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ANESTHESIOLOGY

Effect of Polyethylene-glycolated Carboxyhemoglobin on Renal Microcirculation in a Rat Model of Hemorrhagic Shock

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- The optimal fluid for resuscitation of hemorrhagic shock (crystalloid, colloid, or hemoglobin-containing) and mitigation of acute kidney injury is unknown

What This Article Tells Us That Is New

- In a rat model of hemorrhagic shock comparing fluid resuscitation with blood, diluted blood, hydroxyethyl starch, or polyethylene-glycolated carboxyhemoglobin, all fluids restored urine output and creatinine clearance, but only blood and diluted blood improved renal Po_2
- Postresuscitation histologic renal tubular damage was increased compared with nonresuscitated rats but slightly less with blood, diluted blood, and polyethylene-glycolated carboxyhemoglobin compared with hydroxyethyl starch
- Restoration of circulatory hemodynamics and kidney microcirculatory Po_2 was comparable with polyethylene-glycolated carboxyhemoglobin and balanced hydroxyethyl starch solution

ABSTRACT

Background: Primary resuscitation fluid to treat hemorrhagic shock remains controversial. Use of hydroxyethyl starches raised concerns of acute kidney injury. Polyethylene-glycolated carboxyhemoglobin, which has carbon monoxide–releasing molecules and oxygen-carrying properties, was hypothesized to sustain cortical renal microcirculatory Po_2 after hemorrhagic shock and reduce kidney injury.

Methods: Anesthetized and ventilated rats (n = 42) were subjected to pressure-controlled hemorrhagic shock for 1 h. Renal cortical Po_2 was measured in exposed kidneys using a phosphorescence quenching method. Rats were randomly assigned to six groups: polyethylene-glycolated carboxyhemoglobin 320 mg · kg^{-1}, 6% hydroxyethyl starch (130/0.4) in Ringer's acetate, blood retransfusion, diluted blood retransfusion (~4 g · dl^{-1}), nonresuscitated animals, and time control. Nitric oxide and heme oxygenase 1 levels were determined in plasma. Kidney immunohistochemistry (histologic scores of neutrophil gelatinase-associated lipocalin and tumor necrosis factor-α) and tubular histologic damages analyses were performed.

Results: Blood and diluted blood restored renal Po_2 to 51 ± 5 mmHg (mean difference, –18; 95% CI, –26 to –11; $P < 0.0001$) and 47 ± 5 mmHg (mean difference, –23; 95% CI, –31 to –15; $P < 0.0001$), respectively, compared with 29 ± 8 mmHg for hydroxyethyl starch. No differences between polyethylene-glycolated carboxyhemoglobin and hydroxyethyl starch were observed (33 ± 7 mmHg *vs.* 29 ± 8 mmHg; mean difference, –5; 95% CI, –12 to 3; $P = 0.387$), but significantly less volume was administered (4.5 [3.3–6.2] *vs.* 8.5[7.7–11.4] ml; mean rank difference, 11.98; $P = 0.387$). Blood and diluted blood increased the plasma bioavailability of nitric oxide compared with hydroxyethyl starch (mean rank difference, –20.97; $P = 0.004$; and –17.13; $P = 0.029$, respectively). No changes in heme oxygenase 1 levels were observed. Polyethylene-glycolated carboxyhemoglobin limited tubular histologic damages compared with hydroxyethyl starch (mean rank difference, 60.12; $P = 0.0012$) with reduced neutrophil gelatinase-associated lipocalin (mean rank difference, 84.43; $P < 0.0001$) and tumor necrosis factor-α (mean rank difference, 49.67; $P = 0.026$) histologic scores.

Conclusions: Polyethylene-glycolated carboxyhemoglobin resuscitation did not improve renal Po_2 but limited tubular histologic damages and neutrophil gelatinase-associated lipocalin upregulation after hemorrhage compared with hydroxyethyl starch, whereas a lower volume was required to sustain macrocirculation.

(*Anesthesiology* 2019; 131:1110–24)

Fluid administration is the first step of rescue therapy during severe hemorrhage or hypovolemic shock before blood products become available.[1] However, aggressive volume fluid resuscitation may lead to secondary severe tissue edema and clinical signs of volume overload, which result in unfavorable outcomes.[2,3] Colloidal solutions are used to sustain intravascular

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oncotic pressure and to shorten the circulatory stabilization time while limiting fluid administration. Hydroxyethyl starch solutions remain the most used synthetic colloids for volume replacement. However, no definitive evidence exists for outcome advantage of starches over crystalloids in various settings, and concerns were raised for kidney function. Therefore, the European Medicines Agency (Amsterdam, The Netherlands) restricts the use of hydroxyethyl starch solutions across the European Union.[4,5] Hyperosmotic and hypertonic saline fluids are also considered alternatives, but the fluids produce uncertain outcomes and possible harm.[6] Therefore, crystalloids remain the sole resuscitation fluid for the treatment of hemorrhage and severe hypovolemia before blood product transfusion. Current resuscitation fluids of crystalloids or synthetic colloids possess no oxygen-carrying capacity and may promote inflammation.

Acute kidney injury is one of the most frequent organ failures in perioperative and critically ill patients.[7] The pathophysiology of acute kidney injury is not fully understood to date, but major contributors have been identified, such as renal microcirculatory hypoxemia, tissue inflammation, and fluid overload.[8–10] The early reestablishment of oxygenation and kidney perfusion during hemorrhage is essential to limit the subsequent development of acute kidney injury.[9,10]

Polyethylene-glycolated carboxyhemoglobin (Sanguinate, Prolong Pharmaceuticals, USA) is a novel agent that exhibits a dual action of carbon monoxide release and oxygen carrying and transfer.[11] Carbon monoxide is poisonous at high concentrations because it inhibits cellular respiration and interacts tightly with hemoglobin to limit oxygen delivery, but it also produces beneficial effects at low concentrations. Carbon monoxide's homeostatic effects occur *via* multiple cellular and molecular mechanisms of action, including redox control, antiapoptosis and antiinflammation, modulation of vasoactive responses (vasodilation), and modulation of the innate immune response.[12,13] There is compelling preclinical data on the use of carbon monoxide as a therapeutic agent *via* carbon monoxide–releasing molecules in models of acute lung injury,[14] sepsis,[15] hemorrhagic shock,[16] and ischemia and reperfusion injury.[17] Carbon monoxide mitigated the inflammatory response and ischemic damage in all of these settings.

The present study hypothesized that a molecule with the ability to release carbon monoxide molecules to injured tissues and carry oxygen to hypoxic tissue would sustain kidney function by increasing renal microcirculatory oxygen tension and limiting the damage of ischemia and hypoxia after resuscitation from hemorrhagic shock while providing a low volume of resuscitation fluid. The primary outcome of our study was improvement of cortical renal microcirculatory oxygen tension. Secondary outcomes included the volume of fluid resuscitation, macrohemodynamics, renal immunohistochemistry and histologic damages, acid–base status, and levels of plasma biomarkers of injury. We sought to compare the renal microcirculatory impact of polyethylene-glycolated carboxyhemoglobin, 6% hydroxyethyl starch balanced in Ringer's acetate, and blood transfusion as resuscitation strategies in a blood pressure–targeted resuscitation model of hemorrhagic shock in the rat.

Materials and Methods

Animals

The Animal Research Committee of the Academic Medical Centre of the University of Amsterdam (Amsterdam, The Netherlands) approved all experiments in this study (DFL 103073). Care and handling of the animals were performed in accordance with the guidelines from the Institutional and Animal Care and Use Committees. This study and the following reporting adhere to the applicable Animal Research: Reporting of In Vivo Experiments guidelines.[18] All experiments were performed on male Wistar albino rats (Charles River, The Netherlands), aged 10 ± 3 weeks with a mean ± SD body weight of 320 ± 26 g, at 9:30 AM (weekdays) at the Department of Experimental Surgery, University Medical Center, Amsterdam, The Netherlands.

Surgical Preparation

The rats were anesthetized using an intraperitoneal injection of a mixture of 100 mg · kg^{-1} ketamine (Nimatek; Eurovet, The Netherlands), 0.5 mg · kg^{-1} dexmedetomidine (Dexdomitor; Orion Group, Finland), and 0.05 mg · kg^{-1} atropine-sulfate (Centrafarm, The Netherlands). Anesthesia was maintained with 50 mg · kg · h^{-1} ketamine. A tracheotomy was performed. The animals were connected to a ventilator (Babylog 8000, Dräger, The Netherlands) and ventilated with tidal volumes of 6 ml · kg^{-1} with a positive end-expiratory pressure of 3 cm H_2O and a fractional inspired oxygen tension of 0.4. A heating pad under the animal allowed the body temperature to be controlled and maintained at 37 ± 0.5°C. The end-tidal carbon dioxide was maintained between 35 and 42 mmHg by adjusting respiratory rate (CapnoMac, Datex-Ohmeda, USA).

The carotid (pressure) and femoral (for blood shedding and samples) arteries and jugular (anesthesia) and femoral (fluid resuscitation) veins were cannulated using polyethylene catheters (outer diameter, 0.9 mm; Braun, Germany). Fluid maintenance during surgery was sustained using Ringer's acetate (Baxter, The Netherlands) administration at a rate of 10 ml · kg^{-1} · h^{-1}. The left kidney was exposed without decapsulation and immobilized in a Lucite kidney cup (K. Effenberger, Germany) *via* a 3-cm incision in the left flank. Renal vessels were carefully separated to preserve the nerves and adrenal gland. An ultrasonic flow probe was placed around the left renal artery (type 0.7 RB; Transonic Systems Inc., USA) and connected to a flow meter (T206; Transonic Systems Inc., USA) to continuously measure renal blood flow. The left ureter was isolated, ligated, and cannulated using a polyethylene catheter for urine collection. The animals rested for 30 min after completion of surgery (~1 h).

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Experimental Protocol

The animals were randomized according to a unique code that was generated by an internet website (Sealed Envelope Ltd., 2016, simple randomization service; available at: https://www.sealedenvelope.com/simple-randomiser/v1/, accessed September 17, 2016) and placed in sealed envelopes. A technician prepared the resuscitation fluid according to the generated code on the day of the experiment. The investigator performing the experiments was partially blinded to group assignment or treatment. Control and hemorrhage groups received no fluids. Another investigator who was blinded to the type of randomization performed the data analyses.

The animals underwent stabilization period (30 min) and were bled from the left femoral artery catheter at a rate of 1 ml · min^{-1} using a syringe pump (Harvard 33 syringe pump; Harvard Apparatus, USA) until reaching a mean arterial pressure (MAP) of ~30 mmHg. This pressure was maintained for 1 h *via* reinfusing or withdrawing blood. Coagulation of the shed blood was prevented with the addition of 200 IU of heparin in the syringe. The animals were randomized into six groups: (1) polyethylene-glycolated carboxyhemoglobin with a maximum dosage of 320 mg · kg^{-1}, (2) 6% hydroxyethyl starch (130/0.4) balanced with Ringer's acetate (Volulyte, Fresenius Kabi, Germany), (3) diluted blood with polyethylene-glycolated carboxyhemoglobin's buffer (to the same hemoglobin content as polyethylene-glycolated carboxyhemoglobin: 4 g · dl^{-1}; diluted blood), (4) blood transfusion (blood), (5) nonresuscitated animals, and (6) control, in which surgery was performed without hemorrhage. Fluid resuscitation was divided into two periods of time: a rescue therapy period of 30 min (rescue period, 30 min after resuscitation), in which the fluid tested was administered alone until reaching MAP of 80 mmHg and stopped, and a maintenance period of 30 min (60 min after resuscitation), in which the resuscitated groups all received 6% hydroxyethyl starch. This design allowed comparisons between groups with a similar target (fig. 1).

Measurement of Renal Cortical Microcirculatory Po_2

An optical fiber with a tip diameter of 5 mm was placed ~1 mm above the exposed kidney to measure oxygenation using a phosphorescence lifetime technique described elsewhere.[19] Palladium(II)-meso-tetra(4-carboxyphenyl)-porphyrin at a concentration of ~5 mg · ml^{-1} was used as a phosphorescent dye, injected at a dose of 1.5 ml · kg^{-1} and excited at 530 nm. The phosphorescent decay time signal corresponds to the microcirculatory Po_2 in the kidney, and it was continuously recorded and processed using software developed in LabView 2014 (National Instruments, USA) as described previously.[19] The depth of penetration of green light and catchment depth were estimated to be within the renal cortex. We excluded any interference between palladium(II)-meso-tetra(4-carboxyphenyl)-porphyrin and polyethylene-glycolated carboxyhemoglobin (*in vitro*) before the *in vivo* experiment.

Determination of Oxygen-carrying Capacity of Polyethylene-glycolated Carboxyhemoglobin and Spectrophotometric Analyses of Its Derivatives

Because polyethylene-glycolated carboxyhemoglobin is bound with carbon monoxide molecules, and its oxygen-carrying capacity is reduced. The release of carbon monoxide likely increases the oxygen-carrying capacity of the molecule. The oxygen-binding/carrying capacity of polyethylene-glycolated carboxyhemoglobin was determined at 37°C under normal atmospheric conditions using similar previously described methods (Supplemental Digital Content 1, http://links.lww.com/ALN/C41). A second set of experiments determined the oxygen-binding/carrying capacity of the molecule after full saturation of polyethylene-glycolated carboxyhemoglobin in 100% oxygen (for 2 h) to induce carbon monoxide release. However, complete carbon monoxide release was not achieved.

The unknown spectrum was determined spectrophotometrically in UV-visible light ranging from 500 to 650 nm with a 1-nm increment at 37°C (Synergy HTX, BioTek,

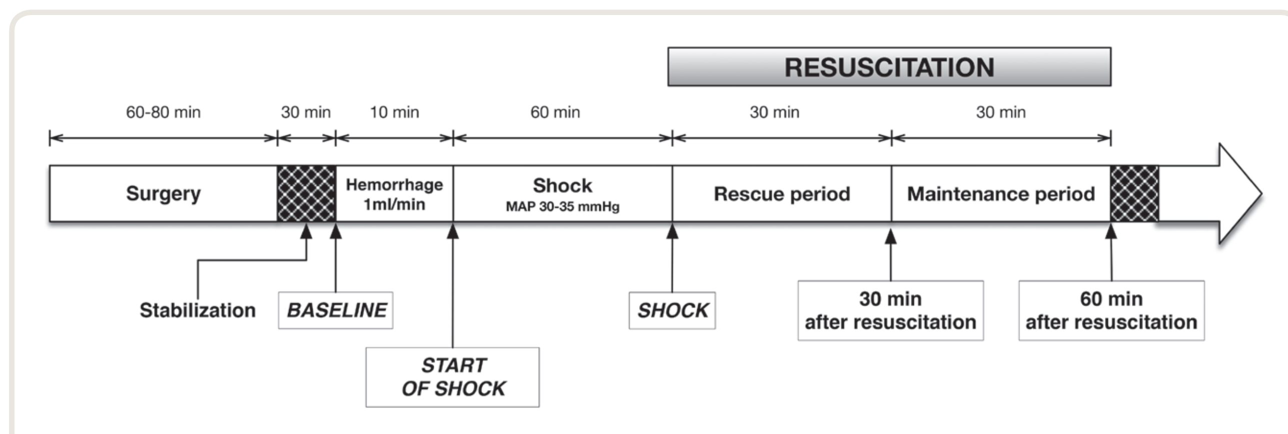

Fig. 1. Design and timeline of the experiment. Control rats were subjected to the same surgery but did not undergo the hemorrhage protocol. Nonresuscitated hemorrhagic shock rats were observed during the same time of observation. MAP, mean arterial pressure.

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Germany) to determine the concentration of each derivative (carboxyhemoglobin, oxyhemoglobin, and deoxyhemoglobin) of polyethylene-glycolated carboxyhemoglobin in the plasma. The observed total absorption spectrum of each plasma sample was then compared with the known spectra of the different derivatives of bovine hemoglobin[20] with their absorption values. With the use of the classical least squares' method (mixture of three components with 150 wavelengths, see Supplemental Digital Content 1, http://links.lww.com/ALN/C41), the different concentrations of each component were recovered.

The concentration of polyethylene-glycolated carboxyhemoglobin in the plasma was obtained after the addition of 5 µl of both 2% KCN and 2% $K_3Fe(CN)_6$ to 40 µl of plasma to convert all polyethylene-glycolated carboxyhemoglobin derivatives to hemiglobincyanide. The endpoint absorbance was read at 540 nm against a calibration curve.

Measurements of Arterial Blood Gas and Biochemistry

Arterial blood samples (250 µl) were collected at four time points (fig. 1). Arterial blood gas parameters were determined using a blood gas analyzer (ABL80 Flex, Radiometer, Denmark), and total hemoglobin concentration, hemoglobin oxygen saturation, base excess, and lactate levels were measured. The arterial oxygen content (Cao_2; ml $O_2 \cdot dl^{-1}$) was calculated considering the oxygen-carrying capacity of polyethylene-glycolated carboxyhemoglobin in the plasma: $Cao_2 = (Sao_2 \times Hb_{RBC} \times \gamma_{Hb}) + (S_{polyethylene\text{-}glycolated\ carboxyhemoglobin}O_2 \times Hb_{polyethylene\text{-}glycolated\ carboxyhemoglobin} \times \gamma_{polyethylene\text{-}glycolated\ carboxyhemoglobin}) + Pao_2 \times 0.0031$, where Hb_{RBC} is the hemoglobin concentration in erythrocyte ($g \cdot dl^{-1}$), S is the arterial oxygen saturation of Hb_{RBC} or polyethylene-glycolated carboxyhemoglobin, and γ is the oxygen-carrying capacity of Hb_{RBC} (1.34 ml $O_2 \cdot g^{-1}$ Hb for rat blood) or polyethylene-glycolated carboxyhemoglobin, as previously determined. Renal delivery of oxygen (Do_2; ml $O_2 \cdot min^{-1}$) was determined at the end of the experiment as follows: renal $Do_2 = Cao_2 \times$ renal blood flow.

Plasma creatinine and urine creatinine were measured using an automatic analyzer (c702, Roche Diagnostics, Switzerland). We established a calibration curve for creatinine levels before measurements to correct for a significant interference because of the presence of polyethylene-glycolated carboxyhemoglobin. Plasma osmolarity was determined using the freezing point method with an osmotic pressure meter (OSMOstation OM-6050, Menarini, Benelux, Belgium). The glomerular filtration rate was estimated at the end of the experiment using the measurement of creatinine clearance (Cl_{crea}): $Cl_{crea} = (U_{Crea} \times V)/(time \times P_{Crea})$, where U_{Crea} is the concentration of creatinine in urine ($mmol \cdot l^{-1}$), V is the urine volume (ml) per unit time of urine collection, and P_{Crea} is the concentration of creatinine in plasma ($mmol \cdot l^{-1}$).

Plasma Nitric Oxide and Heme Oxygenase 1 Levels

The samples were placed in the reducing agent vanadium (III) chloride (VCl_3) in 1 mol $\cdot$ l^{-1} HCl at 90°C to reduce the nitrate and nitrite in the plasma samples to nitric oxide. The vanadium (III) chloride reagent converts nitrite, nitrate, and *S*-nitroso compounds to nitric oxide gas that is guided toward a nitric oxidechemiluminescence signal analyzer (Sievers 280i analyzer, GE Analytical Instruments, USA) to allow the direct detection of nitric oxide.[21] Nitric oxide within the reaction vessel reacts with ozone to generate oxygen and excited-state nitric oxide species, and the decay is associated with the emission of weak near-infrared chemiluminescence. A sensitive photodetector detects this signal and converts it to millivolts. The area under the curve of the detected chemiluminescence (mV · s) represents the amount of nitrix oxide–ozone reactions in real time and thus the amount of bioavailable nitric oxide in the tested samples.

Heme oxygenase 1 constitutes an essential cytoprotective component that ameliorates hemorrhagic shock–induced oxidative tissue injuries.[22] Plasma heme oxygenase 1 levels were measured at the end of the experiment using an enzyme-linked immunosorbent assay (ELISA) kit based on the sandwich assay principle (rat HMOX1/HO-1 ELISA kit [sandwich ELISA], LS-F4085, LSBio LifeSpan BioSciences Inc., USA).

Immunohistochemical Analysis and Histology

The animals were euthanized at the end of the experiment using 40% Euthasol (Produlab Pharma BV, The Netherlands). The kidneys were harvested, fixed in 4% formalin, and embedded in paraffin. Ten tubular areas (n = 10) on each slice per animal were analyzed. A minimum of 40 tubules were examined per group, with n = 6/kidney per animal minimum. The complete description of immunohistochemical analyses and histology are detailed in Supplemental Digital Content 1 (http://links.lww.com/ALN/C41). A histologic score value for neutrophil gelatinase–associated lipocalin and tumor necrosis factor-α was derived for each sample *via* summing the percentages of cells that stained at each intensity multiplied by the weighted intensity of the staining (histologic score = S Pi [i + 1], where i is the intensity score, and Pi is the corresponding percentage of the cells) under a light microscope at ×400 magnification. Histologic changes (brush border, vacuolar degeneration, cast formation, and invagination) in the cortex were assessed using quantitative measurements of tissue damage.

Statistical Analysis

The values are expressed as means ± SD when normally distributed (Kolmogorov–Smirnov test) or as median [interquartile range] otherwise. Repeated-measures two-way ANOVA (two factors: time as a related within-animal

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factor and group as a between-animal factor) and *post hoc* Bonferroni correction test for multiple analyses were used to determine intergroup and/or intragroup differences in hemodynamics, cortical renal Po_2, and biochemical data. We reported the simple main effects of group (type of fluid) compared with the control and to the hydroxyethyl starch groups at each time point when a significant interaction was observed between time and group, and the simple main effect of time *versus* baseline within the same group. Ordinary one-way ANOVA with Bonferroni correction was used for the analyses of the oxygen-binding capacity of polyethylene-glycolated carboxyhemoglobin, renal oxygen delivery, ELISA heme oxygenase 1, and volume of blood withdrawn. Analyses of creatinine clearance, total fluid volume, plasma nitric oxide, histologic damages to the kidney, and tumor necrosis factor-α and neutrophil gelatinase–associated lipocalin histologic scores were performed using a Kruskal–Wallis test with Dunn correction test because of their non-Gaussian distribution. Outliers were evaluated, but no action was necessary. Statistical analyses were performed using GraphPad Prism version 7.0a for Mac (GraphPad Software, USA). The overall significance level for each hypothesis was 0.05. Adjusted P values are reported throughout the manuscript in *post hoc* tests.

We assumed that the microcirculatory Po_2 in the kidney would be of 26 ± 3 mmHg (means ± SD) at 30 min after resuscitation of hemorrhagic shock using 6% balanced hydroxyethyl starch based on previous experiments lead by our group. Therefore, a sample size of n = 6/group was needed to detect a 20% increase in kidney microcirculatory Po_2 (estimated to be 31 mmHg, Δ of 5 mmHg) associated with the administration of polyethylene-glycolated carboxyhemoglobin (compared with balanced hydroxyethyl starch) and provide a power (1-β) of 80% with a type I error rate (α) of 5% using a two-group Satterthwaite two-sided t test.

Results

A total of 42 animals were included in the present study (n = 6–8/group). Eight animals were included in the polyethylene-glycolated carboxyhemoglobin group, and four served to established reliability in the protocol. No animal died during the experiment. The volume of blood withdrawn to induce hemorrhagic shock at T1 (6.7 ± 0.8 ml) was similar in all groups. The withdrawn volume represented 32.2 ± 4.4% of the total blood volume. The hemorrhagic shock produced similar alterations in hemodynamics (MAP and renal blood flow) and similar reductions in kidney microcirculatory Po_2 associated with a metabolic acidosis and hyperlactatemia in all groups compared with controls (P < 0.0001). The hemodynamic data throughout the experiment are presented in table 1. MAP was restored to ~80 mmHg in all animals that underwent resuscitation after hemorrhagic shock, according to the protocol. At the end of experiment, the renal blood flow recovered similarly to controls except

Table 1. Systemic and Renal Hemodynamics and Urine Excretion

	Baseline	Shock	Rescue Phase (30 min after Resuscitation)	Maintenance (60 min after Resuscitation)
MAP, mmHg				
Control	94 ± 10	81 ± 5	84 ± 6	90 ± 6
Nonresuscitated	92 ± 5	32 ± 2*	32 ± 1*	30 ± 1*
6% balanced hydroxyethyl starch	92 ± 10	32 ± 1*	81 ± 4	83 ± 6
PEGylated carboxyhemoglobin	91 ± 7	32 ± 2*	76 ± 11	81 ± 3
Blood transfusion	89 ± 4	32 ± 2*	86 ± 8	87 ± 10
Diluted blood transfusion	90 ± 6	33 ± 2*	81 ± 1	79 ± 4*
Renal blood flow, ml · min^{-1}				
Control	9.8 ± 2.3	7.9 ± 2.6	7.4 ± 2.4	7 ± 2.4
Nonresuscitated	10.5 ± 3.2	3.0 ± 1*	3.2 ± 1.1*	3.0 ± 1*
6% balanced hydroxyethyl starch	9.7 ± 1.3	3.9 ± 2.4*	7.4 ± 2.4	8.4 ± 2.9
PEGylated carboxyhemoglobin	11.6 ± 2	2.8 ± 0.5*	6.3 ± 1.2	8.3 ± 1.6
Blood transfusion	10.0 ± 1.7	2.3 ± 0.3*	5.0 ± 0.3	5.4 ± 0.5†
Diluted blood transfusion	11.6 ± 1.2	3.1 ± 1.5*	7.0 ± 1.7	7.3 ± 1.6
Urine output, ml · h^{-1}				
Control	2.80 ± 0.73	2.2 ± 1.42	2.46 ± 0.94	1.87 ± 0.53
Nonresuscitated	4.68 ± 1.8	0 ± 0‡	0 ± 0‡	0 ± 0‡
6% balanced hydroxyethyl starch	2.77 ± 0.91	0 ± 0‡	2.55 ± 0.75	3.93 ± 2.35
PEGylated carboxyhemoglobin	4.01 ± 2.3	0 ± 0‡	0.76 ± 0.55‡	3.97 ± 1.43
Blood transfusion	3.29 ± 1.94	0 ± 0‡	0.85 ± 0.52‡	2.2 ± 1.43
Diluted blood transfusion	4.04 ± 1.31	0 ± 0‡	1.63 ± 1.20‡	3.63 ± 1.75

The values are presented as means ± SD. Repeated measures two-way ANOVA test used with Bonferroni correction to adjust for multiple comparisons.

*Adjusted P < 0.01 *versus* control group at the same time point. †Adjusted P < 0.05 *versus* 6% balanced hydroxyethyl starch group at the same time point. ‡P < 0.01 *versus* baseline value within the same group.

MAP, mean arterial pressure; PEGylated, polyethylene glycolated.

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Table 2. Acid–Base Balance and Arterial Lactate Levels during Experiment

	Baseline	Shock	Rescue Phase (30 min after Resuscitation)	Maintenance (60 min after Resuscitation)
pH				
Control	7.41 ± 0.04	7.38 ± 0.04	7.38 ± 0.02	7.37 ± 0.01
Nonresuscitated	7.39 ± 0.02	7.23 ± 0.02*	7.16 ± 0.05*	7.13 ± 0.05*
6% balanced hydroxyethyl starch	7.40 ± 0.04	7.22 ± 0.05*	7.36 ± 0.03*	7.40 ± 0.04
PEGylated carboxyhemoglobin	7.38 ± 0.02	7.20 ± 0.06*	7.29 ± 0.04*†	7.35 ± 0.02
Blood transfusion	7.40 ± 0.03	7.22 ± 0.03*	7.26 ± 0.04*†	7.33 ± 0.06
Diluted blood transfusion	7.39 ± 0.04	7.22 ± 0.06*	7.28 ± 0.05*†	7.32 ± 0.05†
Base excess, mmol · l⁻¹)				
Control	−4.5 ± 2	−4.6 ± 1	−4.4 ± 0.9	−3.3 ± 0.6
Nonresuscitated	−2.4 ± 1.3	−12.5 ± 1.9*	−15 ± 3.0*	−16.6 ± 2.7*
6% balanced hydroxyethyl starch	−3.5 ± 2.0	−13.6 ± 0.8*	−5.6 ± 1.4*	−2.2 ± 1.4
PEGylated carboxyhemoglobin	−3.7 ± 0.8	−13.4 ± 2.3*	−9.3 ± 2.0*†	−4.5 ± 0.8
Blood transfusion	−2.5 ± 1.7	−13.2 ± 1.4*	−9.5 ± 1.3*†	−6.6 ± 1.6
Diluted blood transfusion	−1.7 ± 1.9	−12.2 ± 3.8*	−8.7 ± 3.1*	−6.9 ± 2.5*
HCO_3^-, mmol · l⁻¹)				
Control	19.3 ± 1.1	19.5 ± 0.5	19.8 ± 1.1	20.8 ± 0.8
Nonresuscitated	21.6 ± 1.8	13.8 ± 1.9*	12.7 ± 2.4*†	11.4 ± 2.2*
6% balanced hydroxyethyl starch	20.0 ± 1.9	12.9 ± 1.1*	18.5 ± 1.4	22.1 ± 1.3
PEGylated carboxyhemoglobin	20.1 ± 1.2	13.6 ± 1.2*	16.3 ± 1.9*	20.2 ± 0.9†
Blood transfusion	21.1 ± 1.9	13.2 ± 1.8*	16.6 ± 1*	18.3 ± 0.9†
Diluted blood transfusion	22.4 ± 1.4	14.2 ± 2.8*	16.9 ± 2.6	18.0 ± 2.5†
Lactate⁻, mmol · l⁻¹				
Control	0.9 ± 0.2	1.4 ± 0.3	1.9 ± 0.4	2.1 ± 0.5
Nonresuscitated	0.8 ± 0.2	5.1 ± 0.5*	5.8 ± 0.6*	7.2 ± 1.1*
6% balanced hydroxyethyl starch	1.1 ± 0.4	5.3 ± 1.3*	3.3 ± 0.6*	2.7 ± 0.5
PEGylated carboxyhemoglobin	0.8 ± 0.2	4.5 ± 0.7*	3.9 ± 0.8*	3.6 ± 0.8*
Blood transfusion	0.8 ± 0.1	5.1 ± 0.5*	3.0 ± 0.2	2.3 ± 0.3
Diluted blood transfusion	0.7 ± 0.1	5.0 ± 1.4*	3.0 ± 0.8	1.9 ± 0.4

The values are presented as means ± SD. Repeated measures two-way ANOVA test was used with Bonferroni's correction to adjust for multiple comparisons.
*Adjusted $P < 0.01$ *versus* control group at the same time point. †Adjusted $P < 0.01$ *versus* 6% balanced hydroxyethyl starch group at the same time point.
PEGylated, polyethylene glycolated.

for the blood transfusion group, which was lower than in the hydroxyethyl starch group ($P = 0.015$). The changes in acid–base status and lactate levels differed according to the resuscitation fluid administered (table 2). Balanced hydroxyethyl starch was the most efficient fluid at restoring the acid–base status at the end of the rescue phase compared with other therapies. Polyethylene-glycolated carboxyhemoglobin corrected the pH, but base excess, bicarbonate levels, and lactate levels remained significantly higher than controls (mean difference, −1.5; 95% CI, −2.6 to −0.4; $P = 0.0018$; table 2).

Significant differences in urine output at baseline were observed between groups. Therefore, only intragroup comparisons were performed. Notably, urine output after the rescue phase in the blood transfusion group was lower than at baseline (mean difference, 2.44; 95% CI, 0.93 to 3.95; $P = 0.0006$), and urine output was readily restored 30 min after resuscitation in the hydroxyethyl starch group (mean difference, 0.23; 95% CI, −1.28 to 1.74; $P = 0.969$; table 1).

Kidney Microcirculatory Po_2 and Renal Function

Figure 2 shows that hemorrhagic shock significantly reduced baseline renal cortical Po_2 in all groups compared with baseline and controls. The mean renal cortical Po_2 was 13 ± 5 mmHg 60 min after hemorrhagic shock induction. Similar MAP and renal blood flow were observed between groups, but none of the fluid therapies restored renal cortical Po_2 to levels similar to controls 30 or 60 min after resuscitation. Whole blood transfusion and diluted blood transfusion outperformed 6% balanced hydroxyethyl starch in increasing renal cortical Po_2 at the end of experiment to 51 ± 5 mmHg (mean difference, −19; 95% CI, −26 to −11; $P < 0.0001$) and 47 ± 5 mmHg (mean difference, −23; 95% CI, −31 to −15; $P < 0.0001$) *versus* 29 ± 8 mmHg, respectively. However, there were no differences between polyethylene-glycolated carboxyhemoglobin and balanced hydroxyethyl starch in renal cortical Po_2 in the early rescue phase (32 ± 7 mmHg *vs.* 27 ± 4 mmHg; mean difference, −6, 95% CI, −13 to 2; $P = 0.209$) or at the end of the experiment (33 ± 7 mmHg *vs.* 29 ± 8 mmHg; mean difference, −5; 95% CI, −12 to 3; $P = 0.387$).

Hemorrhagic shock induced acute kidney injury with a significant increase in plasma creatinine levels (greater than twofold increase of baseline values; fig. 3A) compared with controls ($P < 0.0001$). Creatinine levels remained higher 60 min after resuscitation compared with the control

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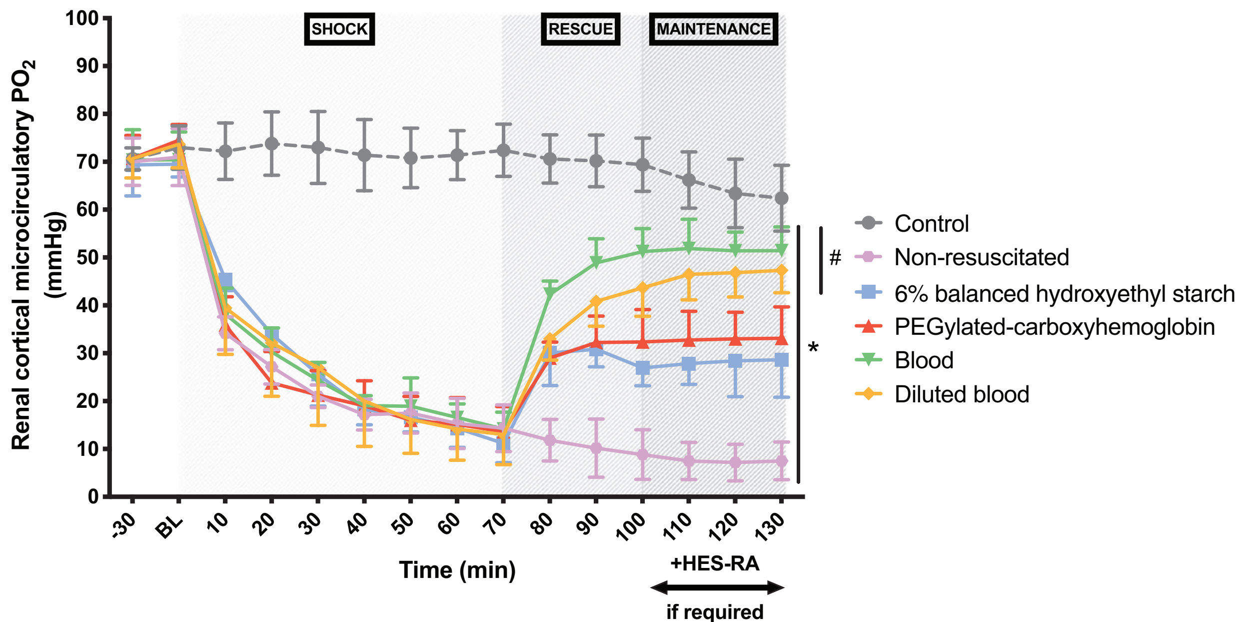

Fig. 2. Renal cortical microcirculatory PO_2 during the experiment. For the sake of clarity, only significant differences were shown at the end of the experiment. Repeated measures two-way ANOVA test was used with Bonferroni correction to adjust for multiple comparisons. *Adjusted $P < 0.005$ *versus* control group. #Adjusted $P < 0.05$ compared with 6% balanced hydroxyethyl starch at the same time point. The data are presented as means ± SD (n = 8 for PEG-carboxyhemoglobin group and n = 6/group for other groups). HES-RA, 6% hydroxyethyl starch balanced in ringer acetate; PEGylated, polyethylene glycolated.

rats, regardless of the therapy used ($P < 0.0001$). There were no differences regarding plasma creatinine between hydroxyethyl starch and polyethylene-glycolated carboxyhemoglobin 60 min after resuscitation (44.0 ± 6.7 μmol/l *vs.* 49.9 ± 6.4 μmol/l, respectively; mean difference, 5.9; 95% CI, −3.0 to 14.8; $P = 0.230$). Conversely, the measured creatinine clearance (fig. 3B) was not different at 60 min between groups.

The CaO_2 (table 3) was calculated for all time points. However, the contribution of polyethylene-glycolated carboxyhemoglobin's hemoglobin to arterial oxygen content was only considered at the last time point of the experiment (60 min after resuscitation) for technical reasons. Therefore, we did not investigate the contribution of polyethylene-glycolated carboxyhemoglobin's hemoglobin to CaO_2 30 min after resuscitation. Blood and diluted blood transfusions produced CaO_2 values similar to controls. The CaO_2 was superior in the polyethylene-glycolated carboxyhemoglobin group compared with balanced hydroxyethyl starch only after the rescue period (30 min; 12.5 ± 1.3 g · dl^{-1} *vs.* 9.4 ± 1.1 g · dl^{-1}; mean difference, −3.0; 95% CI, −5.4 to −0.6; $P = 0.005$). This difference was offset at the end of the experiment. The renal oxygen delivery was significantly lower in all groups at the end of experiment compared with controls except in the diluted blood transfusion group (Supplemental Digital Content 2, http://links.lww.com/ALN/C42).

Fluid Volume

The stacked (total) fluid volume and separate volumes of each therapy plus balanced hydroxyethyl starch are presented in figure 4. The fluid volumes needed to achieve similar MAP targets varied significantly between groups, 8.5 (7.7 to 11.4), 4.5 (3.3 to 6.2), 7.3 (6.4 to 7.8), and 13.2 (11.9 to 15.35) ml, for the balanced hydroxyethyl starch, polyethylene-glycolated carboxyhemoglobin, blood transfusion, and diluted blood transfusion groups, respectively ($P < 0.0002$). The polyethylene-glycolated carboxyhemoglobin group required significantly less volume than balanced hydroxyethyl starch (mean rank difference, −11.98; $P = 0.011$) to achieve similar MAP and renal cortical PO_2. No additional volume of balanced hydroxyethyl starch was required in the diluted blood transfusion group to maintain MAP over the observation time of the experiment. However, this latter group required the largest amount of fluid administration.

Fate of Polyethylene-glycolated Carboxyhemoglobin

Polyethylene-glycolated carboxyhemoglobin partially released its carbon monoxide molecules after injection in the cardiovascular system (table 3). The percentage of carboxyhemoglobin decreased from ~85% measured *in vitro* (manufacturer data) to 23.6 ± 9% ($P < 0.0001$; as determined in the Supplemental Digital Content 1, http://links.lww.com/ALN/C41).

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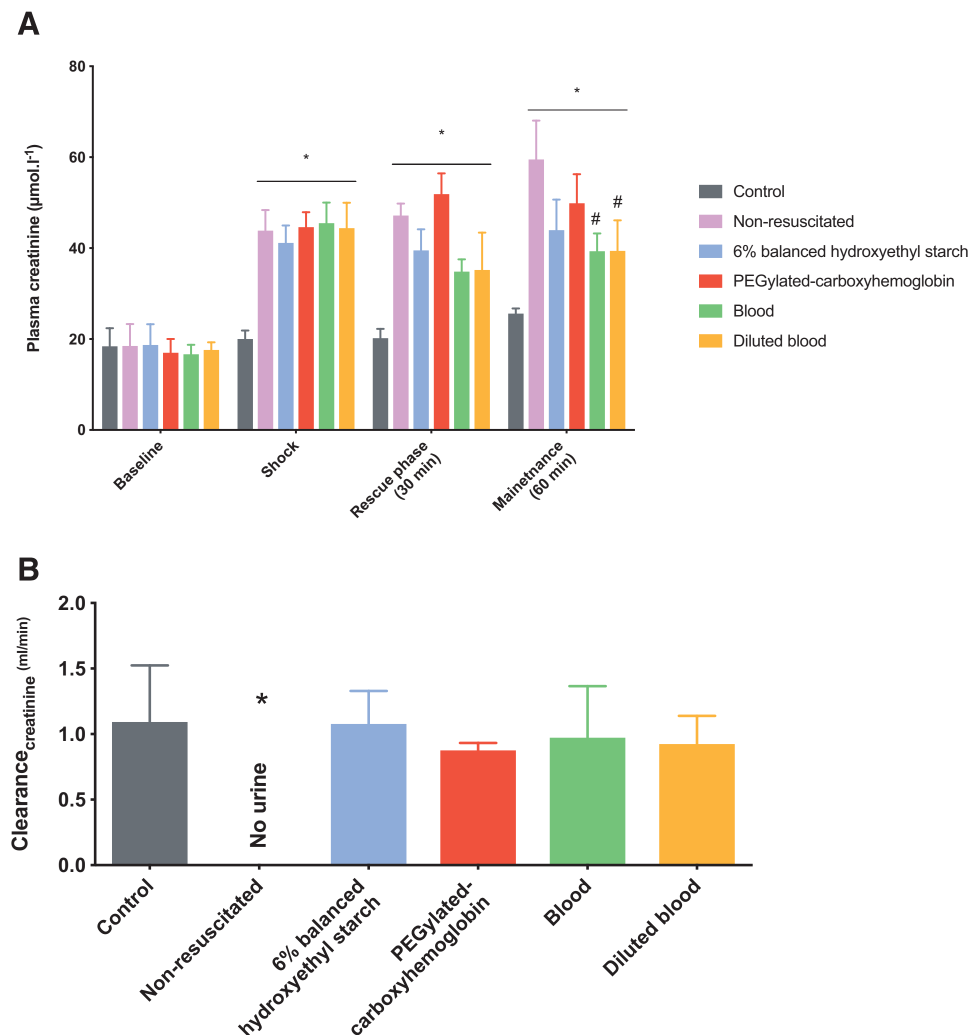

Fig. 3. Time course of changes in plasma creatinine (*A*) and creatinine clearance at the end of the experiment (*B*). (*A*) Repeated measures two-way ANOVA test was used with Bonferroni correction to adjust for multiple comparisons. *Adjusted $P < 0.001$ *versus* control group. #Adjusted $P < 0.05$ compared with 6% balanced hydroxyethyl starch at the same time point. The data are presented as means ± SD. (*B*) Kruskal–Wallis test with Dunn posttest was used. *Adjusted $P < 0.0001$ compared with control group. The data are presented as medians [interquartile range] (n = 8 for PEG-carboxyhemoglobin group and n = 6/group for other groups). PEGylated, polyethylene glycolated.

The oxygen-binding capacity of polyethylene-glycolated carboxyhemoglobin *in vitro* was determined to be 0.29 ± 0.09 ml $O_2 \cdot g^{-1}$ of hemoglobin (seven measurements) in its original form. This oxygen-binding capacity increased significantly after full oxygenation of the molecule to induce carbon monoxide release, reaching 0.92 ± 0.02 ml $O_2 \cdot g^{-1}$ of hemoglobin ($P < 0.0001$).

Nitric Oxide and Heme Oxygenase 1 in Plasma

The administration of polyethylene-glycolated carboxyhemoglobin did not result in a decrease in plasma nitric oxide concentrations, excluding a nitric oxide–scavenging effect. Plasma nitric oxide levels were higher in the blood transfusion, diluted blood transfusion, and nonresuscitated hemorrhagic shock groups compared with balanced hydroxyethyl starch ($P < 0.05$). However, there was no significant difference between the control and balanced hydroxyethyl starch or polyethylene-glycolated carboxyhemoglobin groups (Supplemental Digital Content 2A, http://links.lww.com/ALN/C42). Blood and diluted blood increased the bioavailability of NO in the plasma compared with balanced hydroxyethyl starch (mean rank difference, −20.97; $P = 0.004$; and mean rank difference, −17.13; $P = 0.029$, respectively).

CO is produced *via* the heme oxygenase 1 pathway. Therefore, we investigated the levels of heme oxygenase

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Table 3. Hemoglobin Levels, Arterial Oxygen Content, and Fractions of PEGylated Carboxyhemoglobin's Derivatives

	Baseline	Shock	Rescue Phase (30 min after Resuscitation)	Maintenance (60 min after Resuscitation)
Total hemoglobin concentration, g · dl^{-1}				
Control	14.4 ± 0.9	14.5 ± 0.5	14.3 ± 0.8	13.0 ± 0.3
Nonresuscitated	15.1 ± 0.9	10.5 ± 2.0*	10.3 ± 1.2*†	9.3 ± 0.7*†
6% balanced hydroxyethyl starch	14.8 ± 1.5	9.7 ± 1.0*	6.6 ± 0.8*	5.5 ± 0.5*
PEGylated carboxyhemoglobin	15.6 ± 0.9	11.2 ± 1.0	8.6 ± 0.9	7 ± 1
Blood transfusion	15.3 ± 0.5	9.9 ± 0.8	13.5 ± 1.3	12.4 ± 0.9
Diluted blood transfusion	14.8 ± 0.2	10.1 ± 0.7	11.0 ± 0.6	10.9 ± 0.2
Hematocrit, %				
Control	44 ± 3	45 ± 1	43 ± 3	40 ± 1
Nonresuscitated	46 ± 3	32 ± 6*	32 ± 4*†	29 ± 2*†
6% balanced hydroxyethyl starch	45 ± 5	30 ± 3*	21 ± 2*	17 ± 2*
PEGylated carboxyhemoglobin	48 ± 3	35 ± 3	28 ± 3	22 ± 3
Blood transfusion	47 ± 1	31 ± 3	41 ±4	38 ± 3
Diluted blood transfusion	45 ± 0.5	31 ± 2	34 ± 1.7	34 ± 0.7
PEGylated carboxyhemoglobin and derivatives				
Plasma PEGylated carboxyhemoglobin concentration, g · dl^{-1}			ND	0.6 ± 0.1
PEGylated carboxyhemoglobin fraction (%)			ND	23.6 ± 9
Cao_2, ml O_2 · dl^{-1}				
Control	19.8 ± 1.2	20.1 ± 0.6	19.8 ± 1	18.1 ± 0.4
Nonresuscitated	20.8 ± 1.2	14.7 ± 2.7*	14.5 ± 1.6*†	13.1 ± 0.9*†
6% balanced hydroxyethyl starch	20.4 ± 2.10	13.6 ± 1.40*	9.5 ± 1.10*	8.0 ± 0.7*
PEGylated carboxyhemoglobin	21.6 ± 1.1	15.7 ± 1.2	13 ± 1.3	10.4 ± 1.5
Blood transfusion	21.1 ± 0.6	13.9 ± 1.1	18.7 ± 1.7	17.2 ± 1.2
Diluted blood transfusion	20.4 ± 0.2	14.2 ± 1	15.3 ± 0.8	15.3 ± 0.3

The values are presented as means ± SD. Repeated measures two-way ANOVA test was used with Bonferroni correction to adjust for multiple comparisons.
*Adjusted $P < 0.01$ *versus* control group at the same time point. †Adjusted $P < 0.01$ *versus* 6% balanced hydroxyethyl starch group at the same time point.
Cao_2, arterial oxygen content; ND, not determined; PEGylated, polyethylene glycolated.

1 in the plasma. We were unable to detect any significant changes in plasma heme oxygenase 1 between groups, but there was a trend toward higher heme oxygenase 1 levels in the blood transfusion group (Supplemental Digital Content 2B, http://links.lww.com/ALN/C42).

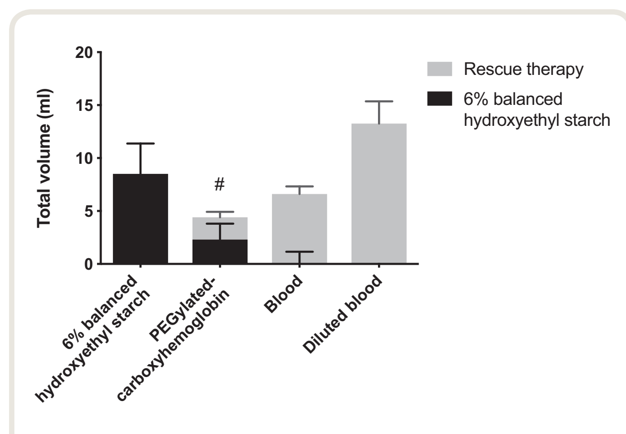

Fig. 4. Total volume of fluid administered in each group. The volumes are stacked for the sake of clarity. The height of the stacked bins represents the total volume administered. The total volumes (rescue therapy + maintenance) were compared between groups. #Adjusted $P < 0.05$ compared with 6% balanced hydroxyethyl starch. Kruskal–Wallis test with Dunn posttest was used. The data are presented as median [interquartile range] (n = 8 for PEG-carboxyhemoglobin group and n = 6/group for other groups). PEGylated, polyethylene glycolated.

Kidney Immunohistochemistry and Histology

Figure 5 depicts the tumor necrosis factor-α (fig. 5A) and neutrophil gelatinase–associated lipocalin (fig. 5B) histologic scores in the kidney. Hemorrhagic shock produced a significant increase in neutrophil gelatinase–associated lipocalin histologic score compared with control rats (270 [210 to 300] *vs.* 225 [197.5 to 251]; mean rank difference, −46; $P = 0.006$, respectively). Only polyethylene-glycolated carboxyhemoglobin and blood transfusion prevented the alterations in neutrophil gelatinase–associated lipocalin histologic score after hemorrhagic shock and outperformed fluid resuscitation with balanced hydroxyethyl starch ($P < 0.0005$). Consistently, the damage scores in kidney (brush border loss, vacuolization, and luminal casts) were markedly decreased in the polyethylene-glycolated carboxyhemoglobin, diluted blood transfusion, and blood transfusion groups (4 [3.3 to 4.3], 4 [3.7 to 4.7], and 3.7 [3.3 to 4], respectively) compared with balanced hydroxyethyl starch (5 [4.7 to 5]; $P < 0.001$) but remained significantly higher than the controls ($P < 0.001$; fig. 5C).

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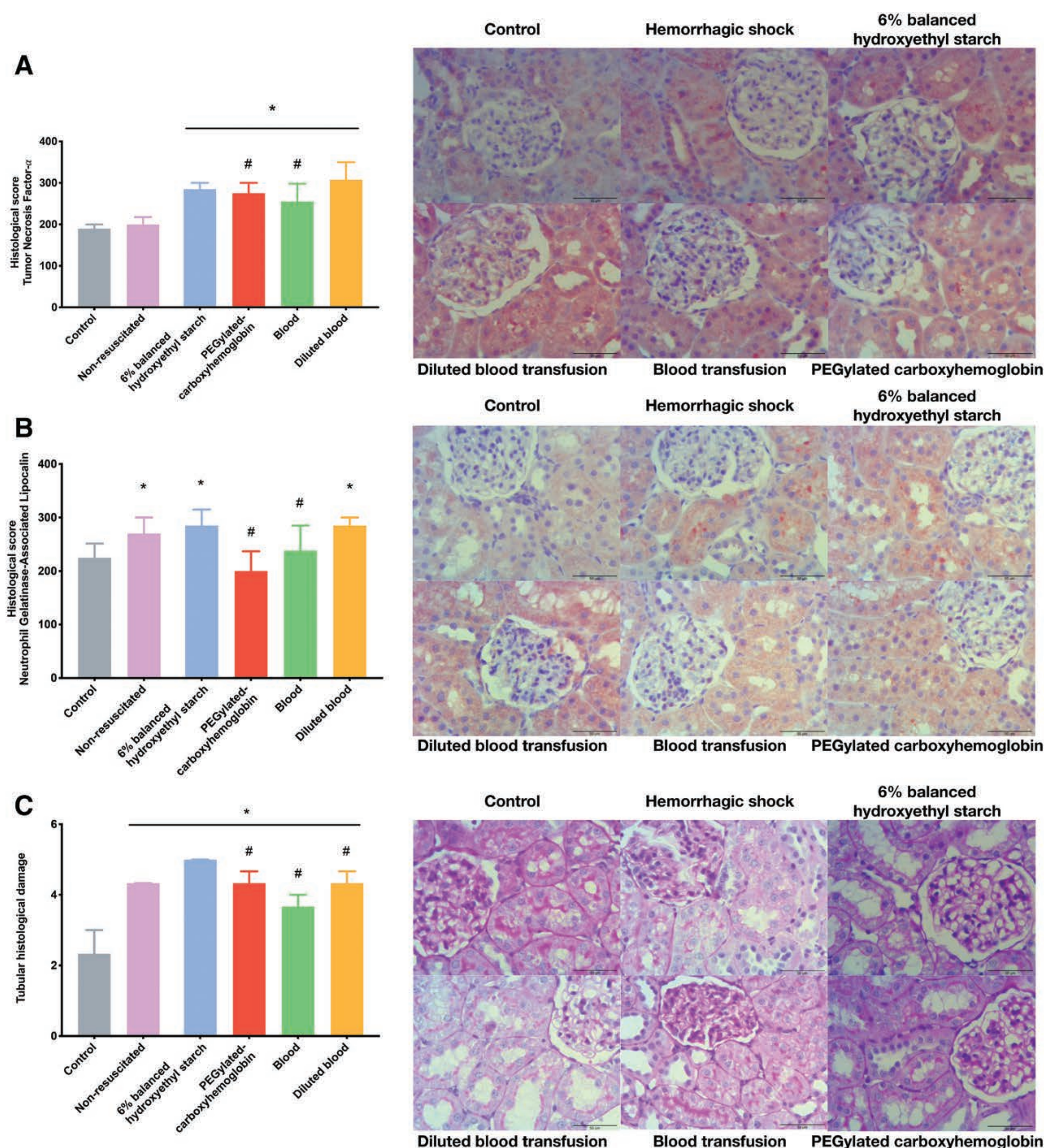

Fig. 5. Kidney immunochemistry and tubular histologic damage scores. (*Left panels*) The histologic scores of tumor necrosis factor-α (*A*) and neutrophil gelatinase-associated lipocalin (*B*) are presented (see Supplemental Digital Content 1, http://links.lww.com/ALN/C41, for additional information). Tubular histologic damage graph (*C*) was drawn by calculating mean of damages of tubular brush border loss, presence of luminal cast and vacuoles. (*Right panels*) Observation of the corresponding histologic images are shown at magnification ×400. The *scale bar* is 50 µm. (*A*) Kidney sections were incubated overnight at 4°C with rabbit polyclonal tumor necrosis factor-α (1:100; Abcam 6671) or (*B*) incubated for 1 h at room temperature with Lipocalin 2 antibody (neurophil gelatinase-associated lipocalin, 1/150; Abcam 41105). (*C*) The kidney sections were stained with periodic acid–Schiff reagent and hematoxyline to depict tubular damage defined as loss of brush border, vacuolar degeneration, and cast formation. *Adjusted $P < 0.05$ compared with control group. #Adjusted $P < 0.05$ compared with 6% balanced hydroxyethyl starch. Kruskal–Wallis test with Dunn's posttest was used. A minimum of 40 tubules were examined per group, with n = 6/kidney per animal minimum. The data are presented as the median [interquartile range]. PEGylated, polyethylene glycolated.

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Discussion

The present study demonstrated that low-volume rescue therapy with polyethylene-glycolated carboxyhemoglobin provided efficient restoration of the macrohemodynamics and maintained a similar kidney microcirculatory Po_2 compared with synthetic balanced hydroxyethyl starch after resuscitation of pressure-controlled hemorrhagic shock in a rodent model. Polyethylene-glycolated carboxyhemoglobin also modulated tubular damages in the kidney, which was associated with a mitigated expression of neutrophil gelatinase–associated lipocalin, because of its embedded carbon monoxide molecules. Polyethylene-glycolated carboxyhemoglobin may blunt the risk for acute kidney injury in this setting. The small increase in oxygen-carrying capacity provided by this molecule did not affect kidney tissue oxygenation or improve lactate or creatinine clearance, but the low volume administered sustained urine output and produced very stable hemodynamic conditions for 60 min after hemorrhagic shock. We also demonstrated that diluted blood transfusion at the same level as polyethylene-glycolated carboxyhemoglobin provided significantly higher renal cortical Po_2 in the kidney but required markedly more volume to reach the targeted MAP.

Low-volume strategies were considered for decades to limit aggressive fluid resuscitation using large uncontrolled fluid boluses that increased bleeding, hemodilution, coagulopathy, hemodynamic decompensation, fluid overload, and excess in mortality.[1,2,23] Our group already published several studies on fluid resuscitation after hemorrhagic shock comparing crystalloids with colloids, balanced or unbalanced, and their consequences on renal cortical Po_2.[24–26] Balanced hydroxyethyl starch solution was found more efficient at restoring macrohemodynamics and renal cortical Po_2 than crystalloids. In the present study, we sought to evaluate an alternative colloid in the context of ongoing controversies about hydroxyethyl starch use. The European Medicines Agency recently issued several restrictions on the use of hydroxyethyl starch solutions in different clinical settings across the European Union.[5] Hydroxyethyl starch solutions are contraindicated in patients with renal impairment and should be administered to manage hemorrhage only when crystalloids alone are not considered sufficient. Therefore, new fluid therapies that outperform crystalloids and compete with the latest synthetic colloid solutions are desired to improve and sustain volume status to promptly restore blood pressure and flow in the context of hemorrhage. However, blood product transfusions remain the gold standard treatment of hemorrhage, but this treatment is not always readily available. Transfusion thresholds have been raised during the last decade with the implementation of restrictive transfusion strategies,[27,28] and fewer patients are likely to receive blood transfusions in the perioperative setting.

Previous generations of hemoglobin-based oxygen carriers failed in clinical practice primarily because of major side effects, such as hypertension and myocardial injury, that were overlooked in preclinical models.[29,30] These effects were presumably related to the extravasation of tetrameric hemoglobin and nitric oxide-scavenging effects. The formulation of hemoglobin-based oxygen carriers has changed to comply with more physiologic prerequisites. Polyethylene-glycolated carboxyhemoglobin provides a larger expansion volume than balanced hydroxyethyl starch partially because of its oncotic effect.[11] The increase in MAP was likely due to the PEGylation process of the carboxyhemoglobin rather than a nitric oxide-scavenging effect. Polyethylene-glycolated carboxyhemoglobin infusion produced similar plasma nitric oxide levels as blood transfusion in our experiment. However, the administration of hydroxyethyl starch alone as a resuscitation fluid significantly decreased the bioavailability of nitric oxide. This result suggests a decreased shear stress–mediated nitric oxide release from the endothelium.[31]

Our group previously demonstrated that a low volume of diaspirin–cross-linked hemoglobin restored epicardial and gut serosal and mucosal microvascular oxygenation.[32,33] Similarly, diaspirin–cross-linked hemoglobin restored pancreatic functional capillary density in a rodent model of hemorrhagic shock.[34] However, increasing the dose of diaspirin–cross-linked hemoglobin produced two main consequences: hyperoxia in the gut serosa and mucosae and severe hypertension. The latter effect was caused by vasoconstriction *via* the inhibition of nitric oxide. Similarly, the extensive literature on bovine hemoglobin–based oxygen carrier (HBOC-201, Hemopure, HbO_2 Therapeutics LLC, USA) reported improvements in tissue oxygenation and macrohemodynamics with induced vasoconstriction.[35] Little data on hemoglobin-based oxygen carriers and kidney oxygenation and function are available. Polyethylene-glycolated carboxyhemoglobin should not be regarded as a hemoglobin-based oxygen carrier *per se*, but more as a colloid with additional beneficial effects. Our experiment highlighted that polyethylene-glycolated carboxyhemoglobin cannot compete with blood transfusion (even diluted at the same hemoglobin concentration) in terms of quality of oxygen-carrying capacity and delivery to hypoxic tissue. In contrast, polyethylene-glycolated carboxyhemoglobin may be an interesting alternative when blood is not an option or in cases of delayed blood transfusion. First, the P_{50} is lower than the one of whole blood (8 to 16 mmHg *vs.* 26 to 30 mmHg, respectively),[11] which impedes oxygen release to moderately hypoxic tissue. Second, we demonstrated that (1) the oxygen-binding capacity of native polyethylene-glycolated carboxyhemoglobin was significantly lower than rat (or human) hemoglobin oxygen-binding capacity even after carbon monoxide release and (2) the concentration of polyethylene-glycolated carboxyhemoglobin in the plasma was too low to expect any significant increase in oxygen delivery compared with whole blood. To test whether polyethylene-glycolated carboxyhemoglobin can release more oxygen, its P_{50} could be theoretically altered by the use of RSR13 (2-[4-[[(3, 5-dimethylanilino)carbonyl]methyl]

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phenoxyl]-2-methylproprionic acid),[36] a synthetic allosteric modifier of oxygen–hemoglobin affinity that increases oxygen release (rightward shift of oxygen-hemoglobin dissociation curve) to tissue by allosterically stabilizing deoxyhemoglobin. However, two limits have to be considered: (1) RSR13 will also alter the P_{50} of native hemoglobin, and (2) it could exacerbate acute renal dysfunction.[36,37]

The renal damage was mitigated with the use of polyethylene-glycolated carboxyhemoglobin compared with the administration of hydroxyethyl starch alone. These effects may be attributed to carbon monoxide release. Previous studies demonstrated possible anti-inflammatory effects of carbon monoxide mediated *via* heme oxygenase 1 activation.[38] Nassour *et al.*[16] demonstrated that carbon monoxide-releasing molecules protected against hemorrhagic shock and resuscitation-induced organ injury and inflammation in a similar rodent model of hemorrhagic shock. However, the carbon monoxide-releasing molecules used could not carry oxygen, which highlights the central role of carbon monoxide and heme oxygenase 1 in ischemia and reperfusion injury after hemorrhage.

The off-loading of carbon monoxide molecules from polyethylene-glycolated carboxyhemoglobin is not instantaneous. Approximately 23% of polyethylene-glycolated carboxyhemoglobin remained saturated with carbon monoxide 60 min after the start of resuscitation in our study. Vandegriff *et al.*[39] demonstrated that the percentage of carboxyhemoglobin of coboxyhemoglobin maleimide-activated poly(ethylene) glycol (another polyethylene-glycolated carboxyhemoglobin) decreased to 20 to 25% 30 min after mixing coboxyhemoglobin maleimide-activated poly(ethylene) glycol with erythrocyte *in vitro* which is similar to our observations. In contrast, Zhang *et al.*[40] observed different results in an *in vivo* top-load rodent model. Whole blood and plasma gas analyses revealed a low percentage (5 to 7%) of carboxyhemoglobin 1 and 3 min after polyethylene-glycolated carboxyhemoglobin injection, which suggests a rapid exchange of carbon monoxide between polyethylene-glycolated carboxyhemoglobin and native erythrocyte.[40] Overall, these results demonstrated a disassociation of carbon monoxide molecules from polyethylene-glycolated carboxyhemoglobin, which subsequently re-equilibrates with native erythrocyte. The remaining polyethylene-glycolated hemoglobin (without carbon monoxide) binds oxygen or may be oxidized in the context of hemorrhagic shock. However, whether this chemical process persists after 60 min is not known.

Parrish *et al.*[41] previously brought evidence that the cell-impermeant polyethylene glycol–20 kDa significantly affected colloid pressure and improved survival in low volume resuscitation hemorrhagic shock models. However, the effects of the administration of this polyethylene glycol–20 kDa on the kidney were not assessed. Polyethylene-glycolated carboxyhemoglobin provides additional benefits with its ability to release carbon monoxide molecules and oxygen-binding capacity.

Limitations

First, we focused only on the golden hour after resuscitation after hemorrhagic shock, and long-term results on the use of polyethylene-glycolated carboxyhemoglobin therefore cannot be anticipated. Second, lactate and creatinine levels were higher in the polyethylene-glycolated carboxyhemoglobin group after 1 h because of low-volume resuscitation even when the preset target of resuscitation, namely MAP, was reached. The changes in creatinine clearance may not be reflected at 1 h. However, there is evidence that low-volume resuscitation may improve survival, although it may delay the correction of biologic parameters.[42] The delay in correction of biologic parameters is unlikely to be related to carbon monoxide toxicity because of the very small amount released. However, we cannot exclude a direct toxicity of polyethylene-glycolated hemoglobin. Third, it may be difficult to distinguish the cytoprotective effects of carbon monoxide from the colloidal effect of polyethylene-glycolated carboxyhemoglobin that restores blood flood and pressure. Nevertheless, previous works demonstrated that the administration of hemoglobin-based oxygen carrier in the carboxy state protected against focal ischemia.[39,40] Fourth, 6% balanced hydroxyethyl starch was used in each group to maintain the targeted MAP after the rescue phase, and it may be considered a bias. However, this study design provided a more realistic approach. The use of a crystalloid or colloid to maintain blood volume in a context of hemorrhage occurs daily in clinical practice. Fifth, the observation time was set at 1 h after resuscitation, which may be short. However, the present study sought to specifically assess the effects of polyethylene-glycolated carboxyhemoglobin on renal oxygenation and function in the early phase of resuscitation after hemorrhagic shock. A survival benefit of polyethylene-glycolated carboxyhemoglobin was demonstrated previously.[43] Further research is warranted to determine the mid- to long-term effects on the kidney. An observation period of 3 to 7 days of recovery after resuscitation of hemorrhagic shock with polyethylene-glycolated carboxyhemoglobin would be relevant in this context.

Conclusions

Polyethylene-glycolated carboxyhemoglobin exhibited interesting properties for the resuscitation of hemorrhagic shock, especially because of the limited volume administered. Biomarkers of shock were not corrected as quickly as expected, but there is evidence that polyethylene-glycolated carboxyhemoglobin's dual action protects the kidney against ischemia and reperfusion damages after resuscitation through its carbon monoxide molecules and sustained urine output, which partially contribute to an increase in the oxygen delivery to tissues during the first hour of resuscitation. Further research on colloidal solutions should be intensified to provide the anesthesiologists and intensive care unit physicians an interesting option for meeting unmet clinical needs. Finally, whether polyethylene-glycolated carboxyhemoglobin exhibits long-term protective

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effects on the kidney without adverse effects on the other organs should be determined in future studies.

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Competing Interests

Dr. Ince owns an Internet site microcirculationacademy.org, which offers services (*e.g.*, training, courses, analysis) related to clinical microcirculation and has received honoraria and independent research grants from Fresenius-Kabi (Bad Homburg, Germany), Baxter Health Care (Deerfield, Illinois), Prolong Pharmaceuticals (South Plainfield, New Jersey), and AM-Pharma (Bunnik, The Netherlands); has developed Sidestream Dark Field imaging; is listed as an inventor on related patents commercialized by MicroVision Medical (Amsterdam, The Netherlands) under a license from the Academic Medical Center; and has been a consultant for MicroVision Medical in the past but has not been involved with this company for more than 5 yr. The company that developed the CytoCam-IDF imaging system, Braedius Medical (Huizen, The Netherlands), is owned by a relative of Dr. Ince. Dr. Ince has no financial relationship with Braedius Medical (*i.e.*, never owned shares or received consultancy or speaker fees). Dr. Jubin is an employee of Prolong Pharmaceuticals. Dr. Jan Bakker is a consultant for Prolong Pharmaceuticals. The other authors declare no competing interests.

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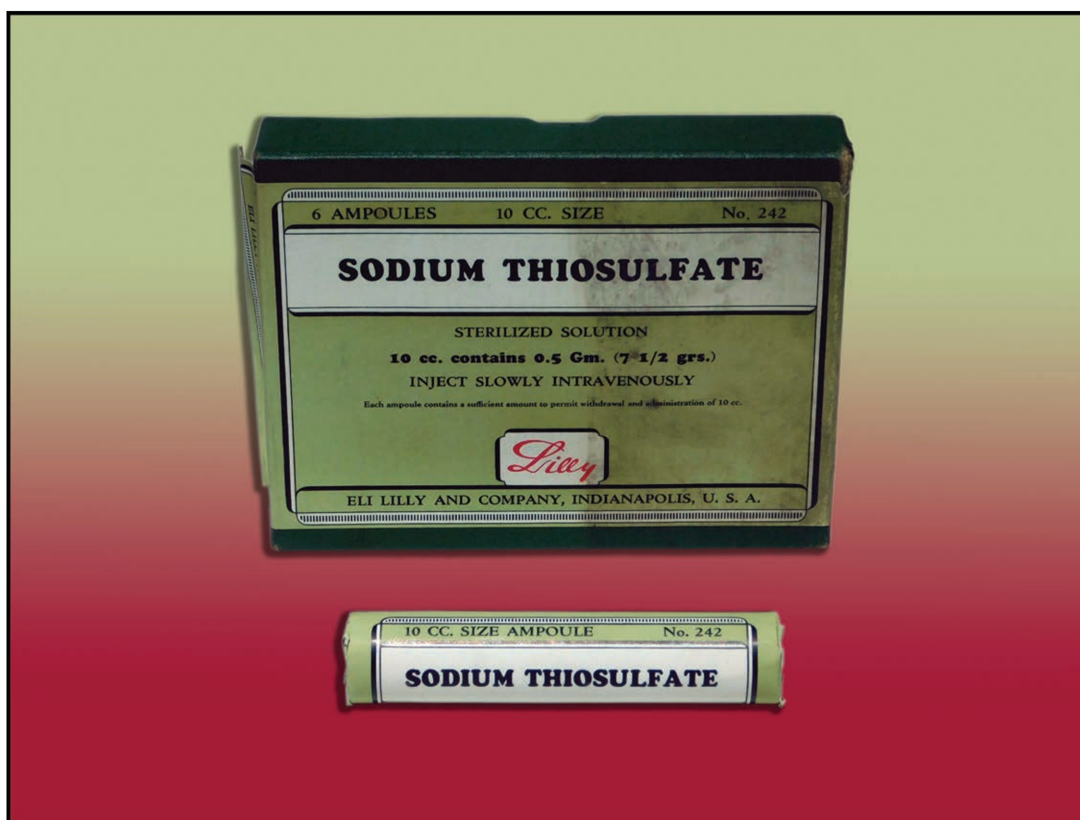
ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

From Fixing Photos to Fixing Cyanide Poisoning: Herschel's "Hypo" or Sodium Thiosulfate



In 1819, a soon-to-be-knighted John Herschel (1792 to 1871) discovered that hyposulfite of soda could "fix" or make photographic images permanent by dissolving away unexposed and otherwise insoluble silver salts. This British genius soon coined terms for his astronomical or botanical images as "negatives" or "positives" and for the art as "photography." Even after it was renamed sodium thiosulfate, hyposulfite of soda retained photographers' nickname for it: "hypo." An antidote for cyanide poisoning, sodium thiosulfate was supplied in ampoules to physicians by Eli Lilly (*above*) and other pharmaceutical firms. Eventually, an editor-in-chief of ANESTHESIOLOGY, Dr. John Michenfelder, would help popularize the antidotal availability of sodium thiosulfate for treating cyanide toxicity resulting from overdoses of antihypertensive infusions of sodium nitroprusside. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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