blood in a Newtonian regime, it would be more desirable to use a shear rate $>500 \text{ s}^{-1}$; however, this would require a larger blood sample than is available from these small animals. The average shear rate selected is a compromise between the need to characterize blood viscosity at a shear rate that is high enough so that complex rheology is not a principal factor and the small blood samples that can be obtained from these animals. Wall shear stress was estimated following *Eq. 1* and was found to be significantly higher after the HV than with the LV protocol. The significantly higher wall shear stress in arterioles stemmed from the increased blood plasma viscosity after the HV protocol since there were not differences in wall shear rate. The already increased venular wall shear rate after the HV protocol compounded with the increased blood viscosity to substantially increase wall shear stress in the venules relative to that in the LV protocol.

Changes in FCD reflect mechanisms that modulate the entrance of RBCs into the capillaries and occur both in normal and diseased states. Perfusion pressure has been shown to directly affect FCD (25). Arteriolar vasoconstriction is a mechanism that could lead to capillary shutdown (40). The combined effects of arteriolar vasodilation and the relatively high level of perfusion pressure observed after the HV protocol are mechanisms leading to sustained capillary perfusion during extreme hemodilution.

Our systemic and microvascular observations after *level 1* exchange are supported by previous studies of moderate or limited hemodilution. Animals in this study compensated for the progressive reduction in oxygen-carrying capacity with hyperventilation. Increasing arterial P_{O_2} and pH along with the concomitant decrease in arterial P_{CO_2} as a function of progressive hemodilution agrees with previous findings in hamsters (24) and other species (28). In our study, both HR and MAP were unchanged, a finding that concurs

with previous investigations in this animal model (29). The decline in MAP after *level 3* LV and HV may in part be due to the critically low oxygen carrying capacity of the blood at this exchange level. In the same model and tissue, Mirhashemi et al. (29) found that a 50% exchange with Dextran 70 did not affect arteriolar diameter, which is similar to our finding after *level 1* exchange. In various anesthetized animal models and different tissue beds, large feeding arterioles similar to those in our investigation resulted in arteriolar vasoconstriction after moderate hemodilution (17, 24, 26, 38). This discrepancy may be partially due to their use of anesthetics, which has been shown to affect vascular tone in our tissue (7) and other regulatory mechanisms in different tissues. In our study, the increase in arteriolar and venular blood velocity observed after *level 1* and *2* exchange is primarily a consequence of reduced blood viscosity due to blood replacement with a less viscous fluid. This is similar to the previously reported observations by investigators from this laboratory (29) and in various tissues by others (17, 24, 26, 38). Our findings show that arteriolar blood flow was still increased from baseline after *level 2* exchange as these vessels remained vasodilated and exhibited increased RBC velocity above baseline levels. This increase in volumetric flow was not apparent in all venular flow measurements, and on the average blood flow was only slightly increased from baseline. This discrepancy is most likely a consequence of redistribution of blood in this subcutaneous vascular network system, which characteristically has more venules than arterioles.

A significant finding in the present study is that local tissue oxygenation levels are maintained even after reduction of the systemic Hct by 60% corresponding to an absolute Hct of 19.5%. The shift of the intravascular PO_2 frequency distributions to higher and lower PO_2 in arterioles and venules, respectively, is indicative of an increased oxygen extraction. The slight rise in arteriolar PO_2 is a result of the elevated systemic arterial PO_2 brought on by hyperventilation and the increase in blood flow, decreasing circulatory transit time resulting in the delivery of blood with higher oxygen content. Oxygen tension measurements made by others using surface multiwire platinum electrodes in the same tissue and model also did not detect any difference in oxygen levels from baseline after isovolemic hemodilution with Dextran 60 to a Hct of 30% (13, 32). However, the multiwire electrode technique provides only a lumped indicator of surface oxygen levels and does not enable one to resolve between the actual oxygen distribution in the vasculature and the interstitium. Oxygen tension histograms in the multiwire electrode study showed a more homogenous tissue PO_2 level after hemodilution with low-molecular-weight dextran to a systemic Hct of 30% (13, 32). Our results after *level 1* exchange show a right shift and narrowing of the extravascular and arteriolar PO_2 histograms, which when combined with the left shift in the venous PO_2 distribution would not result in the more homogeneous microvascular PO_2 distribution observed by others (13).

Multiwire electrode measurements can only be performed after lifting the cover glass of the skinfold window and thereby exposing the tissue to atmospheric oxygen. Moreover, superfusion of the exposed tissue with saline could be another source of oxygen for the tissue and could mask the oxygen delivered by blood perfusion. Repositioning of the surface electrode would also exacerbate the oxygen diffusional fields between the tissue, superfusate, and electrode surface. These factors would lead to higher PO_2 measurements; paradoxically, only 4% of the measurements reported after moderate exchange with Dextran 70 were >30 mmHg after hemodilution (32). Our findings do concur on the concept that moderate hemodilution does not cause regions of hypoxia (PO_2 levels <5 mmHg).

Our analysis of oxygen tension did not include the two animals that had no tissue perfusion after the *level 3* LV protocol, since the phosphorescence probe needed for PO_2 measurements could not reach the tissue under study. It is likely that the intravascular and perivascular PO_2 levels in a nonperfused tissue are close to 0 mmHg. Correction of the PO_2 histograms for *level 3* LV to include these two animals that had no blood flow in the skinfold chamber would result in a slight left shift toward lower oxygen tensions. However, this minor readjustment would not alter our finding that local PO_2 levels fall after 75% hemodilution regardless of the viscosity of the dilutant and the state of tissue perfusion and is a consequence of a reduction in oxygen-carrying capacity beyond the limit of compensatory mechanisms. Because of the large volume of blood exchanged in each hemodilution step, we are not able to determine if tissue oxygenation above baseline levels can be achieved in this tissue during moderate hemodilution as has been suggested by theoretical studies (16, 31). However, others have suggested that the oxygen sensitivity of the vasculature may evoke autoregulatory mechanisms (20), thereby restricting oxygen levels to within a narrow range and thus making higher-than-baseline tissue oxygen levels unattainable.

FCD is an indicator of tissue perfusion and the homogeneity of tissue oxygenation (40). In the current study, we found that a higher FCD could be achieved with the HV than with the LV protocol. Our method for evaluating FCD tends to underestimate the number of capillaries perfused during extreme hemodilution, since, as the hemodilution progresses, the number of RBCs transiting decreases and may lead to a low-Hct vessel being labeled as a nonfunctional capillary. However, because the same method is used in both groups and the systemic Hct levels are the same, the finding that the HV fluid can better maintain the FCD during extreme hemodilution than the LV fluid should not be affected by this limitation in the methodology.

In a study on severe hemorrhagic shock by Kerger et al. (21), maintenance of FCD was the sole critical microvascular parameter that separated surviving from nonsurviving animals. Systemic Hct was reduced to 22.5% due to autotransfusion. Thus our results suggest that reperfusion with HV plasma expanders may be

beneficial in volume restitution, since the higher plasma viscosity may aid in restoring or maintaining FCD.

The present study demonstrates that replacing blood with a HV fluid at extreme hemodilution (systemic Hct of 12%) and thus returning blood viscosity toward normal results in levels of tissue perfusion that would not be achieved with lower-viscosity fluid. It is proposed that increasing plasma viscosity led to an increase in wall shear stress and along with the maintained blood flow triggered vasodilation in the microvascular bed. Direct PO₂ measurements using palladium-porphyrin phosphorescence quenching microscopy document for the first time that local tissue oxygenation in subcutaneous tissue is maintained after a 60% isovolemic hemodilution.

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