Alginate plasma expander maintains perfusion and plasma viscosity during extreme hemodilution

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Cabrales, Pedro, Amy G. Tsai, and Marcos Intaglia. Alginate plasma expander maintains perfusion and plasma viscosity during extreme hemodilution. Am J Physiol Heart Circ Physiol 288: H1708–H1716, 2005. First published December 9, 2004; doi:10.1152/ajpheart.00911.2004.—Extreme hemodilution was performed in the hamster chamber window model using 6% Dextran 70, lowering systemic hematocrit to 60%. Animals were subsequently divided into three groups and hemodiluted to a hematocrit of 11% using 6% Dextran 70, 6% Dextran 500, and a 4% Dextran 70 + 0.7% alginate solution (n = 6 each group). Final plasma viscosities were 1.4 ± 0.2, 2.2 ± 0.1, and 2.7 ± 0.2 cp, respectively, (P < 0.05, high viscosity vs. low viscosity). Blood viscosities were 2.1 ± 0.2, 2.9 ± 0.4, and 3.9 ± 0.3 cp, respectively. The lowest blood and plasma viscosity groups had a significantly lower functional capillary density, 37 ± 16%, whereas the two high-viscosity solutions were 71 ± 15% and 76 ± 12% (P < 0.05, high viscosity vs. low viscosity), respectively. Arteriolar and venular flow in the Dextran 500 and alginate groups was higher than baseline (i.e., normal nontreated animals), whereas the low-viscosity group showed a reduction in flow. These microvascular changes were paralleled by changes in base excess, which was negative for the Dextran 70 group and positive for the other groups. However, tissue Po2 was uniformly low for all groups (average of 1.4 mmHg). Calculation of tissue oxygen consumption in the window chamber based on the microvascular data, flow, and intravascular Po2 showed that only the alginate + Dextran 70 solution-exchanged animals returned to baseline oxygen consumption, whereas the other groups were lower than baseline (P < 0.05). These results show that hemodilution performed with high-viscosity plasma expanders yields systemic arterial pressures and functional capillary densities that are significantly higher (P < 0.05) than those obtained with 6% Dextran 70, a fluid whose viscosity is similar to that of plasma. A condition for obtaining these results is that the oncotic pressure of the plasma expander be titrated to near normal, so that autotransfusion of fluid from the tissue into the vascular compartment does not reduce the effects of increasing plasma viscosity and increased shear stress on the microvascular wall.

HEMODILUTION, or the isovolemic reduction of hematocrit (Hct), is usually implemented with plasma expanders with similar or lower viscosity than plasma, which in normal conditions is about 1.2 cp. The rationale is that blood viscosity can be lowered without compromising oxygen carrying capacity, because decreasing peripheral resistance leads to an increase in cardiac output (17, 18, 21). It is generally perceived that by lowering blood viscosity there is an improvement in tissue perfusion. Because the practical way to accomplish this is by decreasing Hct and therefore intrinsic blood oxygen carrying capacity, the demonstration of this effect is not conclusive (29). The practice of hemodilution has been promoted to maintain circulatory volume without restoring oxygen carrying capacity after extensive blood losses. This postpones the need for blood transfusions to the threshold identified as the “transfusion trigger” where a number of clinical observations converge to indicate that the correction of circulatory volume deficit also requires the restitution of oxygen carrying capacity.

The study of Tsai et al. (28) showed that extending hemodilution beyond the transfusion trigger in an experimental protocol was safely accomplished if, upon reaching this threshold, plasma viscosity was increased. Specifically, the extension of isovolemic isooncotic hemodilution beyond the transfusion trigger led to normal microcirculatory conditions during extreme hemodilution (Hct 11%) if plasma viscosity was increased to 2.3 cp. Notably, high-viscosity plasma also produced increased microvascular perfusion above baseline levels and maintained a positive acid-base balance, which was opposite of the findings for the same protocol when plasma viscosity was normal.

In the study of Tsai et al. (28), plasma viscosity was increased using Dextran 500 to a level that did not simultaneously increase colloid oncotic pressure (COP) and showed no evidence of red blood cell (RBC) aggregation ex vivo and in the venular circulation. Increasing the concentration of Dextran 500 could, in principle, further increase plasma viscosity. However, this increase is self-limiting because autotransfusion due to the higher COP dilutes the material introduced.

We recently developed a new formulation for plasma expanders based on alginates (4). Alginates are produced by brown seaweed (Phaeophyceae; mainly, Laminaria), resulting in a high-viscosity solution at a comparatively low concentration with an extremely low oncotic pressure. This material, when diluted in normal saline at a concentration of 0.7 g/dl, has a viscosity of 7.6 cp and a COP of 2.1 mmHg. Its mixture with a conventional plasma expander, such as Dextran 70, allows the design of a plasma expander with specific viscosity and COP properties.

The present study was carried out to determine the suitability of alginate as a viscogenic material. Alginate was used to determine whether extending plasma viscosity in extreme hemodilution beyond the previously established threshold of 2.2 cp (leading to near-normal blood viscosity) allows the animals to maintain stable hemodynamic conditions.
METHODS

Animal preparation. Investigations were performed on 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 has been widely used for microvascular studies in the unanesthetized state, and the complete surgical technique has been described in detail elsewhere (6, 7). Briefly, the animal was prepared for chamber implantation with a 50 mg/kg ip injection of pentobarbital sodium anesthesia. After hair removal on the back, 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 skin fold was removed following the outline of the window until only a thin layer of retractor muscle and the intact subcutaneous skin of the opposing side remained. Saline and then a coverglass 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; its chamber was then assessed under the microscope for any signs of edema, bleeding, or unusual neovascularization. Barring these complications, 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 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 of catheter implantation.

Inclusion criteria. Animals were suitable for the experiments if 1) systemic parameters were within normal range, namely, heart rate (HR) > 340 beats/min, mean arterial pressure (MAP) > 80 mmHg, systemic Hct > 45%, and arterial Po2 (PaCO2) > 50 mmHg; and 2) microscopic examination of the tissue in the chamber observed under ×650 magnification did not reveal signs of edema or bleeding.

Systemic parameters. MAP 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 PaO2, arterial PaCO2 (PaCO2), base excess, and pH (Blood Chemistry Analyzer 248, Bayer; Norwood, MA). The comparatively low PaO2 and high PaCO2 of these animals are consequences of their adaptation to a fossorial environment. Blood samples for viscosity and COP 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 COP 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) (31). The viscosity of plasma and whole blood was 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).

Functional capillary density. Capillaries were considered functional if RBCs transit through the capillary segments during a 45-s period. Functional capillary density (FCD) was tabulated from the capillary lengths with RBC transit in an area comprising 10 successive microscopic fields (420 × 320 µm²). FCD (cm⁻¹) is the total length of RBC-perfused capillaries divided by the area of the microscopic field of view (5, 14, 28).

Microhemodynamics. Arteriolar and venular blood flow velocities were measured on-line by using the photodiode cross-correlation method (model 102B, Photo Diode/Velocity Tracker, Vista Electronics; San Diego, CA). The measured centerline velocity (V) was corrected according to vessel size to obtain the mean RBC velocity (16). A video image-shearing method was used to measure vessel diameter (D). Blood flow (Q) was calculated from the measured values as $Q = V \times \pi(D/2)^2$. 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 = WSR × Hct, where WSR is the wall shear rate given by 8VD⁻¹ and Hct is blood viscosity.

In conditions of extreme hemodilution with Hct ~11%, the contribution of RBCs to the total viscosity of blood was linear and amounted to about 0.70 cp, which is the difference between blood and plasma viscosity. According to Lipowsky and Firrell (15), the ratio between arteriolar-venular and systemic Hct in extreme hemodilution converges to 0.7 for a Hct ~ 10%. Therefore, we corrected our extreme hemodilution viscosity data by linearly reducing the viscosity contribution by 70%. We used the same procedure for normal blood data, where the Hct reduction was 0.58 for arterioles and 0.68 for venules (15). However, because at normal Hct blood viscosity was not linearly proportional to Hct, we used actual viscosity versus Hct data from dilution of hamster blood to obtain the corrected value for blood viscosity (3).

Infusion solutions. The solutions used for the study were Dextran 70 in 0.9% saline [6% (wt/vol), 70 kDa; Pharmacia], Dextran 500 in 0.9% saline [6% (wt/vol), 500 kDa; Pharmacia], and alginate [FMC Biopolymer; Braknøy, Norway; formulated 0.7% (wt/vol) in 0.9% saline]. A mix of 0.7% (wt/vol) alginate and 4% (wt/vol) Dextran 70 produced a plasma expander with a viscosity of 8.8 cp and COP of 32 mmHg. It was necessary to mix dextran and alginate to obtain the necessary COP for hemodilution.

Acute isovolemic hemodilution. Hemodilution to a final systemic Hct level of 25% of baseline was accomplished with three isovolemic exchange steps. This protocol has been described in detail in our previous studies (2, 5, 25). 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 isovolemic hemodilution steps using 6% Dextran 70, referred to as exchange levels 1 and 2. Level 1 exchange was 40% of blood volume, and levels 2 and 3 were each 35% of blood volume (2, 5).

After level 2 exchange, 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 was hemodiluted with 6% Dextran 70 to a Hct of 11%. Experimental group 2 was hemodiluted using 6% Dextran 500 to a Hct of 11%. Experimental group 3 was hemodiluted with a mixture of 0.7% alginate and 4% Dextran 70 to decrease Hct to 11%.

Table 1 lists the physical characteristics of the solutions.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Viscosity, cp</th>
<th>COP, mmHg</th>
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</thead>
<tbody>
<tr>
<td>Dextran 70 (6%)</td>
<td>2.68 ± 0.42</td>
<td>48.9 ± 0.86</td>
</tr>
<tr>
<td>Dextran 500 (6%)</td>
<td>5.92 ± 0.70*</td>
<td>30.9 ± 0.92*</td>
</tr>
<tr>
<td>Alginate (0.7%) + Dextran 70 (4%)</td>
<td>8.79 ± 0.56*†</td>
<td>32.2 ± 0.96*†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 samples. Shear rate was 160 s⁻¹ at 37°C. COP, colloid osmotic pressure at 27°C. *P < 0.05 compared with Dextran 70; †P < 0.05 compared with Dextran 500.
solutions were infused into the jugular vein catheter after passing through an in-line, 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 (2, 5, 25). This slow rate of exchange provided a stable MAP immediately after the exchange. The animal was allowed a 10-min stabilization period before data acquisition.

**Experimental setup.** The unanesthetized animals were placed in a restraining tube with a longitudinal slit 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 intravitral 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 (LUMPFL-WIR, numerical aperture 0.8, Olympus) water immersion objective. During micropressure measurements, observations were made with either a ×10 (Leitz, numerical aperture 0.22) or ×20 (Leitz, 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 observation 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 with exchanges beginning 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 COP.

**Hb oxygen saturation.** The oxygen saturation of Hb was investigated by deoxygenation of oxygen-equilibrated oxy-Hb in the Hemox buffer (pH 7.4) at 37.6°C using a Hemox Analyzer (TCS). The analyzer measured the O2 pressure with a Clark-type O2 electrode (Yellow Springs Instruments) and simultaneously calculated the Hb saturation via a dual-wavelength spectrophotometer. The oxygen equilibrium curve (OEC) for hamster RBCs was determined from freshly collected blood, and the OEC for PEG-Hb was measured from fresh material.

**Microvascular PO2 distribution.** High-resolution microvascular PO2 measurements were made using phosphorescence quenching microscopy (24), a method based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation. Phosphorescence microscopy is not dependent on the level of dye within the tissue, and the decay time is inversely proportional to the PO2 level. The phosphorescence decay curves were converted to oxygen tensions using a fluorescence decay curve fitter (model 802, Vista Electronics; Ramona, CA) (13). This technique has been used in this animal preparation and others for both intravascular and extravascular oxygen tension measurements, as albumin exchange between plasma and tissue allows for sufficient concentrations of albumin bound dye within the interstitium to achieve an adequate signal-to-noise ratio. Animals received a slow intravenous injection of 15 mg/kg body wt at a concentration of 10.1 mg/ml of a Palladium-meso-tetra(4-carboxyphenyl)porphyrin (Porphyrin Products; Logan, UT). The dye was allowed to circulate for 20 min before PO2 measurements.

In our system, intravascular measurements were made by placing an optical rectangular window (~5 × 40 μm longitudinally) within the vessel of interest, with the longest side of the rectangle slit positioned parallel to the vessel wall. Tissue PO2 was measured in regions void of large vessels within intercapillary spaces with an optical window size of about 10 × 10 μm. Thus the exact localization of the PO2 measurements is known (27). The phosphorescence decay due to quenching at a specific PO2 yields a single decay constant, and in vitro calibration has been demonstrated to be valid for in vivo measurements. Intravascular and perivascular PO2 measurements were made in the arterioles and venules studied. Interstitial tissue PO2 was measured within the interstitium far away from visible underlying and adjacent vessels.

**Tissue oxygen delivery and extraction.** The microvascular methodology used in our studies allowed a detailed analysis of oxygen supply in the tissue. Calculations of oxygen delivery (defined as the amount of oxygen per unit time delivered by the arterioles to the microcirculation normalized relative to control) and extraction (defined as the amount of oxygen released by blood in the microcirculation per unit time normalized relative to control) were made using Eqs. 1 and 2 (3, 5, 23):

\[
O_2 \text{ delivery} = \left[ \frac{\text{RBC}_{\text{Hb}} \times \gamma \times S_A}{(1 - \text{Hct}) \times \alpha} \right] \times \text{arteriolar PO}_2 \times Q \tag{1}
\]

\[
O_2 \text{ extraction} = \left[ \frac{\text{RBC}_{\text{Hb}} \times \gamma \times S_A}{(1 - \text{Hct}) \times \alpha} \right] \times \text{arterio-venular difference in PO}_2 \times Q \tag{2}
\]

where RBCHb is the Hb in RBCs (expressed in g/dl blood), γ is the oxygen carrying capacity of Hb at 100% saturation or 1.34 ml O2/g Hb, S A% is the arteriolar oxygen saturation of RBCs, SA-V% is the arterio-venular difference in oxygen saturation of RBCs, (1 – Hct) is the fractional plasma volume and converts the equation from per decoliter of plasma to per decoliter of blood, α is the solubility of oxygen in plasma equal to 3.14 × 10⁻³ ml O2/dl plasma mmHg, and Q is the microvascular flow for each microvessel as percentage of baseline. The OECs were determined as described before.

**Data analysis.** Results are presented as means ± SD unless otherwise noted. Data within each group were analyzed using one-way ANOVA, and, when appropriate, post hoc analyses were performed with the Bonferroni’s multiple-comparison test. All data have been presented as absolute values and ratios relative to baseline values. A ratio of 1.0 signifies no change from baseline, whereas lower and higher ratios are indicative of changes proportionally higher and lower than baseline. The same vessels and functional capillary fields were followed so that direct comparisons to their baseline levels could be performed allowing for more robust statistics for small sample populations. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software; San Diego, CA). Changes were considered statistically significant if P < 0.05.

**RESULTS**

Eighteen animals were entered into this study, and all animals tolerated the entire hemodilution protocol without visible signs of discomfort. The animals were assigned randomly to the following experimental groups: Dextran 70 hemodilution (n = 6), Dextran 500 hemodilution (n = 6), and alginate hemodilution (n = 6).

**Data groups.** The baseline data set was obtained by combining data from all three experimental groups (n = 18). Similarly, level 1 and level 2 data sets were obtained by combining data from all three experimental groups in the hemodilution protocol. One-way ANOVA on these data showed no significant differences in any of the systemic nor microcirculatory parameters, therefore allowing for the grouping of the data into one representative group for each of the following three states: baseline (n = 18), level 1 (n = 18), and level 2 (n = 18).

**Systemic parameters.** The three experimental groups showed a significant reduction in Hct after each exchange (49.8 ± 1.6% for baseline, 28.2 ± 1.8% for level 1, 18.0 ± 1.3% for...
level 2, 11.1 ± 0.9% for level 3 Dextran 70, 11.8 ± 0.8% for level 3 Dextran 500, and 11.1 ± 1.1% for level 3 alginate, P < 0.001 for all level 3 groups compared with baseline. Hb showed the same trend (15.6 ± 0.8 g/dl for baseline, 9.3 ± 0.5 g/dl for level 1, 5.9 ± 0.7 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 3.8 ± 0.8 g/dl for level 3 alginate, P < 0.001 for all level 3 groups compared with baseline).

MAP at baseline was 101.2 ± 6.2 mmHg and was reduced to 92.4 ± 9.8 mmHg after the level 1 exchange. Upon further hemodilution with Dextran 70, MAP decreased to 87.8 ± 9.6 mmHg at level 2. At level 3, MAP decreased to 64.4 ± 7.5 mmHg (P < 0.05 compared with baseline) in the group that received Dextran 70. The groups that received Dextran 500 and alginate likewise had a decrease in MAP to 79.6 ± 5.4 mmHg (P < 0.05 compared with baseline and P < 0.05 compared with level 3 Dextran 70) and 84.3 ± 6.2 mmHg (P < 0.05 compared with baseline and P < 0.05 compared with level 3 Dextran 70), respectively. HR was not affected significantly compared with baseline and level 3 (Dextran 70, 39.1 ± 2.6 mmHg at level 3).

Table 2. Macrohemodynamic parameters before and after blood exchange

<table>
<thead>
<tr>
<th>Level 3 Hemodilution</th>
<th>Baseline</th>
<th>Dextran 70</th>
<th>Dextran 500</th>
<th>Alginite</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Hct, %</td>
<td>49.8 ± 1.6</td>
<td>11.1 ± 0.9*</td>
<td>11.8 ± 0.8*</td>
<td>11.1 ± 1.1*</td>
</tr>
<tr>
<td>[Hb], g/dl</td>
<td>15.6 ± 0.8</td>
<td>3.7 ± 0.6*</td>
<td>3.8 ± 0.6*</td>
<td>3.8 ± 0.8*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>101.2 ± 6.2</td>
<td>64.4 ± 7.5*</td>
<td>79.6 ± 5.4*</td>
<td>84.3 ± 6.2*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>401.4 ± 27.6</td>
<td>417.8 ± 40.9</td>
<td>422.8 ± 28.7</td>
<td>433.8 ± 38.2</td>
</tr>
<tr>
<td>Pao2, mmHg</td>
<td>56.7 ± 5.9</td>
<td>105.3 ± 16.7*</td>
<td>101.8 ± 12.5*</td>
<td>99.3 ± 11.2*</td>
</tr>
<tr>
<td>Paco2, mmHg</td>
<td>54.2 ± 6.1</td>
<td>39.1 ± 7.8*</td>
<td>41.2 ± 8.6*</td>
<td>42.3 ± 5.8*</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.35 ± 0.04</td>
<td>7.32 ± 0.09</td>
<td>7.35 ± 0.06</td>
<td>7.36 ± 0.08</td>
</tr>
<tr>
<td>Base excess, mmol/l</td>
<td>4.0 ± 1.6</td>
<td>-4.6 ± 2.6*</td>
<td>0.2 ± 1.6*</td>
<td>0.8 ± 1.2*</td>
</tr>
</tbody>
</table>

Microhemodynamics. Changes in diameter, RBC velocity, and blood flow of large feeding and small arcading arterioles (range 40–90 μm) and small collecting and large venular vessels (range 40–100 μm) were measured after each hemodilution step. Figure 1A shows that arteriolar diameter was unchanged after level 1 exchange. After level 2 exchange, however, arterioles dilated to 1.12 ± 0.28 of baseline [number of vessels (N) = 56, P < 0.05 compared with baseline]. This trend reversed after level 3 exchange with Dextran 70, resulting in a slight arteriolar vasoconstriction to 0.93 ± 0.25 of baseline (N = 18, P < 0.05 compared with baseline). After the level 3 exchange with Dextran 500 or alginate, arteriolar diameter remained dilated at 1.20 ± 0.31 of baseline (N = 18, P < 0.05 compared with baseline) and 1.29 ± 0.32 of baseline (N = 20, P < 0.05 compared with baseline), respectively. Arteriolar diameter after level 3 exchange with alginate showed a statistically significant difference from level 3 exchange with Dextran 70.

Venular changes due to the hemodilution protocol are shown in Fig. 1B. Venules responded to level 1 exchange by constricting to 0.91 ± 0.16 of baseline (N = 60, P < 0.05 compared with baseline) and returned to baseline after level 2 exchange, 1.11 ± 0.20 of baseline (N = 60, P < 0.05 compared with baseline). When the exchange protocol was continued to level 3 using Dextran 70, the venules constricted to 0.83 ± 0.18 of baseline (N = 20, P < 0.05 compared with baseline). Level 3 exchange with Dextran 500 or alginate did not change venular diameters from baseline levels, 1.04 ± 0.26 (N = 20) and 1.09 ± 0.24 of baseline (N = 24), respectively. Venular diameter after level 3 exchange with alginate showed a statistically significant difference compared with level 3 exchange with Dextran 70.

Figure 1, C and D, shows 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 1.57 ± 0.45 (P < 0.05 compared with baseline) and 1.40 ± 0.51 (P < 0.05 compared with baseline) of baseline, respectively. After level 2 exchange, arteriolar RBC velocity remained increased from baseline (1.47 ± 0.54 of baseline, P < 0.05 compared with baseline), whereas venular RBC velocity returned to baseline levels. Level 3 exchange with Dextran 70 reduced arteriolar flow velocity to 0.80 ± 0.41 of baseline (P < 0.05 compared with baseline). Venular RBC velocity decreased after level 3 exchange with Dextran 70 to 0.76 ± 0.49 of baseline (P < 0.05 compared with baseline). Level 3 exchange using Dextran 500 or alginate did not present changes in arteriolar and venular RBC velocity.

The relationships between arteriolar and venular blood flow after the hemodilution protocols are presented in Fig. 2. 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 were not sustained, and blood flow was statistically reduced from baseline in both arterioles and venules. Arteriolar blood flow after level 3 exchange with Dextran 500 was above baseline and significantly above level 3 exchange with Dextran 70. The
Fig. 1. Changes in arteriolar and venular hemodynamics after level 3 exchange with Dextran 70 (Dx70), Dextran 500 (Dx500), and alginate. The dashed line represents the baseline level. A: arteriolar diameter; B: venular diameter; C: arteriolar velocity; D: venular velocity. Values are means ± SD; N, no. of vessels studied. Diameters in each animal group were as follows: level 1 arterioles: 56.3 ± 15.2 μm, N = 56; venules: 63.1 ± 21.7 μm, N = 64; level 2 arterioles: 59.1 ± 15.2 μm; venules: 68.4 ± 26.2 μm; level 3 Dx70 (arterioles: 52.1 ± 14.6 μm, N = 18; venules: 71.7 ± 21.6 μm, N = 20); level 3 Dx500 (arterioles: 54.9 ± 18.2 μm, N = 18; venules: 69.6 ± 28.3 μm, N = 20); and level 3 alginate (arterioles: 57.2 ± 17.2 μm, N = 20; venules: 65.2 ± 17.3 μm, N = 24). Red blood cell (RBC) velocities in each animal group were as follows: level 1 arterioles: 4.4 ± 2.6 mm/s, venules: 1.6 ± 0.8 mm/s; level 2 arterioles: 5.0 ± 2.5 mm/s, venules: 1.4 ± 1.1 mm/s; level 3 Dx70 (arterioles: 3.9 ± 2.0 mm/s, venules: 1.1 ± 0.7 mm/s); level 3 Dx500 (arterioles: 4.3 ± 2.2 mm/s, venules: 1.3 ± 0.7 mm/s); and level 3 alginate (arterioles: 4.5 ± 2.7 mm/s, venules: 1.5 ± 0.8 mm/s). *P < 0.05 vs. baseline.

The same trend was maintained for Dextran 70 + alginate. In venules only, level 3 exchange with Dextran 70 + alginate was significantly above level 3 exchange with Dextran 70.

WSR and WSS. The calculation of WSR in arterioles and venules is shown in Fig. 3. WSS for the microvasculature was calculated following the scheme set forth in METHODS using data on diameter and velocity for each vessel and the average plasma and whole blood viscosity (Table 3) for each study group and vessel type. As expected, there was a statistically significant difference in WSS between arterioles and venules. This relationship was found within each experimental group (P < 0.05). Level 3 exchange with Dextran 70 caused a statistically significant drop from baseline in both arteriolar and venular WSS, which was not observed after level 3 exchange with Dextran 500 or alginate, where the WSS levels were not different from baseline. Accordingly, the direct comparison between arterioles and venules after level 3 exchange with Dextran 70 showed significantly reduced WSS relative to level 3 exchange with alginate.

FCD. FCD was reduced after level 1 and level 2 exchange in all groups to 92 ± 6% and 85 ± 8% of baseline, respectively (level 2 exchange, P < 0.05 compared with baseline; Fig. 4). FCD was further reduced after level 3 for all test solutions: Dextran 70 to 37 ± 16% (P < 0.05 compared with baseline), Dextran 500 to 71 ± 15% (P < 0.05 compared with baseline and P < 0.05 compared with Dextran 70), and alginate to 76 ± 12% (P < 0.05 compared with baseline and P < 0.05 compared with Dextran 70).

Fig. 2. Arteriolar and venular blood flow after level 3 exchange. Level 3 exchange with Dx500 maintained blood flow at baseline levels, whereas exchange with Dx70 resulted in the reduction of flow. Data are presented as means ± SE relative to baseline levels (dashed lines). Flows in each animal group were as follows: level 1 arterioles: 11.2 ± 2.6 nl/s, venules: 4.7 ± 1.9 nl/s); level 2 arterioles: 13.0 ± 2.4 nl/s, venules: 5.3 ± 1.5 nl/s); level 3 Dx70 (arterioles: 8.4 ± 2.1 nl/s, venules: 4.4 ± 1.4 nl/s); level 3 Dx500 (arterioles: 9.6 ± 2.3 nl/s, venules: 4.9 ± 1.5 nl/s); and level 3 alginate (arterioles: 9.9 ± 2.3 nl/s, venules: 5.1 ± 1.8 nl/s). *P < 0.05 compared with baseline; †P < 0.05 compared with Dx70.
HIGH PLASMA VISCOSITY DURING EXTREME HEMODILUTION

Intravascular PO2. PO2 measurements after the level 3 exchange-transfusion protocol showed that all materials produced similar distributions of microvascular PO2. Exchange with Dextran 70 yielded an arteriolar PO2 of 33 mmHg, whereas the venules had a PO2 of 3 ± 2 mmHg, which was statistically significantly lower from the exchanges with Dextran 500 or alginate (Fig. 5).

Tissue PO2s after level 3 exchange with Dextran 70, Dextran 500, and alginate were 1.3 ± 1.2, 1.6 ± 1.2, and 1.5 ± 1.2 mmHg, respectively. Tissue PO2s were not statistically different between groups but were statistically different from non-hemodiluted animals [21.7 ± 3.5 mmHg according to previous studies in this species (26)].

Calculations of oxygen delivery and extraction in the microcirculation after level 3 hemodilution with different test solutions are shown in Fig. 6. Delivery of oxygen to the microcirculation was significantly reduced for all exchange groups. However, oxygen extraction in the microcirculation was identical to baseline for the alginate group. Premicrocirculatory oxygen delivery was higher than baseline for all groups, being particularly elevated for the Dextran 500 and alginate groups.

DISCUSSION

The principal finding of this study was that in conditions of extreme hemodilution where Hct was reduced to 11%, using a mixture of alginate and Dextran 70 provided an increase in plasma viscosity to 2.7 cp and improved microvascular conditions over those attained using Dextran 70 and 500 (plasma viscosity of 2.2 cp). The same level of hemodilution attained with Dextran 70 and a plasma viscosity of 1.4 cp did not result in a level of FCD needed for survival, a condition that was probably generalized to most tissues, because base excess was negative. The results obtained with Dextran 70 and 500 were similar to those previously reported by Tsai et al. (28). The experiments we repeated in this study were to ensure that we had the same baseline data as previously studied, to extend our investigation, and to determine oxygen delivery and consumption for the different plasma viscosity conditions.

Systemic parameters were significantly improved in extreme hemodilution with high-viscosity solutions relative to normal plasma viscosity. Because this was accompanied by the increase in microvascular flow, this effect could be related to the action of the heart, indicating that the improved microvascular perfusion observed in the tissue of the window chamber was paralleled with normalization of perfusion in the heart and other organs. The increase in blood pressure to near-baseline values obtained in the alginate group was somewhat lower than that expected from the increase in blood viscosity. In the case of Dextran 500, blood pressure was 79% of baseline and blood viscosity was 68% of baseline, a ratio of 1.16, whereas for

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>4.19±0.44</td>
<td>1.20±0.09</td>
<td>17.7±0.7</td>
</tr>
<tr>
<td>Level 3 exchange</td>
<td>Dextran 70 (6%)</td>
<td>2.11±0.24*</td>
<td>1.39±0.21</td>
</tr>
<tr>
<td></td>
<td>Dextran 500 (6%)</td>
<td>2.88±0.36††</td>
<td>2.21±0.12††</td>
</tr>
<tr>
<td></td>
<td>Alginate (0.7%)</td>
<td>3.86±0.31†††</td>
<td>2.72±0.18††</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 animals. Hcts are presented in Table 2. Shear rate was 160 s⁻¹ at 37°C. *P < 0.05 compared with no diluted blood; †P < 0.05 compared with Dextran 70; ‡P < 0.05 compared with Dextran 500.
alginate, the respective changes were 92% and 83%, a ratio of 1.10, suggesting that the pressor effect for alginites was lower.

Combining information on microvascular blood flow, PO2, local oxygen carrying capacity, and data from the oxygen dissociation curve for hamster blood allowed us to determine premicrovascular and microvascular oxygen delivery and consumption. The results shown in Fig. 6 demonstrate that the reflex vasoconstriction associated with low-viscosity blood and normal plasma viscosity in extreme hemodilution cause oxygen to be preferentially delivered to the premicrovascular circulation, whereas increasing levels of plasma viscosity progressively shifts oxygen delivery to the capillary circulation. The extreme hemodilution with alginate increased plasma viscosity to 2.7 cp and yields a high oxygen extraction in the microcirculation and sustained blood perfusion. A plasma viscosity of 2.7 cp seems to be the limit for the process where an increase in plasma viscosity is able to compensate for the decrease of blood viscosity by the loss of RBCs (5, 9, 12, 22, 28 –30).

Although oxygen extraction in the microcirculation with alginate hemodilution was identical to control, tissue PO2 was significantly lower than control at levels that are considered anoxic. This apparent paradox shows that the values of tissue PO2 were not necessarily representative of the level of oxygen availability and consumption in the tissue and were more closely related to the oxygen level in venules. The extraction of oxygen from blood before its arrival to the microcirculation was greater for Dextran 70 hemodilution than for the other groups, even though the blood oxygen carrying capacity was the same in all cases. This is a direct consequence of the lower arteriolar PO2 in this group (Fig. 5), which corresponded to the steepest portion of the oxygen dissociation curve for Hb, causing a significant lowering of the amount of oxygen carried by blood. This phenomenon was, in part, due to the increased rate of oxygen release due to the lowering of blood flow velocity (19, 20) and increased rate of vessel oxygen consumption associated with vasoconstriction (11, 32).

**Fig. 5.** Microvascular oxygen distribution (means ± SD) at control and after level 3 hemodilution. No statical significance in venular and tissue oxygen levels was achieved. However, small differences in the arteriolar PO2 were found but probably not important in a biological context. Tissue PO2 attained with Dx70, Dx500, and alginate was not different after extreme hemodilution. §P < 0.05 compared with Dx70.

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**Fig. 6.** Microcirculation oxygen delivery and extraction at baseline and after level 3 hemodilution. †P < 0.05 compared with baseline; §P < 0.05 compared with Dx70.
Hamsters are adapted to a fossorial environment and in normal conditions have low central PaO₂, namely, 57 mmHg, corresponding to a Hb O₂ saturation of 84%. Because arteriolar pO₂ is 54 mmHg (Hb O₂ saturation 81%), calculation of the premicrovascular oxygen consumption shows that this species is very efficient in delivering oxygen to the tissue because very little oxygen exits the circulation before arrival at the microcirculation, with the change in blood saturation only 3%. The present experiments showed that when the oxygen supply limitation was reached, oxygen uptake in the lungs increased, raising blood PaO₂ to near-normal levels in most species, ~100 mmHg (Table 2). In this pO₂ distribution, a substantial amount of oxygen exits the circulation before arrival at the microcirculation, because the change in blood saturation for Dextran 70 was 54%, Dextran 500 was 38%, and alginate was 32%. When we include information on changes in blood flow and the intrinsic oxygen carrying capacity due to the change in Hb, we obtain the data presented in Fig. 6, which showed that in extreme hemodilution a significantly greater amount of oxygen was delivered to the nontissue portions of the circulation than in normal conditions. Regardless of the shift in oxygen distribution, the efficiency of extraction increased in extreme hemodilution because the oxygen extraction ratio (O₂ extraction/O₂ delivery) for nonhemodiluted animals was 38%, and, after extreme hemodilution, it increased to 89% (Dextran 70), 86% (Dextran 500), and 84% (alginate), respectively. This calculation showed that in conditions of extreme hemodilution, due to hyperventilation, the overall oxygen uptake from the lungs as well as oxygen consumption by the organism was greater. However, this increased oxygen capture in the lungs does not appear to benefit the tissue, because it exits the circulation before arrival at the microcirculation, suggesting that this oxygen may be consumed by the vasculature per se, because this oxygen does not appear in the tissue.

In these experiments, we used alginites as a biocompatible material appropriate for increasing plasma viscosity. Alginites are a family of polysaccharides extracted from algae, which are used extensively in biotechnology due to their water-binding and viscosic properties, with agar being the most widely used product. A 1.0% solution has a viscosity higher than 8 cp, and its oncotic pressure is essentially zero at this concentration. The compound used in this study was a highly purified material of average molecular mass (200–5,000 kDa) to suit the application. It provided excellent results in terms of microvascular perfusion in resuscitation from hemorrhagic shock in our hamster window chamber model with 24-h survival having led to the development of the concept of hyperoncotic-hyperviscous resuscitation (4). Alginites present a mixture of manuronide (M) and guluronic acid (G) compounds, and their applicability depends on optimizing their relative composition (8). Alginites are not random copolymers but, according to the source algae, consist of blocks of similar and strictly alternating residues (i.e., MMMMMM, GGGGGG, and GMGMG-MGM), each of which have different conformational preferences and behavior.

The present study confirmed that replacing blood with a high-viscosity fluid in extreme hemodilution conditions (Hct 11%) resulted in levels of tissue perfusion that cannot be achieved with normal plasma viscosity. The effects were directly related to an increase in WSS that results from the combined increase in plasma viscosity and increased blood flow velocity. Therefore, it would appear to be related to the increased production of vasodilator substances by the endothelium via biochemical mechanotransduction as postulated by the findings of Frangos et al. (10). Increasing plasma viscosity to 2.7 cp showed a trend of increased FCD, microvascular flow, and pO₂ over that attained previously, when plasma viscosity was increased to 2.2 cp. The changes were not statistically significant. However, their combined effect caused oxygen consumption in the microcirculation to return to baseline levels. Finally, alginites lacking in oncotic effects and being effective at concentrations that are significantly lower than most other plasma expanders may constitute a new type of plasma expander that allows us to tailor viscosity and oncotic properties.

GRANTS

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REFERENCES

14. Kerger H, Saltzman DJ, Menger MD, Messmer K, and Intaglietta M. Systemic and subcutaneous microvascular Po₂ dissociation during 4-h


32. Ye JM, Colquhoun EQ, and Clark MG. A comparison of vasopressin and noradrenaline on oxygen uptake by perfused rat hind limb, intestine, and mesenteric arcade suggests that it is part due to contractile work by blood vessels. Gen Pharmacol 21: 805–810, 1990.