Figure 7 Differences in oxygen saturation profiles between hRBC (solid line) and αNO-hRBC (broken line). Solid and broken arrows indicate estimations of differences in SaO_2 for hRBC and αNO-RBC during transition across hepatic microcirculation, respectively. The estimation is based on observations that mean PO_2 values in periportal regions are 70 mmHg in both groups and those in central venules are 28 mmHg and 33 mmHg in the groups treated with αNO-hRBC and normal hRBC, respectively (See Figure 6E).

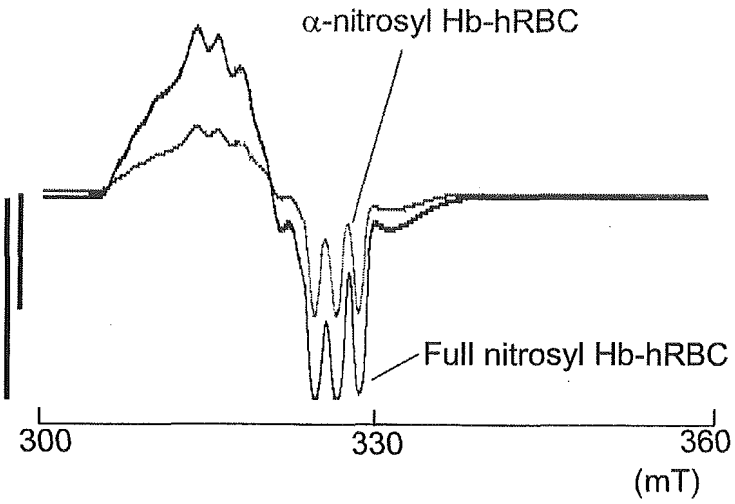
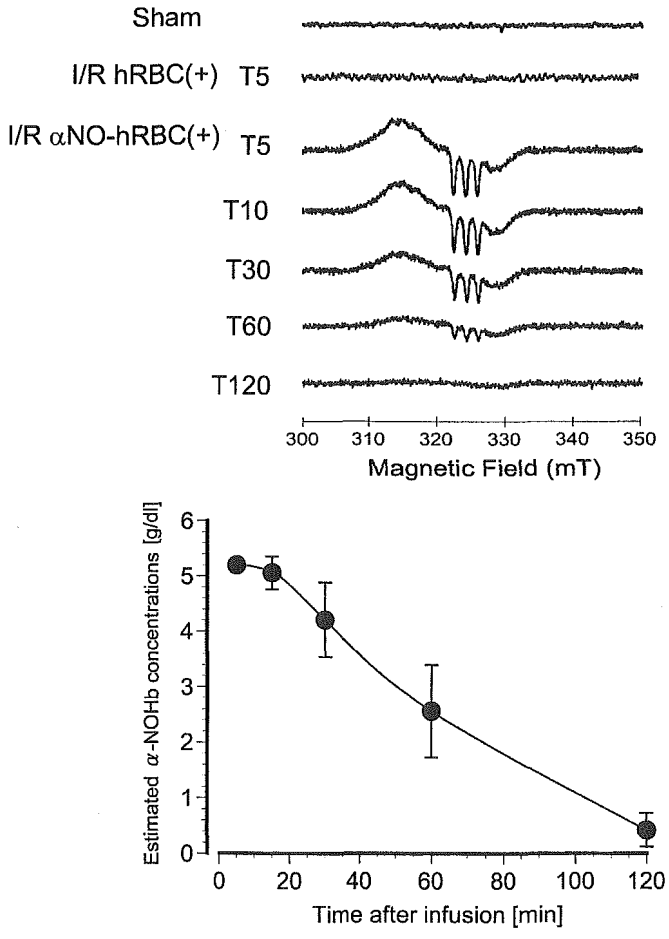


Figure 1



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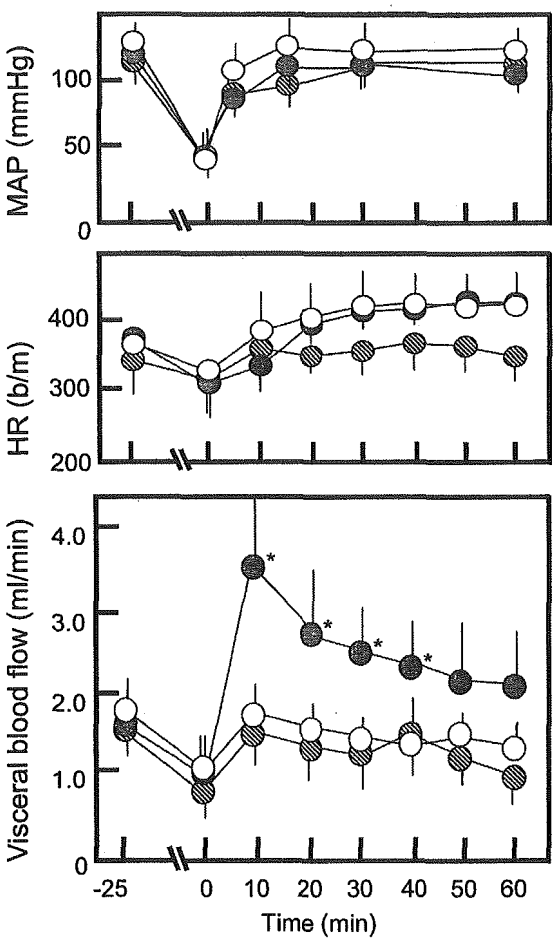

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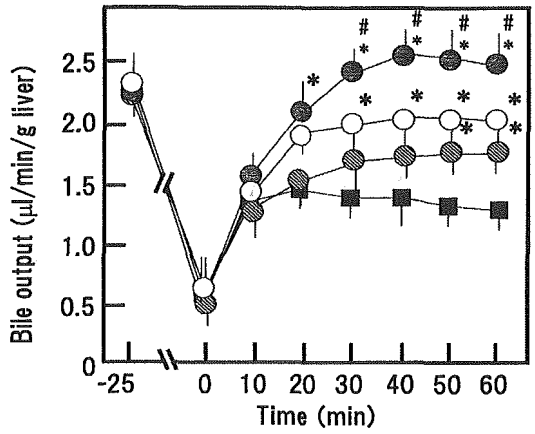


Figure 4

Figure 5

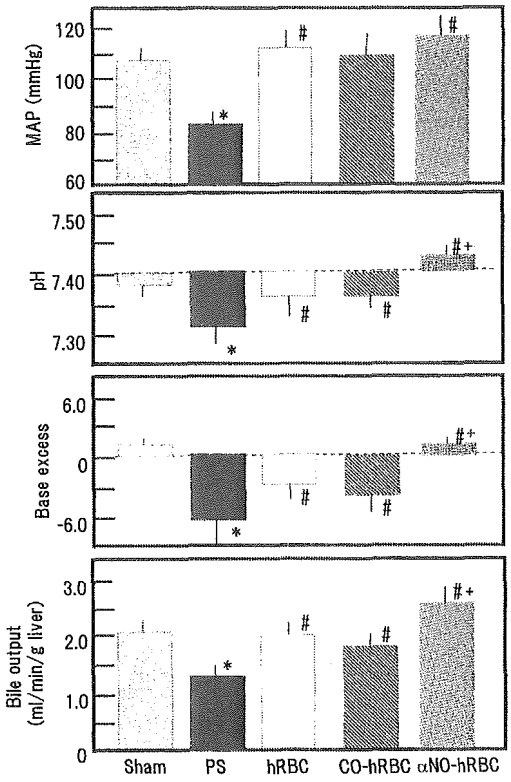
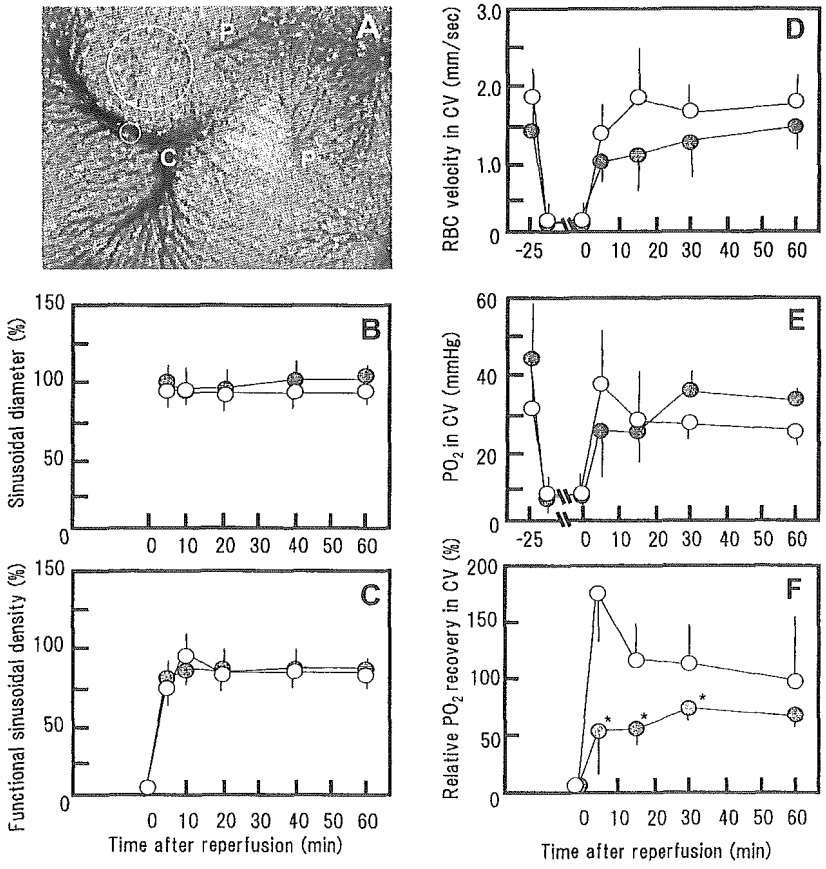


Figure 6



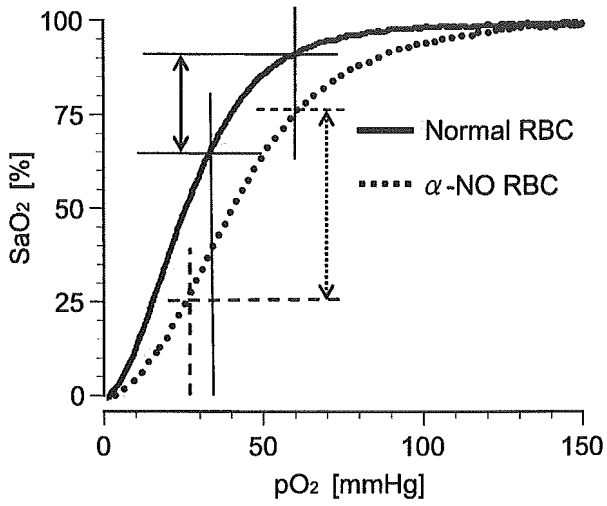


Figure 7

New generation of hemoglobin-based oxygen carriers evaluated for oxygenation of critically ischemic hamster flap tissue

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Objectives: **The aim of this study was to investigate and compare the effects of a traditionally formulated, low-viscosity, right-shifted polymerized bovine hemoglobin solution and a highly viscous, left-shifted hemoglobin vesicle solution (HbV-HES) on the oxygenation of critically ischemic peripheral tissue.**

Design: **Randomized, prospective study.**

Setting: **University laboratory.**

Subject: **A total of 40 male golden Syrian hamsters.**

Interventions: **Island flaps were dissected from the back skin of anesthetized hamsters. The flap included a critically ischemic, hypoxic area that was perfused via a collateralized vasculature. One hour after completion of the preparation, the animals received a 33% blood exchange with 6% hydroxyethyl starch 200/0.5 (HES, n = 9), HbV suspended in HES (HbV-HES, n = 8), or polymerized bovine hemoglobin solution (n = 9).**

Measurements and Main Results: **Three hours after the blood exchange, microcirculatory blood flow (laser-Doppler flowmetry) was increased to 262% of baseline for HbV-HES ($p < .01$) and 197% for polymerized bovine hemoglobin solution ($p < .05$ vs. baseline and HbV-HES). Partial tissue oxygen tension (bare fiber probes) was only improved after HbV-HES (9.4 torr to 14.2 torr, $p < .01$ vs. baseline and other groups). The tissue lactate/pyruvate ratio (microdialysis) was elevated to 51 in the untreated control animals, and to 34 ± 8 after HbV-HES ($p < .05$ vs. control) and 38 ± 11 after polymerized bovine hemoglobin solution (not significant).**

Conclusions: **Our study suggests that in critically ischemic and hypoxic collateralized peripheral tissue, oxygenation may be improved by normovolemic hemodilution with HbV-HES. We attributed this improvement to a better restoration of the microcirculation and oxygen delivery due to the formulation of the solution. (Crit Care Med 2005; 33:806–812)**

Key Words: arterial occlusive diseases; blood substitutes; collateral circulation; energy metabolism; microcirculation; surgical flaps

Occlusion of the anatomic blood supply may lead to critical ischemia in a variety of organs and tissues. Functionality and survival of these tissues depend on the maintenance of adequate oxygen delivery via a collateral vasculature, which is determined by its perfusion and oxygen content.

Artificial oxygen carriers, first developed >30 yrs ago, were created to increase the oxygen content of arterialized blood without risking the adverse effects associated with blood transfusions (1–3). These products were designed to meet the physicochemical properties of normal blood as closely as possible. However, a number of drawbacks have hindered their introduction into clinical practice. One major problem was the vasoconstrictor effect of cell-free hemoglobins, presumably due to their scavenging of nitric oxide (NO) (1–3). This adverse effect has been circumvented by chemical modification of the hemoglobin molecules. Hemopure (Biopure, Cambridge, MA) is one of two chemically modified hemoglobin products currently awaiting U.S. Food and Drug Administration approval after having been extensively tested in clinical trials as a replacement for blood transfusions (4). Hemopure has been approved for routine clinical use in South Africa. Oxyglobin, its veterinary equivalent, was introduced to the U.S. market in 1998. It is a polyionic colloidal fluid consisting of glutaraldehyde-polymerized, ultrapurified bovine hemoglobin (PBHb) suspended in a modified Ringer's solution. Oxyglobin contains 13 g/dL hemoglobin (Table 1). Hemoglobin concentration, osmolarity, and viscosity are in a physiologic range, whereas colloid osmotic pressure was set higher and oxygen affinity was right-shifted (P_{50} = 54 torr) (5) with the scope of blood volume expansion and facilitated oxygen release.

In past years, a new concept has emerged in which oxygen carriers are regarded as oxygen therapeutics rather than as blood substitutes (2, 3, 5–8). Still, in an attempt to avoid blood transfusions, the new generation of artificial oxygen carriers are used to improve the microcirculation and redistribute oxygen in favor of the tissues in need without necessarily augmenting total hemoglobin concentration. For this purpose, increased colloid osmotic pressure, viscosity, and oxygen affinity of the oxygen-carrying solutions proved to be advantageous and more relevant than

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Table 1. Physicochemical characteristics of hamster blood and the diluents

	Hamster Blood	Hamster Plasma	HES	HbV-HES	PBHb
Hb concentration, g/dL	18		0	7.5	13
Mean particle diameter, nm	7,000			250	10
Mean molecular mass, kDa				3,000,000	180
P_{50}, torr	28			15	54
metHb concentration, %				5.2	5.0
Oncotic pressure, mm Hg		18	36	36	38
Viscosity of solution, cP	4.5	1.2	1.9	11.5	1.8
Viscosity in circulation, cP		1.34	1.31	1.68[a]	1.42[a,b]

HES, 6% hydroxyethyl starch; HbV-HES, hemoglobin vesicles suspended in HES; PBHb, polymerized bovine hemoglobin; Hb, hemoglobin; metHb, methemoglobin.

[a] $p < .01$ vs. hamster plasma; [b] $p < .01$ vs. HbV-HES. Viscosity of the solutions was measured at 37°C and at 150 s^{-1}; plasma viscosity was measured at 25°C; data are mean values.

raising the hemoglobin concentration in blood, as evidenced by a series of experimental shock studies (5, 7, 8). It seems conceivable that the concept of targeting oxygen delivery to ischemic tissues may be particularly applicable in an acute situation of local tissue ischemia due to peripheral arterial occlusion, serving as a bridge until adequate perfusion can be re-established either by spontaneous neovascularization or surgical revascularization. In previous studies, we were able to demonstrate that oxygenation in collateralized, ischemic hamster flap tissue was improved after isovolemic hemodilution with highly viscous, highly oncotic solutions containing left-shifted, encapsulated human hemoglobins (9, 10). Encapsulation represents a new alternative to chemical modification and was achieved by a phospholipid bilayer membrane coated with polyethylene glycol (11).

The aim of this study was to compare the effects of a traditionally formulated polymerized bovine hemoglobin solution (Oxyglobin) and a highly viscous solution containing a low concentration of left-shifted encapsulated hemoglobins (the new generation of oxygen carriers), both administered in the course of intentional isovolemic hemodilution, on the oxygenation of acutely and critically ischemic peripheral tissues in the well-established hamster-flap model described previously (9, 10, 12). Due to its widespread clinical use, hydroxyethyl starch (HES) was chosen as the diluent in which the encapsulated hemoglobins (hemoglobin vesicles, HbV) were suspended. Microcirculatory blood flow, partial tissue oxygen tension, tissue lactate concentration, and lactate/pyruvate ratio were taken as end points.

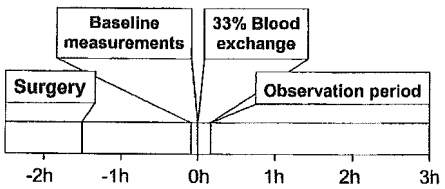

Figure 1. Diagram illustrating sequence of interventions.

MATERIALS AND METHODS

Animals and Solutions. Experiments were performed according to the National Institutes of Health guidelines for the care and use of laboratory animals and with the approval of the local animal ethics committee. A total of 40 male Syrian golden hamsters (Charles River, Sulzfeld, Germany) weighing 75–110 g were included in this study. The animals were randomly assigned and equally distributed to the control group and three groups subjected to normovolemic hemodilution with 6% HES 200/0.5 (Fresenius, Stans, Switzerland), HbV suspended in HES (HbV-HES), and PBHb, respectively. HbV was prepared as described previously (11). PBHb (Oxyglobin) was purchased from Biopure. The physicochemical characteristics of the solutions are described in Table 1.

Animal and Flap Preparation. A hamster skin-flap model was used as previously described in detail (9, 10, 12). Anesthesia was induced by pentobarbital injected intraperitoneally (100 mg/kg body weight, Nembutal, Abbott Laboratories, Chicago, IL). The carotid artery and external jugular vein were cannulated for administration of anesthesia, blood exchange, laboratory analysis, and monitoring blood pressure. Catheterization and flap dissection were performed with the aid of an

Table 2. Systemic and laboratory data at baseline and at 1 and 3 hrs after blood exchange

	Baseline	1 hr	3 hrs
MAP, mm Hg			
Control	91 ± 6	90 ± 5	83 ± 11[a]
HES	93 ± 6	92 ± 6	84 ± 7[a]
HbV-HES	94 ± 3	92 ± 3	80 ± 7[b]
PBHb	95 ± 5	92 ± 3	93 ± 6
Hematocrit			
Control	0.55 ± 0.04	0.54 ± 0.03	0.52 ± 0.03
HES	0.59 ± 0.04	0.31 ± 0.04[b]	0.31 ± 0.04[b]
HbV-HES	0.59 ± 0.04	0.31 ± 0.02[b]	0.33 ± 0.04[b]
PBHb	0.58 ± 0.05	0.32 ± 0.05[b]	0.31 ± 0.04[b]
Total Hb concentration, g/dL			
Control	17.8 ± 1.2	17.2 ± 1.0	17.0 ± 1.1
HES	17.8 ± 1.3	9.7 ± 0.9[b]	10.5 ± 1.0[b]
HbV-HES	18.8 ± 0.7	11.2 ± 1.3[b]	12.1 ± 1.0[b]
PBHb	18.0 ± 1.8	13.6 ± 0.9[b]	11.9 ± 1.1[b]
Po_2, torr			
Control	43 ± 3	44 ± 6	43 ± 4
HES	43 ± 13	56 ± 10	64 ± 13[b]
HbV-HES	43 ± 4	51 ± 13	68 ± 9[b]
PBHb	43 ± 8	53 ± 9	61 ± 11[b]
Pco_2, torr			
Control	58 ± 8	58 ± 9	56 ± 8
HES	57 ± 3	56 ± 3	47 ± 13[a]
HbV-HES	53 ± 4	51 ± 4	41 ± 3[b]
PBHb	57 ± 5	53 ± 4[a]	47 ± 5[b]
pH			
Control	7.28 ± 0.06	7.29 ± 0.06	7.29 ± 0.06
HES	7.23 ± 0.03	7.27 ± 0.03[a]	7.31 ± 0.07[b]
HbV-HES	7.27 ± 0.03	7.30 ± 0.03[a]	7.34 ± 0.02[b]
PBHb	7.25 ± 0.02	7.30 ± 0.05[a]	7.34 ± 0.06[b]

MAP, mean arterial pressure; HES, 6% hydroxyethyl starch; HbV-HES, hemoglobin vesicles suspended in HES; PBHb, polymerized bovine hemoglobin.

[a] $p < .05$ and [b] $p < .01$ vs. baseline. Values displayed as mean ± SD.

Table 3. Microvascular diameters (in micrometers) at baseline

	Control	HES	HbV-HES	PBHb
Flap artery	112 ± 15	122 ± 18	114 ± 8	118 ± 17
Anatomically perfused tissue				
Conduit arterioles	57 ± 8	58 ± 14	54 ± 12	56 ± 15
End arterioles	8.4 ± 3.2	8.9 ± 3.4	7.7 ± 2.7	7.8 ± 2.3
Ischemic tissue				
Conduit arterioles	56 ± 9	54 ± 15	60 ± 10	54 ± 17
End arterioles	8.4 ± 3.2	8.9 ± 3.4	7.7 ± 2.7	7.8 ± 2.3

HES, 6% hydroxyethyl starch; HbV-HES, hemoglobin vesicles suspended in HES; PBHb, polymerized bovine hemoglobin.

Values are mean ± SD.

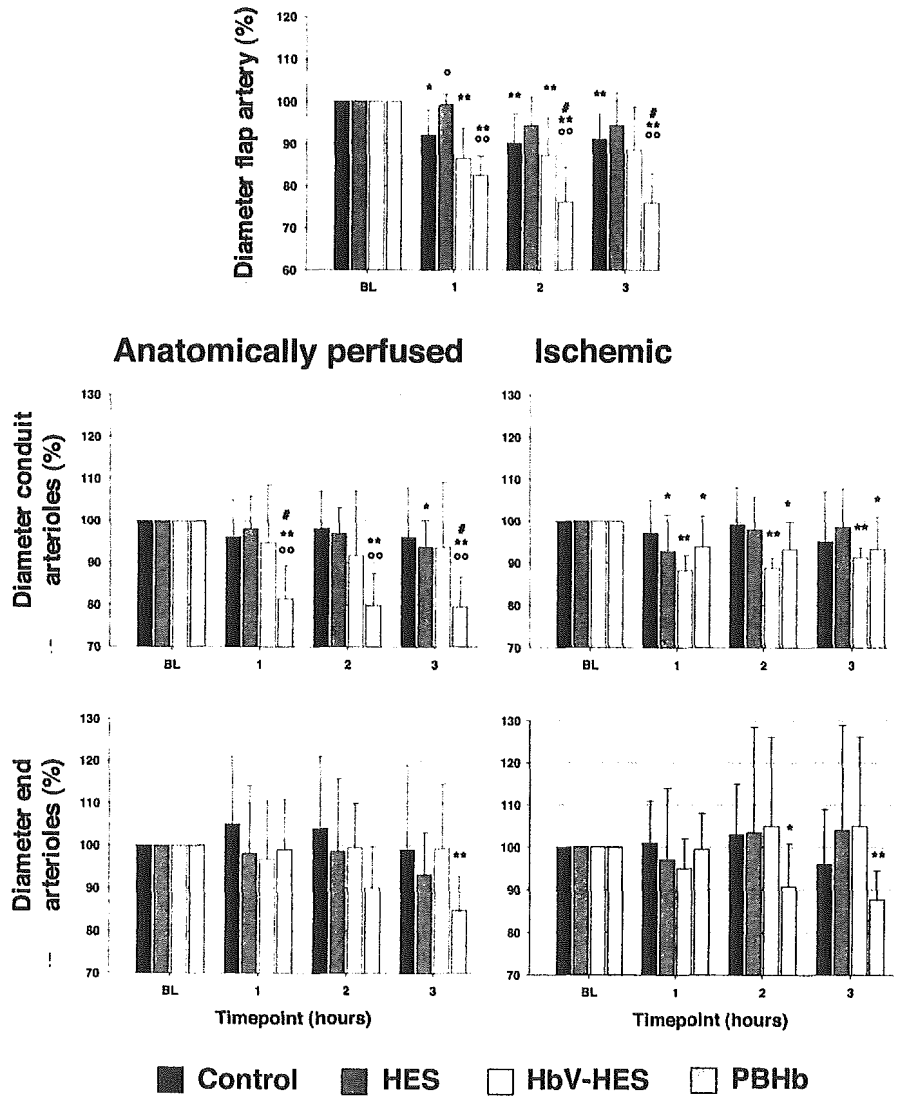

Figure 2. Microvascular diameters in flap artery, conduit arterioles, and end arterioles in the anatomically perfused and ischemic tissues at baseline (*BL*) and after hemodilution with 6% hydroxyethyl starch (*HES*), hemoglobin vesicles suspended in HES (*HbV-HES*), and polymerized bovine hemoglobin (*PBHb*). Data are given as a percentage of baseline and represent mean values and SD. $*p < .05$, $**p < .01$ vs. baseline; $°p < .05$, $°°p < .01$ vs. control; $\#p < .05$ vs. HbV-HES.

operating microscope at ×10 magnification (Wild, Heerbrugg, Switzerland). An island flap measuring 3 × 2 cm was dissected from the shaved and epilated back skin of the animal. The flap consisted of skin and a thin layer of panniculus carnosus muscle and was perfused by one vascular axis that bifurcated into two equal-sized branches within the flap, each of them supplying a separate vascular territory. One of the branches was transected after being secured with microsurgical ligatures, thus rendering the corresponding vascular territory ischemic. This tissue was perfused by a collateral vasculature connecting the two vascular networks. During surgery, the flap was irrigated with 0.9% NaCl to prevent the flap from drying out. The animal was placed on a specially designed Plexiglas stage including a platform for fixation of the flap.

Laboratory Analysis. Blood samples were taken from the carotid artery catheter and collected in heparin-washed microtubes for immediate measurements of total hemoglobin concentration, pH and systemic arterial Po_2 and Pco_2 (ABL 625, Radiometer, Copenhagen, Denmark). Hematocrit was determined by centrifugation. The colloid osmotic pressure of the diluents was measured with a colloid osmometer (model 4420, Wescor, Logan, UT) with a 30,000 d cutoff membrane. The viscosity was measured with a cone-plate viscometer (PVII+, Brookfield Engineering, Middleboro, MA) or a capillary rheometer (Anton Parr DCS 300, Parr Physica, Graz, Austria) at 37°C. Viscosity of blood and plasma were measured 3 hrs after hemodilution with a Höppler-type viscosimeter (HAAKE Messtechnik, Karlsruhe, Germany).

Microhemodynamics and Partial Tissue Oxygen Tension. Investigations were performed using an intravital microscope (Axioplan 1, Zeiss, Jena, Germany). Microscopic images were captured by a television camera (Intensified CCD camera, Kappa Messtechnik, Gleichen, Germany), recorded on video (50 Hz, Panasonic, Osaka, Japan), and displayed on a television screen (Trinitron PVM-1454QM, Sony, Tokyo, Japan). The preparation was observed visually with a ×40 objective resulting in a total optical magnification of ×909 on the video monitor. Transilluminating with a green filter produced a well-defined image of the width of the erythrocyte column, which could then be measured manually on the television screen. A mapping of the vasculature was made before the baseline measurements were taken to allow for repeated measurements of diameters on exactly the same vessel and location. The arterioles were classified according to physiologic and anatomic features into conduit arterioles and end arterioles (9, 10, 12).

We used combined bare fiber probes (Oxylite probes, Oxford Optronix, Oxford, UK) to measure tissue oxygen tension, temperature, and microvascular blood flow continuously. Microvascular blood flow was measured with two 230-μm fibers. The sensitive tip of the oxygen probe (100 μm in diameter) consisted of Ruthenium-III-(tris)-chloride, which measured Po_2 by fluorescence quenching of the dye. A T-type thermocouple was attached to the probe, which was coated with a biocompatible sleeve of polyurethane. According to

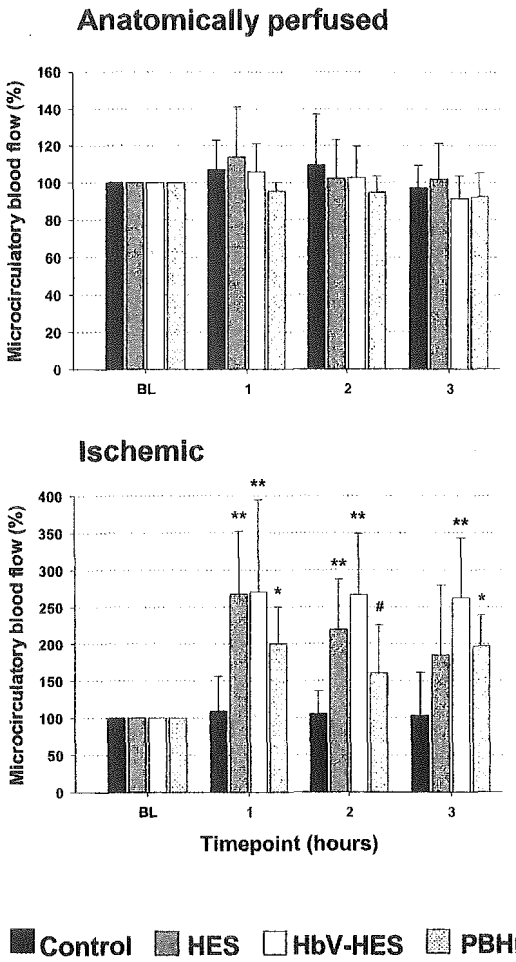

Figure 3. Microcirculatory blood flow in the anatomically perfused and ischemic tissues at baseline (*BL*) and after hemodilution with 6% hydroxyethyl starch (*HES*), hemoglobin vesicles suspended in HES (*HbV-HES*), and polymerized bovine hemoglobin (*PBHb*). Data are given as a percentage of baseline and represent mean values and SD. $*p < .05$,$**p < .01$ vs. baseline and control; $\#p < .05$ vs. HbV-HES.

the manufacturer, the bare fiber probe provides resolutions of <1 torr and 0.1°C for partial oxygen tension and temperature, respectively. The data on blood flow were displayed in arbitrary perfusion units and further processed into percentages of the baseline.

Carbohydrate Metabolite Concentrations. The interstitial concentrations of glucose, pyruvate, and lactate were assessed by microdialysis, as previously described (13, 14). The system used in our study included microprobes (CMA/20, CMA Microdialysis AB, Stockholm, Sweden) carrying a microcell that was perfused by a microinjection pump (CMA/100, CMA Microdialysis AB). The molecular cutoff of the membrane was 20,000 d. This pore size does not allow the tested Hb compounds to penetrate into the microcell, as confirmed by preliminary pilot experiments. The outlet tube was connected to a refrigerated fraction collector (CMA/200 F, CMA Microdialysis AB) in which the dialysates were collected in microvials, stored at 4°C, and further processed for laboratory analysis (CMA 600, CMA Microdialysis AB). The microcell was continuously perfused with isotonic Ringer's solution at a flow rate of 0.75 μL/min, which resulted in a time delay from the membrane to the microvial of 7 mins. The sampling time was set at 60 mins. Before each experiment, the probes were equilibrated according to the guidelines of the supplier.

Protocol. The animals were kept under light anesthesia with a continuous infusion of 50 mg/mL pentobarbital given at a rate of approximately 0.5 $mg \cdot min^{-1} \cdot kg$ body $weight^{-1}$ throughout the experiment. The depth of anesthesia was regulated by tolerating a noxious reflex due to pinching of the hind paw, but no nonaversive reflexes (palpebral, corneal, and jaw reflex) (12). With a heating pad and the room's temperature at 28°C, the temperatures of the animals were kept constant at 32°C, which was verified with a microthermometer placed on the abdominal skin.

Microdialysis and Oxylite probes were inserted subcutaneously in the middle of each vascular territory of the flap. The timing of the interventions is illustrated in Figure 1. The collection of the microdialysates started after a 30-min stabilization period. Another hour later, the baseline values were obtained. Thereafter, one third of the total blood volume was exchanged with HES or the oxygen-carrying solutions. This was achieved by simultaneous blood withdrawal via the carotid catheter and infusion via the jugular catheter over 15 mins. Exclusion criteria were abnormalities of the vascular anatomy, insufficient optical clarity, mean arterial pressure of <60 mm Hg, and systemic arterial pH, Po_2, and Pco_2 outside the normal ranges at baseline (7.19–7.29, 35–55 torr, and 45–65 torr, respectively). The animals were killed with an overdose of pentobarbital at the end of the experiment.

Statistical Analysis. The InStat version 3 program (Graph Pad Software, San Diego, CA) was used for statistical analysis. The data were presented as mean ± SD. The time-related differences between repeat measurements were assessed by the paired analysis of variance, followed by the Dunnett's posttest. Differences between the groups were assessed by unpaired analysis of variance and Tukey's posttest. A value of $p < .05$ was taken to represent statistical significance.

RESULTS

Six animals (two control animals, one in the HES group, two in the HbV-HES group, and one in the PBHb group) did not fulfill the inclusion criteria and were excluded from this study. The systemic data are presented in Table 2. Mean arterial pressure gradually declined during the experiment in all groups ($p < .05$) except the Oxyglobin group, in which mean arterial pressure remained virtually unchanged ($p < .05$ vs. other groups). Similar hematocrits were obtained in all hemodiluted animals. After the 33% blood exchange with HES, Hb concentration was reduced to 9.7 ± 0.9 g/dL, whereas the addition of HbV to the diluent enhanced the total Hb concentration to 11.2 ± 1.3 g/dL ($p < .05$ vs. HES), and hemodilution with PBHb resulted in a total Hb concentration of 13.6 ± 0.9 g/dL ($p < .05$ vs. HbV-HES). However, this difference became less pronounced over time. Hemodilution increased mean Po_2 from 43 torr to mean values of >60 torr and mean pH from 7.23–7.27 to mean values of 7.31–7.34, whereas mean Pco_2 decreased from 53–57 torr to 41–47 torr (all $p < .01$ vs. baseline).

At baseline, the microvascular diameters were similar in all groups (Table 3). The behaviors of the microvascular diameters are shown in Figure 2. The diameters were gradually reduced over time in

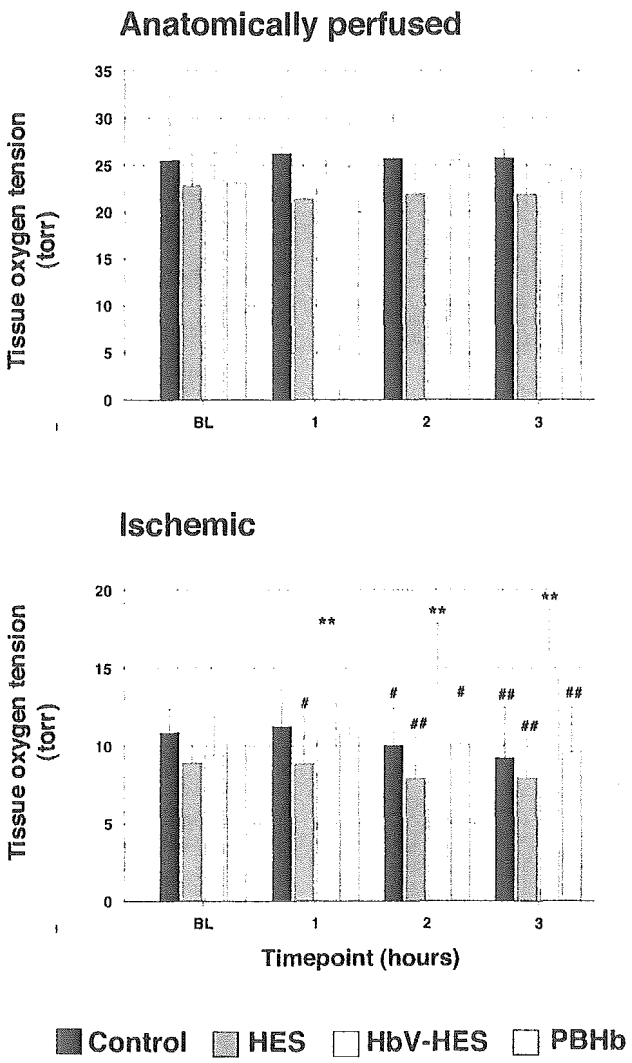

Figure 4. Po_2 in the anatomically perfused and ischemic tissues at baseline (*BL*) and after hemodilution with 6% hydroxyethyl starch (*HES*), hemoglobin vesicles suspended in HES (*HbV-HES*), and polymerized bovine hemoglobin (*PBHb*). Data represent mean values and SD. $**p < .01$ vs. baseline and control; $\#p < .05$, $\#\#p < .01$ vs. HbV-HES.

the flap artery in all animals (not significant for HES, $p < .01$ for other groups) but most substantially in the PBHb group, where they reached 76% ± 7% of baseline ($p < .01$ vs. control, $p < .05$ vs. HbV-HES). In the anatomically perfused tissue, diameters were decreased to 79% ± 7% in the conduit arterioles ($p < .01$ vs. baseline and control, $p < .05$ vs. HbV-HES) and 85% ± 8% ($p < .01$ vs. baseline) in the end arterioles after receiving PBHb, whereas no significant differences were observed between the other groups. A similar yet less pronounced pattern was seen in the collateralized arterioles ($p < .01$ vs. baseline for PBHb).

The mean baseline laser-Doppler signals ranged between 61 and 114 perfusion units in the anatomically perfused tissue and between 3 and 17 perfusion units in the ischemic part. Microcirculatory blood flow remained virtually unchanged in the anatomically perfused tissue in all groups (Fig. 3), whereas mean blood flow was maximally increased after hemodilution by 168% for HES ($p < .01$), 170% for HbV-HES ($p < .01$), and 100% for PBHb ($p < .05$ vs. baseline and HbV-HES).

Oxygen tension was significantly reduced in the ischemic tissue compared with the anatomically perfused part ($p < .01$) (Fig. 4). It remained at baseline levels in both parts of the flap and in all groups except for HbV-HES, which showed a Po_2 increase from 9.4 ± 2.5 torr to 14.2 ± 4.5 torr in the ischemic tissue ($p < .01$ vs. baseline and other groups).

Glucose concentrations were gradually decreased in the anatomically perfused tissue over time (Fig. 5), reaching 1.5 ± 1.1 mmol/L in the control group ($p < .01$), 2.7 ± 1.2 mmol/L for HES (not significant), 3.1 ± 1.4 mmol/L for HbV-HES (not significant, $p < .05$ vs. control), and 2.2 ± 0.9 mmol/L for PBHb ($p < .01$). The reductions were more accentuated in the ischemic tissue, at 0.8 ± 0.6 mmol/L in the control group ($p < .01$), 1.4 ± 0.9 mmol/L in the HES group ($p < .01$), 1.6 ± 1.4 mmol/L in the HbV-HES group (not significant), and 1.3 ± 0.7 mmol/L in the PBHb group ($p < .01$). At baseline, the mean lactate concentrations ranged between 1.7 and 2.2 mmol/L in both parts of the tissue in all groups. The values remained virtually stable in the anatomically perfused tissue. In the ischemic tissue, lactate concentrations were raised to 2.8 ± 0.6 mmol/L in control ($p < .01$) and to 3.4 ± 1.4 mmol/L in HES ($p < .01$) but only to 2.4 ± 1.0 mmol/L in HbV-HES and to 2.2 ± 0.7 mmol/L in PBHb (both not significant). At baseline, the mean lactate/pyruvate ratio was higher in the ischemic tissue (24–27) compared with the anatomic part (19–22). No relevant changes occurred in the anatomic part. Lactate/pyruvate ratio was increased to 51 ± 23 in the control group and 48 ± 12 in the HES group (both $p < .01$) but only to 34 ± 8 and 38 ± 11 for HbV-HES (not significant vs. baseline, $p < .05$ vs. control) and PBHb ($p < .05$), respectively.

DISCUSSION

The principal findings of the this study were that the elevation in lactate concentration and lactate/pyruvate ratio in the critically ischemic tissue could be attenuated by isovolemic hemodilution with the oxygen-carrying solutions. This suggests that oxidative energy metabolism was improved in the hypoxic cells, which is crucial for their survival and functional outcome. The effect was superior for HbV-HES, which also yielded markedly higher oxygen tension in the ischemic flap tissue. Both tissue oxygen tension and oxidative energy metabolism are dependent on the oxygen supply to this tissue, which is determined by the oxygen content of the blood entering the collateralized tissue and the perfusion of the latter.

As expected, microcirculatory blood flow was substantially improved in all hemodiluted animals due to the hematocrit reduction; however, microcirculatory blood flow was improved to a lesser ex-

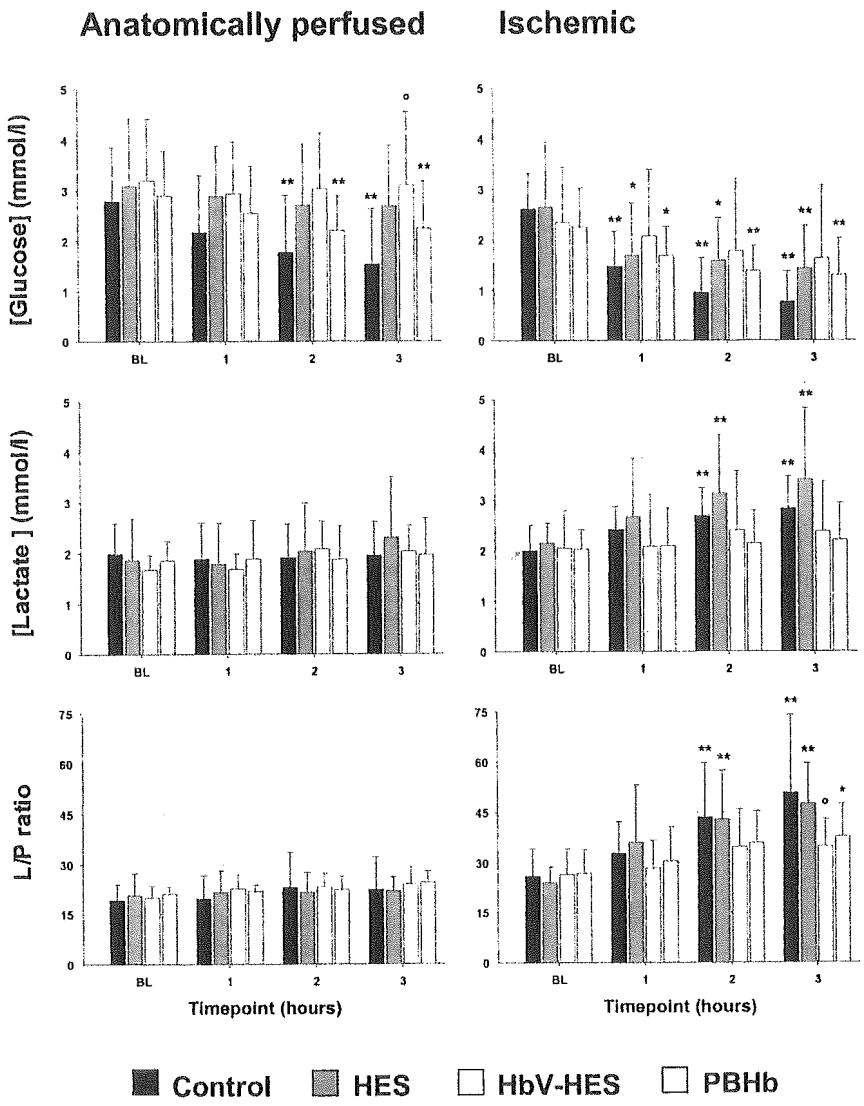

Figure 5. Carbohydrate metabolite concentrations in the anatomically perfused and ischemic tissues at baseline (*BL*) and after hemodilution with 6% hydroxyethyl starch (*HES*), hemoglobin vesicles suspended in HES (*HbV-HES*), and polymerized bovine hemoglobin (*PBHb*). Data represent mean values and SD. $*p < .05$,$**p < .01$ vs. baseline; $°p < .05$ vs. control.

tent for PBHb, which also yielded diminished microvascular diameters. In previous studies we demonstrated that microvascular blood flow and diameter were closely related to the rheologic formulation of the diluent, thus advocating solutions with both high colloid osmotic pressure and high viscosity (9).

The colloid osmotic pressure of the solutions used in the present study was approximately twice as high as that of hamster plasma, thus causing volume expansion, as evidenced by hematocrit values that were markedly lower than the theoretical value expected after a normovolemic 33% blood exchange. The volume expansion was similar for both oxygen-carrying solutions, whereas hemodilution with HbV-HES resulted in markedly higher viscosity in the plasma phase. Plasma viscosity affects shear stress on the vascular lining, thus stimulating the NO-mediated relaxation of the vascular tone in small arteries and large arterioles (15). NO may be scavenged by cell-free hemoglobin, which may cause a strong vasopressor effect and subsequent hypertension (1, 16). It has been shown that the degree of NO-scavenging is correlated with the size of the hemoglobin compound (16), the HbV being more than ten times larger than the PBHb. NO-scavenging was most likely the reason for the microvascular narrowing found after the administration of PBHb in our study. This vasoconstriction was most pronounced in the flap artery. This vascular segment was considered most sensitive to NO-mediated regulation of vascular tone (17) and vasopressor effect of acellular hemoglobins (16), which may explain the higher mean arterial pressure we obtained in the animals receiving PBHb compared with HbV-HES. Taken together, our data suggest that HbV-HES provides a better balance of oxygen carrier-related viscosity increase and NO-scavenging.

Our study suggests that in critically ischemic and hypoxic collateralized peripheral tissue, oxygenation may be improved by normovolemic hemodilution with hemoglobin vesicles suspended in hydroxyethyl starch.

However, the prevention of metabolic deterioration in the ischemic flap tissue after hemodilution with the oxygen-carrying solutions cannot be solely related to the improved microcirculatory blood flow because no metabolic benefit was observed in the animals receiving HES. One possible explanation is that due to their small size, the supplemented hemoglobins may perfuse capillaries that are no longer accessible by red blood cells and that are therefore relieved of their function. Although not measured in this study, impaired functional capillary density, a common feature in the compromised microcirculation (5, 7, 18), must be assumed in the ischemic flap tissue. Indeed, circulating HbVs were observed in capillaries showing a cessation of red blood cell flux (18).

The improved oxygen tension found in the ischemic tissue after hemodilution with HbV-HES is indicative of a larger amount of oxygen being brought to the collateralized vasculature than is the case with the PBHb group. According to the oxygen dissociation curve for hamster blood (5, 19), hemodilution with both oxygen-carrying solutions led to an in-

crease in arterial oxygen saturation of the cellular hemoglobin from approximately 80% at baseline to 90% at 3 hrs after blood exchange, whereas the estimated oxygen saturation was approximately 95% for the HbV but only 60% for PBHb, thus resulting in a slightly higher arterial oxygen content in the HbV-HES animals. Furthermore, it may be postulated that HbVs prevented the transmural diffusion of oxygen during the passage through the upstream vasculature. This mechanism may influence oxygen delivery to collateralized tissues substantially, because up to 40–50% of the systemic arterial oxygen was estimated to be lost from the upstream circulation before reaching the collateral vasculature nourishing the ischemic flap tissue (12). Upstream oxygen loss may be influenced in many ways by the addition of oxygen-carrying solutions. According to the Stokes-Einstein equation, the diffusivity of oxygen through the plasma is inversely proportional to the size of the plasma-bound oxygen carrier and the viscosity of the suspension, which were both greater for HbV-HES. Facilitated diffusion has been reported for PBHbs in static (20) and dynamic (21) *in vitro* models, whereas virtually no such effect has been obtained with HbVs (20, 22). Enhancing the oxygen affinity of the added oxygen carrier shifts transmural oxygen diffusion downstream, as has repeatedly been demonstrated *in vivo* (5, 19), *in vitro* (22), and in mathematical models (23). In a recent study, we were able to show that the oxygenation in the critically ischemic hamster flap tissue could substantially be improved by left-shifting of the HbV (10). Taken together, it may be assumed that with HbV-HES, there was less unwanted oxygen loss in the upstream vasculature before reaching the collateralized, ischemic flap tissue than with PBHb. This was likely due to the larger size of HbV-HES, the higher oxygen affinity of its hemoglobin compound, and the higher viscosity of the solution.

CONCLUSIONS

From our data, we conclude that hypoxia in the critically ischemic hamster flap tissue was substantially attenuated after hemodilution with HbV-HES and PBHb due to improved microcirculation and the presence of the supplemented oxygen carriers. It seemed that due to its rheologic formulation and physiochemical properties, HbV-HES provided a superior oxygen supply to the ischemic flap tissue. Therefore, our data suggest that the concept of targeting oxygen delivery to where it is most needed may find a particular, clinically most relevant application in the treatment of hypoxia in critically ischemic peripheral tissues. However, these findings may not be extrapolated to vital organs. Moreover, the beneficial effect obtained with the artifical oxygen carriers may be further enhanced by adding antioxidant enzymes to attenuate any possible ischemia-reperfusion injury (1).

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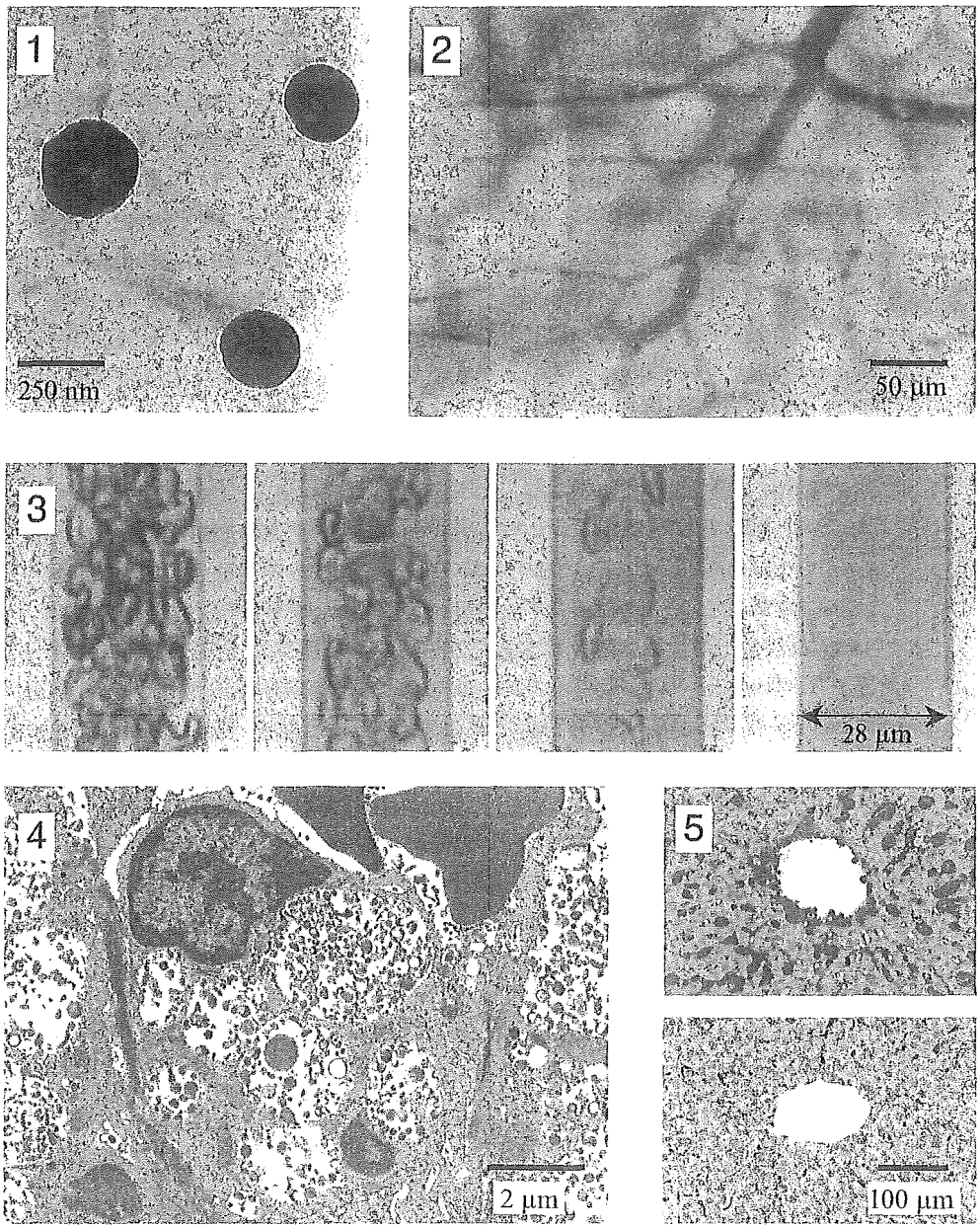
微小血管内を均一に流れる人工赤血球とその運命



＊写真左上の数字は図の番号です。

図1　人工赤血球（Hb小胞体）の透過型電子顕微鏡像

2　循環血液量の80%をHb小胞体で交換した後のハムスター背部皮下微小循環の光学顕微鏡像　波長420nm付近の光（Hbの吸収帯）を照射して撮影。

3　赤血球とHb小胞体を混合し、プラスチック製のモデル血管内を流動させたときの光学顕微鏡像　混合容積比は左から、赤血球/Hb小胞体＝100/0, 50/50, 10/90, 0/100。

4　Hb小胞体投与1日後のラット脾臓マクロファージの透過型電子顕微鏡像

5　Hb小胞体投与1日後（上）、7日後（下）の肝臓組織の抗ヒトHb抗体免疫染色像

目で見るバイオ

ヘモグロビン小胞体は、高純度ヒトヘモグロビン(Hb)をリン脂質小胞体(リポソーム)に内包した人工赤血球である。何と、脂質成分とHbが分子間相互作用だけで形成している分子集合体である。透過型電子顕微鏡写真(図1)から、精密制御された粒子径(250nm)と、Hb(約30000個)が濃度高く内包されている様子が解る。粒子表面は約6000本のpoly(ethylene glycol)鎖で修飾してあり、室温で2年間の保存も可能な優れた安定度を有する。光学顕微鏡では粒子の形状は観察できないが、ハムスターの循環血液量の80%を交換したのちの皮下微小循環の観察では、Hb小胞体は血漿中に均一に分散し、通常は見えない毛細血管の形状までもがコントラストよく見える(図2)。愛媛大学医学部との共同で、赤血球と種々の比率で混合し、モデル血管内(28 μm径)を1mm/sの流速で流動させた実験では、赤血球(8 μm)は管の中心側を流動するのに対し(図3)、血漿層の濁りからHb小胞体は均一分散して管壁側を流動する様子が解った。慶応義塾大学医学部ほかとの共同によるHb小胞体の交換輸血試験、出血ショック蘇生試験では、赤血球と同等の酸素運搬機能が実証されている。加えて、赤血球に比較して小粒径で均一分散するので、血管狭窄部の透過、あるいは側副経路を経由した虚血性低酸素領域への酸素運搬にも有効であることが解ってきた(Univ. California, San DiegoおよびInselspital Univ. Hospital, Bernとの共同)。Hb小胞体は酸素運搬の機能を終えたのち、最終的には老化赤血球と同様に細網内皮系(主に脾、肝)に捕捉される運命をたどる。Hb小胞体をラットに投与し(20mL/kg)、1日後の脾臓を電子顕微鏡観察すると、マクロファージ食胞内にHb小胞体が多数捕捉されていたが(図4)、7日後には完全に消失した。また、抗ヒトHb抗体を用いた肝臓の免疫染色(図5)では、投与1日後にはKupffer細胞に捕捉されたHb小胞体が赤染部位として認められたが、7日後には完全消失しており、Hb小胞体は蓄積することなく、速やかに分解排泄される様相まで明らかになった。

血液型物質を含まず、感染の心配がなく、しかも長期間備蓄可能な人工赤血球の実現は、医療技術に変革をもたらすことは間違いない。現在は、担当企業に技術移転を行い、夢を膨らませて臨床試験に向けた最終作業を進めている。

(酒井宏水、土田英俊)

キーワード：人工赤血球、微小循環、ヘモグロビン、リポソーム、輸血代替

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4. 酸素輸液（人工赤血球）の臨床応用

4.1 酸素輸液（人工赤血球）とその重要性

我が国では安全な輸血用血液が医療機関に常備され、国民の健康福祉に大きく貢献している。肝炎やエイズなど輸血に伴う感染もかつて大きな社会問題となったが、献血血液の厳重な検査と管理が強調され、常に被害を最小限に留める尽力により安全になってきている。特に核酸増幅法（NAT検査）の採用効果は多大であるが、Window Periodにおける検査漏れ、検査項目から外れる病原体の存在、それに未知感染源の存在の脅威にも常に曝されている。その他、赤血球の保存期限は採血後わずか3週間、血小板に至ってはわずか3日間である。それに過誤による血液型不適合の医療事故もある。赤血球輸血回避のため、術中輸血開始目安のヘモグロビン（Hb）濃度10 g/dLの低減、低温無輸血手術、また赤血球造血因子の投与による術前貯血も普及してきてはいるが、症例によっては適用できない。献血1回当たりの採血量は400 mLになったが、人口の高齢化に伴い献血者総数は低下し続けている。

このような状況に鑑み、我が国では厚生労働科学研究・医薬安全総合研究事業として、「人工血液」の研究が推進されている。人工赤血球、人工血小板、人工抗体の3部門から構成され、現行の献血・輸血システムの問題点を克服するさまざまな試みが継続されている。

本稿では酸素輸液（人工赤血球）の話題に絞って概説する。急性出血ショック患者の蘇生には、一般的に循環血液量の補給（電解質輸液）が先決で、その後に体組織内の全細胞の呼吸を満足する酸素供給（赤血球輸血）、さらに血漿増量剤の投与による循環血液量の保持が必要とされる。このうち赤血球の酸素運搬機能を代替できる輸液（酸素輸液）はいまだ実現されていない。我が国のように地震など自然災害が危惧される場合、緊急需要に対応してもっぱら酸素を運搬する輸液製剤を安全に大量供給できることは重要な国家的施策でもある[1]。

歴史的には酸素溶解度の高いパーフルオロカーボン（FC）をリン脂質で乳化したFluosol-DAが我が国（ミドリ十字社）で開発され、米国FDAの認可（1990年）を受けた唯一の製剤であることは誇るべき事実でもある。しかし、用途が経皮経管冠動脈拡張術後の灌流に制限された。実質的には相対的酸素溶解度が低いこと、形態の不安定度、肺胞膜表面の活性物質への影響、蓄積性の問題、それに必要量の酸素量が十分輸送できないなど用途が確定せず、生産を停止（1993年）した。

我が国ではその後、ヒトヘモグロビンを利用するHb小胞体が実現間近になってきている[2]。また、アルブミンに合成ヘムを担持させた完全合成系の酸素輸液も具体的になってきているので、ここに紹介する。

4.2 ヘモグロビン小胞体

4.2.1 分子集合体としてのHb（ヘモグロビン）小胞体の構成

赤血球は直径約8 μmの中窪み円盤状粒子であり、酸素を結合できる蛋白質ヘモグロビン(分子量64,500)の高濃度溶液（約35 %）を赤血球膜に被覆された構造をしている。膜で覆われている理由は、①35 %濃厚Hb溶液の高い粘度と膠質浸透圧の抑制、②毒性のあるHb逸脱の抑制、③Hb機能維持のための各種リン酸類（エネルギー分子）、また解糖および還元—酵素系保持の役割もある。それに、④血液（血球分散系）は非Newton流体で、体内循環、特に末梢血管内における特色ある流動形式と生理作用(ホメオスターシス)特性による。これら赤血球本来の構造と機能の関係が初めから解明されていれば、小胞体（細胞型）構造が無害投与の必要条件との結論が容易に想定できたはずである。

1960年代後半に両親媒性分子であるリン脂質が水相系で二分子膜を形成、これが小胞構造になること、1970年代初めにイリノイ大学のDjordjevichらは、リン脂質二分子層膜で覆った小胞構造“Synthetic Erythrocyte”の研究を開始した[3]。ただし、粒径制御など調製の困難さ、血漿蛋白質との相互作用に起因する凝集阻止に十分な手段が得られなかったので具体化しなかった。リン脂質二分子膜で高濃度Hb溶液を被覆する技術、特に毛細血管を容易に通過できる粒径の制御、また血中分散安定度の向上は、高分子科学と分子集合科学に立脚した工夫（1980年代）を組み込んで、ようやくHb小胞体として完成（図16.4.1）した。

高純度・高濃度Hb溶液（濃度35 %以上）を脂質分子二層膜（厚さ5 nm）で被覆させた小胞体（平均

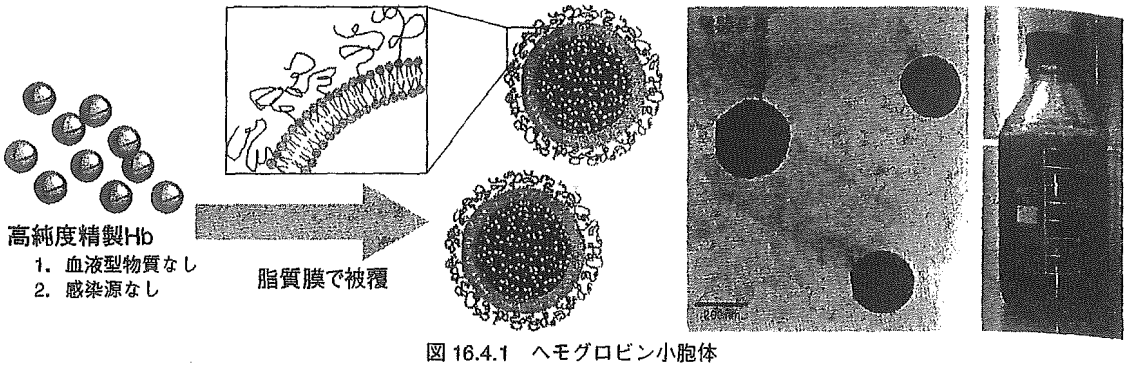


図 16.4.1　ヘモグロビン小胞体
高純度 Hb をリン脂質二分子膜で被覆。粒子表面はポリオキシエチレン鎖で修飾。右側の TEM 写真から、制御粒子径（250 nm）と Hb が濃度高く充填されている様子がわかる。

粒径 250 nm）を可能とした[2]。脂質成分と Hb が分子間相互作用（2 次的相互作用：疎水的相互作用、静電的相互作用、水素結合など）だけで形成されている分子集合体である。この粒子には 1 粒当たり 3 万個の Hb が濃度高く充填されている。原料 Hb は NAT 検査済みの献血血液の期限切れ赤血球由来で、精製に際し 60 ℃の加熱処理（pasteurization）とウイルス除去膜処理（ultrafiltration）の組み合わせで、感染に対する安全を確保している[4]。約 1500 本のポリオキシエチレンを粒子表面に配置して小胞体粒子間の凝集抑制と分散安定度向上が得られ[5]、溶液のまま室温にて 2 年以上の保存が可能である[6]。

4.2.2　輸血代替としての Hb 小胞体

Hb 小胞体粒子分散液は膠質浸透圧を持たないので、血液交換率が高い場合には循環血液量を維持するために、血漿増量剤の添加が必要となる。例えば、認可間近とされる 5 % γ Albumin（rHSA）水溶液に分散させた場合の膠質浸透圧は 20 mmHg、粘度と浸透圧は血液とほぼ同等に調節、循環動態の恒常性に寄与する[7]。Hb 小胞体の粒径は赤血球の約 1/30 と小さく、血漿中に均一分散できる。

Hb 小胞体の動物投与試験では、交換輸血法で循環血液量の 90 %超過の交換でも血圧が維持され、また血液ガス組成も腎皮質の酸素分圧も正常値を推移することを確認している。臨床現場で想定される最大の交換率 40 %の交換輸血において、ラットは全例が生存し、ヘマトクリット（赤血球体積分率）が 1 週間で完全に回復する。Hb 小胞体は最終的に細網内皮系に捕捉され、安全に分解、排泄される[8]。

したがって、臨床では術前血液希釈、術中出血分の補給、さらに胸部外科手術における人工心肺（ECMO）体外循環回路の補充液としての利用が十分に期待できる。特に小児患者の体外循環では、無輸血充填とした場合（血漿増量剤の充填）、術中短時間の Hb 濃度低下が脳に後遺症を与え術後の知能発達に影響するともされており、Hb 小胞体を充填液として用いることの利点も期待できる。

出血ショック時の蘇生液としても検討され[7]、赤血球と同等の酸素運搬機能を実証している。例えば、Sevoflurene 麻酔下、ラットの循環血液量の 50 %を脱血して 15 分後に Hb 小胞体を 5 % rHSA に分散させて投与し 6 時間観察する。rHSA 単独の投与では 8 匹中 2 匹の死亡に対し、Hb 小胞体の投与では、循環動態も血液ガス組成も脱血液の投与と同等に推移し、全例が生存（図 16.4.2）できる[7]。現在、ウサギやビーグル犬を用いた出血ショック蘇生試験も開始しており、概ね良好な成績が得られている。

これから Hb 小胞体が医療現場にて使用できるようになれば、特に救急医療や外科的手術において、血液型不一致や感染の心配をせずにいつでも要求に応じて投与し、同種血輸血の回避または必要量の低減が可能となることも示唆できた。

4.2.3　Hb 小胞体の安全度

①循環動態の恒常性

欧米で開発が進められてきた Hb に化学的修飾を加えた形式（分子内架橋、重合型、PEG 結合型）では、投与に際し血圧の異常亢進あるいは食道蠕動の運動障害など副作用が明らかとなり、開発中断を余儀なくされたものもある。これは NO の高い親和度に起因すると考えられる[1]。ハムスター皮下微小循環系の抵抗血管径と血圧変動の相関追跡では、修飾 Hb（特に分子内架橋 Hb、粒径 7 nm）で抵抗血管径が最大収縮とな

り、同時に顕著な血圧亢進を示す。血管収縮は末梢循環を阻害し、組織へ十分量の酸素供給が疎外される。粒子径が大きくなるにつれこれらの変動値は小さくなり、直径 250 nm の Hb 小胞体では血管収縮も血圧亢進も生起しないことが確認されている。現在のところ、分子状の修飾 Hb は、血管内皮細胞層を透過し血管内平滑筋近傍に到達、内皮細胞が産生する NO を捕捉するので、血管弛緩機能が低下する。直径 250 nm の Hb 小胞体では赤血球と同様に平滑筋までは到達できず血管内腔に留まるため、血管収縮も血圧亢進も起こらない。

肝臓中では肝実質細胞にある hemeoxygenase がヘムを分解する。実はこの際に産生する一酸化炭素（CO）が、血管弛緩因子として血管内壁（Ito 細胞）に作用するのが明確に示された。摘出肝灌流中の微小循環動態の検討は、類洞血管の孔（穿孔篩：fenestration、孔径約 100 nm）よりも小さい修飾 Hb（7 nm）では、これを容易に通過し Disse 腔に侵入、肝実質細胞で代謝され bilirubin 排泄の亢進と同時に CO を放出する。CO 親和度の高い血中 Hb に捕捉され、結果として 20 % の血管抵抗増大と同時に、類洞の不連続的狭窄と流動停止領域の存在が観測されている。他方は Hb 小胞体は粒径が 250 nm と大きいため、類洞血管孔を透過できず肝実質細胞に到達しないためこの現象は生起せず、この間灌流圧は一定値に保たれる（図 16.4.3）。

この結果は臓器移植の際に Hb 小胞体を灌流液として安全使用できることも意味し、摘出から移植までの所要時間を延長できる。移植臓器を従来よりも遠隔地に運搬が可能となる。

②体内動態と代謝過程

酸素輸液の投与は血液量の大半を置換する大量投与も前提となるので、成分の体内動態と代謝過程の詳細検討が必要となる。これまでの検討では、投与 Hb 小胞体は最終的に貪食細胞が多く存在する脾、肝、骨髄など、いわゆる細網内皮系（RES）への移行が同位元素（^{99m}Tc）修飾した Hb 小胞体の体内動態観測から解明され[9]、RES 機能への影響と Hb 小胞体の構成成分の代謝確認も実施された。

これら詳細検討は、貪食細胞に捕捉された Hb 小胞体が 7 日以内に分解消失（図 16.4.4）し[10]、また脾臓重量も一過性増大を示すものの 7 日後には正常値に戻り、血液生化学検査でも異常値は認めないので、老化赤血球の代謝経路と同様と考えられる。また、40 % の血液を急速交換した後の生存試験（ラット）

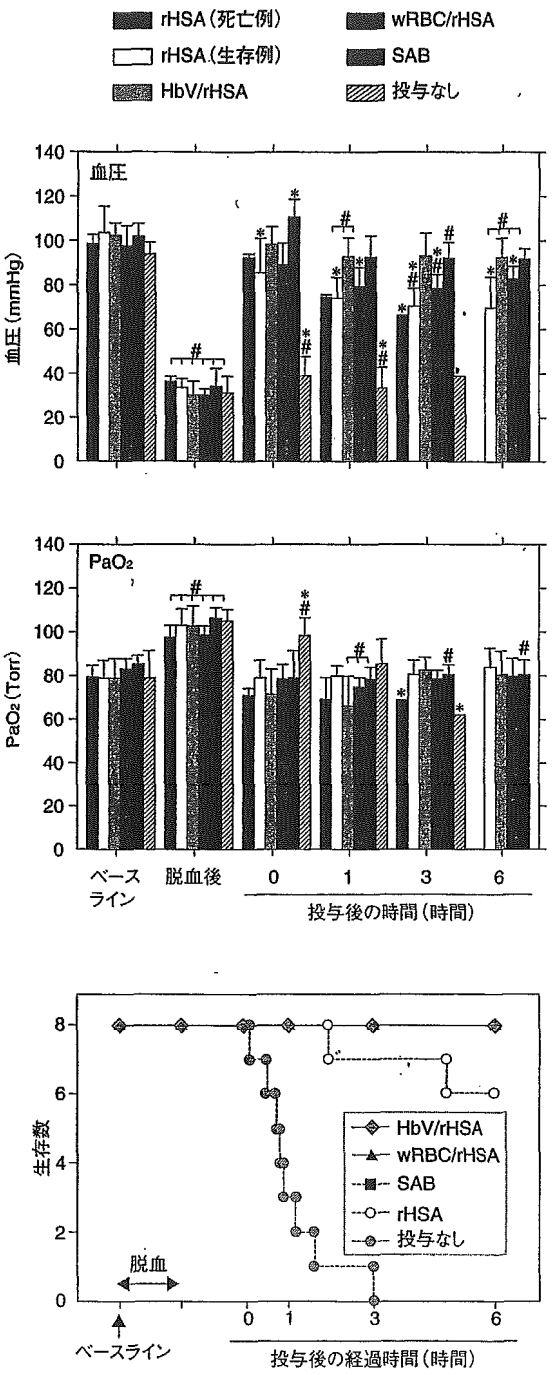
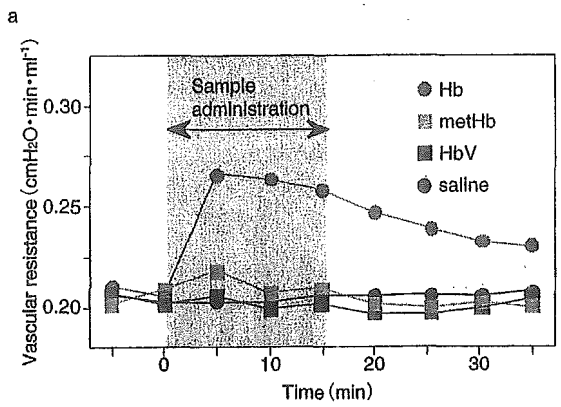


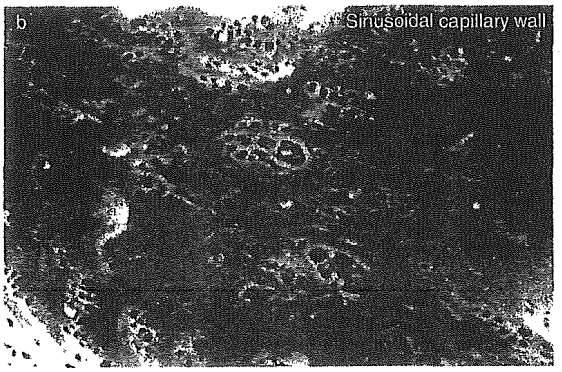
図 16.4.2　ヘモグロビン小胞体による出血ショック蘇生試験

#p < 0.05 vs baseline

*p < 0.05 vs the HbV/rHSA group

Sevoflurene 吸入麻酔下、ラット循環血液量の 50 % を脱血、15 分経過後、Hb 小胞体を rHSA に分散させて投与。ショック状態では血圧低下、代償機能により過呼吸、動脈血酸素分圧（PaO_2）の上昇、pH と塩基余剰の低下が観測できる。蘇生液の投与がないと全例が死亡。rHSA 群は 8 匹中 2 匹が死亡、HbV/rHSA 群は全例生存。





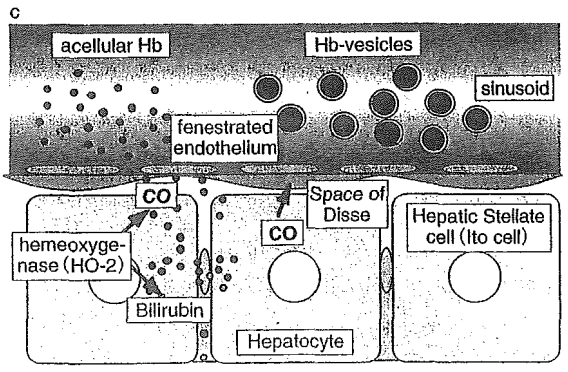


図 16.4.3　肝の微小循環動態と Hb 代謝

a：Hb 溶液で摘出肝を灌流すると灌流圧が上昇するが、Hb 小胞体と metHb の場合には灌流圧上昇はない。

b：肝類洞血管には無数の孔（fenestration）が開いている。

c：Hb 分子はこの孔を通って Disse 腔に拡散、ヘムは肝実質細胞の hemeoxygenase-2 により代謝され、ビリルビンと CO を排泄。

CO は血管弛緩因子として作用するが、Disse 腔で Hb に捕捉され弛緩作用が低下し、灌流圧が増大。Hb 小胞体は孔より大きいので通過なし、CO 結合なしのため肝臓の微小循環低下はほとんどない。また metHb は Disse 腔に拡散するが CO 結合ないため変化なし。

では、約1週間後には赤血球量は正常値に回復していることから[8] Hb 小胞体成分は造血に有効利用されていると想定され、詳細検討中である。

新薬非臨床試験の場合、安全度確認の項目として、GMP 基準での製造試料について齧歯類とその他の動物を対象とした反復投与試験がある。予備的にラットに対して Hb 小胞体の反復投与試験（投与量 10 mL/kg/日を 14 日間投与）を実施、循環血液量の 2.5 倍の分散液を投与したが、体重は継続して増加を続け、血液生化学的、組織病理学的検討でも顕著な副作用はなく、結果として Hb 小胞体成分が速やかに代謝される過程が得られ、安全度がきわめて高い製剤と証明されている[11]。

4.2.4　低酸素領域への酸素輸送

ヒト赤血球の酸素親和度（P_{50}：酸素が 50 %結合飽和するときの酸素分圧）は、37 ℃で約 28 Torr である。全身の酸素消費量は、動脈血酸素分圧（110 Torr）と静脈血酸素分圧（40 Torr）の間の酸素飽和度較差

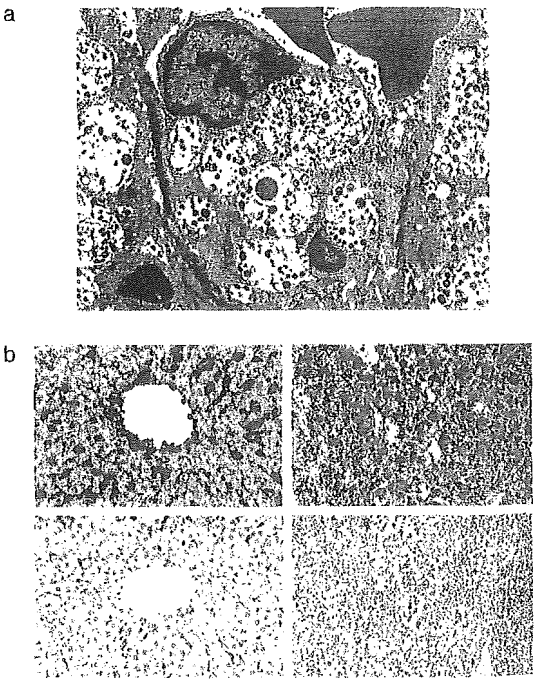


図 16.4.4　ヘモグロビン小胞体の代謝過程（b：口絵 50 参照）

a：Hb 小胞体投与 1 日後のラット脾臓 macrophage の透過型電子顕微鏡写真。食胞（phagosome）中に Hb 小胞体粒子を多数認める。7 日後にはほとんど消失。

b：Hb 小胞体投与後のラット肝臓（左）、脾臓（右）組織の顕微鏡写真。抗ヒト Hb 抗体染色による赤染はヒト Hb の存在部位。7 日後にはほとんど消失、蓄積はまったく認めず。

(A-V較差、ヒト赤血球の場合は25％程度）とHb濃度、それに心拍出量の積として算出される。赤血球から精製単離したHbのP_{50}は8 Torr程度と低く、静脈血酸素分圧（40 Torr）では酸素放出は生起しない。また、Hbを利用する人工酸素運搬体のP_{50}も、赤血球と同等あるいはそれ以上に調節すべきと考えられてきた。

正常組織であればこの理論が成立する。しかし、血管性障害により十分な血流が行き届かない組織（虚血性領域）の場合、血流速度も組織酸素分圧も極度に低下しており、動脈血はこの領域に到達する前に酸素を放出してしまう。つまり、酸素親和度を赤血球よりも大きく（P_{50}値を小さく）すれば、虚血性領域に到達してからの酸素放出可能と考えた[12]。このとき赤血球よりも小粒径の人工赤血球（250 nm径）は血漿中に均一に分散しているので、赤血球では届かない狭窄部を経由して到達できる。

この仮説の下では、これまでにハムスター有茎皮弁虚血モデルにおいて、高酸素親和度Hb小胞体（P_{50}：15 Torr）での血液希釈により虚血領域の酸素分圧が有意に上昇できることが証明された[13]。したがって、輸血代替としての利用以外に酸素治療剤としての適応症、例えば脳や心筋など虚血領域の酸素化、腫瘍組織の酸素化など、低酸素領域へ選択的な酸素ターゲッティングとして利用できる。Hb小胞体の酸素親和度はアロステリック因子により自在に調節できるので、各々の適応にふさわしいP_{50}を有するテーラーメード人工赤血球が可能となる。

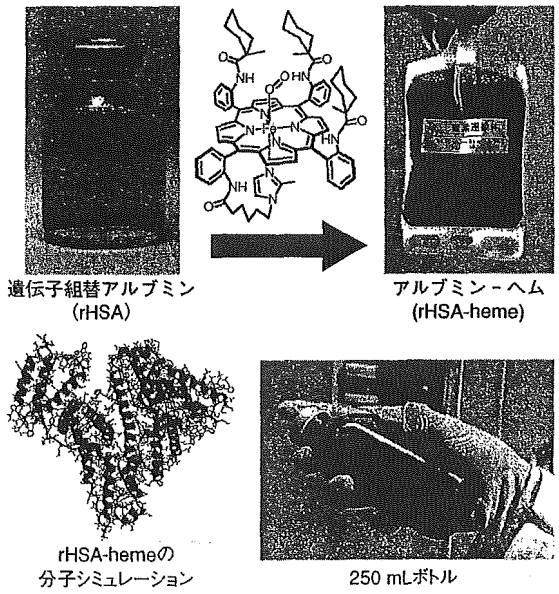


図16.4.5　アルブミン－ヘム
組替ヒト血清アルブミンに合成ヘムを包接させたアルブミン－ヘムは、酸素輸送のできる血漿蛋白質となる。最大8分子のヘムを包接させても、アルブミンの構造、物性、表面電荷は変化なし。酸素輸送能はヒト赤血球と同等、2年以上棚置き保存も可能。アルブミン－ヘムの生理食塩水溶液はHbを一切必要としない完全合成系酸素輸液。

4.3　アルブミン－ヘム

4.3.1　酸素を輸送できる赤色のアルブミン

他方、Hbをまったく利用しない完全合成系酸素輸液の開発も進んでいる。血清アルブミン（Mw. 66.5 kD）は血漿蛋白質の約70％を占める単純蛋白質であり、コロイド浸透圧の維持／各種内因性物質・薬物の運搬／血液pHの調整などの役割を果たしているが、我が国では世界に先駆けて組替ヒト血清アルブミン（rHSA）の大量発現に成功しており、世界初の上市を間近に控えている。年間100万バイアルの生産ラインの稼動体制も整い、血漿増量剤としてだけでなく、各種製剤の基材としての活用にも国際的注目が集まっているので、このアルブミンの非特異的多分子結合能を利用して、rHSAに酸素配位能を有するヘム誘導体を包接させ、従来類例のない新しい合成ヘム蛋白質「アルブミン－ヘム」が創製できる（図16.4.5）[14,15]。

アルブミン－ヘムは、①完全合成系酸素輸液であり、感染の危険性がなく、②一切のヒト（および動物）由来の血液資源を必要としない。また、③P_{50}はヘム構造の調整により調節可能、④アルブミンが血管内皮を透過しないためNO捕捉に伴う血管収縮・血圧亢進は惹起されない、などの多くの優れた特長を持つ。すでに酸素輸液としての酸素結合能／溶液物性／血液適合性が実証されており、安全性と効果の解明を中心とした前臨床評価試験も進められている。

4.3.2　構造と酸素結合能

アルブミン1分子当たりに結合するヘムの数は最大（8個／分子）であり、これはHbの2倍に相当する。ヘムの結合定数は$1.2 \times 10^6 \sim 1.3 \times 10^4$ (M^{-1})、包接の駆動力は疎水性相互作用であるため、結合後もアルブミンの2次構造や表面電荷に変化はない。しかし、1998年インペリアルカレッジのCurryらが、脂肪酸包接rHSAのX線結晶構造解析に初めて成功し、基質結合後のrHSAは幅8 nmから9 nmへ膨張することが明らかにされた[16]（図16.4.6）。この発見はヘムがrHSA内部に包接されると数nmの範囲では微細な構造変化が誘起されることを示唆しており、酸素配位

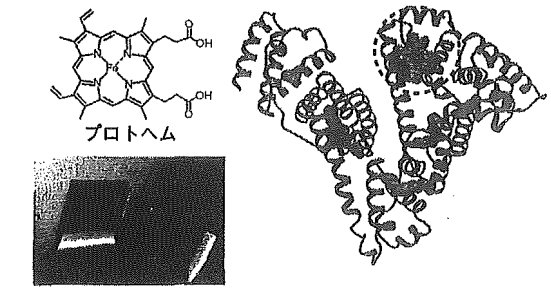


図 16.4.6　アルブミン－プロトヘムの単結晶の写真と分子構造（口絵 51 参照）

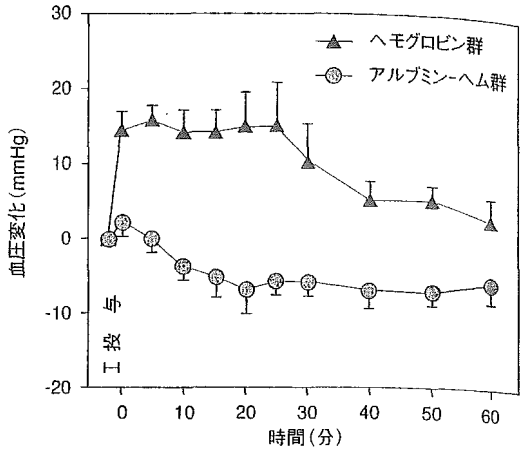


図 16.4.7　アルブミン－ヘム投与後の血圧の変化
分子状 Hb 溶液をラットに静注、瞬時に血圧亢進が生起。Hb が血管内皮誘導弛緩因子（NO）を捕捉するため。しかし、同条件でアルブミン－ヘム溶液を投与しても血圧値に変化はない。アルブミン分子は等電点（PH_I 4.8）で分子雰囲気が負帯電のため、Hb に比べ血管透過性が低いことに起因。

特性の変化とも関連してアルブミン－ヘム結晶構造の解明が待たれている。

また、酸素配位結合部位であるヘムの化学構造（酸素配位座近傍置換基、分子内軸塩基など）を変化させた一連の誘導体群が合成され、rHSA 包接体とした系についてヘム構造と酸素配位能の相関が定量的に明らかにされている[15]。Hb と同じヒスチジン残基を軸塩基としたモデルの場合、非常に安定な酸素錯体が観測できる点は大変興味深い[15]。

4.3.3　体内酸素輸送能

アルブミン－ヘム水溶液の血液適合性は高く、全血液と混合してもヘムの解離・転移を起こさない。また、赤血球数、白血球数、血小板数のみならず血液凝固系パラメーターにも変動がないことが確認されている。溶液安定度は高く、室温で 2 年間以上の保存も可能である。

一方、アルブミン－ヘムもヘム蛋白質であるから、NO 親和度は O_2 親和度の 7.6×10^6 倍と高いが、それを体内へ投与しても、Hb 製剤にみられる血管内皮からの漏出、NO 捕捉に伴う血圧亢進はまったく認められなかった（図 16.4.7）[17]。これは、アルブミンの表面電荷が負に帯電していることに起因するためと考えられており、本製剤の最大の利点である。

このようなアルブミン－ヘムの特徴を巧みに利用すると、閉塞部位への効率の高い酸素供給も可能となる。一般に悪性腫瘍は放射線療法や化学療法に抵抗を示すが、その原因として腫瘍組織内低酸素細胞（hypoxic cell）の存在がある。細胞の異常増殖は新生血管の生成が追いつかない湿潤性腫瘍細胞では十分な血流および酸素化が得られず、それが上記療法の妨げとなっている。アルブミン－ヘムを腫瘍組織の患部近傍へ投与し、腫瘍組織内低酸素細胞の酸素化を試みた。アルブミン－ヘム投与により患部の酸素分圧は投与前の 2.5 倍に増大[18]。これは従来報告の Hb 製剤による処置に比べ格段に高い値、粒子径の小さいアルブミン－ヘムが腫瘍内部へ容易に到達できるためと考えられる。今後、抗癌剤治療や放射線治療との併用により、酸素輸液投与による抗癌作用の増強効果が期待されている。

生体内へ投与され酸素輸送の役割を終えた合成ヘムは肝臓で捕捉され、緩慣代謝に入ることも最近明らかになった。安全度の高いアルブミン－ヘム製剤を出血ショックからの蘇生液として利用する評価実験が、ラット、ビーグル犬を用い進行している[19]。

4.3.4　アルブミン－ヘムの新展開

分子化学的手法を駆使した新しいアルブミン－ヘムの展開も進んでいる。アルブミンが還元型 Cys を 1 つ（Cys^{34}）しか持たない特徴に着目して、アルブミン分子を上手に連結すると、構造明確なアルブミン二～四量体が合成できる[20]。カラム分離により単離された二、三、四量体は MALDI-TOF 質量分析からその分子量が正確に決定され、間違いなくアルブミンが繋がったクラスター構造であることが示されている。アルブミン四量体にヘム 32 分子を結合したアルブミン－ヘム四量体の 20 wt％水溶液は、コロイド浸透圧を生理条件に保ったまま、血液の 2.6 倍量の酸素を溶解できる酸素輸液となる。

ごく最近、アルブミンに天然のプロトヘムを包接させたアルブミン－プロトヘム錯体の X 線結晶構造解析