

Novel Starches

Single-dose Pharmacokinetics and Effects on Blood Coagulation

Caveh Madjdipour, M.D.,* Caroline Thyges, M.D.,* Thierry Buclin, M.D.,† Philippe Frascarolo, Ph.D.,‡ Inès von Roten, M.D.,* Andreas Fisch, Ph.D.,§ Marc Burmeister, M.D.,|| Thomas Bombeli, M.D.,# Donat R. Spahn, M.D., F.R.C.A.**

Background: Carboxymethyl starch (CMS) and carboxymethylated hydroxyethyl starch (CM-HES) might offer advantages over hydroxyethyl starch (HES) with regard to their volume expansion effect and their pharmacokinetic characteristics. The goal of the current study was to determine the pharmacokinetics of CMS and CM-HES and to investigate their influence on blood coagulation in comparison with the standard low-molecular, low-substituted HES (130/0.42) used in Europe.

Methods: The study was conducted as a randomized, blinded, parallel three-group study in 30 pigs. Twenty ml/kg of 6% HES (control), 6% CMS, or 6% CM-HES was infused as a single dose, and serial blood sampling was performed over 20 h to measure plasma concentration and molecular weight and to assess blood coagulation. Concentration–effect relations were assessed by pharmacokinetic–pharmacodynamic analysis.

Results: CMS and CM-HES showed significantly higher plasma concentrations and molecular weights over 20 h (P for both < 0.001) with smaller volumes of distribution and longer elimination rates during the terminal phase (P for both < 0.01) when compared with HES. CMS and CM-HES impaired whole blood coagulation more than HES as assessed by Thrombelastograph® analysis (Haemoscope Corporation, Niles, IL). However, similar effects of all three starch preparations on blood coagulation were found when related to the plasma concentrations in mass units.

Conclusions: Carboxymethylation of starch results in an increased intravascular persistence and a slower fragmentation compared with HES. The greater impairment of blood coagulation by CMS and CM-HES seems to be caused by the higher plasma concentrations.

MODIFIED biopolymers such as gelatin, dextran, and hydroxyethyl starch (HES) have colloid osmotic properties and are therefore widely used as plasma substitutes.¹

One of the most frequently used colloids is HES, a derivative of the waxy maize starch amylopectin.² After partial hydrolysis of the high-molecular amylopectin, hydroxyethyl groups are introduced and define thereby the physical and chemical properties of the resulting HES. HES is currently the only starch derivative used in surgery or critically ill patients. However, its clinical use is limited because of its potential to cause adverse effects, which consist mainly of an impairment of blood coagulation and development of pruritus after prolonged administration.³⁻⁶ This has prompted extensive research aimed at minimizing these harmful effects. The effects of HES on blood coagulation could be reduced by the modification of the molecular weight and of the degree and pattern of hydroxyethylation, although it simultaneously affects the pharmacokinetic behavior of the molecule.^{2,7}

Albumin is the most important physiologic contributor to the maintenance of plasma colloid osmotic pressure (COP).⁸ The excess osmotic pressure that is related to the negative charges of the albumin molecule is referred to as the *Gibbs-Donnan effect*.⁸ In contrast, HES represents a neutral colloid and does not show the Gibbs-Donnan effect. Negatively charged starches could, however, be formed through the linkage of carboxymethyl rather than hydroxyethyl groups to the starch backbone. Such a polyanionic starch, *i.e.*, carboxymethyl starch (CMS), resembles the natural colloid albumin with respect to its water binding capacity and is more hydrophilic than the conventional HES. It may thus result in a higher COP and consequently exert a more pronounced volume expansion effect compared with starch derivatives with nonionic side chains such as HES. In addition, the polar properties of CMS suggest a more complete elimination and lower tendency for accumulation in the body. Starch molecules can also be processed to produce mixed derivatives substituted with both hydroxyethyl and carboxymethyl residues, *i.e.*, carboxymethylated HES (CM-HES). This preparation would be expected to lie between those of HES and CMS regarding its oncotic and pharmacokinetic properties.

We therefore considered CMS or CM-HES as promising colloids and possible alternatives to HES. Up to now, there exist no reports on the pharmacokinetics and pharmacodynamics of CMS or CM-HES that would allow precise statements about their potential use as plasma substitutes. Therefore, the primary aim of this study was to determine the pharmacokinetic profile of these novel

* Research Fellow, ‡ Research Associate, ** Professor and Chairman, Department of Anesthesiology, † Private Docent and Consulting Physician, Division of Clinical Pharmacology and Toxicology, University Hospital Lausanne. § Head of Research Development Liquids, B. Braun Melsungen SA, Crissier, Switzerland. || Private Docent and Associate Professor of Anesthesiology, Vice President Research and Development, B. Braun Melsungen AG, Melsungen, Germany. # Professor, Head of Coagulation Laboratory, Division of Hematology, University Hospital Zurich, Zurich, Switzerland.

Received from the Department of Anesthesiology, University Hospital Lausanne, Switzerland. Submitted for publication February 10, 2006. Accepted for publication September 3, 2006. Supported by departmental funds and B. Braun Medical SA, Crissier, Switzerland. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Atlanta, Georgia, October 24, 2005; the Annual Meeting of the European Society of Anaesthesiologists, Vienna, Austria, May 29, 2005; the Annual Meeting of the Swiss Society of Anesthesiology and Resuscitation, Interlaken, Switzerland, November 4, 2005; and the 2nd International Congress Sepsis and Multiorgan Dysfunction, Weimar, Germany, September 9, 2005. Drs. Burmeister and Fisch are employees of B. Braun AG, Melsungen and Crissier, Switzerland, and Dr. Spahn is a paid consultant for B. Braun Medical AG, Switzerland. The Department of Anesthesiology of the University Hospital Lausanne has done other research projects in collaboration with B. Braun Melsungen AG and has thereby received other funding in the past as well as educational funds from competitor companies.

Address correspondence to Dr. Spahn: Department of Anesthesiology, University Hospital Zurich, CH-8091 Zurich, Switzerland. donat.spahn@usz.ch. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

starches. The secondary aim was to investigate any potential interference with blood coagulation.

Materials and Methods

This study was performed as a randomized, blinded, parallel three-group trial in pigs. Animals received humane care according to the guidelines of the Swiss Federal Veterinary Office. The protocol was approved by the institutional review board of the Veterinary Office of the Canton de Vaud, Lausanne, Switzerland.

Animal Preparation

The pigs, weighing 39.5 ± 5.3 kg, received intramuscular premedication with xylazine (2 mg/kg), ketamine (20 mg/kg), and atropine (1 mg). Once sedated, anesthesia was induced by inhalation of 3% halothane by mask, followed by tracheal intubation. Controlled ventilation was started with tidal volumes of 10 ml/kg and a ventilatory rate of 15/min (Ventilator Dräger Sulla 909 V; Dräger, Luebeck, Germany). Anesthesia was maintained with 0.8–1.5% halothane in oxygen. The internal jugular vein and the carotid artery were catheterized for infusion of HES, CMS, or CM-HES and for blood sampling and invasive blood pressure measurement. A urinary bladder catheter was placed for collection of urine. Electrocardiogram, invasive blood pressure, capillary oximetry, end-tidal carbon dioxide, and esophageal temperature were monitored continuously. After the final blood sampling, the pigs were killed with 150 mg/kg intravenous pentobarbital and cessation of mechanical ventilation.

HES, CMS, and CM-HES Synthesis and Characterization

The compounds investigated in this study were synthesized and analyzed by B. Braun Medical SA (Crissier, Switzerland). The analytical methods for determination of molecular weight, molar substitution, and concentration (see Plasma and Urine Measurements) used in this study are validated according to the guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Food and Drug Administration and have been used for the analysis of numerous commercially available but also experimental products in the past.

For the synthesis of CMS and CM-HES, thin-boiling waxy maize starch was suspended in water, activated by means of sodium hydroxide, and allowed to react with dichloroacetic acid for 2 h at 40°C. The amounts of waxy maize starch and dichloroacetic acid were chosen to yield a molar substitution with carboxymethyl groups of 0.30 for CMS and 0.06 for the CM-HES precursor. Hydroxyethylation of the CM-HES precursor and native starch to synthesize CM-HES and HES, respectively, was

conducted under the same reaction conditions as the carboxymethylation by introducing ethylene oxide in quantities to yield a molar substitution with hydroxyethyl groups of 0.34 for CM-HES and 0.42 for HES. All these starch derivatives were hydrolyzed stepwise thereafter, by treatment with hydrochloric acid, to obtain molecular weights of approximately 130 kd. These were treated with activated carbon, purified by ultrafiltration, diluted to a final concentration of 6% (wt/vol) in 0.9% saline, filled in glass bottles of 500 ml each, and heat-sterilized at 121°C for 20 min. For determination and verification of molecular weights, HES, CMS, and CM-HES sample solutions were analyzed in duplicate by gel permeation chromatography equipped with multiple angle laser light scattering (Wyatt Technology, Woldert, Germany) at a flow rate of 1 ml/min in a 70-mm phosphate buffer, pH 7.0, using serial gel permeation chromatography columns HEMA Bio 40, 100, and 1000 (PSS, Mainz, Germany). The mean weight-average molecular weight (M_w) was calculated using ASTRA Software (Wyatt Technology). M_w is the average molecular weight of a polydisperse polymer sample, averaged to give higher statistical weight to larger molecules, calculated as $M_w = \frac{\sum_i (M_i^2 N_i)}{\sum_i (M_i N_i)}$. In contrast, the number-average molecular weight (M_n) is the average molecular weight of a polydisperse polymer sample, averaged to give equal statistical weight to each molecule, calculated as $M_n = \frac{\sum_i (M_i N_i)}{\sum_i N_i}$. The width of the molecular weight distribution is characterized by its polydispersity, defined by the quotient M_w/M_n . The closer this quotient comes to a value of 1, the narrower the molecular weight distribution is.

The degree of molar substitution with respect to hydroxyethyl groups of HES and CM-HES was determined and verified in duplicate according to the method described by Hodges *et al.*⁹ and Lee *et al.*¹⁰ The degree of molar substitution of CM-HES and CMS by carboxymethyl groups was determined using a colorimetric method according to Eyer *et al.*¹¹ Physicochemical parameters of the three starches used in this study are summarized in table 1, and their molecular structures are shown in figure 1.

Experimental Protocol

Animals were randomly assigned into three groups of 10 pigs each, receiving HES, CMS, or CM-HES. We infused 20 ml/kg body weight of the respective 6% solution (blinded to the investigators) during 30 min as a top-load dose. Blood samples were taken before (baseline) and 5, 20, 40, 60, 120, 240, 480, 720, 960, and 1,200 min after the end of the colloid infusion. For Thrombelastograph® analysis (TEG®; Haemoscope Corporation, Niles, IL) and plasma measurements, separate blood samples were collected in 3-ml tubes containing 0.3 ml (0.106 M) buffered (pH 5.5) sodium citrate (S-Monovette; Sarstedt, Nuernbrecht, Germany).

Table 1. Physicochemical Properties of HES, CMS, and CM-HES In Vitro

	HES	CMS	CMS-HES
Mw, kd	139	129	138
Mw 10%, kd	21	18	26
Mw 90%, kd	335	299	318
Mn, kd	49	48	61
Mw/Mn	2.8	2.7	2.3
MS with CM	—	0.30	0.06
MS with HE	0.42	—	0.34
Concentration, %	6	6	6

CM = carboxymethyl groups; CMS = carboxymethyl starch; CM-HES = carboxymethylated hydroxyethyl starch; HE = hydroxyethyl groups; HES = hydroxyethyl starch; Mn = number-average molecular weight; MS = molar substitution; Mw = weight-average molecular weight; Mw 10% = molecular weight at which 10% of the molecules are smaller; Mw 90% = molecular weight at which 90% of the molecules are smaller; Mw/Mn = polydispersity.

Laboratory Measurements

Native Blood Measurements. TEG[®] permits monitoring of the process of blood coagulation by assessing the kinetics of the integrated coagulation process.¹² It has been previously shown that TEG[®] in conjunction with conventional coagulation assays provides a complementary approach suitable for detection of coagulation abnormalities in the course of hemodilution by colloids.^{7,13} The method of TEG[®] has been extensively described previously.^{14,15} In brief, we evaluated the following TEG[®] variables: Reaction time (r) is the latency time from placing blood in the cup until the clot starts to form (defined by a TEG[®] tracing amplitude of 2 mm), which corresponds to initial fibrin formation. Coagulation time (k) is referred to as the time between a tracing amplitude of 2 and 20 mm, representing the rate at which a specific level of clot strength is attained. The angle (α) is measured by drawing a slope from r to the point on the TEG[®] tracing that corresponds to k characterizing thus the rapidity of clot strengthening. The maximal amplitude (MA) is the greatest amplitude of the tracing and represent the ultimate strength of the fibrin clot. Similarly, the shear elastic modulus (G) is a viscoelastic measurement for clot firmness. The coagulation index (CI) gives the sample's overall coagulation and is calculated from r , k , MA, and α .

Before TEG[®], blood samples were incubated for 1 h in a 37°C water bath.¹⁶ Blood recalcification and TEG[®] measurements have been performed according to the manufacturer's instructions.

Hemoglobin concentrations were determined on a Radiometer ABL 700 blood gas analyzer (Radiometer Medical ApS, Bronshøj, Denmark).

Plasma and Urine Measurements. Blood samples were transported on ice and immediately centrifuged at 3,000 rpm for 15 min at 4°C for separation of plasma and the blood cellular components (Rotanta/RP; Hettich, Bäch, Switzerland).

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined on an automated coagulation analyzer (BCS; Dade Behring, Marburg, Germany) using a PT reagent containing recombinant tissue factor (Innovin[®]; Dade Behring) and an aPTT reagent containing ellagic acid (Actin FS[®]; Dade Behring), respectively. Functional activity of von Willebrand factor (vWF) was determined in a ristocetin cofactor assay (Dade Behring) on an automated coagulation analyzer (BCS[®]; Dade Behring). Briefly, vWF activity was assessed by the ability to agglutinate fixed human platelets in the presence of ristocetin. Agglutination was turbidimetrically measured by the coagulation analyzer. Antigenic vWF levels were assayed by a commercial enzyme-linked immunosorbent assay kit (Asse-rachrom vWF antigenic; Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions. Factor VIII (FVIII) was assessed functionally using FVIII-deficient plasma according to the manufacturer's instructions (Dade Behring).

Hydroxyethyl starch, CMS, and CM-HES concentrations were quantified after extraction from plasma and urine, respectively, and total hydrolysis to substituted and unsubstituted glucose monomers. Briefly, plasma and urine samples (1 ml) were incubated at 100°C for 60 min after addition of 0.5 ml KOH solution 35% (wt/wt) (Fluka, Buchs, Switzerland). HES was then precipitated by adding 10 ml ice-cold absolute ethanol (Fluka) to the supernatant of the reaction mixture and subjected to acid hydrolysis in 2N hydrochloric acid (Fluka) for 60 min at 100°C. Glucose determination was performed using an enzymatic test kit based on hexokinase/glucose 6-phosphatase (Boehringer Mannheim, Darmstadt, Germany) using a calibration curve for each colloid separately. For *ex vivo* molecular weight determination of the colloids, plasma proteins were eliminated by trichloroacetic acid precipitation (6.4% [wt/wt] end concentration) and neutralized supernatants were analyzed by gel permeation chromatography equipped with multiple angle laser light scattering as described above.

Plasma COP before and after infusion of HES, CMS, or CM-HES, respectively, was measured using an osmometer Osmomat 050 (Gonotec GmbH, Berlin, Germany) and 20-kd cutoff membranes according to the instruction of the supplier.

Pharmacokinetic Analysis

The concentration data of the colloids were expressed both in g/l and in mM. The analysis was thus performed twice, using both types of data. The pharmacokinetic parameters were obtained through individual fitting of the plasma concentration data with a two-compartment model using Kinetica, version 4.3 (InnaPhase Corp., Philadelphia, PA). The fitting was performed by nonlinear regression, using weights of $1/Y_{\text{obs}}^2$ to accommodate for data heteroscedasticity. A two-compartment model proved appropriate for the description of the plasma

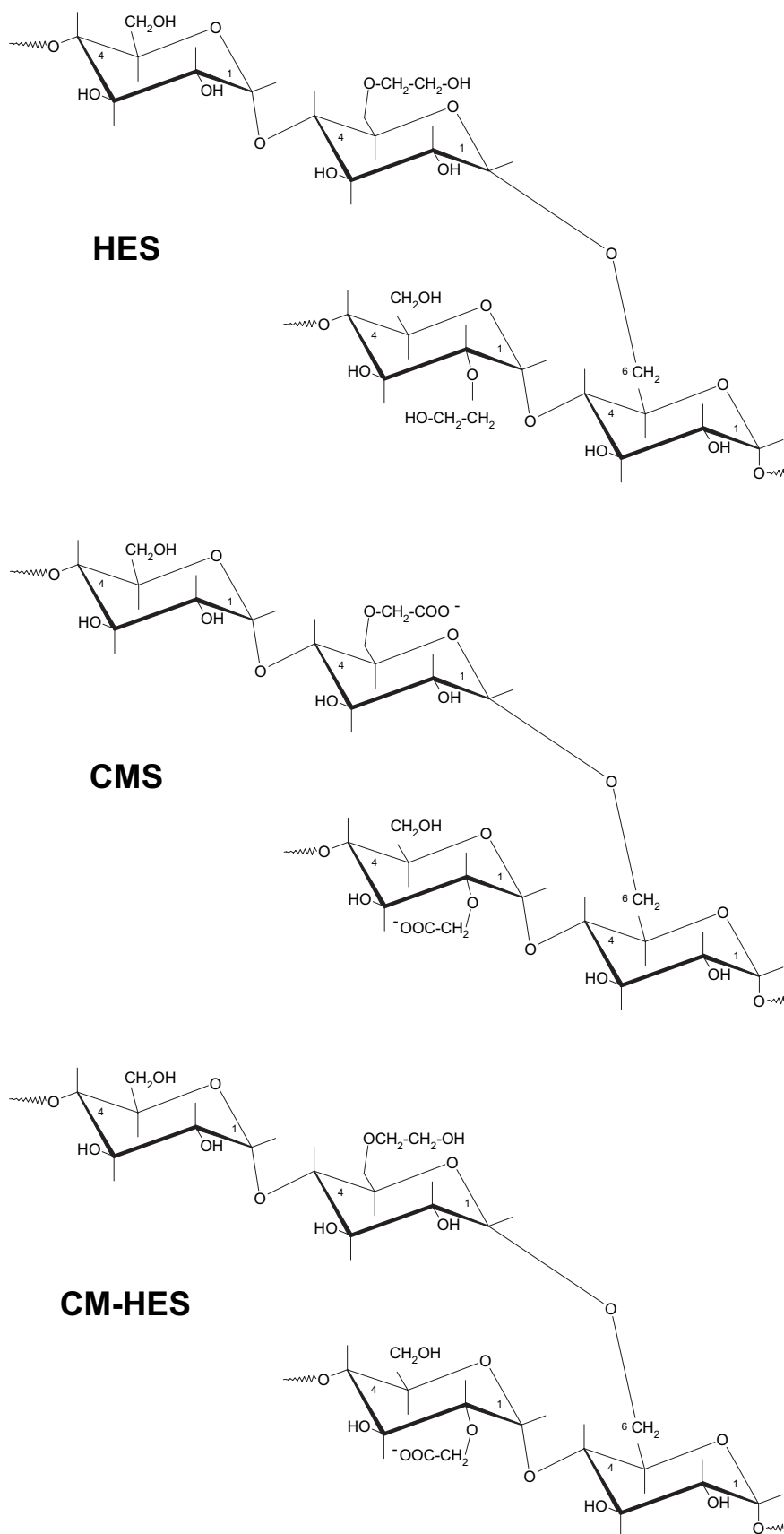


Fig. 1. Molecular structure of hydroxyethyl starch (HES), carboxymethyl starch (CMS), and carboxymethylated HES (CM-HES). Glucose units are linked by 1:4 and 1:6 α -glycosidic bonds. Substitution with the respective groups at carbons 2 and 6 of the glucose molecule are shown.

concentration curves: A one-compartment model provided significant misfit, whereas a three-compartment model was overparameterized, with a high coefficient of variation on estimates, indicating that—based on the available data—pharmacokinetic parameters could not be precisely estimated.

Pharmacodynamic Analysis

In a first step, observed effects of CMS and CM-HES were compared with HES as described in the Statistical Analysis section.

In a second step, pharmacokinetic–pharmacodynamic (PK-PD) analysis was performed. The pharmacodynamic parameters were obtained through the individual fitting of concentration–effect relations, using a simple instantaneous linear model, relating the predicted concentration values with the observed effects: Thus, for each pharmacodynamic variable, a slope (corresponding to the change of the respective parameter per g/l or mM of the colloid plasma concentration) and an intercept (baseline effect for a zero concentration) were obtained. The weighting of effect data were $1/Y_{\text{obs}}$. An instantaneous linear model was appropriate for fitting concentration–effect relations to obtain pharmacodynamic parameters. Models more complicated such as log-linear or E_{max} (maximal effect)–type relations did not improve the data description: In the E_{max} model, the parameters EC_{50} (half maximum effective concentration) and E_{max} showed huge intercorrelation and exceedingly high SEM values. The application of a PK-PD model including an equilibration delay between concentration and effect profile did not improve the description of the results, and the equilibration parameter k_{e0} could not be estimated with any reasonable precision in most cases, while displaying very high values in selected cases (*i.e.*, almost instantaneous equilibrium, confirming the lack of utility of this model extension).

Statistical Analysis

In this study, HES served as the control solution, *i.e.*, two comparisons were made: CMS *versus* HES and CM-HES *versus* HES.

Baseline values of HES, CMS, and CM-HES were compared by means of one-way analysis of variance (ANOVA) followed by pairwise comparisons of CMS *versus* HES and CM-HES *versus* HES by unpaired Student *t* test.

To compare time courses of CMS and CM-HES solutions with the HES solution, data were analyzed using a two-way ANOVA for repeated measures on one way (time) with Greenhouse-Geisser correction including solution and time effects.

Pharmacokinetic and pharmacodynamic parameters obtained from model fitting were compared between all three treatments by means of one-way ANOVA. When this analysis showed a significant ($P < 0.05$) global effect

for treatment, pairwise comparisons of CMS *versus* HES and CM-HES *versus* HES were performed by unpaired Student *t* test. All data were tested for normality and analyzed by the Wilcoxon Mann–Whitney test when not normally distributed.

The resulting *P* values of these comparisons of CMS and CM-HES solutions with the HES solution were considered to be statistically significant if less than 0.05. Therefore, no Bonferroni correction was applied to correct for multiplicity testing (*i.e.*, correction for the two comparisons: CMS *vs.* HES and CM-HES *vs.* HES). This was done to minimize the risk of a type II error, which must be avoided in studies on drug development when investigating side effects. The statistical analyses were performed using the JMP 5.1 statistical package (SAS Institute, Cary, NC). Results are expressed as mean \pm SD.

Results

At baseline, no significant differences were found among the CM-HES, CMS, and HES groups in terms of body weight, coagulation parameters (TEG[®] and plasma coagulation), hemoglobin concentration, and COP (P for all ≥ 0.100).

Colloid Pharmacokinetics

Time courses of colloid plasma concentrations expressed in g/l (fig. 2A) and mM (fig. 2B) were significantly different over the entire experimental period, with higher concentrations for CM-HES and CMS compared with HES (P for both solutions and concentrations < 0.001). Time courses of mean Mw of CM-HES and CMS were significantly different from HES (P for both < 0.001), with higher values at early time points (fig. 2C). The time course of polydispersity was not significantly different between CM-HES and HES ($P = 0.913$), whereas CMS showed a significantly greater polydispersity compared with HES ($P = 0.031$) (fig. 2D). The pharmacokinetic parameters showed highly significant differences between CM-HES/CMS and HES with CM-HES/CMS showing higher values for maximal concentration in g/l, area under the curve, and half-life (P for both solutions and all parameters < 0.001) and smaller values for maximal concentration in mM (P for CMS < 0.001 , P for CM-HES = 0.008), central distribution volume (P for both < 0.001), volume of distribution at steady state (P for CMS < 0.001 , P for CM-HES = 0.003), total clearance (P for both < 0.001), and transfer constants k_{12} (P for both < 0.001), k_{21} (P for CMS = 0.012, P for CM-HES = 0.002), and k_{e1} (P for both < 0.001) (table 2). Urine recovery was significantly higher with CMS ($P = 0.003$), whereas CM-HES showed no difference ($P = 0.379$) compared with HES (table 2).

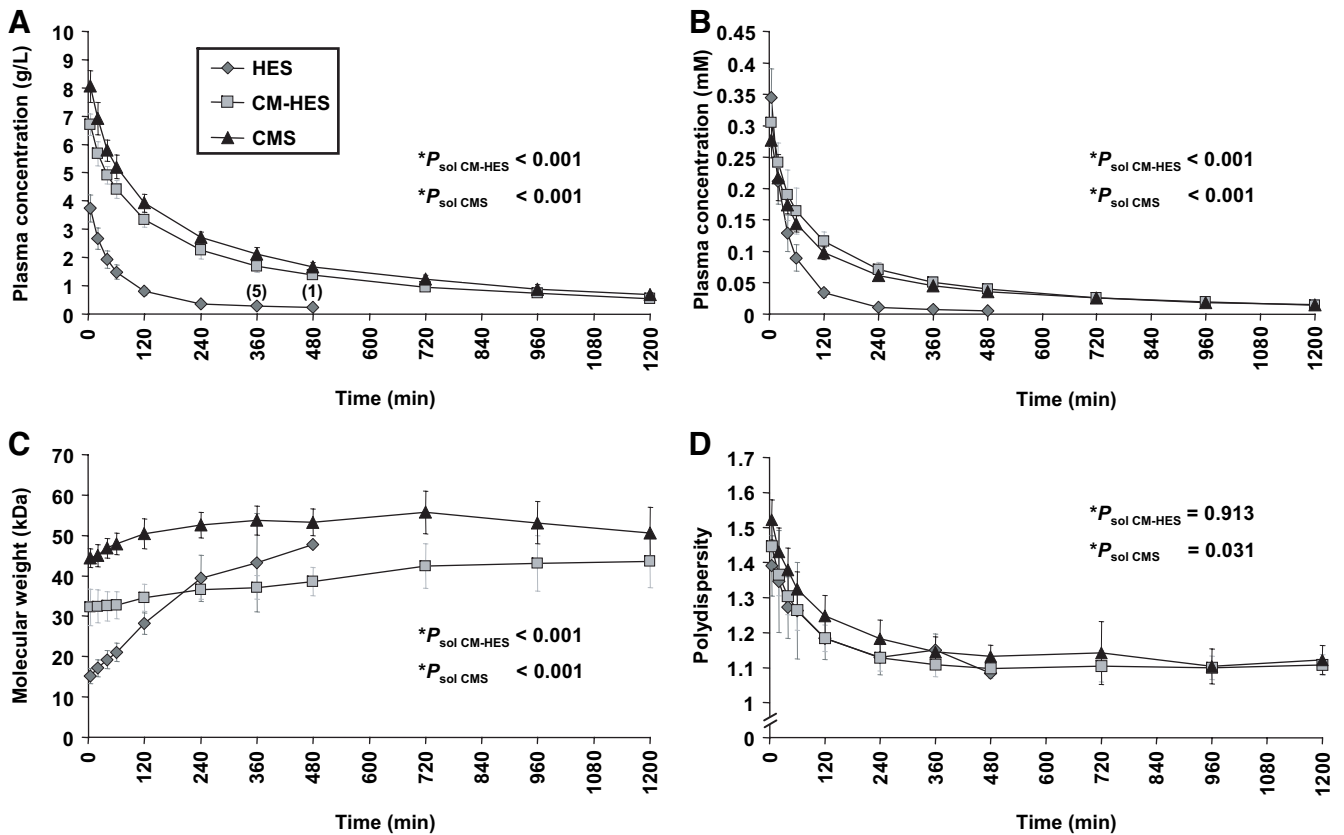


Fig. 2. Time course of hydroxyethyl starch (HES), carboxymethylated HES (CM-HES), or carboxymethyl starch (CMS) plasma concentration in g/l (A) and mM (B), weight-average molecular weight (C), and polydispersity (D). Results are shown as mean (SD). * Solution effect of CM-HES versus HES ($P_{\text{sol CM-HES}}$) and CMS versus HES ($P_{\text{sol CMS}}$) as determined by two-way analysis of variance. Numbers in parentheses indicate the number of animals in which HES plasma concentration and molecular weight were still detectable at the respective time points.

Colloid Pharmacodynamics

Coagulation Analyses.

$TEG^{\text{®}}$. Compared with HES, CM-HES showed a stronger decrease of CI, G, MA, and α (P for all < 0.001) (figs. 3A-D), whereas k and r did not differ significantly from control ($P = 0.115$ and 0.713 , respectively) (figs. 3E and

F). CMS showed significantly lower values for CI, G, MA (P for all < 0.001 , α ($P = 0.032$), and k ($P = 0.037$) (figs. 3A-E), whereas r ($P = 0.552$) did not differ significantly from HES (fig. 3F).

Plasma Coagulation. Carboxymethylated hydroxyethyl starch induced a significantly stronger reduction of

Table 2. Pharmacokinetic Parameters of HES, CMS, and CM-HES

	HES	CMS	CM-HES
Cmax, g/l	4.0 (0.5)	8.0* (0.6)	6.6* (0.4)
Cmax, mM	0.37 (0.06)	0.27* (0.03)	0.30† (0.04)
AUC, min · g · l ⁻¹	442 (94)	2,959* (287)	2,469* (283)
Vc, l	9.1 (1.4)	5.2* (0.6)	6.5* (0.7)
Vss, l	18.7 (5.4)	10.2* (1.5)	12.7† (1.5)
CL, l/min	0.115 (0.032)	0.016* (0.001)	0.019* (0.003)
$t_{1/2}$, min	185 (117)	525* (75)	561* (78)
k_{12} , min ⁻¹	0.009 (0.002)	0.005* (0.001)	0.004* (0.001)
k_{21} , min ⁻¹	0.011 (0.006)	0.005‡ (0.002)	0.004† (0.001)
k_{el} , min ⁻¹	0.013 (0.003)	0.003* (0.0003)	0.003* (0.0003)
Urine recovery, % of g	51 (5)	58† (4)	53§ (7)

A two-compartmental analysis was applied for the pharmacokinetic calculations. The following parameters are reported: maximal concentration (Cmax), area under the curve (AUC), central distribution volume (Vc), volume of distribution at steady state (Vss), total clearance (CL), half-life ($t_{1/2}$), rate constant of transfer from the central to the peripheral compartment (k_{12}), rate constant of transfer from the peripheral to the central compartment (k_{21}), and rate constant of elimination (k_{el}). Results are shown as mean (SD).

* $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$, § not significant compared with hydroxyethyl starch (HES).

CMS = carboxymethyl starch; CM-HES = carboxymethylated hydroxyethyl starch.

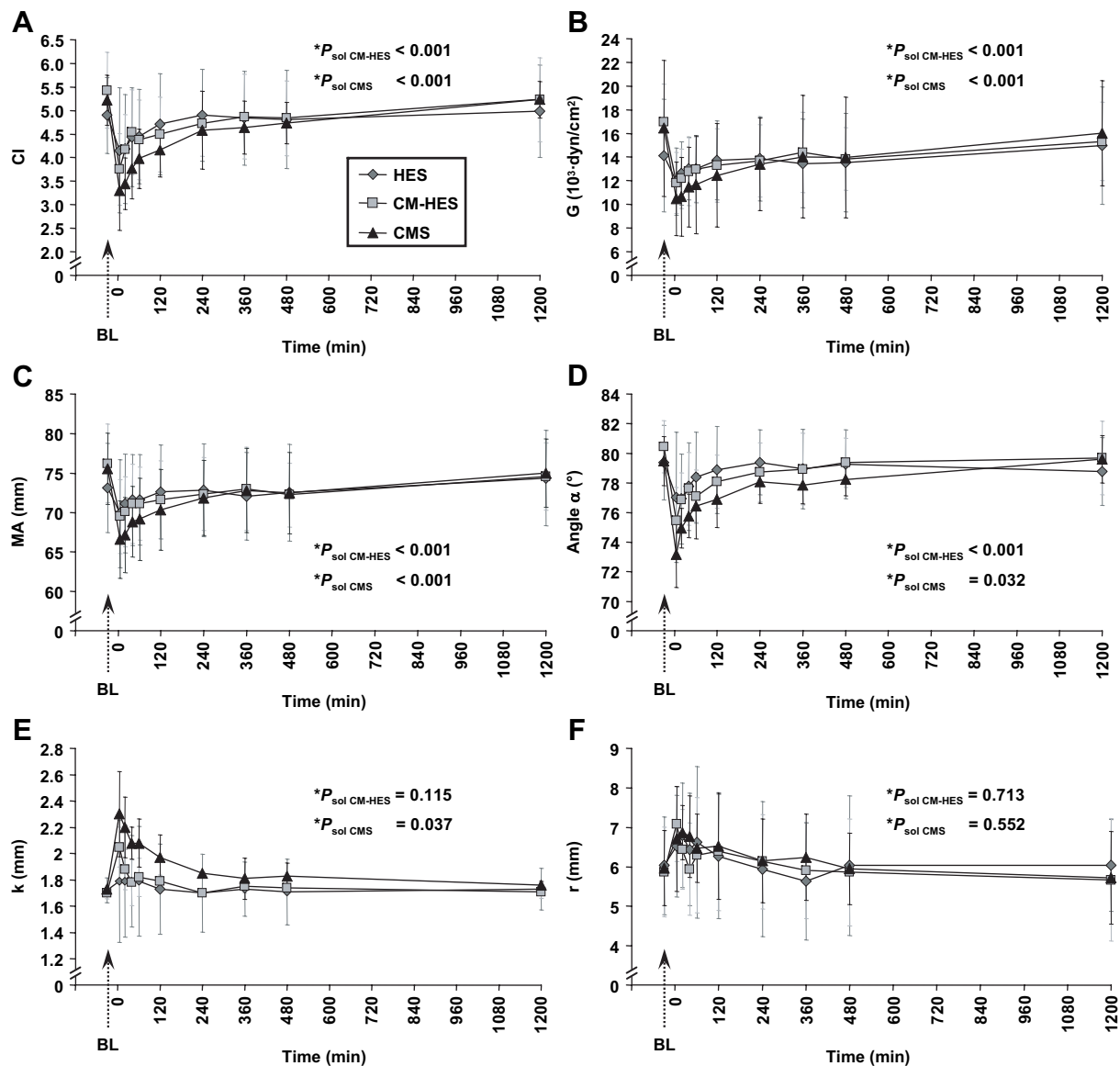


Fig. 3. Time course of Thrombelastograph® analysis (TEG®) parameters: coagulation index (CI; *A*), shear elastic modulus (*G*; *B*), maximal amplitude (MA; *C*), angle (α ; *D*), coagulation time (*k*; *E*), and reaction time (*r*; *F*). TEG® was determined before (baseline [BL]; arrow) and 5, 20, 40, 60, 120, 240, 360, 480, and 1,200 min after the end of the colloid infusion. Results are shown as mean (SD). * Solution effect of carboxymethylated hydroxyethyl starch (CM-HES) versus hydroxyethyl starch (HES) ($P_{\text{sol CM-HES}}$) and carboxymethyl starch (CMS) versus HES ($P_{\text{sol CMS}}$) as determined by two-way analysis of variance.

vWF antigen levels compared with HES ($P = 0.013$) (fig. 4A), whereas vWF activity ($P = 0.278$), FVIII activity ($P = 0.054$), aPTT ($P = 0.851$), and PT ($P = 0.704$) did not differ from HES (figs. 4B–E). CMS caused a significantly stronger decrease of vWF antigen levels ($P < 0.001$), vWF activity ($P = 0.011$), FVIII activity ($P = 0.038$), and aPTT ($P = 0.034$) compared with HES (figs. 4A–D). CMS did not evoke a PT response different from HES ($P = 0.698$) (fig. 4E).

Hemoglobin Time Course. The decrease in hemoglobin concentration was significantly more pronounced after CMS than after HES infusion ($P = 0.048$), whereas the decrease in hemoglobin concentration was similar after HES and CM-HES infusions ($P = 0.426$) (fig. 5).

Colloid Osmotic Pressure. No change in plasma COP was observed after HES infusion, whereas CM-HES and CMS infusions caused a significant increase in COP at 5 min (P for both < 0.001) (table 3). COP at 60 min after CM-HES infusion was not different from baseline ($P = 0.096$). In contrast, COP at 60 min after CMS infusion stayed significantly increased ($P = 0.002$).

Pharmacokinetic–Pharmacodynamic Analysis

When PK-PD analysis was based on plasma concentration in g/l (table 4, left half), pharmacodynamic parameters for TEG® of the CMS and CM-HES groups were not significantly different from HES: P of overall ANOVA was greater than 0.05 for CI ($P = 0.397$), G ($P = 0.360$), α

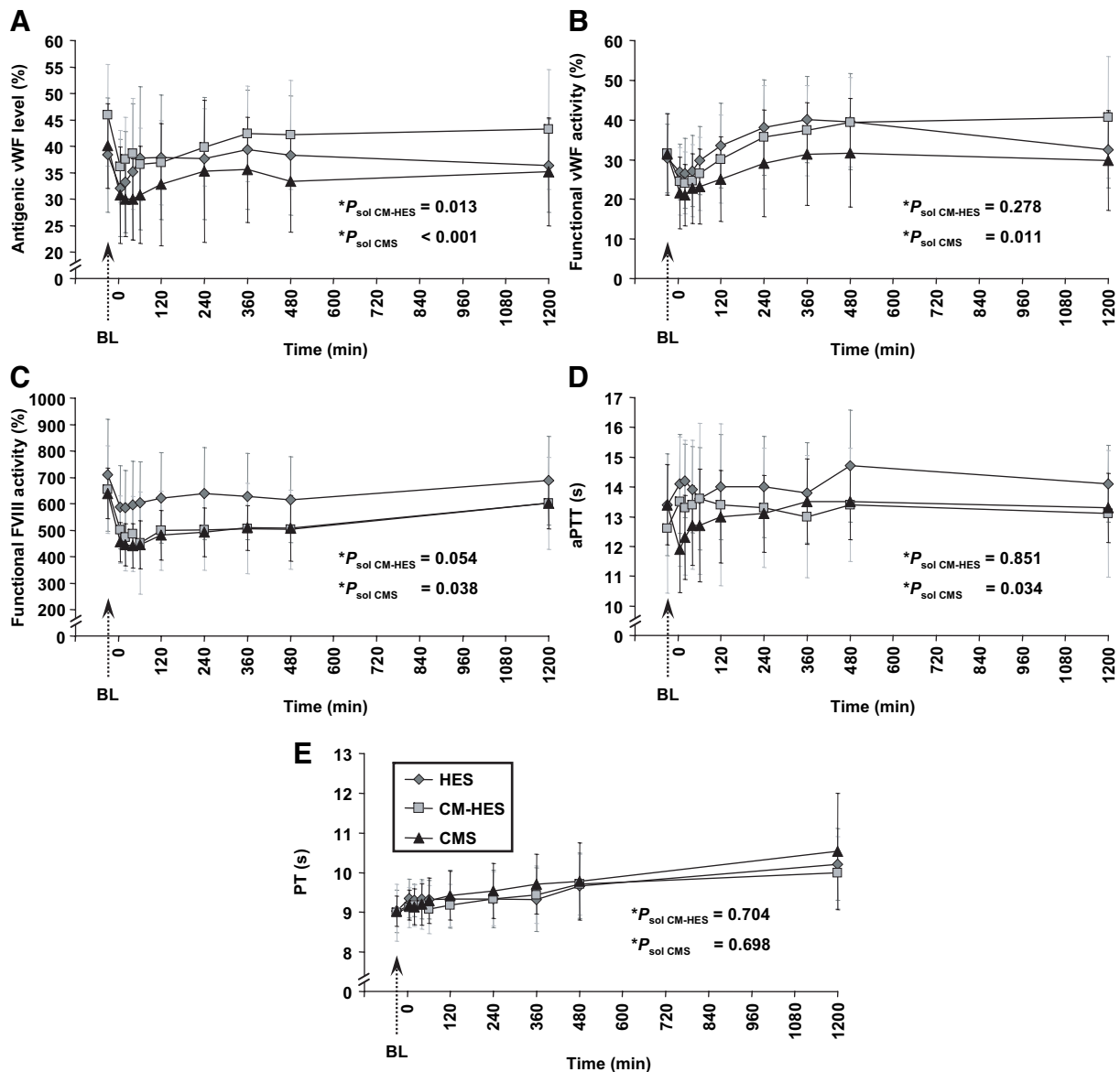


Fig. 4. Time course of plasma coagulation parameters: antigenic von Willebrand factor (vWF) level (A), functional vWF activity (B), functional factor VIII (FVIII) activity (C), activated partial thromboplastin time (aPTT; D), and prothrombin time (PT; E). Parameters were determined before (baseline [BL]; arrow) and 5, 20, 40, 60, 120, 240, 360, 480, and 1,200 min after the end of the colloid infusion. Results are shown as mean (SD). * Solution effect of carboxymethylated hydroxyethyl starch (CM-HES) versus hydroxyethyl starch (HES) ($P_{\text{sol CM-HES}}$) and carboxymethyl starch (CMS) versus HES ($P_{\text{sol CMS}}$) as determined by two-way analysis of variance.

($P = 0.744$), k ($P = 0.259$), and r ($P = 0.655$); P of overall ANOVA was less than 0.05 for MA ($P = 0.034$), but not significant after between-group comparison of CMS and CM-HES versus HES ($P = 0.057$ and 0.424 , respectively). There were also no significant differences with any of the pharmacodynamic parameters for plasma coagulation after comparison of CMS and CM-HES with HES: P of overall ANOVA was greater than 0.05 for vWF antigen levels ($P = 0.117$), vWF activity ($P = 0.067$), FVIII activity ($P = 0.961$), aPTT ($P = 0.128$), and PT ($P = 0.113$). The decrease in hemoglobin concentration was significantly smaller with both CMS and CM-HES compared with HES (P for both < 0.001).

When PK-PD analysis was based on plasma concentra-

tion in mm (table 4, right half), CMS showed a significantly more pronounced change of TEG[®] parameters CI ($P < 0.001$), G ($P < 0.001$), MA ($P < 0.001$), α ($P = 0.003$), and k ($P = 0.007$) when compared with HES. CM-HES had a higher impact on TEG[®] parameters CI ($P = 0.007$), α ($P = 0.011$), and MA ($P = 0.039$) but did not result in pharmacodynamic parameters for G and k significantly different from HES ($P = 0.104$ and 0.097 , respectively). Overall ANOVA detected no differences for r ($P = 0.288$). The plasma coagulation parameters vWF antigen level, vWF activity, FVIII activity, and aPTT showed no differences between CMS/CM-HES and HES: P of overall ANOVA was greater than 0.05 for vWF antigen levels ($P = 0.908$), vWF activity ($P = 0.153$),

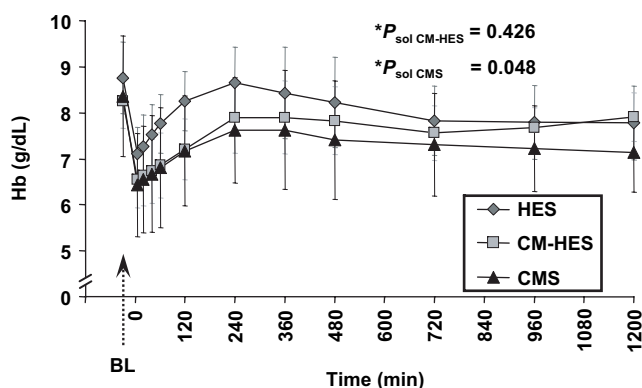


Fig. 5. Time course of hemoglobin (Hb) concentration: hemoglobin was determined before (baseline [BL]; arrow) and 5, 20, 40, 60, 120, 240, 360, 480, 720, 960, and 1,200 min after the end of the colloid infusion. Results are shown as mean (SD). * Solution effect of carboxymethylated hydroxyethyl starch (CM-HES) versus hydroxyethyl starch (HES) ($P_{\text{sol CM-HES}}$) and carboxymethyl starch (CMS) versus HES ($P_{\text{sol CMS}}$) as determined by two-way analysis of variance.

FVIII activity ($P = 0.225$), aPTT ($P = 0.053$), and PT ($P = 0.113$). PT showed a significantly stronger decrease with increasing molar plasma concentrations in the CMS group compared with HES ($P = 0.008$), whereas the difference between CM-HES and HES was not significant ($P = 0.059$). There was no significant difference in hemoglobin decrease between CMS or CM-HES and HES (P of overall ANOVA = 0.199).

Discussion

The primary aim of the current trial was to investigate carboxymethylated starches (CMS and CM-HES) regarding their pharmacokinetic characteristics after single-dose administration. CMS and CM-HES were compared with HES 130/0.42, which served as a control solution. Low-molecular and low-substituted starches such as HES 130/0.42 and HES 130/0.4 are the current standard hydroxyethyl starches being used in Europe, whereas high-molecular and high-substituted starch such as hetastarch (HES 450/0.7 or 670/0.75) is the only starch in the United States approved for volume replacement. In contrast to the latter, the former are free of major cumulative

effects and cause only limited impairment of blood coagulation.¹⁷ Therefore, in the authors' view, it is likely that the use of hetastarch as a control solution may have resulted in a failure to detect the observed impairment of blood coagulation by CMS and CM-HES or may even have led to the conclusion that these new compounds are less harmful than the control solution because hetastarch compromises blood coagulation more than HES 130/0.4.¹⁷

For the sake of appropriate comparison with the control group, a total molar substitution ratio of 0.42 was also aimed at for CM-HES. To this, carboxymethylation contributed with a substitution ratio of 0.06 considering antecedent laboratory work of our group showing that substitution with carboxymethyl groups in a molar ratio of 0.06 would provide the resulting starch derivative with the same anionic net charge as albumin (data not shown). As far as pure CMS is concerned, foregoing *in vitro* studies had shown that the originally intended substitution ratio of 0.42 led to an extremely high colloid osmotic pressure of the resulting 6% solution of CMS and very low *in vitro* degradation rates (data not shown). Finally, a molar substitution ratio of 0.3 was chosen for pure CMS to avoid any excessive expander effects.

We showed that CMS and CM-HES have pharmacokinetic properties (based on plasma concentrations in g/l) that are strikingly different from HES: much smaller volumes of distribution, a markedly longer elimination rate during the terminal phase, and a significantly lower clearance. These results indicate that carboxymethylation of starch or, in the case of CM-HES, even a very low substitution of HES with carboxymethyl groups leads to a much slower degradation *in vivo* compared with HES.

This study was designed to determine the pharmacokinetics of a single top-load dose leading to hypervolemic hemodilution. This is a common design in characterizing new classes of volume expanders. The authors are aware of the fact that this experimental setting does not correspond to the usual clinical use, *i.e.*, administration of colloids to replace blood loss. However, the initial investigation of these new compounds in a study of blood volume replacement would have rendered it difficult to attribute the changes in blood coagulation solely to the side effects of the colloids because blood replacement itself leads to hypocoagulation caused by the loss of coagulation factors. In addition, assessing pharmacokinetics in a hemodilution protocol is highly complex. Therefore, as a first step, we have chosen the current design to compare carboxymethylated starches with HES before going into more complicated but clinically more relevant models such as isovolemic hemodilution.

The pharmacokinetic and pharmacodynamic analysis of infused starch solutions is complicated by the fact that starches are heterogenous solutions with respect to molecular weight and polydispersity.² Furthermore, mean

Table 3. Colloid Osmotic Pressure

	COP, mmHg			P Value
	BL	5 min	60 min	
HES	12.5 (1.2)	12.5 (0.8)	11.7 (0.9)	
CMS	12.8 (0.7)	17.3 (1.3)	14.2 (0.9)	0.001
CM-HES	12.0 (0.9)	14.6 (0.7)	12.7 (0.8)	0.001

Colloid osmotic pressure (COP) response after infusion of hydroxyethyl starch (HES), carboxymethyl starch (CMS), or carboxymethylated hydroxyethyl starch (CM-HES). Results are shown as mean (SD). P values refer to differences in response over time compared with HES.

BL = baseline.

Table 4. Pharmacodynamic Parameters

PD Parameter	Change per g/l Plasma Concentration			Change per mm Plasma Concentration		
	HES	CMS	CM-HES	HES	CMS	CM-HES
TEG [®] CI	-0.23 (0.08)	-0.26§ (0.09)	-0.20§ (0.09)	-2.26 (0.67)	-7.06* (2.55)	-4.23† (1.95)
TEG [®] G, kdyn/cm ²	-0.56 (0.33)	-0.72§ (0.29)	-0.50§ (0.40)	-5.7 (2.8)	-20.1* (8.5)	-10.0§ (7.4)
TEG [®] MA, mm	-0.83 (0.33)	-1.12§ (0.29)	-0.70§ (0.38)	-8.6 (2.9)	-31.6* (9.7)	-14.7‡ (8.1)
TEG [®] angle α , °	-0.77 (0.43)	-0.74§ (0.38)	-0.65§ (0.23)	-7.9 (4.2)	-21.4† (11.6)	-14.0‡ (5.4)
TEG [®] k, mm	0.04 (0.04)	0.08§ (0.05)	0.06§ (0.05)	0.40 (0.49)	2.31† (1.38)	1.16§ (0.94)
TEG [®] r, mm	0.21 (0.20)	0.15§ (0.15)	0.17§ (0.10)	2.03 (1.87)	3.86§ (3.91)	3.72§ (2.33)
vWF level, %	-3.4 (1.9)	-1.5§ (1.5)	-3.1§ (1.7)	-33.7 (21.5)	-40.6§ (42.5)	-65.2§ (37.3)
vWF activity, %	-1.88 (1.67)	-0.69§ (0.57)	-1.11§ (1.11)	-20.8 (19.5)	-18.6§ (16.2)	-22.7§ (24.4)
FVIII activity, %	-15.7 (23.3)	-16.3§ (9.9)	-13.8§ (24.0)	-159 (266)	-435§ (275)	-275§ (441)
aPTT, s	0.06 (0.37)	-0.21§ (0.30)	-0.07§ (0.26)	0.53 (3.90)	-5.69§ (8.72)	-0.79§ (4.72)
PT, s	-0.01 (0.14)	-0.14§ (0.13)	-0.12§ (0.15)	-0.24 (1.41)	-3.67† (3.26)	-2.41§ (3.11)
Hb, g/dl	-0.45 (0.11)	-0.12* (0.08)	-0.24* (0.07)	-4.59 (1.28)	-3.85§ (2.02)	-5.21§ (1.46)

Pharmacodynamic (PD) parameters as obtained by pharmacokinetic–pharmacodynamic analysis of Thrombelastograph[®] (TEG[®]) and plasma coagulation parameters and hemoglobin (Hb) concentration, based on colloid plasma concentration in g/l (left half) or mm (right half), respectively. Pharmacodynamic parameters indicate the change of the respective parameter per g/l and per mm increase in colloid plasma concentration. Results are shown as mean (SD). All pharmacodynamic parameters give *absolute* changes per g/l or per mm; *i.e.*, the decreases for von Willebrand factor (vWF) level, vWF activity, and factor VIII (FVIII) activity given in percent describe absolute and not relative numbers.

Normal baseline values calculated as means (SD) from all animals ($n = 30$) are as follows: TEG[®] coagulation index (CI): 5.2 (0.7); TEG[®] shear elastic modulus (G; kdyn/cm²): 15.8 (4.7); TEG[®] maximal amplitude (MA; mm): 75.0 (5.1); TEG[®] angle α : 80.0 (2.0); TEG[®] coagulation time (k; mm): 1.7 (0.1); TEG[®] reaction time (r; mm): 6.0 (1.1); vWF level (%): 41.5 (9.8); vWF activity (%): 31.1 (9.4); FVIII activity (%): 668 (162); activated partial thromboplastin time (aPTT; s): 13.1 (1.8); prothrombin time (PT; s): 9.0 (0.5); Hb (g/dl): 8.5 (0.9).

* $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$, § not significant compared with hydroxyethyl starch (HES).

CMS = carboxymethyl starch; CM-HES = carboxymethylated hydroxyethyl starch.

molecular weight, together with polydispersity and the number of circulating starch molecules, is constantly changing over time *in vivo*, mainly because of degradation by α -amylases and renal elimination.^{2,18}

Accordingly, time courses of plasma concentrations expressed in mass units (g/l) or molar units (mm) may differ, clearly as observed in this study. We restricted the presentation of colloid pharmacokinetics in table 2 to the parameters based on concentration data in g/l, with the exception of maximal concentration, which is shown both in g/l and in mm.

Interestingly, urine recovery was significantly higher with CMS than with HES, although there was only a slight difference. Accordingly, because the plasma concentration of CMS at 20 h after the end of infusion was still detectable in contrast to HES concentration, which had decreased between 4 and 8 h below the detection threshold, other pathways of clearance from the intravascular compartment must exist. Rehm *et al.*¹⁹ showed in surgical patients that approximately 60% of a loading dose of 20 ml/kg of 6% HES left the intravascular space within 30 min during volume loading, with a considerable amount of HES passing the endothelial barrier. This may be due to altered properties of the endothelial surface layer mediated by atrial natriuretic peptide which is released after volume loading.^{20–22} Because the carboxymethyl side chains give CMS a polyanionic character, one might argue that CMS is relatively more repelled by the negatively charged endothelial glycocalyx, which results in lesser extravasation than with HES. This difference may be enhanced by the much smaller mean

molecular weight of HES compared with CMS at early time points (fig. 2C), thereby possibly facilitating extravasation even more. The significantly smaller distribution volumes (central and total) for CMS and CM-HES clearly support this hypothesis, which is in accordance with current opinion on colloid pharmacology.^{1,17} Thus, carboxymethylated starch seems to exhibit a lower tendency for extravascular accumulation and tissue storage. However, it may be possible that extravasated degradation products of HES (oligosaccharides) are metabolized and cleared from the extravascular space. Therefore, only direct measurement of extravasation or a comparative tissue analysis could prove a potential benefit of carboxymethylated starches with respect to tissue storage and the related development of pruritus after administration of starches.⁶

Because inhibition of blood coagulation is the major side effect of starches, the analysis of their influence on blood coagulation by means of TEG[®] and plasma coagulation tests was the secondary aim of this study. Most of the TEG[®] parameters showed a significantly more pronounced impairment of blood coagulation with CMS compared with HES. Similarly, most of the plasma coagulation tests performed were significantly changed toward hypocoagulation with CMS compared with HES. CM-HES showed a similar but less marked effect on blood coagulation. PK-PD analysis based on plasma concentration in g/l did not show a significantly more pronounced alteration of any of the coagulation parameters with CMS and CM-HES compared with HES. In contrast, when PK-PD analysis was performed with molar plasma

colloid concentration, most TEG[®] parameters showed significant differences between HES and CMS, with a stronger anticoagulant effect of the latter colloid. This indicates that molecular weight (and/or molecular structure)—which is the uncontrolled variable in PK-PD analysis based on molar concentration—may be the critical factor on the blood coagulation-impairing effect. However, because the size simultaneously changes over time together with the number (moles) of circulating starch molecules, it is difficult to determine the isolated effects of each of these two parameters by PK-PD analysis. Accordingly, it is the plasma concentration in g/l—integrating both number and size of circulating molecules—that primarily determines the final effect on blood coagulation *in vivo*.

Surprisingly, although most coagulation tests showed changes toward hypocoagulation after CMS infusion compared with HES, there were also procoagulant effects of CMS: aPTT was shortened after CMS infusion, whereas it was prolonged initially after HES infusion (fig. 4D). These responses were significantly different ($P = 0.034$), demonstrating a procoagulant effect of CMS in this test. In addition, PK-PD analysis based on molar concentration showed a statistically significant decrease of PT after CMS infusion compared with HES (table 4). The explanation for this seemingly contradictory effect remains to be elucidated.

The current results do not allow a definitive statement about the mechanisms of the observed changes of blood coagulation after CMS infusion. Both vWF concentration and activity were decreased more with CMS compared with HES (figs. 4A and B), indicating an impairment of primary hemostasis. Although the classic tests of secondary hemostasis, PT and aPTT, did not show an anticoagulant effect of CMS (but a procoagulant effect as discussed above), the fact that almost all TEG[®] parameters and FVIII activity were significantly changed toward hypocoagulation indicates an impairment of secondary hemostasis as well.

The porcine model is an established animal model for blood coagulation research.²³ However, there are some species-specific differences between blood coagulation of humans and swine, including increased FVIII activity in swine and shortened aPTT, as we have found in our study.²³⁻²⁶ Importantly for our study, coagulation changes in response to colloid infusion and hemodilution are similar between the human and porcine coagulation systems.¹³

The changes in COP after administration of the colloids (table 3) deserves some comment: HES did not change COP 5 min after the end of the 30-min infusion, whereas CMS and CM-HES infusion resulted in an increase of COP of 35% and 22%, respectively. This difference in favor of the carboxymethylated starches may be explained by the fact that maintenance of COP is governed by molecular size, degradation of larger molecules

to smaller ones, and negative net charge, which causes repulsion from the negatively charged endothelium *in vivo*.^{1,27} However, the physiologic relevance of this finding may be limited because it depends on the pore size of the used membrane. The measurement of COP with membranes that have a smaller cutoff (*e.g.*, 10 kd) may have resulted in an increase of COP with HES 5 min after the end of the infusion because mean molecular weight was higher than 10 kd at this time point (fig. 2C). However, the pore size of the membrane chosen in this study (20 kd) is close to the 10-kd selectively permeable membrane, which allows a good estimate of intravascular COP.²⁸

In accordance with higher COP values reached after infusion, hemoglobin concentration as an indirect indicator of the volume effect decreased significantly more with CMS compared with HES. In contrast, when the effects of the different colloids compared in this study were analyzed by PK-PD analysis, infusion of HES resulted in a significantly stronger decrease of hemoglobin concentration per g/l colloid concentration *in vivo* compared to CMS and CM-HES. Differences in hemoglobin decrease were not significant when PK-PD analysis was based on molar colloid plasma concentration. This indicates that rather than the size of starch molecules (which is different between HES and CMS/CM-HES if PK-PD analysis is adjusted for the molar plasma concentration), the number of starch molecules may be the crucial factor for the volume effect. This may be important from a scientific point of view, but the clinician may draw more attention to the observation that after infusion of equal amounts (grams) per kilogram body weight, CMS shows a trend to decrease hemoglobin concentration more than HES. However, the authors are aware of the fact that a change in the hemoglobin concentration is only an indirect indicator of plasma expansion and that other techniques that measure blood and plasma volume simultaneously before and after infusion may be more appropriate for determination of the volume effect of infused colloids.²⁹

In summary, carboxymethylation not only seems to cause a stronger volume effect, but also results in a longer circulatory persistence and possibly a longer volume effect. In addition, significantly smaller distribution volumes may indicate less extravasation of CMS and CM-HES compared with HES. However, these novel starches exert a more pronounced inhibition on blood coagulation than conventional HES. The results of this study do not allow prediction of the relation between primary effects (volume expansion) and side effects (influence of blood coagulation) at equal primary effects or at equal side effects: At an equal influence on blood coagulation, there still may be a stronger volume effect of carboxymethylated starches compared with HES. Therefore, the clinical significance of this side effect is not clear and must be addressed in the future with

clinically more relevant models as, for example, acute normovolemic hemodilution, hemorrhagic shock models, or redosing studies.

The authors thank Marco Burki (Research Associate, Department of Experimental Surgery, University Hospital Lausanne, Lausanne, Switzerland) for excellent technical assistance during animal experiments.

References

- Vercueil A, Grocott MP, Mythen MG: Physiology, pharmacology, and rationale for colloid administration for the maintenance of effective hemodynamic stability in critically ill patients. *Transfus Med Rev* 2005; 19:93-109
- Treib J, Baron JF, Grauer MT, Strauss RG: An international view of hydroxyethyl starches. *Intensive Care Med* 1999; 25:258-68
- Warren BB, Durieux ME: Hydroxyethyl starch: Safe or not? *Anesth Analg* 1997; 84:206-12
- Treib J, Haass A, Pindur G: Coagulation disorders caused by hydroxyethyl starch. *Thromb Haemost* 1997; 78:974-83
- Deusch E, Gamsjäger T, Kress HG, Kozek-Langenecker SA: Binding of hydroxyethyl starch molecules to the platelet surface. *Anesth Analg* 2003; 97:680-3
- Bork K: Pruritus precipitated by hydroxyethyl starch: A review. *Br J Dermatol* 2005; 152:3-12
- Madjdipour C, Dettori N, Frascarolo P, Burki M, Boll M, Fisch A, Bombeli T, Spahn DR: Molecular weight of hydroxyethyl starch: Is there an effect on blood coagulation and pharmacokinetics? *Br J Anaesth* 2005; 94:569-76
- Nicholson JP, Wolmarans MR, Park GR: The role of albumin in critical illness. *Br J Anaesth* 2000; 85:599-610
- Hodges KL, Kester WE, Wiederrich DL, Grover JA: Determination of alkoxy substitution in cellulose ethers by zeisel-gas chromatography. *Anal Chem* 1979; 51:2172-6
- Lee YC, Baaske DM, Carter JE: Determination of the molar substitution ratio of hydroxyethyl starches by gas chromatography. *Anal Chem* 1983; 55:334-8
- Eyler EW, Klug ED, Diephuis F: Determination of degree of substitution of sodium carboxymethylcellulose. *Anal Chem* 1947; 19:24-7
- Pivalizza EG, Abramson DC: Thromboelastography: Another point of view. *Anesth Analg* 2001; 93:517-8
- Jamnicky M, Bombeli T, Seifert B, Zollinger A, Camenzind V, Pasch T, Spahn DR: Low- and medium-molecular-weight hydroxyethyl starches: comparison of their effect on blood coagulation. *ANESTHESIOLOGY* 2000; 93:1231-7
- Chandler WL: The thromboelastography and the thromboelastograph technique. *Semin Thromb Hemost* 1995; 21 (suppl 4):1-6.
- Traverso CI, Caprini JA, Arcelus JI: The normal thromboelastogram and its interpretation. *Semin Thromb Hemost* 1995; 21 (suppl 4):7-13
- Camenzind V, Bombeli T, Seifert B, Jamnicky M, Popovic D, Pasch T, Spahn DR: Citrate storage affects Thromboelastograph analysis. *ANESTHESIOLOGY* 2000; 92:1242-9
- Jungheinrich C, Neff TA: Pharmacokinetics of hydroxyethyl starch. *Clin Pharmacokinet* 2005; 44:681-99
- Quon CY: Clinical pharmacokinetics and pharmacodynamics of colloidal plasma volume expanders. *J Cardiothorac Anesth* 1988; 2:13-23
- Rehm M, Haller M, Orth V, Kreimeier U, Jacob M, Dressel H, Mayer S, Brechtelsbauer H, Finsterer U: Changes in blood volume and hematocrit during acute preoperative volume loading with 5% albumin or 6% hetastarch solutions in patients before radical hysterectomy. *ANESTHESIOLOGY* 2001; 95:849-56
- Rehm M, Zahler S, Lotsch M, Welsch U, Conzen P, Jacob M, Becker BF: Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. *ANESTHESIOLOGY* 2004; 100:1211-23
- Bruegger D, Jacob M, Rehm M, Loetsch M, Welsch U, Conzen P, Becker BF: Atrial natriuretic peptide induces shedding of endothelial glycocalyx in coronary vascular bed of guinea pig hearts. *Am J Physiol Heart Circ Physiol* 2005; 289:H1993-9
- Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger T: Atrial natriuretic factor: A circulating hormone stimulated by volume loading. *Nature* 1985; 314:264-6
- Olsen AK, Hansen AK, Jespersen J, Marckmann P, Bladbjerg EM: The pig as a model in blood coagulation and fibrinolysis research. *Scand J Lab Anim Sci* 1999; 26:214-24
- Karges HE, Funk KA, Ronneberger H: Activity of coagulation and fibrinolysis parameters in animals. *Arzneimittelforschung* 1994; 44:793-7
- Hahn N, Popov-Cenic S, Dorer A: Basic values of blood coagulation parameters in pigs (*Sus scrofa domestica*) [in German]. *Berl Munch Tierarztl Wochenschr* 1996; 109:23-7
- Roussi J, Andre P, Samama M, Pignaud G, Bonneau M, Laporte A, Drouet L: Platelet functions and haemostasis parameters in pigs: Absence of side effects of a procedure of general anaesthesia. *Thromb Res* 1996; 81:297-305
- Vink H, Duling BR: Capillary endothelial surface layer selectively reduces plasma solute distribution volume. *Am J Physiol Heart Circ Physiol* 2000; 278:H285-9
- Webb AR, Barclay SA, Bennett ED: *In vitro* colloid osmotic pressure of commonly used plasma expanders and substitutes: A study of the diffusibility of colloid molecules. *Intensive Care Med* 1989; 15:116-20
- Jacob M, Rehm M, Orth V, Lotsch M, Brechtelsbauer H, Weninger E, Finsterer U: Exact measurement of the volume effect of 6% hydroxyethyl starch 130/0.4 (Voluven) during acute preoperative normovolemic hemodilution [in German]. *Anaesthesist* 2003; 52:896-904