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Compatibility of Different Colloid Plasma Expanders with Perflubron Emulsion

An Intravital Microscopic Study in the Hamster

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Background: **Perfluorocarbon-based oxygen carriers have been proposed as an adjunct to autologous blood conservation techniques during elective surgery. To date, the effects of perfluorocarbon emulsions at the microcirculatory level have not been studied extensively. In this study the effects of perflubron emulsion on the microcirculation after acute normovolemic hemodilution (ANH) were investigated using different colloid plasma expanders.**

Methods: **The dorsal skin fold chamber model and intravital fluorescence microscopy were used for analysis of the microcirculation in the thin striated skin muscle of conscious hamsters (body weight, 40–60 g). Measurements of microvascular perfusion and leukocyte adhesion (n = 6 animals per experimental group) were made before and at 10, 30, and 60 min after ANH (to hematocrit 0.3) with either 6% hydroxyethyl starch 200/0.6 (HES), 3.5% gelatin, 5% human serum albumin (HSA), or 6% dextran 60 (DX-60) followed by intravenous injection of 3 ml/kg body weight of a 60% weight/volume perfluorocarbon emulsion based on perflubron (perfluorooctyl bromide) emulsified with egg yolk lecithin.**

Results: **Acute normovolemic hemodilution with HES, gelatin, or HSA followed by injection of perflubron emulsion elicited no alterations of local microvascular perfusion or leukocyte–endothelium interaction as assessed in arterioles and postcapillary venules. However, ANH with DX-60 followed by injection of perflubron emulsion led to a significant reduction of erythrocyte velocity in postcapillary venules and an increase in venular leukocyte sticking that was never observed with DX-60 alone.**

Conclusions: **Hydroxyethyl starch, gelatin, and HSA are compatible with perflubron emulsion in the setting of ANH. Only DX-60 appeared to be incompatible with perflubron emulsion, as evidenced by impairment of capillary perfusion. (Key words: Blood substitute; microcirculation; oxygen carrier; perfluorocarbons.)**

THE development of blood substitutes capable of carrying and delivering oxygen to the tissue has proceeded with two different approaches. One relies on the use of hemoglobin-based oxygen-carrying solutions,[1] whereas the other relies on the use of perfluorocarbon-based oxygen-carrying solutions.[2] The latter are often referred to as perfluorocarbons or perfluorochemicals. The intrinsic ability of perfluorocarbons to dissolve and deliver oxygen has been highlighted in an experiment by Clark and Gollan,[3] who demonstrated that mice could be completely immersed into these liquids and survive for extended periods of time.[3] The first commercially available perfluorocarbon emulsion (Fluosol-DA 20%; Green Cross Corp., Osaka, Japan) was registered for clinical use as an oxygen carrier during percutaneous transluminal coronary angioplasty (balloon angioplasty).[2]

The elucidation of the pharmacokinetic profile of perfluorocarbon emulsions has greatly contributed to the understanding of the biologic effects occurring after intravenous injection in humans.[4,5] The perfluorocarbon emulsion particles are first cleared from the circulation through phagocytic cells of the reticulo-endothelial system, followed by liposolubilization of intracellular perfluorocarbon back into the circulation and elimination of the perfluorocarbon as a vapor through the lung in expired air.

The biologic interactions of perfluorocarbon emulsions with macrophages both *in vitro* and *in vivo* led us to investigate whether the potential clinical application of perfluorocarbons in perioperative hemodilution for autologous blood collection in elective surgery is associated with any activation of intravascular leukocytes, which might impair microvascular perfusion. To address this issue, we investigated the effects of perflubron emulsion on the microcirculation of striated skin muscle in conscious golden hamsters by analyzing microvascular leukocyte–endothelium interaction and perfusion during the clinically relevant situation of acute normovolemic hemodilution (ANH). Different colloids clinically applied as plasma expanders were investigated to rule out any unwanted interactions of these solutions with perflubron emulsion.

Materials and Methods

Fluorocarbon Solution

Perflubron emulsion (Oxygent, AF0144; Alliance Pharmaceutical Corp., San Diego, CA) is a 60% weight/vol-

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Table 1. Properties of Perflubron Emulsion

Parameter	Values
Osmolality	300–310 mOsm/kg
pH	7.0–7.2
Median particle size	0.16–0.18 μm
Endotoxin*	Negative
Emulsifying surfactant	Egg yolk phospholipid
Oxygen solubility (Po_2 760 mmHg; 37°C)	~27 ml O_2/100 g PFC
Viscosity (at a shear of 1 s^{-1})	~4 cP

* The product was terminally heat sterilized and tests for pyrogens and endotoxin yielded negative results (Dr. Keipert, Alliance Pharmaceutical Corp., San Diego, CA).

Po_2 = partial pressure of oxygen; PFC = perfluorocarbon.

ume perfluorocarbon emulsion containing perfluorooctyl bromide ($C_8F_{17}Br$) as the primary perfluorocarbon, egg yolk phospholipid as an emulsifying surfactant, phosphate-buffered saline for osmolality in the aqueous phase, and a small quantity of a secondary perfluorocarbon, perfluorodecyl bromide ($C_{10}F_{21}Br$), to stabilize particle size and slow down Ostwald ripening (growth of particle size over time caused by molecular diffusion of perfluorocarbon from one particle into another). The properties of perflubron emulsion are summarized in table 1.

Colloidal Solutions

As plasma volume substitutes, the following solutions were used to perform the ANH to a final hematocrit of 0.3: (1) 6% hydroxyethyl starch 200/0.6 (HES; Schiwa, Glandorf, Germany); (2) 3.5% gelatin (Haemaccel; Behring, Marburg, Germany); (3) 5% human serum albumin (HSA; Immuno, Heidelberg, Germany); and (4) 6% dextran 60 (DX-60; Schiwa, Glandorf, Germany).

Animal Model

The experiments were approved by the Local Ethics Committee and were performed according to the National Institutes of Health guide for the care and use of laboratory animals. Intravital fluorescence microscopy was performed in the dorsal skin fold chamber preparation of conscious Syrian golden hamsters as first described by Endrich *et al.*[6] and subsequently modified.[7,8] With this setup, intravital microscopic analysis of microhemodynamic parameters, including sequential measurements of vessel diameter and erythrocyte velocity, quantitative assessment of leukocyte–endothelial cell interactions, macromolecular leakage, and functional capillary density can be performed in the thin striated skin muscle (fig. 1A). During the intravital microscopic investigations, the awake hamsters are immobilized in specially designed tubes (fig. 1B) in which they had been acclimatized before start of the experimental design. The wall of the tubes was perforated with multiple 5-mm-diameter holes allowing for adequate breathing of room air. Oxygen could also be administered through an inlet

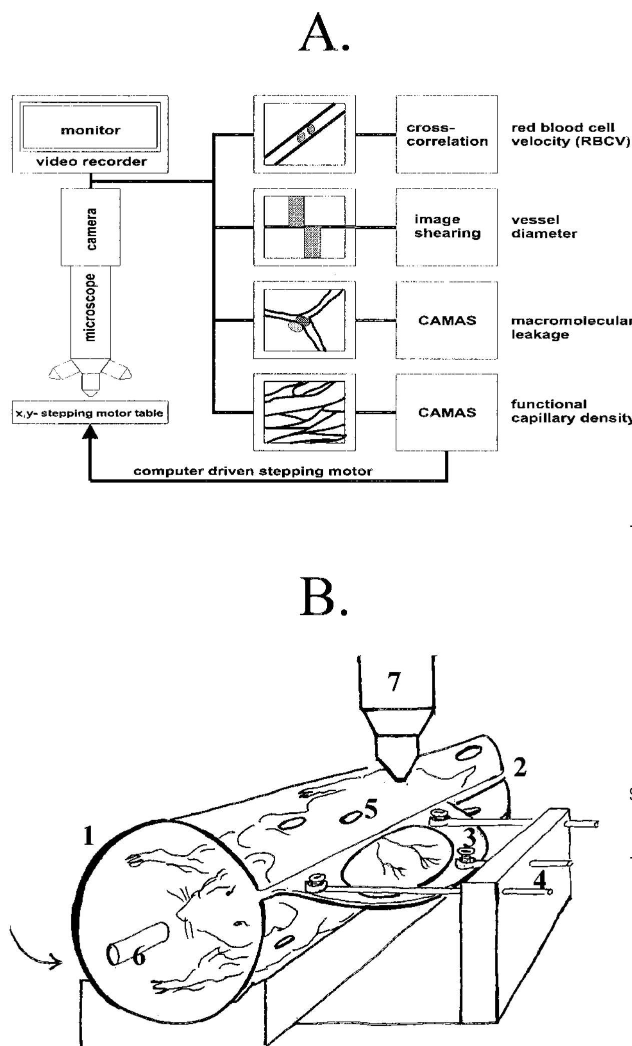

Fig. 1. Animal model and intravital microscopic analysis setup (for technical details, see reference 7). (*A*) Intravital microscopic setup with observation stage connected to a computer-driven stepping motor permitting the recall of the previously defined vessel segments in the microcirculation of the striated skin muscle of the hamster (for details, see Methods). The microcirculatory parameters are recorded on videotape and are analyzed offline using the Computer-assisted Microcirculation Analysis Software (CAMAS).[10] (*B*) Awake hamster immobilized in a specially designed tube (1) with a slit at the top (2) allowing the horizontal positioning and rigid fixation of the chamber (3) achieved by a holder (4). Also shown are holes in the tube (5) and inlet at the anterior side of the tube for connection to oxygen line (→) (6), as well as the objective of the fluorescence microscope (7).

at the end of the tube (fig. 1B). The animal model, the handling under the intravital microscope, and the intravital microscopic setup are shown in figures 1A and 1B.

Surgical Technique

Male Syrian golden hamsters (age, 6–8 weeks; weight, 40–60 g) were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body weight; Abbott, Wiesbaden, Germany). Implantation of the chambers was performed according to Endrich *et al.*[6] with minor mod-

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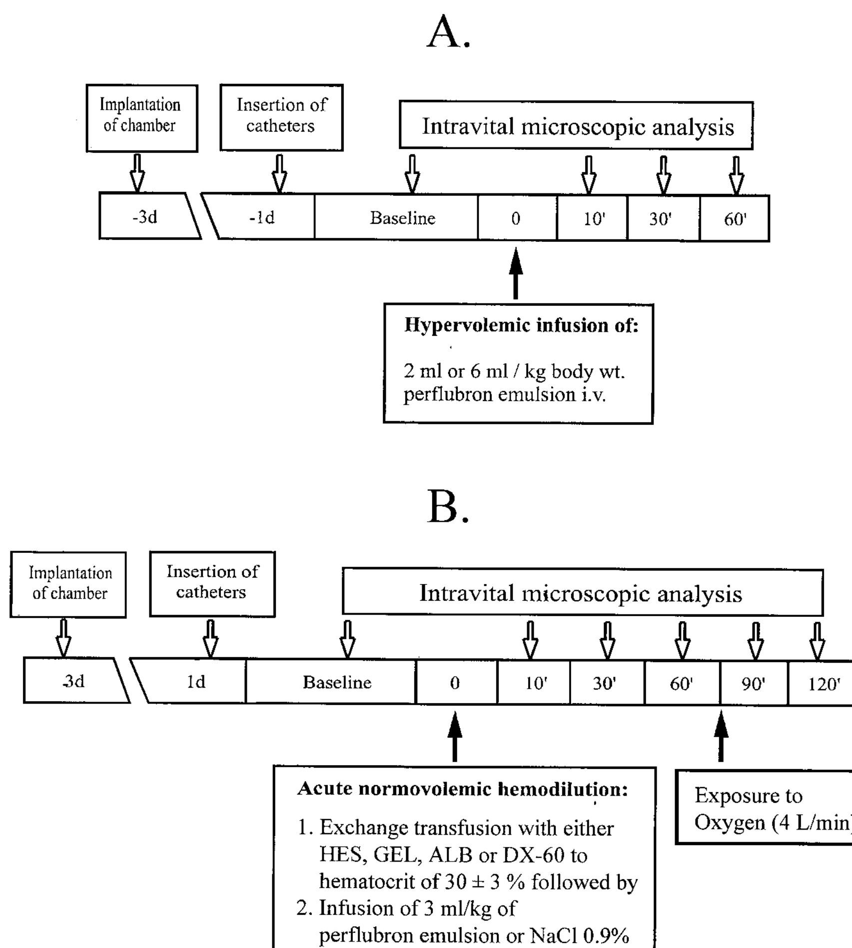

Fig. 2. Experimental designs. Hypervolemic top load infusion of perflubron emulsion (*A*) and acute normovolemic hemodilution with the different colloidal solutions in the presence or absence of perflubron emulsion used in the present study (*B*). Wt. = weight; i.v. = intravenous; HES = hydroxyethyl starch 200/0.6; GEL = gelatin 3.5%; ALB = human serum albumin; DX-60 = dextran 60 kd 6%.

ifications. Briefly, the entire back of the animal was shaved, and two titanium frames were implanted so as to sandwich the extended double layer of the skin. One layer was completely removed in a circular area of 15 mm in diameter, and the remaining layer, consisting of epidermis, subcutaneous tissue, and thin striated skin muscle, was covered with a cover slip incorporated in one of the frames. Fine polyethylene catheters (PE10, 0.28-mm ID) were inserted into the jugular vein and the carotid artery, respectively, passed subcutaneously to the dorsal side of the neck, and sutured to the titanium frames. The animals tolerated the dorsal skin fold chambers well and showed no signs of discomfort. In particular, no effects on sleeping or feeding habits were observed. A recovery period of 48–72 h was allowed to elapse between implantation of chambers and subsequent microscopic investigations, to eliminate effects of anesthesia and surgical trauma on the microcirculation.

Intravital Fluorescence Microscopy

A 25-fold water immersion objective (total magnification, 560-fold; E. Leitz, Inc., Wetzlar, Germany) was used to identify six to eight sites of interest per chamber, each containing one to two arterioles, four to six postcapillary venules, and one region of interest, consisting of six to eight video frame shifts of neighboring capillary fields for assessment of functional capillary density. By means of a computer-controlled stepping motor, the identical vessel segments and regions of interest could be located and investigated. Vessel diameter, erythrocyte velocity, leukocyte–endothelium interaction, functional capillary density, and macromolecular leakage were assessed at preset time points. For the measurement of vessel diameter, erythrocyte velocity, functional capillary density, and macromolecular leakage, the plasma was stained with fluorescein-isothiocyanate–labeled dextran (FITC-dextran, 15 mg/kg body weight, M_r 150,000; Pharmacia, Uppsala, Sweden). Erythrocyte velocity was assessed by a cross-correlation technique using the Capi-Flow software system[9] (Version 3.1; Lawrence Comp., Sulzbach, Germany).

Endothelial Integrity and Capillary Perfusion

As an indicator of endothelial integrity, macromolecular leakage was assessed immediately after the 30-s period of leukocyte visualization by densitometry of extravasated FITC-dextran 150,000 and quantified as the quotient of fluorescence intensity outside *versus* that inside the vessel segment. Functional capillary density was assessed by measuring the length of erythrocyte-

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perfused capillaries per observation area through redrawing of the capillary on the video screen using a mouse pad (expressed as cm^{-1}). The mathematical calculation was performed by a Pythagorean quantification exerted by the Computer-assisted Microcirculation Analysis System (CAMAS).[10,11]

Leukocyte–Endothelium Interaction

As an indicator of leukocyte activation, the interaction of leukocytes with the endothelial lining of postcapillary venules was quantitatively analyzed. Leukocyte–endothelial cell interaction was assessed by *in vivo* staining of leukocytes with the fluorescence marker rhodamine 6G (0.15 mg/kg body weight administered intravenously; Sigma Chemical Company, Deisenhofen, Germany). Because of the different fluorescence spectra of rhodamine 6G and FITC-dextran 150,000, visualization of macromolecular leakage and leukocyte–endothelium interaction was sequentially performed in the identical vessel segments.[12] According to their interaction with the microvascular endothelium, leukocytes were classified as nonadherent, rolling, or sticking leukocytes. Nonadherent leukocytes were defined as cells passing the observed vessel segments without interacting with the endothelial lining and are expressed as cells per microliter per minute. Rolling leukocytes were defined as cells moving along the endothelial lining at a velocity markedly less than that of the surrounding erythrocyte column and are given as the number of cells traversing an observed vessel segment within 1 min. Sticking leukocytes were defined as cells that did not detach from the endothelial lining within a 30-s observation time and are given as cells per squared millimeter of endothelial surface, calculated from diameter and length (200 μm) of the vessel segment under study. Epi-illumination was achieved with a 75-watt HBO mercury lamp attached to a Ploemopak illuminator (E. Leitz, Inc., Wetzlar, Germany) with an I-2/3 filter block and a N-2 filter block for sequential plasma and leukocyte staining, respectively, as described recently.[12] The microscopic images (camera, COHU 44,00; Prospective Measurements, San Diego, CA) were recorded on videotape and analyzed during playback using CAMAS.[10]

Experimental Designs

Hypervolemic Infusion. To first determine whether perflubron emulsion had any direct effects on the microcirculation, animals were given an infusion of either 2 or 6 ml/kg body weight (equivalent to 1.2 or 3.6 g/kg, respectively). These doses were selected to encompass the current dose of 4.5 ml/kg being used in phase III clinical studies. Intravital microscopic measurements were made before the hypervolemic infusion (baseline) and at 10, 30, and 60 min after infusion (fig. 2A).

Acute Normovolemic Hemodilution. Perfluorocarbon (3 ml/kg [1.8 g/kg]) was administered after ANH of the animals to a hematocrit of 27–33%. ANH was performed by drawing blood from the carotid artery and simultaneously infusing one of the different colloidal solutions through the jugular vein as previously described.[13,14] Measurements were made before hemodilution and at 10, 30, 60, 90, and 120 min thereafter (fig. 2B). Because perfluorocarbon emulsions are designed to function optimally in the presence of high arterial oxygen partial pressure (Pa_{O_2}), it was important to address the impact of oxygenation on microvascular, particularly arteriolar, diameters.[15] Awake animals were therefore exposed to 4 l/min of oxygen, which was started immediately after finishing the 60-min recordings (fig. 2B). Thus, values obtained at 120 min after administration of perflubron emulsion take into account the increase in Pa_{O_2} from approximately 60–80 mmHg before to approximately 300–500 mmHg after oxygen breathing (table 2).

Table 2. Quantitative Analysis of Pa_{O_2} and Mean Arterial Blood Pressure (MAP)

Parameter (mmHg)	Treatment	Baseline	10 min before 4 l $O_2 \times min^{-1}$ (60')	30 min after 4 l $O_2 \times min^{-1}$ (120')
Pa_{O_2}	HES	66 ± 8	72 ± 12	272 ± 46*
	HES + PFOB	70 ± 14	81 ± 21	369 ± 53*
	GEL	60 ± 22	76 ± 9	314 ± 164*
	GEL + PFOB	71 ± 4	64 ± 18	388 ± 115*
	HSA	81 ± 36	85 ± 13	463 ± 175*
	HSA + PFOB	68 ± 8	66 ± 5	507 ± 185*
	DX-60	63 ± 13	75 ± 15	282 ± 48*
	DX-60 + PFOB	74 ± 15	82 ± 12	353 ± 82*
MAP	HES	86 ± 5	90 ± 15	85 ± 12
	HES + PFOB	75 ± 14	71 ± 20	69 ± 13
	GEL	68 ± 22	76 ± 19	85 ± 15
	GEL + PFOB	71 ± 13	78 ± 18	89 ± 15
	HSA	81 ± 8	89 ± 14	93 ± 17
	HSA + PFOB	73 ± 12	77 ± 15	67 ± 20
	DX-60	92 ± 7	75 ± 20	78 ± 18
	DX-60 + PFOB	79 ± 16	92 ± 24	75 ± 8

Values are mean ± SD of six animals per experimental group. No statistically significant differences were observed between groups.

* $P < 0.05$ *versus* baseline, Wilcoxon test.

Pa_{O_2} = arterial oxygen tension; HES = hydroxyethyl starch; PFOB = perflubron emulsion; GEL = gelatin; HSA = human serum albumin; DX-60 = dextran, 60 kD.

Measurement of Fluorocrit and Arterial Oxygen Partial Pressure Fluorocrit was assessed according to the principle for measurement of hematocrit (Haematocrit Analyzer; Fa. Hettich, Munich, Germany) and is given as percent of the length of perflubron emulsion (indicated by the white color) per entire length of the centrifuged whole blood (consisting of the following three layers with increasing density: plasma, cells, and perflubron emulsion) in the hematocrit tube. Arterial blood gases were taken by drawing 100 μl of blood from the carotid artery and were analyzed using a standard blood gas analyzer (ABL 300; Radiometer Copenhagen, Radiometer GmbH, Willich, Germany).

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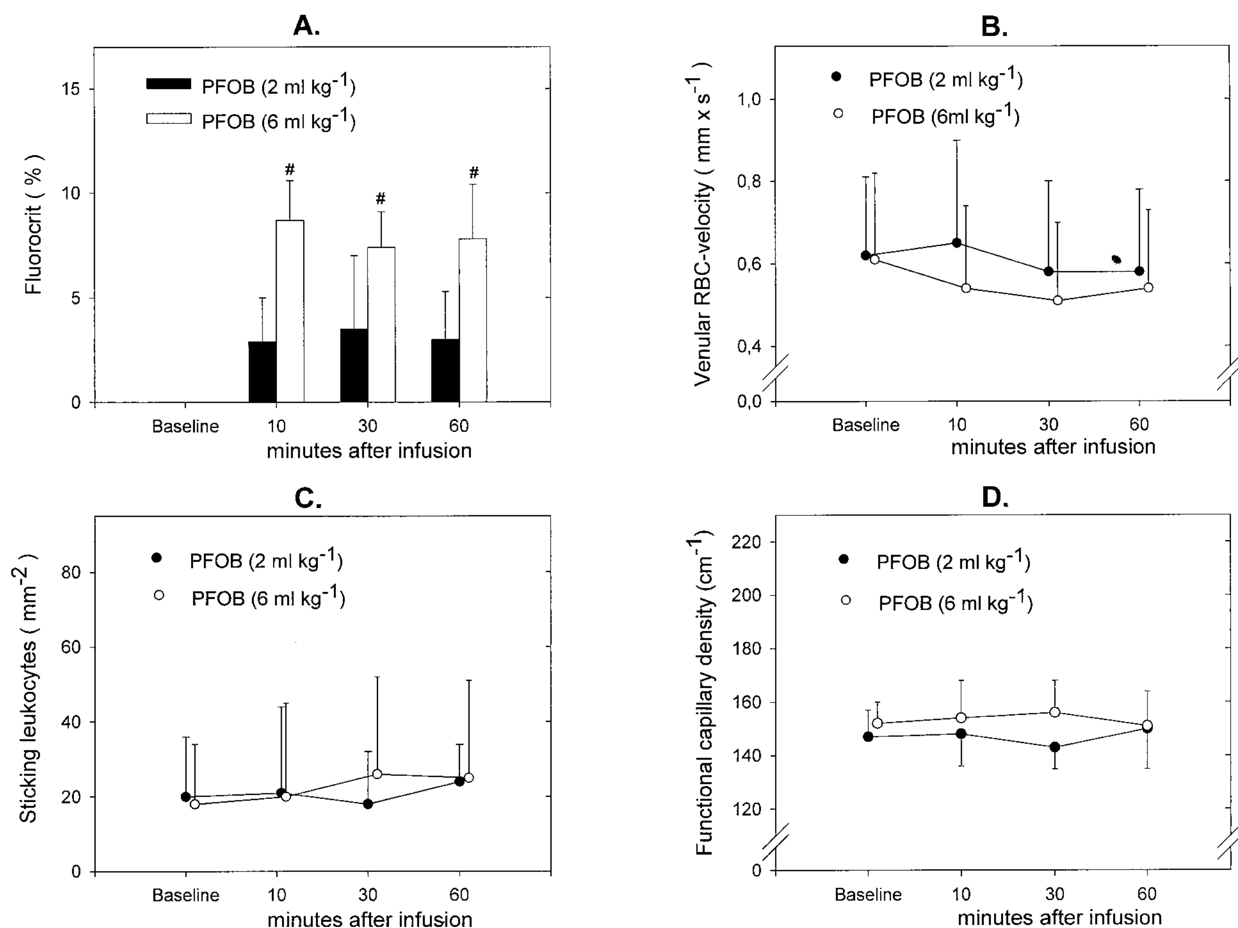

Fig. 3. Changes of arterial fluorocrit (*A*), venular erythrocyte velocity (*B*), leukocyte sticking (*C*), and functional capillary density (*D*) in the striated skin muscle of the awake Syrian golden hamster before and after intravenous (topload) infusion of 2 and 6 ml/kg body weight of perflubron emulsion (PFOB, 60% weight/volume). Fluorocrit indicates the percentage of perflubron emulsion of the total blood drawn from the carotid artery. Values are mean ± SD, n = 6. #*P* < 0.05 *versus* PFOB (2 ml/kg), Mann–Whitney test. RBC = red blood cell.

Statistical Analysis

Paired data of more than two samples were analyzed using Friedman (repeated-measures analysis of variance on ranks) nonparametric test for two-way analysis of variance and comparison on ranks for multiple samples followed by calculation of *P* using the Wilcoxon test. For unpaired data of more than two samples, the Kruskal-Wallis test (analysis of variance on ranks) was used followed by the Mann–Whitney test (Sigma Stat for Windows, Version 1.0; Jandel Corp., San Rafael, CA). *P* values less than 0.05 were considered significant. Despite nonparametric distribution, data are given in tables and figures as arithmetic mean ± 1 SD, unless otherwise indicated.

Results

Hypervolemic Infusion Study

Infusion of 2 and 6 ml/kg of perflubron emulsion resulted in fluorocrits of 3.0 ± 2.3% and 7.8 ± 2.6% at 60 min postinfusion, respectively (fig. 3A).

No significant changes in diameters of arterioles (30–70 μm) and postcapillary venules (20–50 μm) were seen after injection of either dose of perflubron emulsion. Likewise, no changes in local microvascular erythrocyte velocity in postcapillary venules were observed (fig. 3B).

The number of nonadherent and rolling leukocytes remained unaffected. There were no changes in the number of sticking leukocytes in postcapillary venules after infusion of perflubron emulsion (fig. 3C).

In neither of the treatment groups were changes of endothelial permeability observed in arteriolar or postcapillary segments of the microvascular bed. Functional capillary density was not altered after administration of perflubron emulsion 2 or 6 ml/kg (fig. 3D).

Acute Normovolemic Hemodilution Study

Microvascular Diameter and Erythrocyte Velocity. There were no changes in mean arterial blood pressure after ANH (table 2). Diameters of arterioles remained unchanged after infusion of 3 ml/kg of perflubron emulsion (table 3) in hamsters hemodiluted

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Table 3. Quantitative Analysis of Arteriolar Diameter and Rolling Leukocytes in Postcapillary Venules

			Time Points of Investigation				
			10 min	30 min	60 min	90 min	120 min
Parameter	Treatment	Baseline	after Acute Normovolemic Hemodilution				
Arteriolar diameter (μm)	HES	38 ± 6	38 ± 9	37 ± 10	38 ± 9	36 ± 6	38 ± 7
	HES + PFOB	40 ± 9	40 ± 6	38 ± 5	40 ± 6	39 ± 8	38 ± 8
	GEL	50 ± 9	52 ± 10	52 ± 11	56 ± 9	52 ± 6	51 ± 7
	GEL + PFOB	54 ± 5	53 ± 6	54 ± 8	52 ± 7	51 ± 3	52 ± 1
	HSA	63 ± 16	62 ± 11	63 ± 16	59 ± 18	56 ± 8	61 ± 1
	HSA + PFOB	53 ± 15	48 ± 14	48 ± 11	51 ± 13	50 ± 12	50 ± 1
	DX-60	40 ± 3	37 ± 5	40 ± 6	42 ± 4	36 ± 4	42 ± 4
	DX-60 + PFOB	42 ± 4	42 ± 5	44 ± 6	45 ± 7	37 ± 4	41 ± 5
Rolling leukocytes (cells/min^{-1})	HES	23 ± 10	29 ± 13	26 ± 7	24 ± 6	29 ± 7	31 ± 5
	HES + PFOB	29 ± 8	28 ± 11	26 ± 11	30 ± 11	29 ± 11	21 ± 5
	GEL	19 ± 8	9 ± 6	9 ± 3	16 ± 11	17 ± 6	18 ± 9
	GEL + PFOB	15 ± 6	10 ± 4	14 ± 5	15 ± 8	18 ± 7	26 ± 1
	HSA	13 ± 2	13 ± 4	14 ± 3	17 ± 8	15 ± 4	16 ± 7
	HSA + PFOB	18 ± 7	13 ± 4	17 ± 5	15 ± 5	19 ± 7	20 ± 6
	DX-60	25 ± 5	20 ± 3	26 ± 4	28 ± 3	32 ± 3	27 ± 4
	DX-60 + PFOB	18 ± 5	19 ± 3	23 ± 4	31 ± 4	35 ± 8*	48 ± 6

Values are mean ± SD of six animals per experimental group.

* $P < 0.05$ *versus* baseline, Wilcoxon test.

HES = hydroxyethyl starch; PFOB = perflubron emulsion; GEL = gelatin; HSA = human serum albumin; DX-60 = dextran, 60 kD.

with any of the four different colloids used (HES, gelatin, DX-60, and HSA). Likewise, no significant differences in arteriolar diameters were observed in either treatment group on 100% oxygen breathing when compared with the prehemodilution baseline values (table 3). Microvascular diameters and erythrocyte velocities in venules remained unchanged in the HES, gelatin, and HSA groups (figs. 4A–4C), whereas a significant reduction in venular erythrocyte velocity was observed after DX-60 treatment, beginning 30 min after start of the exchange transfusion (fig. 4D). Exposure to 100% oxygen at 60 min after exchange transfusion had no effects in either treatment group.

The further decrease of venular erythrocyte velocity in the DX-60 group at 90 and 120 min after exchange is not a result of the oxygen exposure, because similar observations were made in the absence of 100% oxygen breathing in a separate group of six animals (fig. 4D).

Leukocyte–Endothelium Interaction. Leukocyte rolling was unchanged after hemodilution with HES, gelatin, and HSA, whereas an increase was observed in the animals treated with DX-60 (table 3). This was independent of whether animals were exposed to oxygen 4 l/min. The enhancement of leukocyte rolling in the animals treated with DX-60 was accompanied by increased leukocyte sticking (fig. 5B). There was no enhanced leukocyte sticking observed after hemodilution with any of the other three (*i.e.*, HES, gelatin, and HSA) volume substitutes (figs. 5A and B).

Endothelial Integrity and Functional Capillary Density. In the animals hemodiluted with HES, gelatin, HSA, or DX-60, no changes in endothelial permeability were observed (figs. 5C and 5D). Functional capillary density, *i.e.*, the length of erythrocyte perfused capillaries per squared centimeter of observation area, remained unchanged after hemodiluting the animals with HES, HSA, or gelatin and perflubron emulsion over the entire observation period (fig. 6A). In the DX-60–treated animals, however, functional capillary density was found significantly decreased at 60 min after exchange transfusion in the presence of perflubron emulsion (fig. 6B).

Discussion

The present study provides important new data on the microcirculation of striated skin muscle in conscious Syrian golden hamsters after administration of perflubron emulsion. We have shown that hypervolemic administration of perflubron emulsion up to 6 ml/kg (3.6 g/kg) has no negative effects on microvascular perfusion; that doses of 3 ml/kg induce no unwanted side effects during normovolemic hemodilution (hematocrit 0.3) with either 6% HES, 3.5% gelatin, or 5% HSA as the hemodiluent; and that hemodilution with 6% DX-60 in conjunction with perflubron emulsion leads to a significant deterioration of microvascular perfusion, which may be, at least in part, caused by intravascular activation of leukocytes with ensuing adhesion to the microvascular endothelium.

The use of perfluorocarbon emulsions as oxygen carriers has been suggested to have several advantageous properties in the microcirculation.[16] First, perfluorocarbon emulsions have a much lower viscosity than blood when applied during the experimental conditions of ANH.[4] In this study, hemodilution was performed with different colloids that account for a reduction in whole-blood viscosity.[17] Perflubron emulsion given after ANH

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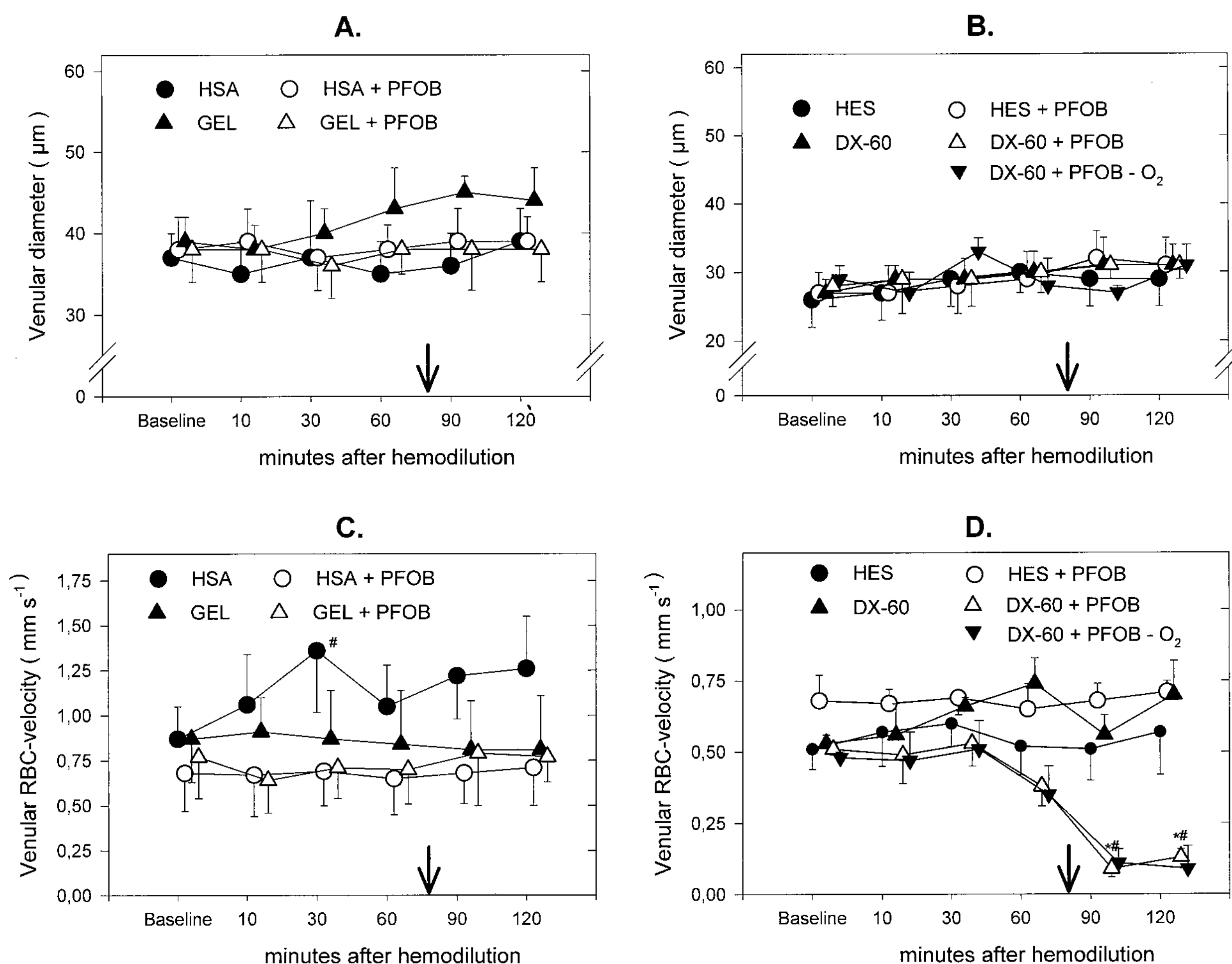

Fig. 4. Venular diameter (*A* and *B*) and erythrocyte velocity (*C* and *D*) in postcapillary venules of the striated skin muscle in the hamster after acute normovolemic hemodiluton (ANH; to hematocrit 0.3) with either 6% hydroxyethylstarch 200/0.6 (HES), 6% dextran-60 (DX-60), 5% human serum albumin (HSA), or 3.5% gelatin (GEL) followed by an additive administration of 3 ml/kg body weight of perflubron emulsion (PFOB, 60% weight/volume). Arrow indicates start of oxygen exposure (4 l/min). DX-60 + PFOB − O_2 indicates absence of additional oxygen exposure. Values shown are means ± SD of n = 6 animals per experimental group. *$P <$ 0.05 *versus* baseline, Wilcoxon test; #$P <$ 0.05 *versus* DX-60, Mann–Whitney test. RBC = red blood cell.

as an additive intravenous injection of 3 ml/kg yielded a fluorocrit of 2–4%, which has been demonstrated not to affect microvascular perfusion (fig. 3).

Second, the size of perfluorocarbon emulsion droplets compared with erythrocytes is approximately 35× smaller (0.2 µm *vs.* 7 µm) and may thus contribute to oxygenation, particularly in the critically ischemic tissue, by bypassing local stenoses where only plasma flow is maintained. This may be facilitated by the reduction of erythrocyte membrane stiffening and thus improved perfusion in the ischemia-endangered tissue during conditions of hypoxia and tissue acidosis.[18] Bajaj *et al.*[19] reported on the limitation of reperfusion injury with Fluosol, which he attributed to a reduction in neutrophil activation. Perfluorocarbon-based oxygen carriers have also proven to maintain organ function during preservation of various organs such as skin flaps, intestine, or hearts.[20–24] However, the interaction of perfluorocarbons on leukocytes in ischemia–reperfusion is controversial depending on the perfluorocarbon emulsions used in earlier studies. The impact of hyperbaric oxygen on the hypoxic or ischemic tissue may result in propagation of oxygen radical–mediated tissue damage.[25,26] Likewise, changes in local oxygen tension have been shown to influence vascular tone.[15,27,28] We therefore exposed the awake, spontaneously breathing animals to 4 l/min of oxygen, yielding Pa_{O_2} levels of 300–500 mmHg, compared with 60–80 mmHg at baseline without affecting mean arterial pressure (table 2). Even at these elevated Pa_{O_2} levels, no enhancement of leukocyte–endothelium interaction or changes in arteriolar or venular diameters were observed. This is in line with previous findings from our laboratory showing that, even during the experimental conditions of ischemia–reperfusion, there is no exacerbation of reperfusion injury in the presence of high oxygen tensions in this animal model.[29]

The incompatibility of dextran and perflubron emulsion, observed 90 min after administration, cannot be attributed to the supplemental oxygen exposure be-

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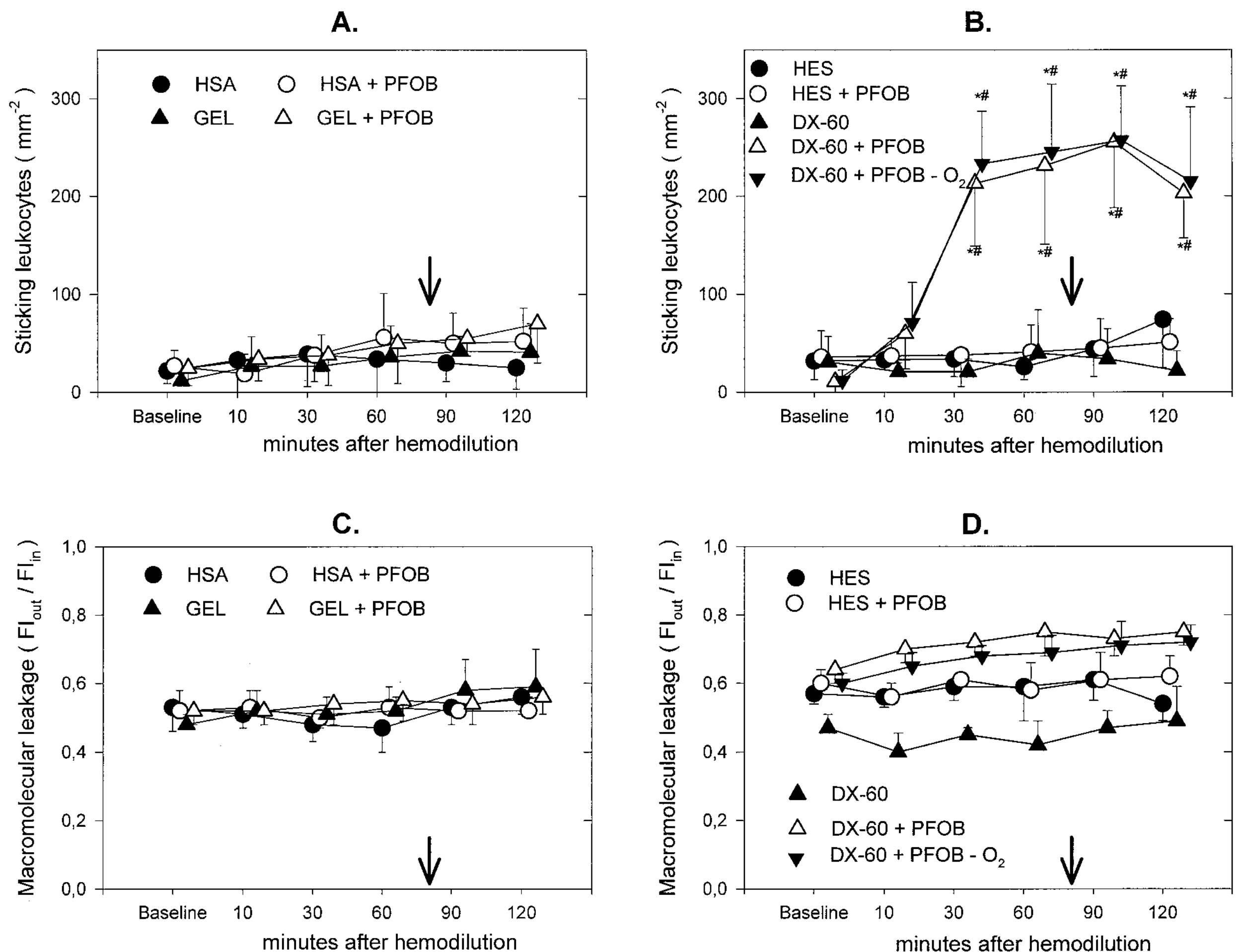

Fig. 5. Leukocyte sticking (*A* and *B*) and macromolecular leakage (*C* and *D*) in postcapillary venules of the striated skin muscle in the hamster after acute normovolemic hemodilution (ANH; hematocrit 0.3) with either 6% hydroxyethyl starch 200/0.6 (HES), 6% dextran-60 (DX-60), 5% human serum albumin (HSA), or 3.5% gelatin (GEL) followed by an additive administration of 3 ml/kg body weight of perflubron emulsion (PFOB, 60% weight/volume). Arrow indicates start of oxygen exposure. DX-60 + PFOB − O_2 indicates absence of additional oxygen exposure. Values shown are means ± SD of n = 6 animals per experimental group. *$P < 0.05$ *versus* baseline, Wilcoxon test; #$P < 0.05$ *versus* DX-60, Mann–Whitney test.

cause similar findings were made in a separate group of animals in the absence of oxygen exposure (fig. 5B). Thus, the enhancement of leukocyte–endothelium interaction in the DX-60–diluted animals was not induced by hyperoxia. Likewise, there was no significant change of mean arterial blood pressure in these treatment groups (table 2). Hence, the observed decrease in venular erythrocyte velocity in the animals treated with DX-60–perflubron emulsion cannot be attributed to a decrease in macrohemodynamic perfusion pressure (fig. 4D), supporting the concept of a rather specific, albeit undetermined, interaction.

Third, Fluosol-DA, the first-generation 20% weight/volume perfluorocarbon emulsion consisting of perfluorodecalin and perfluorotripropylamine, has been suggested to be beneficial for the outcome of hypoxia-endangered tissue by an apparent inhibitory action on neutrophil function.[30] In our study, we observed no inhibitory effect of perflubron emulsion on leukocyte activation or adhesion in the applied microcirculation model. In the hypervolemic infusion study, doses of intravenous perflubron emulsion of 2 and 6 ml/kg were void of unwanted side effects on the microcirculation. Based on these results, we investigated the effects of the second-generation 60% weight/volume perflubron emulsion[31,32] on the microcirculation of striated skin muscle during the conditions of ANH, mimicking the clinical situation of autologous blood collection in elective surgery. Using a canine model of hemodilution followed by dosing with a 90% weight/volume perflubron emulsion, Keipert *et al.*[33] showed a significant increase in oxygen delivery during profound hemodilution even to a hemoglobin level of approximately 4 g/dl. These findings have been substantiated in a recent study in dogs in which perflubron emulsion effectively maintained tissue oxygenation after profound hemodilution to hemoglobin levels ranging as low as approximately 3.0 g/dl.[34] Our study extends these previous findings on oxygen delivery at the microcirculatory level using the hamster dorsal skin fold chamber preparation, which has already been proven suit-

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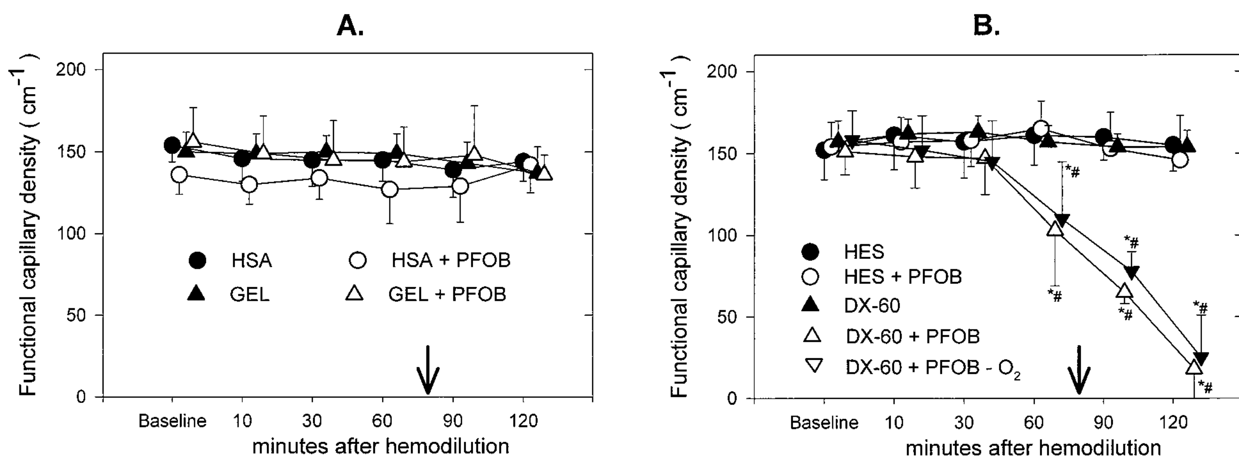

Fig. 6. Functional capillary density (*A* and *B*) in the striated skin muscle of the hamster after acute normovolemic hemodilution (ANH; hematocrit 0.3) with either 6% hydroxyethyl starch 200/0.6 (HES), 6% dextran-60 (DX-60), 5% human serum albumin (HSA), or 3.5% gelatin (GEL) followed by an additive administration of 3 ml/kg body weight of perflubron emulsion (PFOB, 60% weight/volume). Arrow indicates start of oxygen exposure (4 l/min). DX-60 + PFOB − O_2 indicates absence of additional oxygen exposure. Values shown are mean ± SD of n = 6 animals per experimental group. *$P < 0.05$ *versus* baseline, Wilcoxon test; #$P < 0.05$ *versus* DX-60, Mann–Whitney test.

able for investigation of various treatment schedules,[7] including oxygen-carrying solutions.[13,14,35,36] The commonly used colloids HES, gelatin, and HSA were all fully compatible with perflubron emulsion. Only hemodilution with DX-60 followed by dosing with perflubron emulsion (3 ml/kg) led to a significant impairment of microvascular perfusion, as assessed by local erythrocyte velocity (fig. 4D) and functional capillary density (fig. 6). The underlying mechanism may be ascribed to an intravascular activation and adhesion of leukocytes (fig. 5), which was independent of alterations in macrohemodynamic driving forces (table 2). The demonstration that no adverse reactions were observed with HES, gelatin, or HSA strongly suggests a dextran-specific interaction with the injected perflubron emulsion independent of an increase in systemic Pa_{O_2}.

The first demonstration of a direct action of perfluorocarbons on macrophages was described by Bucala *et al.*,[37] who proposed perfluorocarbon-based emulsions as a potential drug for selective removal of macrophages from blood using FC-43 (perfluorotributylamine, 25%) and pluronic F-68 as emulsifier. An experimental study in mice using Fluosol-DA 20% demonstrated impaired neutrophil migration during the inflammatory reaction, which was associated with increased infection mortality and attributed to the emulsifier Pluronic F-68.[38] However, contrary findings on the effects of perfluorocarbons (such as Oxypherol [Green Cross Corp.] or Fluosol-DA) on neutrophil function showed an attenuation of phagocytosis, chemotaxis, and oxygen-free radical formation.[30,39] These reports on perfluorocarbons or their emulsifiers *vis à vis* leukocyte function demonstrate the differences in biologic effects that can occur, and they support the need for careful analysis of immune function studies in the microcirculation. Endrich *et al.*[40] were the first to report that certain perfluorocarbons or their emulsifiers (FC-47 and FC-43 in 5% pluronic) can exert unwanted side effects based on video microscopic studies of the omental microcirculation, which demonstrated augmented venular leukocyte sticking, conglomeration of erythrocytes in capillaries, and damage to endothelial cells. We tested several perfluorocarbon emulsions differing in their capacity to interfere with spontaneous leukocyte–endothelium interaction, of which only a few did not provoke an enhancement of leukocyte–endothelium interaction *in vivo*.[36] Thus, the dose of a particular fluorochemical or its emulsifying agents appears to play a pivotal role in mediating biologic compatibility.

The phenomena seen after hemodilution with DX-60 followed by injection of 3 ml/kg perflubron emulsion may be attributed to an aggregate formation between dextran and perflubron emulsion particles (which is observed *in vitro* when DX-60 is mixed directly with perflubron emulsion). These aggregates would be expected to induce macrophage activation with ensuing recruitment of leukocytes. The alterations could also

Table 4. Hematology Assessment

Parameter	Treatment	Baseline (before ANH)	60 min post-ANH
Leukocytes ($10^3/\mu l$)	DX-60 + PFOB	3.3 ± 1.8	3.5 ± 1.4
	DX-60 alone	3.7 ± 1.2	3.74 ± 1.4
Platelets ($10^3/\mu l$)	DX-60 + PFOB	414.8 ± 64.5	347.9 ± 44.1
	DX-60 alone	413.8 ± 31.9	400.8 ± 18.2
Fibrinogen (mg/dl)	DX-60 + PFOB	197.5 ± 32.7	121.3 ± 25.4
	DX-60 alone	178.0 ± 38.3	109.8 ± 17.9

Values are mean ± SD of six animals per experimental group. No statistically significant differences were observed within or between groups, although there is a trend for platelet count and fibrinogen levels to decrease in the dextran (60 kD) (DX-60) + perflubron emulsion (PFOB)–treated animals 60 min after ANH.

ANH = acute normovolemic hemodilution.

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be caused by an activation of the coagulation cascade because alterations of the platelet count and fibrinogen levels in the blood of the animals treated with DX-60 plus perflubron emulsion were observed (table 4). Hence, a disseminated intravascular coagulation syndrome as transient pathomechanism may be considered. A perflubron emulsion–dependent hypersensitivity reaction of the hamsters to the dextran component as reported in the rat[41] can be ruled out from our studies.[7,42,43]

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