Microvascular pressure and functional capillary density in extreme hemodilution with low- and high-viscosity dextran and a low-viscosity Hb-based O₂ carrier

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Microvascular pressure and functional capillary density in extreme hemodilution with low- and high-viscosity dextran and a low-viscosity Hb-based O₂ carrier. Am J Physiol Heart Circ Physiol 287: H363–H373, 2004. First published February 19, 2004; 10.1152/ajpheart.01039.2003.—Blood losses are usually corrected initially by the restitution of volume with plasma expanders and subsequently by the restoration of oxygen-carrying capacity using either a blood transfusion or possibly, in the near future, oxygen-carrying plasma expanders. The present study was carried out to test the hypothesis that high-plasma viscosity hemodilution maintains perfused functional capillary density (FCD) by preserving capillary pressure. Microvascular pressure responses to extreme hemodilution with low- (LV) and high-viscosity (HV) plasma expanders and an exchange transfusion with a polymerized bovine cell-free Hb (PBH) solution were analyzed in the awake hamster window chamber model (n = 26). Systemic hematocrit was reduced from 50% to 11%. PBH produced a greater mean arterial blood pressure than the nonoxygen carriers. FCD was higher after a HV plasma expander (70 ± 15%) vs. PBH (47 ± 12%). Microvascular pressure spanning the capillary network was higher after a HV plasma expander (16–19 mmHg) compared with PBH (12–16 mmHg) and a LV plasma expander (11–14 mmHg) but lower than control (22–26 mmHg). FCD was found to be directly proportional to capillary pressure. The use of a HV plasma expander in extreme hemodilution maintained the number of perfused capillaries and tissue perfusion by comparison with a LV plasma expander due to increased mean arterial blood pressure and capillary pressure. The use of PBH increased mean arterial pressure but reduced capillary pressure due to vasoconstriction and did not maintain FCD.

BLOOD LOSSES ARE USUALLY CORRECTED initially by the restitution of volume with plasma expanders and subsequently by the restoration of oxygen-carrying capacity by means of either a blood transfusion or possibly, in the near future, oxygen-carrying plasma expanders (OCPEs; also termed blood substitute or artificial blood). This procedure has been validated in many studies and is embodied in the practice of hemodilution, whose basis was established experimentally by Messmer et al. (18). Although there remains some lingering controversy on whether crystalloidal or colloidal solutions provide for optimal short-term restitution of cardiovascular and microvascular function, at present it has been assumed that the viscosity of these fluids (including OCPEs) should be similar to that of plasma and therefore similar to that of water (21).

The presumed universal benefit of instituting hemodilution with low-viscosity plasma expanders was challenged by Tsai et al. (32), who showed that microvascular function can only be maintained in extreme hemodilution if plasma viscosity is increased during the hemodilution process. This approach showed that in the awake hamster window chamber model, hemodilution could be carried to a hematocrit (Hct) of 11% (normally 46%) while preserving normal capillary flow if plasma viscosity was elevated to ~2.2 cP using Dextran 500 (molecular mass = 500,000 Da). Normal microvascular and cardiovascular function were evidenced by the maintenance of capillary perfusion and acid-base balance, microvascular flow maintained above control values, and maintenance of mean arterial blood pressure (MAP). Conversely, neither of these parameters/functions could be maintained at a systemic Hct of 11% if plasma viscosity was ~1.1 cP as attained when Dextran 70 (molecular mass = 70,000 Da) was used as the hemodiluent.

The outcome obtained with the high-viscosity diluent is desirable because it maintains capillary perfusion, also termed functional capillary density (FCD), which is defined as the number of capillaries with passage of red blood cells (RBCs) per unit surface of the field of view of a microscopically observed tissue (20). This microvascular parameter was found to be critical in defining tissue survival by Kerger et al. (12), who showed that in extended hemorrhagic shock in awake hamsters. Maintenance of FCD above a specific threshold was the only functional factor observable at the microvascular level that differentiated surviving and nonsurviving animals (12).

The potential beneficial effect of high plasma viscosity in extreme hemodilution may be due to a combination of factors. High plasma viscosity may restore shear stress in the microcirculation leading to the production of vasodilators as postulated by the study of Frangos et al. (8). Increased plasma viscosity has also been associated with vasodilatation and increased microvascular flow (32). We hypothesize that high plasma viscosity and increased FCD may also be due to the direct transmission of central blood pressure to the periphery and the capillaries when microvascular flow is unimpeded by vasoconstriction.

The present study was carried out to test the hypothesis that the use of high-viscosity plasma expanders in extreme hemodilution maintains FCD by maintaining capillary pressure. Extreme hemodilution can also be attained using OCPEs formulated with modified Hb molecules. Some of these products are
vasoactive and therefore maintain central blood pressure through vasoconstriction, and their ability to carry oxygen may allow extreme hemodilution to be sustained with improved microvascular conditions relative to those realized with non-OCPEs. However, extreme hemodilution with these materials reduced FCD (31); thus, as a corollary to our hypothesis, we propose that this fall of FCD is a direct consequence of a fall in capillary pressure.

The preceding observations indicate that restoration of systemic conditions do not necessarily lead to the restoration of microvascular function. Therefore, the present study was also carried out to show that restoration of central blood pressure in pathophysiological conditions can be achieved by increasing peripheral vascular resistance through vasoconstriction, leading to a negative outcome in terms of capillary perfusion, and through the increase of plasma/blood viscosity, which leads to the restoration of microvascular function. To test these hypotheses, we measured microvascular pressures in extreme hemodilution with high- and low-viscosity plasma expanders and a low-viscosity oxygen-carrying vasoactive plasma expander, under the assumption that capillary pressure is the major determinant of FCD.

METHODS

Animal preparation. Investigations were performed in 55- to 65-g golden Syrian hamsters (Charles River Laboratories; Boston, MA). Animal handling and care were provided following the procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The study was approved by the local Animal Subjects Committee. The hamster window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere (4). Briefly, the animal was anesthetized again with pentobarbital sodium. Arterial and venous catheters (polyethylene-50) were implanted in the carotid artery and jugular vein. The catheters were filled with a heparinized saline solution (30 IU/ml) to ensure their patency at the time of catheter implantation. A video image-shearing method was used to measure vessel diameter (D) (10). Blood flow (Q) was calculated from the measured values as \( Q = V \times \pi D^2 \). Changes in arteriolar and venular diameter from baseline were used as indicators of a change in vascular tone. Wall shear stress (WSS) was defined by \( WSS = \frac{8V}{\pi D} \times \eta \), where \( WSR \) is the wall shear rate given by \( 8V/\pi D \).

Inclusion criteria. Animals were suitable for the experiments if 1) systemic parameters were within normal range, namely, heart rate (HR) > 340 beat/min, MAP > 80 mmHg, systemic Hct > 45%, and arterial PO\(_2\) (Pa\(_{O_2}\)) > 50 mmHg; and 2) microscopical examination of the tissue in the chamber observed under \( \times 650 \) magnification did not reveal signs of edema or bleeding.

Systemic parameters. MAP, mean venular pressure (MVP; jugular catheter), and HR were recorded continuously (MP 150, Biopac Systems; Santa Barbara, CA) except during the actual blood exchange. Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge, Clay Adams, Division of Becton-Dickinson; Parsippany, NJ). Hb content was determined spectrophotometrically from a single drop of blood (B-Hemoglobin, Hemocue; Stockholm, Sweden).

Blood chemistry and rheological properties. Arterial blood was collected in heparinized glass capillaries (0.05 ml) and immediately analyzed for Pa\(_O_2\), arterial FCO\(_2\) (Pa\(_{CO_2}\)), base excess, and pH (Blood Chemistry Analyzer 248, Bayer; Norwood, MA). The comparatively low Pa\(_O_2\), and high Pa\(_{CO_2}\) of these animals is a consequence of their adaptation to a fossorial environment. 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.

Blood samples were centrifuged, and colloid osmotic pressure in the plasma was measured using a membrane colloid osmometer (model 420, Wescor; Logan, UT). Calibration of the osmometer was made with a 5% albumin solution using a 30-kDa cutoff membrane (Amicon; Danvers, MA) (34). The viscosities of plasma and whole blood were determined with a cone and plate viscometer at a shear rate of 160 s\(^{-1}\) at 37°C (DV-II+ Viscometer, Brookfield Engineering Laboratories; Middleboro, MA).

FCD. Capillaries were considered functional if RBCs transit though the capillary segments during a 45-s period. FCD was tabulated from the capillary lengths with RBC transit in an area comprising 10 successive microscopic fields (420 \( \times \) 320 \( \mu \)m\(^2\)). FCD (cm\(^{-1}\)) is the total length of RBC-perfused capillaries divided by the area of the microscopic field of view.

Microhemodynamics. Arteriolar and venular blood flow velocities were measured on-line by using the photodiode cross-correlation method (9) (Photo Diode/Velocity Tracker model 102B, Vista Electronics; San Diego, CA). The measured centerline velocity (V) was corrected according to vessel size to obtain the mean RBC velocity (17) A video image-shearing method was used to measure vessel diameter (D) (10). Blood flow (Q) was calculated from the measured values as \( Q = V \times \pi D^2 \). Changes in arteriolar and venular diameter from baseline were used as indicators of a change in vascular tone. Wall shear stress (WSS) was defined by \( WSS = \frac{8V}{\pi D} \times \eta \), where \( WSR \) is the wall shear rate given by \( 8V/\pi D \).

In conditions of extreme hemodilution with a Hct of \( \sim 11\%\), the contribution of RBCs to the total viscosity of blood is linear and amounts to \( \sim 0.70 \) cP, which is the difference between blood and plasma viscosity. According to Lipowsky and Firrle (16), the “...relationship between systemic arteriolar venular and systemic hematocrit is illustrated by a tendency toward equilibrium during extreme hemodilution,” converging to an average value of the ratio between microvascular and systemic Hct of \( \sim 0.7 \) for Hct \( \sim 10\%\). Therefore, we corrected our extreme hemodilution viscosity data by linearly reducing the viscosity RBC contribution by 70%. The same procedure was used for the normal blood data, where the Hct reduction is 0.58 for arterioles and 0.68 for venules (16); however, because at normal Hct blood viscosity is not linearly proportional to Hct, we used actual viscosity versus Hct (dilution with hamster blood) data to obtain the corrected value for blood viscosity.

Microvascular pressure. Microvascular pressures were measured with the servo-nulling technique developed by Wiederhielm et al. (35). The principal features of this technique have been described in detail by Intaglietta and Tompkins (11). Briefly, the unknown intravascular pressure is compared with a known, controlled pressure, generated by a voltage-to-pressure converter. The comparison was made using the property of glass microneedles filled with saline described by Rubio and Zabelta (26). Alternating current (AC) excitation is used to measure the resistance of the plasma-saline interface in the glass micromembrane. Signal loss due to shunting impedance is compensated by automatically increasing the microcanalume AC current excitation with amplitude and phase that precisely make up for...
the stratify capacitance current loss produced by a feedback system. This voltage controls the amplifier gain, which generates the compensating current that is added to the pipette excitation current (11).

Micropipettes were pulled from glass capillary tubing (1 mm outer diameter (OD) × 0.5 mm inner diameter (ID), Omega dot, Frederick Haer & Bowdoinham) using a P-87 micropipette puller (Sutter Instruments; Novato, CA) and beveled on an Alumina abrasive plate 0.05 μm (Sutter Instruments). The resultant pipettes had two taper tips. The first taper had a relatively gentle slope leading to a barrel length of 10–20 μm (ID = 30 μm). The second taper had a sharp slope leading to a barrel length of 5 μm (ID = 1–4 μm) (5). The pipettes were filled using a MicroFil filling needle (World Precision Instruments; Sarasota, FL) with 2.0 M NaCl to prevent debris from accumulating and were stored at 4°C. The final direct current electrical resistance ranged from 0.5 to 2 MΩ. The pressure system was calibrated as previously described (6). The micropipette was controlled using a hydraulic joystick micromanipulator (MO-102, Narishige Scientific Instruments; Tokyo, Japan) adapted for use with an Olympus BX51WI microscope.

Pressure measurements were accepted only if the following criteria were satisfied: 1) the micropipette could be manipulated inside the vessel lumen without affecting the pressure tracing; 2) the pressure tracing was insensitive to small changes in the gain of the servo-null system; and 3) the pressure tracing returned to zero when the pipette was removed from the vessel but remained submerged into the superfusate (5).

Acute isovolemic hemodilution. Progressive hemodilution to a final systemic Hct level of 25% of baseline was accomplished with three isovolemic exchange steps. This protocol is described in detail in our previous reports (31). Briefly, the volume of each exchange-transfusion step was calculated as a percentage of the blood volume, estimated as 7% of the body weight. An acute anemic state was induced by lowering systemic Hct by 60% with two steps of progressive isovolemic hemodilution using 6% Dextran 70, referred to as exchange levels 1 and 2 (Fig. 1). Level 1 exchange was 40% of blood volume and level 2 and 3 exchanges were each 35% of blood volume.

After the level 2 exchange, the level 3 exchange was performed with animals randomly divided into three experimental groups by sorting a set of random numbers produced in a random ordering scheme (1). Experimental group 1 (low-viscosity group) was hemodiluted with Dextran 70 to a Hct of 25% of baseline. Experimental group 2 (high-viscosity group) was hemodiluted by means of a 10% of the total blood volume exchange using 6% dextran, which was followed by a 25% of the total blood volume exchange using 6% Dextran 500. This procedure was used because a previous study (29) showed that this concentration of Dextran 500 caused no RBC aggregation with a Hct of 11%. The total concentration of dextran achieved in the low- and high-viscosity groups was 4.5% and the concentration of Dextran 500 in the high-viscosity group was 1.5%. Experimental group 3 received 13.1% polymerized bovine cell-free hemoglobin (PBH) solution in a modified lactated Ringer solution (Oxyglobin, Biopure; Boston, MA) in an isovolumetric exchange that reduced Hct to 25% of baseline as described by Tsai (31). Table 1 lists the physical characteristics of the three test solutions.

Because mixed blood is withdrawn during the exchanges, a 110% blood volume exchange was needed to reduce the Hct by 75% of baseline. Test solutions were infused into the jugular vein catheter after passing through an inline, 13-mm-diameter, 0.2-μm syringe filter at a rate of 100 μl/min. Blood was simultaneously withdrawn by a dual syringe pump (33 syringe pump, Harvard Apparatus; Holliston, MA) at the same (isovolemic-normovolemic) rate from the carotid artery catheter (31, 32). This slow rate of exchange provided for a stable MAP immediately after the exchange. The animal was allowed a 10-min stabilization period before data acquisition.

Experimental groups. Animals were randomly divided into three experimental groups based on the test solution used during the final step of the hemodilution. A group which did not undergo the hemodilution protocol served as the control for this study.

Experimental setup. The unanesthetized animals were placed in restraining tube with a longitudinal from which the window chamber projected outward. The animals were 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 (BX51WI, Olympus; New Hyde Park, NY). The tissue image was projected onto a charge-coupled device camera (COHU 4815) connected to a videocassette recorder (AG-7355, JVC) and viewed on a monitor. Measurements were carried out using a ×40 (LUMPEL-WIR, numerical aperture 0.8, Olympus) water immersion objective. During micropressure measurements, observations were made with either a ×10 (Leitz, Germany; numerical aperture 0.22) or a ×20 (Leitz, Germany; numerical aperture 0.33) dry objective. For easier detection of RBC passage, the contrast between RBCs and tissue was enhanced with a BG12 (420 nm) bandpass filter.

Fields of observations and vessels were chosen for study at locations in the tissue where the vessels were in sharp focus. Detailed mappings were made of the chamber vasculature so that the same microvessels were studied throughout the experiment. 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 start of the first exchange. Blood samples were withdrawn from level 3 exchange animals at the end of the experiment for subsequent analysis of viscosity and colloid osmotic pressure.

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**Table 1. Physical characteristics of the solutions**

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Viscosity, cp</th>
<th>COP, mmHg</th>
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<tr>
<td>Dextran 70</td>
<td>2.68±0.42</td>
<td>48.9±0.86</td>
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<tr>
<td>Dextran 500</td>
<td>5.92±0.70</td>
<td>30.9±0.92</td>
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<tr>
<td>PBH</td>
<td>1.82±0.67</td>
<td>38.7±1.22</td>
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Values are means ± SD; n = 5 animals. Viscosity measurements were made at a shear rate of 160 s⁻¹ at 37°C. COP, colloid osmotic pressure at 27°C; PBH, polymerized bovine cell-free Hb.
The cover glass of the window chamber was removed at the completion of the microhemodynamics measurements after the third exchange, and the tissue preparation was superfused (~5 ml/min) with a physiological salt solution of the following composition (in mM): 131.9 NaCl, 4.7 KCl, 2.0 CaCl2, 1.2 MgSO4, and 20.0 NaHCO3, with pH 7.4 at 37°C. The tissue was maintained at 33–34°C by the heated solution. The solution spread on the tissue as a thin film, drained into a platter, and was drawn off by suction. Under control conditions, the solution was equilibrated with 95% N2-5% CO2, which maintained suffusate pH at 7.4 and minimized O2 delivery from the superfusate to the tissue (27). Pressure measurements were initiated 20 min after glass window removal, a period that was found to allow the tissue to stabilize and present unchanged microvascular parameters, as shown in Fig. 2.

Data analysis. Results are presented as means ± SD unless otherwise noted. Data within each group were analyzed using ANOVA for nonparametric repeated measurements, and, when appropriate, post hoc analyses performed with the Dunn’s multiple-comparison test. Microvascular pressure was analyzed using two-way ANOVA (diameter and fluid); post hoc analyses were performed with the Bonferroni post tests. All statistics were calculated using SigmaStat 3.0 (SPSS). Changes were considered statistically significant if P < 0.05.

RESULTS

Twenty-seven animals were entered into this study, and all animals tolerated the entire hemodilution protocol without visible signs of discomfort. In one of the experiments, the arterial catheter clogged during the implementation of the second step of the hemodilution, and the animal was excluded from the study. The animals were assigned randomly to the experimental groups: control (n = 8), low-viscosity hemodilution with Dextran 500 (n = 6), high-viscosity hemodilution with Dextran 500 (n = 6), and PBH hemodilution with 13.1% Hb solution (n = 6).

Data groups. The baseline data set was obtained by combining data from all four experimental groups (n = 26). Similarly, level 1 and level 2 data sets were obtained by combining data from all three experimental groups in the hemodilution protocol (n = 18). One-way ANOVA on these data showed no significant differences in any of the systemic or microcirculatory parameters, therefore allowing for grouping of the data into one representative group for each of the three states: baseline (n = 26), Level 1 (n = 18), and level 2 (n = 18). The control group (n = 8) was used to directly compare parameters that could not be repeated at each level in each animal, namely, micropressure and blood rheology parameters.

Systemic parameters. The three experimental groups with exchange protocol showed a significant reduction of Hct after each exchange (50.4 ± 2.1% for baseline, 27.8 ± 1.9% for level 1, 18.3 ± 1.5% for level 2, 11.1 ± 0.9% for level 3 Dextran 70, 11.8 ± 0.8% for level 3 Dextran 500, and 11.3 ± 1.2% for level 3 PBH; P < 0.001 for all level 3 groups compared with baseline). Hb showed the same trend (16.2 ± 1.2 g/dl for baseline, 9.4 ± 0.6 g/dl for level 1, 6.0 ± 0.8 g/dl for level 2, 3.7 ± 0.5 g/dl for level 3 Dextran 70, 3.8 ± 0.6 g/dl for level 3 Dextran 500, and 6.7 ± 0.7 g/dl for level 3 PBH; P < 0.001 for all level 3 groups compared with baseline). The exchange using PBH did not show a statistically significant decrease in Hb content compared with the level 2 Hb, as expected.

MAP was not changed from baseline (98.9 ± 8.1 mmHg) after the level 1 exchange (91.4 ± 11.9 mmHg), and upon further hemodilution with Dextran 70 MAP decreased to 88.4 ± 10.5 mmHg at level 2 (see Table 2). At level 3, MAP decreased to 64.4 ± 7.5 mmHg (P < 0.05 vs. baseline) in the group that received Dextran 70. The group that received Dextran 500 likewise had a decrease in MAP to 79.6 ± 5.4 mmHg (P < 0.05 vs. baseline). However, the group that received PBH during the third exchange did not show a significant change in MAP (86.8 ± 9.7 mmHg). MVP was not affected during the exchanges through level 1 and level 2, but during the last exchange the Dextran 70 group dropped to 5.8 ± 2.1 mmHg (P < 0.05 vs. baseline), and the PBH group also decreased to 6.8 ± 2.7 mmHg (P < 0.05 vs. baseline). HR was not affected significantly during the hemodilution protocol.

Changes in the systemic and blood gas parameters before hemodilution and for the experimental groups are presented in Table 2. Systemic arterial blood gas analysis showed a statistically significant rise in PaO2 from baseline after level 1 in all of the solutions (Dextran 70, 105.3 ± 16.7 mmHg; Dextran 500, 87.3 ± 9.5 mmHg; PBH, 107.6 ± 5.6 mmHg). Arterial PaCO2, pH, and PCO2 were not different from baseline after the hemodilution protocol (79.9 ± 5.4 mmHg, 7.35 ± 0.07, and 49.6 ± 17.4 mmHg, respectively).

Table 2. Macrohemodynamic parameters before and after blood exchange

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<th>Hemodilution (Level 3)</th>
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<td>Baseline</td>
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<td>n</td>
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<tr>
<td>Plasma</td>
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<td>MAP, mmHg</td>
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<td>Heart rate, beats/min</td>
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<td>MVP, mmHg</td>
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<tr>
<td>Arterial PCO2, mmHg</td>
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<td>Articular pH</td>
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<td>Base excess</td>
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Values are means ± SD; n, no. of animals. Baseline included all the animals in the study. Hct, systemic hematocrit; [Hb], hemoglobin content of blood; MAP, mean arterial blood pressure; MVP, mean venular blood pressure. No significant differences were detected between the baseline values of each group or between the values after level 1 and level 2 exchange before the exchange with test solutions. *P < 0.05, †P < 0.01, and ‡P < 0.001 compared with baseline.
500, 101.8 ± 12.5 mmHg; PBH, 94.6 ± 17.4 mmHg; \( P < 0.05 \) vs. baseline). \( \text{PaCO}_2 \) and blood pH were not statistically changed from baseline in all the experimental groups by the hemodilution protocol. Blood base excess exhibited a statistically significant decrease after the level 3 hemodilution compared with baseline in Dextran 70 (−4.6 ± 2.6 mmol/l; \( P < 0.05 \)) and PBH (−0.3 ± 1.5 mmol/l; \( P < 0.05 \)).

**Microhemodynamics.** The changes in the diameter, RBC velocity, and blood flow of large feeding and small arcading arterioles (range 36–95 \( \mu \)m) and small collecting venules and large venular vessels (range 34–146 \( \mu \)m) were measured after each hemodilution step. Figure 3A shows that arteriolar diameter was unchanged after level 1 exchange. Upon further blood exchange to level 2, arterioles dilated to 1.10 ± 0.30 (\( N = 54 \), where \( N \) is the number of vessels, \( P < 0.05 \)) of baseline. This trend reversed after level 3 exchange with Dextran 70 and PBH, resulting in a slight arteriolar vasoconstriction to 0.93 ± 0.25 (Dextran 70, \( N = 18 \), \( P < 0.05 \)) and 0.90 ± 0.28 (Dextran 70, \( N = 18 \), \( P < 0.05 \)) of baseline. After the level 3 exchange with Dextran 500, arteriolar diameter remained dilated at 1.20 ± 0.31 (\( N = 18 \), \( P < 0.05 \)) of baseline.

Venular changes due to the hemodilution protocol are shown in Fig. 3B as a function of blood Hb content. Venules responded to level 1 exchange by constricting to 0.92 ± 0.19 of baseline (\( N = 60 \), \( P < 0.05 \)) and returned to baseline after level 2 exchange, 1.10 ± 0.24 (\( N = 60 \), \( P < 0.05 \)). When the exchange protocol was continued to level 3 using Dextran 70 and PBH, the venules constricted to 0.83 ± 0.18 (Dextran 70, \( N = 20 \), \( P < 0.05 \)) and 0.91 ± 0.14 (PBH, \( N = 20 \), \( P < 0.05 \)) of baseline. Level 3 exchange with Dextran 500 did not change venular diameters from baseline levels 1.0 ± 0.26 (\( N = 20 \)).

Figure 3, C and D, show the change in RBC velocity in arterioles and venules as a function of blood Hb content. An increase in both arteriolar and venular RBC velocity was detected after level 1 exchange to 1.62 ± 0.47 (\( P < 0.05 \)) and 1.42 ± 0.56 (\( P < 0.05 \)) of baseline, respectively. After level 2 exchange, arteriolar RBC velocity remained increased from baseline (1.43 ± 0.57, \( P < 0.05 \)), whereas venular RBC velocity returned to baseline levels. Level 3 exchange with Dextran 70 and PBH reduced arteriolar flow velocity to 0.80 ± 0.41 (Dextran 70, \( P < 0.05 \)) and 0.69 ± 0.38 (PBH, \( P < 0.05 \)) of baseline, respectively. Venular RBC velocity decreased after
The relationships between arteriolar and venular blood flow after the hemodilution protocols and Hb are presented in Fig. 4. The results are given as means ± 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 exchange with Dextran 500 and PBH caused the return of arteriolar blood flow to baseline levels.

The results of calculating shear stress in arterioles and venules show (Fig. 5) that the high-viscosity plasma produced a significantly higher level of shear stress in extreme hemodilution than that achievable with either the oxygen-carrying or noncarrying blood diluents. It should be noted that the latter level of shear stresses were essentially lower than normal.

**FCD.** FCD (Fig. 6) was reduced after level 1 exchange in all groups by 93 ± 5% (P < 0.05 vs. baseline). Level 2 exchange reduced FCD to 84 ± 9% (P < 0.05 vs. baseline). FCD was further reduced after level 3 for all test solutions (Dextran 70, 38 ± 16%, P < 0.001; Dextran 500, 71 ± 15%, P < 0.05 vs. baseline; PBH 46 ± 12%, P < 0.01 vs. baseline).

**Micropressure.** Microvascular pressure distribution in the awake hamster window chamber preparation during control (nonhemodiluted) conditions in arterioles ranging from 30 to 90 µm and small collecting venular vessels ranging from 20 to 90 µm is shown in Fig. 7.

Figure 7 also shows the microvascular pressure distribution after level 3 hemodilution compared with the pressure during control. All microvessels (arterioles and venules) were grouped according to their diameter, showing a significant decrease in pressure compared with control (P < 0.05 vs. control). Comparing level 3 pressure distributions for the different fluids shows a significant difference between Dextran 70 and Dextran 500 microvascular pressure in both arteriolar and venular vessels (P < 0.05). Comparing level 3 hemodilution with Dextran 70 and PBH shows that there is a significant difference in arterioles of 80 and 60 µm diameter (P < 0.05). Comparing level 3 hemodilution with Dextran 500 and PBH shows that there are significantly different pressures in venules with 60 and 70 µm diameter (P < 0.05).
DISCUSSION

The principal finding of this study is that perfused FCD is directly proportional to capillary pressure, as shown in Fig. 8. Furthermore, capillary pressure in extreme isovolemic hemodilution (Hct 11%) in the hamster window chamber model is reduced from the control range of 22–26 to 11–14 mmHg when plasma viscosity remains normal using Dextran 70 as the plasma expander. Conversely, when an identical level of reduction in Hct is performed while plasma viscosity is elevated to 2.21 × 10⁻⁰.₁₂ from 1.21 × 10⁻⁰.₀₈ cP (at control conditions) the capillary pressure remains in the range of 16–19 mmHg. These differences in capillary pressure parallel changes in FCD, which remains near normal for extreme hemodilution with high plasma viscosity conditions being 80% of control but falling to 40% of control conditions at normal plasma viscosity, as shown in Fig. 6.

In the present study, we did not measure capillary pressure directly because of the inherent technical difficulties of cannulating capillaries. Furthermore, in this awake preparation, we found that in conditions of extreme hemodilution contact of the micropipette tip with 10- to 20-μm arterioles caused a rapid contraction, followed by the complete occlusion of their lumen. Conversely, diameter changes were not seen when contacting and cannulating venules or larger-diameter arterioles. Thus our reported range of values are approximately representative of the hydraulic pressure that straddles the entrance and exit of the capillary circulation and indicative that in this tissue the net driving pressure across the capillary network is on the order of 4 mmHg.

Lipowsky (15) summarized the pressure data available for the mesentery, the cat tenuissimus muscle, and the rat spinotrapezious muscle, showing that the pressure difference between these size microvessels is significantly greater and that pressure distribution is highly asymmetric. An exception was the pressure distribution in the vascular network of the rabbit omentum, where the difference between the same-size vessel was 20 mmHg and the distribution between arterioles and venules was linear, which is similar to the findings in our study. The hamster testis is also a capillary network straddled by a low pressure differential, with pressure in 12.5-μm-diameter arterioles being 12.7 ± 0.7 mmHg and in ~25-μm-diameter venules being 9.9 ± 0.7 mmHg according to Sweeney et al. (30).

The differences in pressure found in the smallest arteriolar and the collecting venules measured in each group was 4.5 ± 7.0 mmHg for control and for the level 3 exchanges were 3.1 ±
4.9 mmHg for Dextran 70, 3.2 ± 4.8 mmHg for Dextran 500, and 4.0 ± 7.0 for PBH. Thus the pressure gradient that drives flow through the capillary network is the same for all conditions, because these results are not statistically different; furthermore, it was comparatively small, ~4.0 mmHg. Therefore, although the distribution of anatomy-dependent vascular resistance is asymmetric with its major fraction being located in the arteriolar side of the network (Fig. 7), capillary pressure can be determined with a small error by simply taking the midpoint pressure measured in the end arterioles and the collecting venules.

The finding that FCD is unaffected by the pressure gradient suggests exploring the relationship between FCD and the average transmural capillary pressure, as shown in Fig. 8, which presents the paired data (FCD vs. interpolated capillary pressure) in the network bracketed by the arterioles and venules measured. This graph shows a strong correlation between FCD and capillary pressure, indicating that a determinant factor in maintaining flow of RBCs in the capillaries at a given pressure gradient is the maintenance of a near-normal transmural pressure.

The phenomenon of capillary flow cessation after the lowering of local hydraulic pressure was documented by Lindbom and Arfors (14) in the rabbit tenuissimus muscle, where they reported that FCD was a direct function of the pressure delivered by the femoral artery, showing that FCD decreased in proportion to the lowering of blood pressure due to the occlusion of this supply vessel.

Capillary vascular hindrance must be inversely proportional to the number of capillaries that participate in carrying the network throughput; therefore, our findings that the measured arteriolar/venular pressure does not change even when FCD decreases by 50% must be due to hindrance of the capillary circulation being a minimal portion of the ~4 mmHg that we measured as the pressure drop between small arterioles and venules. Furthermore, because the venular circulation following the capillaries may have an even smaller vascular resistance than the capillary network, it would appear that the major portion of this ~4-mmHg pressure drop resides in the terminal arterioles.

The results obtained may in part be conditioned by the exposure of the preparation to the environment. To ensure that there were no unusual effects due to exposure, we measured in the same preparation the arteriolar diameter before window removal and during suffusion of the exposed tissue and found no significant differences between diameters, as shown in Fig. 2. The finding that arterioles constricted with the PBH exchange, lowering capillary pressure to the same level as that found in extreme hemodilution with Dextran 70, a condition paralleled by a significant decrease of central blood pressure, supports the contention that relative changes of capillary pressure found are directly related to the effects due to the changes in plasma viscosity and vasoactivity.

Normovolemic hemodilution to level 2 caused improved flow conditions even though plasma viscosity was normal. Extreme hemodilution to level 3 with high-viscosity plasma produced microhemodynamic and systemic conditions that were near normal and a level of shear stress that was ~50% higher than control. In this situation, the endothelium is presumably stimulated to increase nitric oxide (NO) production, a situation in which shear stress, plasma viscosity, NO and prostacyclin production, central and capillary pressure, and FCD are intertwined factors (7). However, given the linear relationship between FCD and capillary pressure, it appears that capillary pressure is the determinant of FCD.

When PBH is present, lowered capillary pressure is due to vasoconstriction and the low blood and plasma viscosity. Microvascular shear stress level 3 hemodilution with PBH was the same as control, whereas capillary pressure was the lowest of all groups, indicating that shear stress per se is not sufficient to maintain FCD. Thus capillary pressure plays a major role in determining FCD, and shear stress alone is not sufficient to ensure microvascular function, if capillary pressure is not maintained above a threshold level.

Our findings indicate that a high-viscosity plasma expander is able to improve tissue perfusion through the restoration of FCD, a phenomenon documented by the measurement of FCD and flow in the study of Tsai et al. (32) and presently by the restoration of capillary pressure in this study. This phenomenon is in part due to the increase in peripheral vascular resistance and concomitant increase in MAP attained in the absence of vasoconstriction. It is notable that increasing or restoring blood pressure by means of a vasoactive material, such as PBH, does not result in either the restoration of capillary pressure or the restoration of FCD, the latter noted in a previous study by Tsai (31). This result is due to vasoconstriction interposing a significant resistance between central blood pressure and the peripheral capillary network via the reduction of arteriolar diameter. Conversely, the increase in vascular resistance caused by the increased plasma viscosity is not a phenomenon localized in a specific circulatory district, and, being a resistance that is uniformly distributed throughout the circulation, it does not necessarily prevent the transmission of central blood pressure to the periphery, as shown by mathematical modeling by Tsai and Intaglia (33).

The direct dependence of capillary pressure on systemic pressure and blood viscosity found in this study is in contrast to the apparent regulation of capillary pressure found in normal subjects whose central blood pressure varies during isometric exercise, as shown by Shore et al. (29). This difference in responses is probably due to extreme hemodilution bringing the circulation beyond the autoregulatory range tested by their experiments (29). Thus, in the present experiments, in extreme hemodilution, the circulation appears on one hand to attempt to compensate for the significantly lowered oxygen-carrying capacity by vasodilatation while ensuing that overall lowering of hydraulic pressure leads to a capillary shutdown, because capillary pressure is no longer sufficient to maintain the patency of these conduits.

The results of the present study also allow us to compare the significance of considering systemic versus microvascular parameters in evaluating the efficacy of transfusion fluids used to restore or maintain circulatory volume. It is undeniable that the primary objective of maintaining circulatory volume is that of maintaining vascular filling and therefore blood pressure; however, the latter can also be attained via vasoconstriction, as shown in this and other studies by the increase of blood viscosity. The two modalities for restoring perfusion pressure, however, have different outcomes in terms of microvascular function, because vasoconstriction, while maintaining central blood pressure, a clinically observable parameter, does not necessarily result in the maintenance of capillary function. The significance of this process was shown by the study of Kerger
et al. (12), who found that the maintenance of capillary function, particularly FCD, is the only critical microvascular parameter that differentiates the outcome between survivors and nonsurvivors in extended hemorrhagic shock. Conversely, restoration of blood pressure via the increase in blood viscosity produces a condition that facilitates the transmission of central blood viscosity to the capillaries, thus pressurizing this compartment and improving tissue perfusion.

These experiments show that tissue perfusion at the capillary level is maintained by maintaining blood viscosity to near-normal levels by increasing plasma viscosity. In the present experiments, this occurs because central blood viscosity is also significantly reduced by the reduction of Hct. Thus blood viscosity in extreme hemodilution with high-viscosity plasma returns to 2.88 ± 0.36 cP, a value that is still significantly lower than the viscosity of whole blood for the normal animals, which is 4.21 ± 0.67 cP. The conditions of extreme hemodilution with low-viscosity plasma brings overall blood viscosity to the level of 2.11 ± 0.24 cP; thus it would appear that the “viscosity threshold,” i.e., the point at which the pressure distribution determined by blood viscosity is no longer able to sustain FCD, is somewhere between 2.10 and 2.90 cP. It should be noted that because high-viscosity plasma does not carry oxygen in significant amounts, this viscosity threshold appears to be independent of oxygen-carrying capacity and delivery and solely dependent on how viscosity and consequently hydraulic pressures are distributed in the circulation (Table 3).

Table 3. Rheological properties and COP

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Blood Viscosity, cp</th>
<th>Plasma Viscosity, cp</th>
<th>COP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (control)</td>
<td>4.21±0.67</td>
<td>1.21±0.08</td>
<td>17.6±0.7</td>
</tr>
<tr>
<td>Level 3 Dextran 70</td>
<td>2.11±0.24</td>
<td>1.39±0.21</td>
<td>16.8±0.8</td>
</tr>
<tr>
<td>Level 3 Dextran 500</td>
<td>2.88±0.36</td>
<td>2.21±0.12†</td>
<td>15.7±0.7*</td>
</tr>
<tr>
<td>Level 3 PBH</td>
<td>1.72±0.27†</td>
<td>1.38±0.18</td>
<td>19.6±1.1*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. Viscosity measurements were made at a shear rate of 160 s⁻¹ at 37°C. Hcts are presented in Table 2. *P < 0.05, †P < 0.01, and ‡P < 0.001 compared with nondiluted blood.

The result of this normalization, plotted in Fig. 9, shows that high plasma viscosity produces a vasodilatory effect, leading to increased capillary pressure.

The pressure data can be additionally analyzed in terms of relative changes of vascular resistance in the different vascular compartments to attempt to differentiate between anatomic and rheological factors as causes for the changes in capillary pressure. To accomplish this, the intravascular micropressures (P) were normalized relative to the inlet and outlet pressures of the network according to the formula

\[ P_{n i} = \frac{P_i - P_{vexit}}{P_{ambrace} - P_{vexit}} \]

where \( P_{n i} \) is the normalized pressure \( P_i \) measured in a given vessel, \( P_{vexit} \) is the lowest pressure measured in the largest vessels, and \( P_{ambrace} \) is the largest arteriolar pressure measured. The result of this normalization, plotted in Fig. 9, shows that blood viscosity for these groups was 2.12 ± 0.35 and 2.80 ± 0.22 cP, respectively, vascular resistance being directly proportional to blood viscosity, the ratio of low- versus high-viscosity vascular resistance was 0.75 (data from Table 3). Conversely, the ratio found experimentally is unity because the calculated values are not statistically significantly different.

These findings show that the presence of high-viscosity plasma does not change systemic vascular resistance, contrary to the change predicted if systemic vascular resistance is a direct function blood viscosity. Such an effect could still be due to RBC aggregation due the addition of Dextran 500 if aggregation decreases blood viscosity, thus compensating for the increased plasma viscosity; however, this is unlikely in extreme hemodilution, where aggregation should have virtually no effect on lowering bulk flow viscosity but could have the opposite effect, i.e., increasing vascular resistance through microvessel occlusion, for which there was no evidence because FCD increased with high-viscosity hemodilution. The conclusion of this analysis is that the lack of difference in macrocirculatory resistance in the presence of a significant increased plasma viscosity in extreme hemodilution suggests that high plasma viscosity produces a vasodilatory effect, leading to increased capillary pressure.
the fraction of vascular resistance contributed by the arterioles is diminished for Dextran 70 and Dextran 500 compared with control, whereas the relative contribution to vascular resistance in the venular circulation is augmented relative to control for Dextran 500, suggesting that rheological factors such as aggregation, although not evident by visual inspection, may have a role in the venous microcirculation when Dextran 500 is used. PBH hemodilution led to the same relative changes in vascular resistance in the arterioles as control, although blood viscosity was 40% of normal due to the hemodilution protocol, highlighting the effect of vasoconstriction due to the presence of molecular Hb in blood.

In conclusion, this study shows that FCD is a linear function of capillary pressure, as shown in Fig. 8, and that this parameter is related to central blood pressure, plasma viscosity, the tone of microvasculature resistance, and shear stress in this vascular compartment. Capillary pressure was significantly decreased in extreme hemodilution with low-viscosity plasma and was maintained at near-normal levels when Dextran 500, a high-viscosity plasma expander, was used. This effect was due to increased blood pressure and vasodilatation because macrocirculatory resistance did not increase. Relative venous resistance was elevated with the high-viscosity plasma; therefore, it is likely that the related increased capillary pressure may be the result of increased viscosity in the venous circulation (2), although RBC aggregation was not apparent. These findings have implications in the design and use of plasma expanders and oxygen-carrying blood substitutes because the hydraulic resistance of the vasculature is dependent on both the diameter of blood vessels and the viscosity of the circulating fluid. Our study shows that in the absence of vasoconstriction, and in conditions of extreme hemodilution, it is possible to pressurize the circulation and the microcirculation using high-viscosity plasma expanders, rendering the microcirculation functional by restoring FCD. In extreme hemodilution, this effect appears to be independent of tissue oxygen on a local basis. Restitution of oxygen-carrying capacity with a vasoactive Hb solution, with low viscosity, results in pathologically low capillary pressure and FCD because vasoconstruction further impairs the transmission of central pressure to the periphery.

GRANTS

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