Importance of the Infusion Rate for the Plasma Expanding Effect of 5% Albumin, 6% HES 130/0.4, 4% Gelatin, and 0.9% NaCl in the Septic Rat

Björn P. Bark, MD; Johan Persson, MD, PhD; Per-Olof Grände, MD, PhD

Objectives: To compare the plasma volume (PV) expanding effect of a fast infusion rate with that of a slow infusion rate of a fixed volume of 5% albumin, of the synthetic colloids, 6% hydroxyethyl starch 130/0.4 and 4% gelatin, and of 0.9% NaCl in a rat sepsis model and to compare the plasma-expanding effect among these fluids.

Design: Prospective, randomized animal study.

Setting: University hospital laboratory.

Subjects: One hundred and twelve adult male rats.

Interventions: Sepsis was induced by cecal ligation and incision followed by closure of the abdomen. After 3 hrs, an infusion of the PV expander under study was started at a volume of 12 mL/kg for the colloids and of 48 mL/kg for 0.9% NaCl, either for 15 mins or for 3 hrs. A control group underwent the same experimental procedure but no fluid was given.

Measurements and Main Results: Three hours after start of the infusion (end of experiment), the plasma-expanding effect was better with a slow than a fast infusion rate for the colloids, especially albumin, but the NaCl groups did not differ significantly from the control group. The PV for the control group was 28.7 ± 3 mL/kg. In the slow and the fast infusion groups, it was 38.9 ± 4.3 and 32.6 ± 4.2 mL/kg for albumin ($p < 0.001$), 32.9 ± 4.3 and 29.5 ± 4.4 mL/kg for hydroxyethyl starch 130/0.4 ($p < 0.05$), 31.8 ± 3.9 and 28.2 ± 4.1 mL/kg for gelatin ($p < 0.05$), and 31.8 ± 5.3 and 30.7 ± 6.6 mL/kg for NaCl (n.s), respectively.

Conclusions: The study showed that the PV expansion by a colloid was greater when given at a slow than at a fast infusion rate, an effect more pronounced for albumin. This difference was not seen for NaCl. The PV-expanding effect was poor for NaCl and better for albumin than for the other colloids. (*Crit Care Med* 2013; 41:0–0)

Key Words: albumin; colloids; gelatin; hydroxyethyl starch; infusion rate; plasma volume; plasma volume expander.

Plasma is continuously transferred from the intravascular space to the extravascular space and returned back to the circulation via the lymphatic system. The rate of the transfer is denoted "the transcapillary escape rate" (TER). TER for albumin is normally 5%–7% of total albumin per hour in man, but it can increase by a factor of 2–3 times during inflammatory conditions such as sepsis/systemic inflammatory response syndrome (SIRS) and after trauma (1–3). A TER above the capacity of the lymphatic system will result in accumulation of interstitial fluid and hypovolemia, with activation of the baroreceptor reflex. This may cause compromised tissue perfusion, increased tissue pressure, and reduced transcapillary oncotic pressure with altered Starling fluid equilibrium, longer diffusion distances, and pulmonary insufficiency (4, 5). Treatment of hypovolemia with plasma volume (PV) substitution under these conditions will cause further accumulation of interstitial fluid. Thus, in the restoration of PV in these patients, it would be favorable to reduce transfer of fluid to the extravascular space.

According to the two-pore theory of transcapillary fluid exchange, the capillary membrane contains small pores that are permeable only to small solutes and the large pores—which are more than 10,000 times less abundant—that are also permeable to proteins (6). Transcapillary leakage of proteins not

Department of Anesthesia and Intensive Care, Lund University and Lund University Hospital, Lund, Sweden.

This study was supported by the Swedish Research Council, Stockholm, Sweden (11581), and Region Skåne (ALF 18401).

The authors have not disclosed any potential conflicts of interest.

Address requests for reprints to: Per-Olof Grände, MD, PhD, Department of Anesthesia and Intensive Care, Lund University Hospital, SE-221 85 Lund, Sweden. E-mail: per-olof.grande@med.lu.se

DOI: 10.1097/CCM.0b013e318274157e

only depends on the permeability of large pores but also on the hydrostatic transcapillary pressure. Hydrostatic capillary pressure can increase through an increase in arterial pressure, an increase in venous pressure, or an increase in postcapillary/precapillary resistance ratio (7). It was recently confirmed, both experimentally in the rat (8) and in man (9), that a moderate increase in arterial pressure from noradrenaline infusion results in a significant decrease in PV under conditions of increased capillary permeability. As a high infusion rate of a PV expander most likely will cause a transient increase in arterial and venous pressures and also a transient decrease in precapillary resistance, it can be expected that there will be greater transcapillary leakage when a PV expander is administered at a high infusion rate than when it is administered at a low infusion rate. A fast infusion will also cause a transient dilution of the red blood cells, which will increase the exposure of the extended plasma column between two erythrocytes to the large pores, with the potential of an increase in plasma leakage. This hypothesis is supported by experimental studies on the dog and the rat, showing increase in PV after the transfusion of erythrocytes (10, 11). Furthermore, the release of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) from the heart may be larger with a bolus infusion than with a continuous infusion, resulting in more urine production and smaller PV (12). There are indications from the literature that these hormones may also cause an increase in microvascular permeability (13).

Correction of hypovolemia is an important therapeutic measure, and both crystalloids and colloids are used (14). Crystalloids are distributed passively within the entire extracellular compartment in relation to the volume of plasma and intersitium. Under normal circumstances, approximately 70%−80% of the infused volume will be distributed relatively quickly to the intersititial space and about 20%−30% acts as PV expander (15). Thus, treatment with crystalloids is associated with a significant increase in interstitial fluid volume. Colloid solutions consist of macromolecules and, according to the two-pore theory, leak mainly through the large pores, a leakage that increases under increased permeability (8, 16). This means that treatment with colloids can be associated with aggravation of adverse interstitial accumulation of macromolecules and fluid, especially in inflammatory conditions such as sepsis/SIRS.

To our knowledge, there are no studies specifically analyzing the importance of the infusion rate for PV expansion. We have only found two studies, which showed some relation to this issue. One study showed a higher survival rate and lower morbidity with a slow infusion rate than with a fast infusion rate in acute pancreatitis (17). The other study showed higher mortality in children suffering from a severe febrile illness if given a bolus infusion at the time of admission to the hospital instead of a standard fluid administration (18). Even though there is no consensus regarding infusion rates, a PV expander is often given at a fast rate to treat a suspected hypovolemia without delay.

According to the considerations above, the smallest possible volumes for PV resuscitation to maintain normovolemia should be used to reduce the risk of simultaneous interstitial fluid accumulation. Using a model of rat sepsis, we tested the hypothesis that a slow infusion rate of a PV expander results in better plasma expansion than a fast infusion rate. This was done by comparing the PVs 3 hrs after start of the infusion of a fixed volume of the natural colloid 5% albumin, the synthetic colloids 6% HES 130/0.4 and 4% gelatin, and the crystalloid 0.9% NaCl when given at a fast and at a slow rate. We also compared the PV expansion for the different fluids for each infusion rate.

MATERIALS AND METHODS

Anesthesia and Set-Up

The study was approved by the Ethical Committee for Animal Research at Lund University, Sweden (application no. M180-10), and the animals were treated in accordance with the guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats ($n = 112$) weighing 354 ± 21 g (mean ± SD) were used. Anesthesia was induced by placing the animals in a covered glass container with a continuous supply of 5% isoflurane in air (Isoba vet; Intervet, the Netherlands). After induction, the animals were removed from the container and anesthesia was maintained with 1.5%–1.8% isoflurane in air using a mask, followed by tracheostomy and connection to a ventilator (Ugo Basile; Biological Research Apparatus, Comerio, Italy). Ventilation was performed in a volume-controlled mode using a positive end expiratory pressure of 4 cm H_2O. End-tidal PCO_2 was continuously monitored and kept between 4.9 and 5.5 kPa (Capstar-100; CWE, Ardmore, PA). Body temperature, measured rectally, was kept at 37.1°C–37.3°C via a feedback-controlled heating pad. The left femoral artery was cannulated to record arterial blood pressure (BP) and to obtain blood samples for analysis of arterial blood gases, electrolytes, lactate, hematocrit (I-STAT; Abbot Point of Care Inc, Abbot Park, IL), and PVs. The left femoral vein was cannulated and used for infusions and kept open with a continuous infusion of saline at 0.2 μL/min. The right internal jugular vein was cannulated and used for injection of ^{125}I-albumin to measure PVs. After the experiments, the animals were killed with intravenous injection of potassium chloride.

Experimental Procedure

A model of severe sepsis as described previously (19, 20) was used in this study. After a longitudinal midline skin incision over the abdominal wall with diathermia, a laparotomy was performed by incision along the linea alba. The cecum was ligated just below the ileocecal valve, and an incision of 1 cm in length was made in the cecum, allowing leakage of fecal material into the abdominal cavity, thereby inducing sepsis/SIRS. The abdominal wall and the skin were then closed with clips. There were no bleedings.

Plasma Volume

PV was determined by measuring the increase in radioactivity in 100 μL of plasma taken 5 mins after an intravenous injection of human ^{125}I-albumin with a known amount of activity. The increase in radioactivity was calculated by subtracting the activity in a blood sample taken just before the injection from that taken 5 min after the injection. Each PV measurement was thereby adjusted for any remaining radioactivity from previous measurements. To calculate the amount of radioactivity given, the remaining activity in the emptied vial, syringe, and needle used was calculated and subtracted from the total activity in the prepared dose. This is a reliable and established technique for PV measurements, giving reproducible results (16, 21, 22). As will be discussed, sources of error are small with the design of the technique used in this study (see Discussion). Free iodine was measured regularly following precipitation with 10% trichloroacetic acid and was found to be less than 1.2% in the prepared samples. Radioactivity was measured with a gamma counter (Wizard 1480; LKB-Wallace, Turku, Finland).

Experimental Protocol

In this study, we compared the PV-expanding effect of a fixed volume of a PV expander when given at a slow or a fast infusion rate. The PV expanders analyzed were 5% albumin (MW 69 kDa; $n = 12$ per group), 6% HES 130/0.4 (MW 20–250 kDa, mean 130 kDa; $n = 10$ per group), 4% gelatin (0–150 kDa, mean MW 30 kDa; $n = 10$ per group), and 0.9% NaCl ($n = 8$ per group) given intravenously. The time scale of the experimental procedure is shown schematically in **Figure 1**. The infusions of 12 mL/kg for the colloids and 48 mL/kg for saline were started 3 hrs after the end of the surgical intervention. Pilot experiments have shown that a systemic inflammation with plasma leakage has developed at this time point, shown by an increase in hematocrit and a marked decrease in PV. An infusion volume of 12 mL/kg was selected in this study as previous experiments have shown a PV reduction 3 hrs after sepsis induction of 7–9 mL/kg, and to this, we then added a few milliliters to compensate for the anesthesia-induced vasodilation, and the blood samples taken just before start of the infusion. A 4 times larger volume for saline was given as saline is quickly distributed to the whole extracellular space, which is about 4 times larger than the PV.

Two groups were formed at random for each fluid. In one group, the fluid was given over 15 mins (the "bolus" group), and in the other group, the same volume was given over 3 hrs (the "continuous" group). The investigators were blinded to the bolus or the continuous treatment. In a "control" group, the animals underwent the same experimental procedure but no PV expander was given. PVs were measured at baseline (PV_1), 3 hrs after the surgical preparation (PV_2), and 3 hrs after the start of the infusion (at the end of the experiment; PV_3). Blood samples for measurements of arterial pH, $PaCo_2$, Pao_2, hematocrit, lactate, sodium, and potassium were taken at the same time points. Urine was collected in a glass vial placed at the external meatus of the urethra from the start of the infusion until the end of the experiment, and the bladder was emptied by external compression at the end of the experiment. Animals, that did not show a decrease in PV 3 hrs after the preparation were considered to be nonseptic and were excluded from the study. These animals and animals that died before the end of the experiment were replaced with new animals. PVs of blood samples were of the same size for all groups and therefore had no influence on the conclusions made.

To evaluate if there was a difference in BP between the bolus and the continuous groups the nearest time after the infusion, which could have contributed to a difference in PV between the groups (see introductory section), the mean of mean arterial BP during the 30-min period just before start of the infusion were compared with that during the 30-min time period just after start of the infusion for the different groups.

To evaluate if there was a difference in hematocrit, between the bolus and the continuous group, which could have contributed to a difference in transcapillary leakage between the groups affecting PV (see introductory section), a special

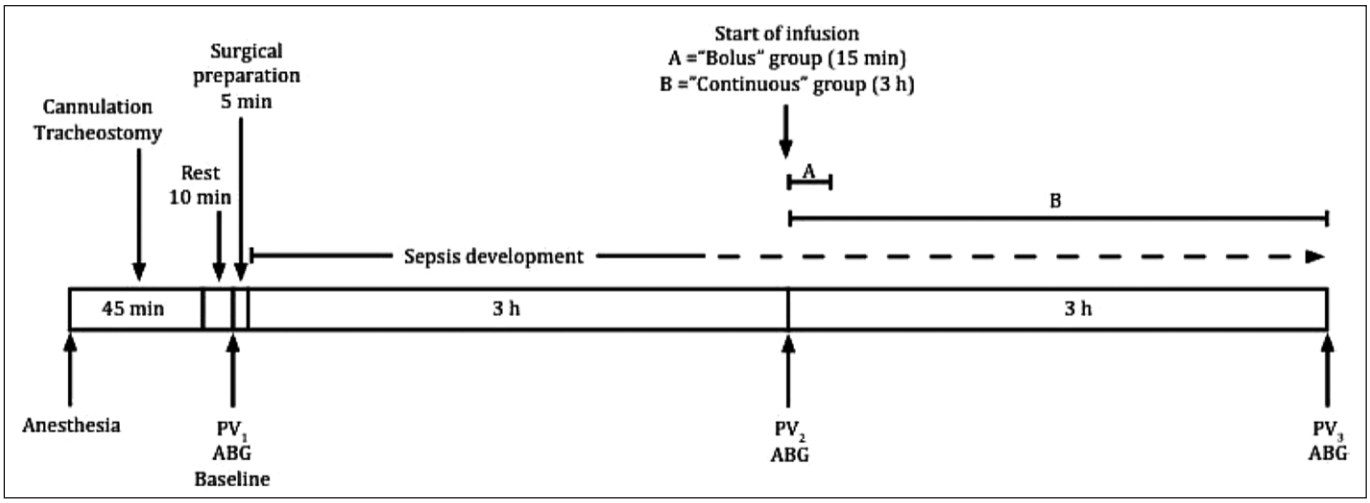

Figure 1 . Time scale of the experimental protocol. PV_1 = plasma volume at baseline; PV_2 = plasma volume 3 hrs after surgical preparation, just before the start of infusion; and PV_3 = plasma volume at the end of the experiment; and ABG = arterial blood sample for analysis of blood gases, hematocrit, and electrolytes.

analysis was performed in 12 separate animals (6 per group) given albumin. In these experiments, hematocrit was measured 20, 40, and 60 mins after start of the infusion and compared with the value just before the start of the infusion.

Our results of a faster loss of albumin in the bolus group initiated additional experiments to evaluate how fast PV was lost after end of the albumin bolus infusion. The results were compared with corresponding results when given as a continuous infusion ($n = 6$ per group). The experiments were similar to the main experiments except that PV was measured only just before the start of the infusion and at 1 and 1.5 hrs thereafter.

Statistical Analysis

Statistical comparisons between the groups regarding difference in PV, difference in physiological variables, and difference in hematocrit were performed with a two-way analysis of variance followed by Bonferroni post hoc test. Student's *t* test for unpaired observations was used to evaluate the difference between groups in the change in mean arterial pressure after start of the infusion. One-way analysis of variance followed by Bonferroni post hoc test was used for analysis of urine production. *p* values of less than 0.05 were considered significant. All data were normally distributed. The results are presented as mean ± SD. GraphPad Prism version 5.0 for Mac OS was used (GraphPad Software, San Diego, CA).

RESULTS

Four animals died in the control group (mortality rate, 33%), three animals died in the HES groups (13%), and one animal died in the gelatin groups (5%) before end of the experiment. Four animals were considered to be nonseptic and were excluded from the study, as they did not show any decrease in PV and any increase in hematocrit and lactate concentration 3 hrs after the preparation.

Physiological Data

Data from arterial blood samples for sodium (Na^+), potassium (K^+), hematocrit, lactate, pH, $PaCO_2$, and PaO_2 are summarized in **Table 1** for the bolus groups, the continuous groups, and the control group. There were no differences among the control group, the continuous groups, and the bolus groups in any of the physiological variables analyzed at baseline and at 3 hrs after the surgical preparation.

For all solutions analyzed, there was a tendency of higher hematocrit in the bolus groups than in the continuous groups at the end of the experiment, but this difference did not reach the stipulated significance level in any group. There was a significant difference in hematocrit between the continuous and the control group for all colloids analyzed ($p < 0.05$), while the differences for the bolus groups and the control group reached significance only for albumin ($p < 0.05$).

Results from the separate experiments, in which the hematocrit values for the bolus and the continuous groups for albumin were compared during a 60-min period just after start of the infusion (at 20, 40, and 60 mins), are presented in Figure 4A ($n = 6$ per group). There was a significantly lower hematocrit at 20- and 40-min period in the bolus group than in the continuous group.

At the end of the experiment, the differences in lactate levels among all groups were small, but they reached the stipulated level of significance between the NaCl continuous group and the HES bolus and continuous groups and between the gelatin bolus group and the control group ($p < 0.05$).

Data for mean arterial BP at baseline, 3 hrs after the preparation, and 1.5 and 3 hrs after the start of the infusion are given in **Table 2**. There were no significant differences in BP between the groups at any of these time points. The difference between mean of mean BP during a 30-min period after the start of the infusion with that during a 30-min period just before the start of the infusion for the bolus and the continuous groups for the solutions analyzed are presented in Figure 4*B*. There was a significant difference between the bolus and the continuous groups for all fluids analyzed. This difference in mean BP between the bolus and the continuous groups was transient as mean BP after 1.5 hrs did not differ between the groups (Table 2).

Plasma Volume

PV at baseline (PV_1), 3 hrs after the preparation (PV_2), and 3 hrs later at the end of the experiment (PV_3) for the fluids analyzed and also for the control group are shown in **Figure 2**. At baseline and 3 hrs after the preparation, there were no differences in PVs among the control group, the continuous groups, and the bolus groups. At the end of the experiment, there were significant differences between the albumin continuous group and the albumin bolus group ($p < 0.001$), between the HES continuous group and the HES bolus group ($p < 0.05$), and between the gelatin continuous group and the gelatin bolus group ($p < 0.05$). There was no significant difference between the NaCl bolus and the NaCl continuous group. There was a significant difference compared with the control group for the albumin continuous group ($p < 0.001$), the HES continuous group ($p < 0.01$), and the albumin bolus group ($p < 0.05$).

A comparison of the change in PVs among the different solutions analyzed from the start of infusion (PV_2) to the end of the experiment (PV_3) for the continuous group and the bolus group is shown in **Figure 3*A*** and ***B***. The plasma expansion was significantly better for the albumin continuous group than for the other groups analyzed ($p < 0.001$). There was a significant difference between the albumin bolus group and the gelatin bolus group ($p < 0.01$) and between the albumin bolus group and the control group ($p < 0.05$).

PVs in the additional experiments analyzing how fast the PV was lost after end of the infusion after a bolus infusion of albumin are shown in Figure 4*C*. As seen, PV was reduced to the same level as in the group with continuous infusion after slightly more than 1 hr after start of the infusion, a time point when only 35%–40% of the continuous infusion volume had been given.

TABLE 1. Data (Mean ± SD) for Sodium, Potassium, Hematocrit, Lactate, pH, $Paco_2$, and Pao_2

	Sodium (mmol/L)	Potassium (mmol/L)	Hematocrit (%)	Lactate (mmol/L)	pH	$Paco_2$ (kPa)	Pao_2 (kPa)
Albumin							
15 min ($n = 12$)							
Baseline	135 ± 2	4.7 ± 0.4	43 ± 2	2.0 ± 0.2	7.52 ± 0.02	4.6 ± 0.2	12.1 ± 1.3
3 h after preparation	132 ± 3	5.0 ± 0.5	44 ± 1	2.4 ± 0.6	7.45 ± 0.04	4.8 ± 0.4	12.1 ± 1.2
3 h after start of infusion	134 ± 2	5.6 ± 0.8	46 ± 3[a]	2.4 ± 0.9	7.43 ± 0.04	4.4 ± 0.5	12.2 ± 1.3
3 h ($n = 12$)							
Baseline	136 ± 1	4.7 ± 0.4	43 ± 2	1.8 ± 0.6	7.51 ± 0.02	4.6 ± 0.3	12.0 ± 0.8
3 h after preparation	134 ± 2	4.9 ± 0.4	44 ± 2	2.3 ± 0.6	7.47 ± 0.04	4.7 ± 0.4	11.5 ± 0.9
3 h after start of infusion	135 ± 1	4.9 ± 0.7	44 ± 3[a]	2.1 ± 0.5	7.45 ± 0.03	4.7 ± 0.3	11.3 ± 0.9
Hydroxyethyl starch							
15 min ($n = 10$)							
Baseline	135 ± 2	4.5 ± 0.5	42 ± 2	1.9 ± 0.4	7.50 ± 0.03	5.1 ± 0.3	11.4 ± 1.1
3 h after preparation	133 ± 2	4.9 ± 0.6	43 ± 4	2.3 ± 0.4	7.45 ± 0.04	5.3 ± 0.4	11.3 ± 0.5
3 h after start of infusion	134 ± 3	5.4 ± 0.8	48 ± 5	2.7 ± 0.9	7.41 ± 0.05	4.8 ± 0.5	11.6 ± 0.9
3 h ($n = 10$)							
Baseline	136 ± 2	4.4 ± 0.6	43 ± 3	2.0 ± 0.7	7.51 ± 0.03	4.9 ± 0.2	11.8 ± 0.6
3 h after preparation	133 ± 3	5.2 ± 0.7	44 ± 3	2.2 ± 0.6	7.47 ± 0.03	5.0 ± 0.5	11.5 ± 0.9
3 h after start of infusion	134 ± 2	5.7 ± 1.1	45 ± 4[a]	2.5 ± 0.9	7.42 ± 0.03	4.7 ± 0.6	11.4 ± 0.7
Gelatin							
15 min ($n = 10$)							
Baseline	136 ± 2	4.9 ± 0.4	43 ± 3	2.1 ± 0.4	7.51 ± 0.03	4.7 ± 0.4	11.5 ± 0.6
3 h after preparation	134 ± 3	5.2 ± 0.6	44 ± 4	2.5 ± 0.5	7.45 ± 0.03	4.9 ± 0.4	11.3 ± 0.5
3 h after start of infusion	135 ± 2	5.8 ± 1.0	47 ± 5	2.5 ± 1.0	7.42 ± 0.04	4.6 ± 0.6	11.7 ± 0.9
3 h ($n = 10$)							
Baseline	135 ± 2	4.6 ± 0.3	43 ± 2	2.1 ± 0.3	7.50 ± 0.03	5.0 ± 0.5	11.5 ± 0.9
3 h after preparation	133 ± 2	5.1 ± 0.7	45 ± 2	2.8 ± 0.3	7.45 ± 0.02	4.9 ± 0.5	11.5 ± 1.0
3 h after start of infusion	134 ± 2	5.6 ± 1.0	46 ± 3[a]	2.6 ± 0.6	7.42 ± 0.05	4.5 ± 0.6	11.6 ± 0.9
NaCl							
15 min ($n = 8$)							
Baseline	134 ± 2	4.9 ± 0.4	43 ± 2	2.0 ± 0.4	7.52 ± 0.02	4.9 ± 0.2	12.1 ± 0.7
3 h after preparation	132 ± 2	5.6 ± 0.6	44 ± 3	2.7 ± 0.4	7.46 ± 0.02	4.9 ± 0.4	11.9 ± 0.7
3 h after start of infusion	135 ± 1	5.8 ± 1.0	47 ± 6	2.2 ± 0.5	7.41 ± 0.04	4.4 ± 0.6	12.3 ± 0.8
3 h ($n = 8$)							
Baseline	135 ± 2	4.9 ± 0.3	42 ± 2	1.9 ± 0.4	7.50 ± 0.02	5.0 ± 0.5	11.5 ± 0.9
3 h after preparation	133 ± 2	5.4 ± 0.9	46 ± 2	2.6 ± 0.6	7.46 ± 0.02	4.9 ± 0.5	11.5 ± 1.0
3 h after start of infusion	136 ± 2	5.5 ± 1.3	45 ± 5[a]	1.8 ± 0.4[b]	7.41 ± 0.04	4.5 ± 0.6	11.6 ± 0.9
Control ($n = 8$)							
Baseline	136 ± 2	4.7 ± 0.5	44 ± 2	1.9 ± 0.3	7.49 ± 0.02	5.3 ± 0.3	11.1 ± 1.2
3 h after preparation	133 ± 2	5.2 ± 0.3	45 ± 3	2.5 ± 0.4	7.46 ± 0.02	5.2 ± 0.1	10.8 ± 1.0
3 h after start of infusion	133 ± 1	5.9 ± 0.4	51 ± 4	2.6 ± 0.6	7.45 ± 0.02	4.6 ± 0.3	12.0 ± 1.1

[a] $p < 0.05$ compared with the control group.

[b] $p < 0.05$ compared with the control group, the 3-h and 15-min HES groups, and the 15-min gelatin group; Two-way analysis of variance with Bonferroni post hoc test were used for the statistical analysis.

TABLE 2. Data (Mean ± SD) for Mean Arterial Blood Pressure (mm Hg) at Baseline, 3 h After the Surgical Preparation, 1.5 hrs After the Start of Infusion, and 3 h After the Start of Infusion With 5% albumin, 6% Hydroxyethyl Starch 130/0.4, 6% Gelatin, 0.9% NaCl, and the Control Group

	Baseline	3 h After Surgical Preparation	1.5 h After Start of Infusion	3 h After Start of Infusion
Albumin				
15 min ($n = 12$)	97 ± 8	90 ± 11	95 ± 11	95 ± 12
3 h ($n = 12$)	100 ± 11	88 ± 8	96 ± 8	103 ± 8
Hydroxyethyl Starch				
15 min ($n = 10$)	104 ± 11	90 ± 14	96 ± 13	97 ± 15
3 h ($n = 10$)	107 ± 10	94 ± 10	101 ± 12	99 ± 18
Gelatin				
15 min ($n = 10$)	95 ± 18	97 ± 10	98 ± 11	99 ± 14
3 h ($n = 10$)	101 ± 14	102 ± 12	108 ± 9	109 ± 8
NaCl				
15 min ($n = 8$)	102 ± 11	95 ± 16	95 ± 16	103 ± 18
3 h ($n = 8$)	100 ± 9	97 ± 15	106 ± 12	108 ± 17
Control ($n = 8$)	101 ± 14	95 ± 10	97 ± 13	98 ± 12

Urine Production

Urine production from the start of the infusion to the end of the experiment was 5.9 ± 1.3 mL/kg in the albumin continuous group, 6.3 ± 0.9 mL/kg in the albumin bolus group, 3.2 ± 0.9 mL/kg in the HES continuous group, 3.8 ± 1.2 mL/kg in the HES bolus group, 3.2 ± 0.9 mL/kg in the gelatin continuous group, 3.3 ± 1.2 mL/kg in the gelatin bolus group, 4.0 ± 0.6 mL/kg in the NaCl continuous group, 4.9 ± 2.2 mL/kg in the NaCl bolus group, and 2.6 ± 1.4 mL/kg in the control group. There was significantly more urine production in the albumin groups and the bolus NaCl group than in the control group ($p < 0.05$).

DISCUSSION

This study has shown that the degree of PV expansion of a fixed volume of a colloid solution measured 3 hrs after start of the infusion is larger when it is given at a slow infusion rate than if it is given at a fast rate. However, this difference was greater for albumin than for HES and gelatin, and albumin was the most effective PV expander. For NaCl, there was no significant difference in PV-expanding effect at the end of the experiment for the bolus group and the continuous group. The NaCl groups did not differ significantly from the control group, even though NaCl was given in a 4 times larger volume than the colloid solutions. The differences in PV at the end of the experiment were reflected in the difference in hematocrit values for all colloids, in the sense that there were lower hematocrit values in all the continuous groups than in the control group.

The dilution technique using ^{125}I-albumin as tracer is well established for the calculation of PV in experimental and clinical studies with reproducible results in both normal and inflammatory states (21, 22). As has been discussed previously (21, 23), this technique, however, means some overestimation of the PV. Free iodine in the tracer injected can result in some overestimation, as free iodine is distributed quickly to the whole extracellular space, but the free iodine was small in this study (< 1.2 %) and therefore must have had minor influence on the results. There might have been overestimation of PV because of transcapillary escape of radioactive albumin during the 5-min period between injection of the tracer and collection of the blood sample and especially at states of increased permeability (21, 23). This means a larger overestimation of the PV after initiation of sepsis than at baseline. This overestimation, however, must be small in this study by the short time period of 5 min between the injection of ^{125}I-albumin and the measurement, a time period shorter than the 10–15 min used in the referred studies (21, 23). A time period of 5 min has previously been shown to be sufficient for complete mixture of the tracer in plasma both in cat and in human (14, 21). Finally, remaining radioactivity of the syringe, the vial, and the needle used was subtracted from the initially calculated radioactivity and therefore will not contribute to an overestimation of the PVs. All this taken together means, that the expected overestimation of the PV measurements with the design of the dilution technique used in this study is small. Independent of this, remaining errors will have no influence on the conclusions made, as they will be of the same magnitude for all groups.

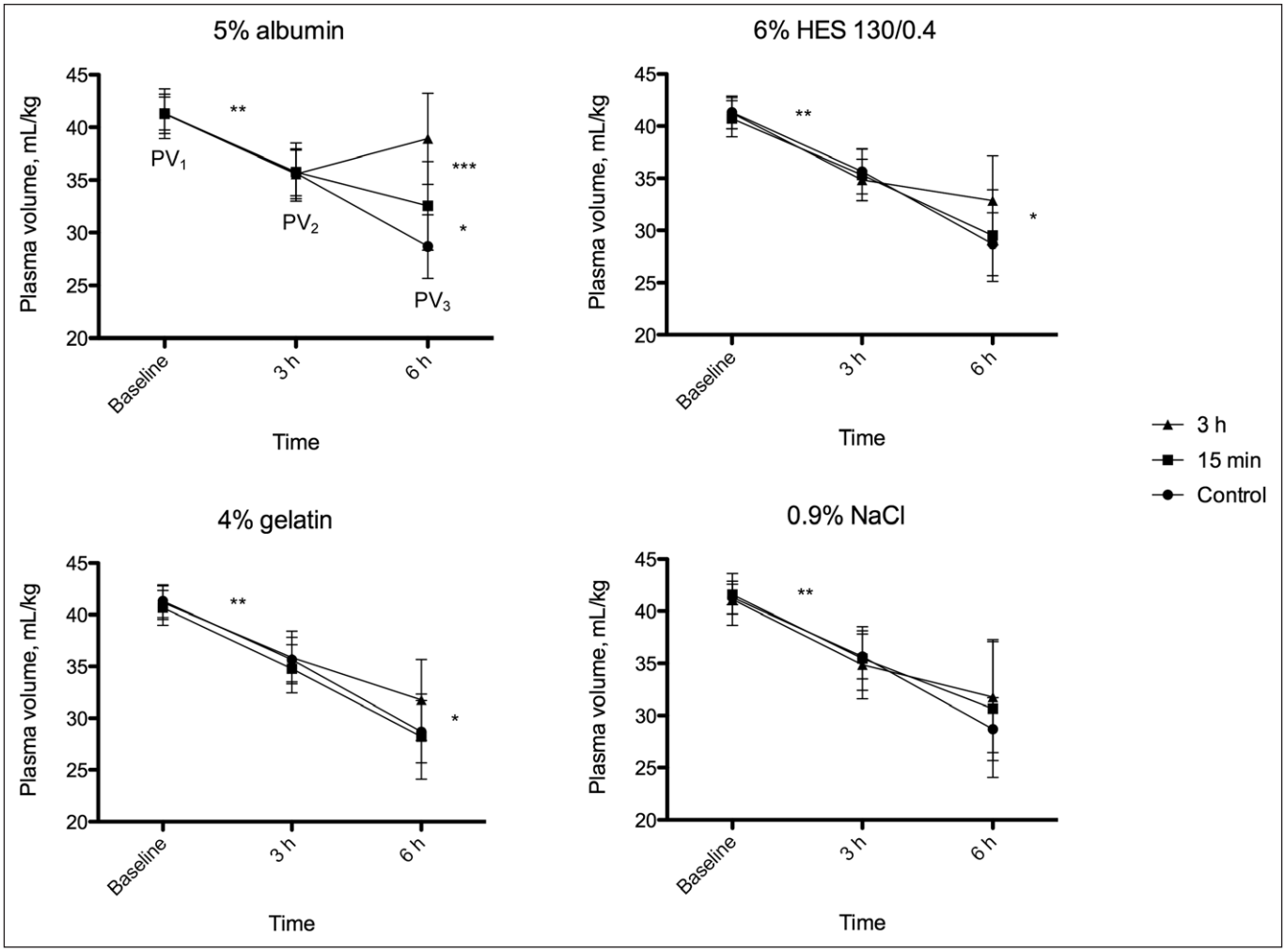

Figure 2. Plasma volumes (PVs) at baseline (PV_1), 3 hrs after the preparation just before the start of infusion (PV_2), and at the end of the experiment (PV_3) given as a continuous (3-hr) infusion or as a bolus (15-min) infusion of 5% albumin ($n = 12$ per group), 6% hydroxyethyl starch 130/0.4 ($n = 10$ per group), 4% gelatin ($n = 10$ per group), or 0.9% NaCl ($n = 8$ per group). Corresponding data for the control group ($n = 8$) are also shown. There was a significant difference between PV_1 and PV_2 for all groups and a significant difference between the continuous group and the bolus group for all solutions except 0.9% NaCl. There was a significant difference between the albumin bolus group and the control group. Two-way analysis of variance with Bonferroni post hoc test was used for the statistical analyses. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

The better PV-expanding effect with a slow infusion rate than with a fast infusion rate is compatible with the two-pore theory of transcapillary fluid exchange. As suggested in the introductory section, a bolus infusion would be expected to cause a transient increase in capillary pressure from the transient increase in systemic arterial pressure and decrease in precapillary resistance and decrease in hematocrit, all of which can be expected to lead to an increase in transcapillary fluid loss (8). Our results of a significantly larger increase in mean arterial pressure and decrease in hematocrit in the bolus groups during the first time period after start of the infusion are compatible with these proposals (**Fig. 4*A*** and ***B***). It is unlikely that the release of ANP and BNP after a bolus infusion, as discussed in the introductory section, would have influenced the results through an increase in urine production, as urine production was very small in these experiments in relation to the volumes infused. However, we cannot exclude the possibility that the permeability-increasing effect of ANP and BNP resulted in PV loss (5).

Tentative explanations can be given regarding the bad plasma expansion, and the smaller differences in PVs between the continuous and the bolus groups for HES and gelatin compared with albumin. HES is degraded by amylase resulting in halving of the molecular weight within 20–30 min (24). The initial half-life of plasma elimination of HES 130/0.4 is thought to be approximately 30–45 mins after infusion in man (24). Degradation of the HES molecules causes an increase in leakage of the smaller degradation products to the extravascular space: The degradation rate can be expected to be even faster in the rat than in man because of a higher plasma concentration of amylase in the rat (25), most likely resulting in extensive degradation within the 3-hr study period. This might be one explanation of the poor PV-expanding effect of HES, and the fact that the bolus group did not even differ significantly from that of the control group. Thus, the results for HES in this study cannot be directly extrapolated to humans.

Gelatin has a relatively low mean MW of 30 kDa. Being a polydisperse colloid, a large part of the molecules are small

enough to pass not only through the large pores but also through the small pores. This, and the fact that there is degradation of the molecules, means that there may be a relatively fast and continuous transcapillary leakage of gelatin during the 3-hr period after the start of the infusion, especially when there is an increase in capillary permeability. This fact might explain the poor PV-expanding effect of gelatin in this study.

The lack of any difference in PV expansion between the bolus and the continuous groups for 0.9% NaCl is to be expected, since the capillaries are freely permeable to crystalloids with a fast distribution of the solution to the whole extracellular space. One would expect, however, that the plasma-expanding effect of 0.9% NaCl would be better than that of HES and gelatin, considering that about 25% of the infused volume of 48 mL/kg should stay intravascularly and that the urine production was small.

Unexpectedly, the PV expansion for 0.9% NaCl did not even differ significantly from that of the control group, and we can

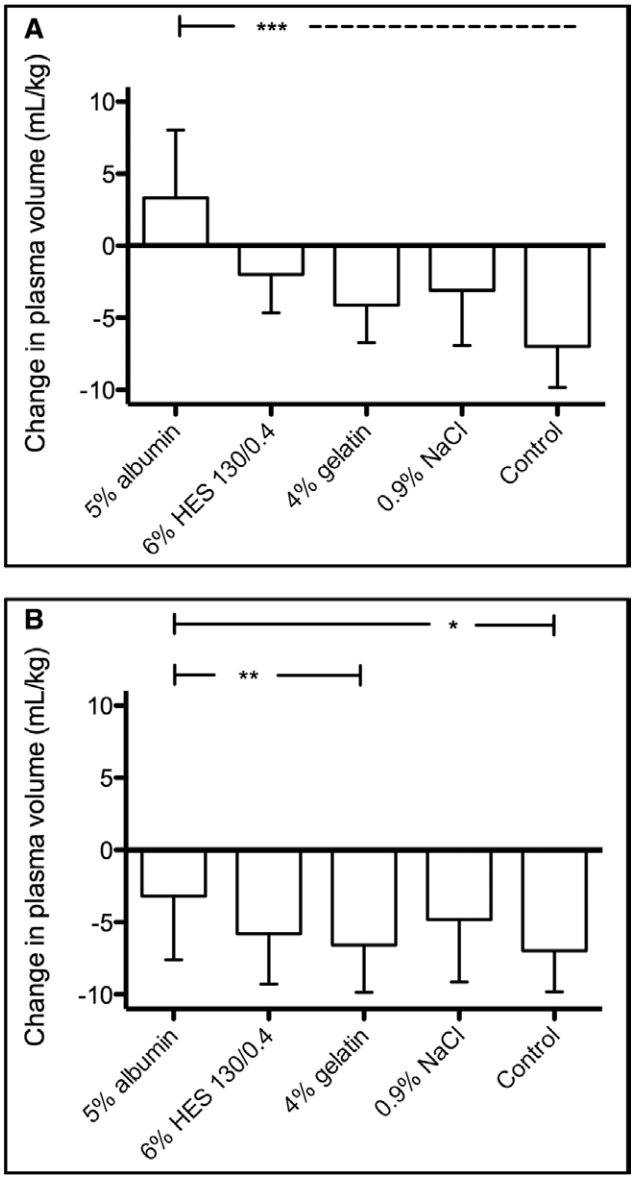

Figure 3. Comparison of the different solutions analyzed regarding change in plasma volume (PV) from the start of infusion (PV_2) to the end of the experiment (PV_3) for the continuous (3-hr) groups (**A**) and the bolus (15-min) groups (**B**). There was a significant difference between the albumin continuous group and the other groups, between the albumin bolus group and the gelatin bolus group, and between the albumin bolus group and the control group. Two-way analysis of variance with Bonferroni post hoc test was used for the statistical analyses. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

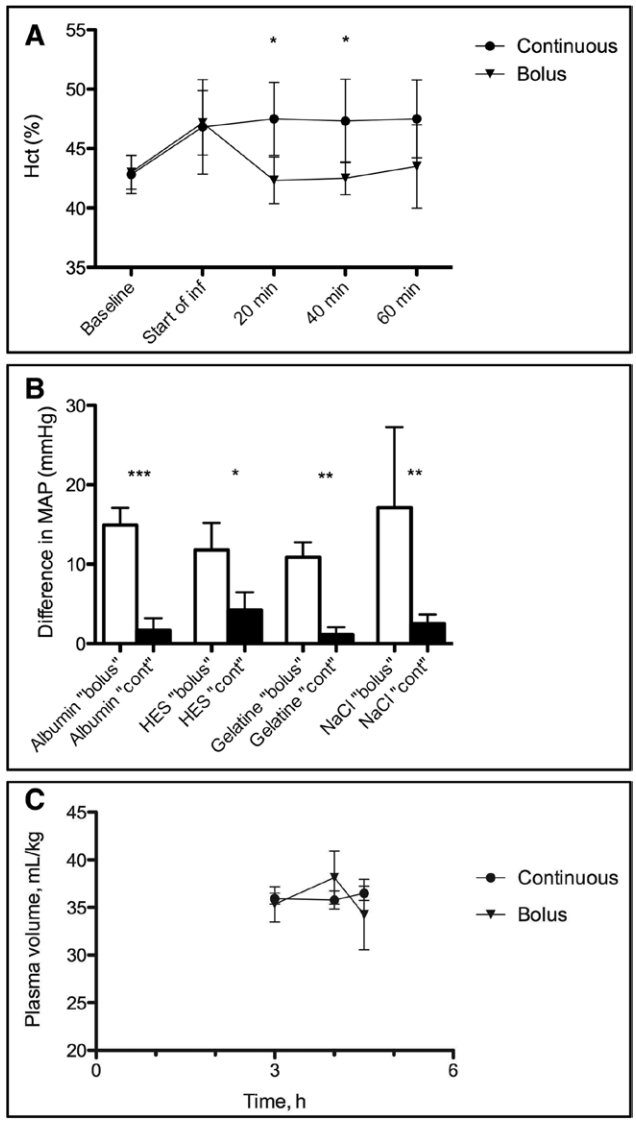

Figure 4. A, Hematocrit at baseline; just before start of the infusion; and 20, 40, and 60 min after start of the infusion for the bolus group and for the continuous group for albumin The analysis was performed in a separate series of animals ($n = 6$ per group). Two-way analysis of variance with Bonferroni post hoc test was used for the statistical analyses (*$p < 0.05$). **B**, Mean of mean arterial blood pressure (MAP) during a 30-min period just after start of the infusion subtracted with that 30 min just before start of the infusion for the bolus and the continuous groups for the different solutions analyzed. Student's *t* test for unpaired observations was used for the statistical analyses (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). **C**, Plasma volume (PV) 3 hrs after initiation of sepsis just before start of the infusion and 1 and 1.5 hrs after start of the infusion for the bolus group and the continuous group. The analysis was performed in a separate series of animals after end of the main study ($n = 6$ per group).

only speculate about possible explanations. When giving a crystalloid solution, under normal circumstances approximately 75% of the infused volume is distributed quickly to the interstitial space. With sepsis, when plasma has already been lost to the extravascular space, the ratio between the PV and the interstitial volume is reduced. This means that relatively more of the infused volume would be distributed to the interstitial space. While the infused saline is passing the large pores of the capillary membrane, there may also be a subsequent loss of proteins via convection (26). The large volumes of saline will transiently dilute plasma proteins, resulting in a reduction in transcapillary oncotic pressure, which will increase fluid transfer to the extravascular space and also reduce the absorbing forces across the capillary membrane (27). Finally, the 4 times higher infusion rate for saline than for the colloids results in a transient increase in hydrostatic capillary pressure. All these mechanisms may lead to increased leakage of fluid through the capillary pores, and increased leakage of proteins by convection through the large pores, especially under a state of increased number of large pores, such as in sepsis/SIRS.

The capacity of a colloid to maintain a normal PV is essential for its effectiveness. However, the relatively fast degradation rates of HES and gelatin can be compensated for by repeated infusions, while the low degradation rate of albumin compared with synthetic colloids may be negative because albumin will linger in the interstitium for a longer time.

As seen from Figure 4C, the PV loss after end of the bolus infusion of albumin was rather fast, reaching the same PV as that obtained when the infusion was given continuously after slightly more than 1 hr. This result supports the hypothesis presented in the introductory section that the PV loss can be related to hemodynamic effects of the bolus infusion, such as the transient decrease in hematocrit (Fig. 4*A*), the transient increase in arterial pressure (Fig. 4*B*) and the precapillary vasodilation.

We cannot tell from this study for how long time the slow rate of albumin infusion is favorable after it has been completed. However, if assuming about the same leakage of albumin after end of the infusion as occurring after initiation of sepsis (the same TER), the low rate of albumin infusion will be favorable during the subsequent 2–3 hrs after end of the infusion. If so, the continuous infusion will be favorable up to 5–6 hrs after start of the infusion, while the bolus infusion is more favorable than the continuous infusion only up to 1–1.5 hrs after start of the infusion (Fig. 2 and 4*C*).

As seen from Table 1, the direction of the changes in hematocrit follows the pattern expected from the articulated hypothesis as presented in the introductory section, in the sense that the continuous groups generally have lower hematocrit values than the bolus groups.

For the colloid groups and the control group, the lactate concentrations follow the expected pattern in relation to the PVs, which means that the lowest concentrations at the end of the experiment were seen for the group with the highest PV, that is, the continuous group of albumin. For 0.9% NaCl, the lactate concentrations were lower at the end of the experiment than in the colloid groups, despite the fact that the PVs were low and did not even differ from the control group. It is most likely that this does not mean that there was less lactate production in the 0.9% NaCl groups but rather that lactate was diluted in a larger interstitial volume in these groups because of the larger (4 times) volumes infused.

As the differences in urinary production were small between the continuous and the bolus groups, it is unlikely that the urine production influenced the difference in PV. The highest production of urine was seen in the albumin groups, which also had the largest PVs—most likely due to a lesser degree of hypovolemia in these groups.

Our finding of a generally better PV-expanding effect with 5% albumin than with the other solutions tested is in agreement with previous studies on rat and cat (16, 22).

The fact that there was a higher mortality rate before the end of the experiment in the control group compared with the other groups, supports the idea that fluid infusion is of importance for outcome in sepsis/SIRS.

Even though this study with its strict protocol has few clear limitations, one limitation is that the experiments were performed on the rat and therefore cannot be directly transferred to man. Especially, the results with HES suffer from limitations as HES is degraded by amylase, the concentrations of which are higher in rat than in man. Further, there may be some variations in the degree of sepsis and thus in degree of increase in microvascular permeability among animals, even if the surgical technique to induce sepsis was carefully standardized. An equal distribution of the intensity of sepsis in the groups is therefore of importance as plasma clearance for colloids is dependent on permeability.

In summary, this study in the septic rat showed that the plasma expansion of 5% albumin, 6% HES 130/0.4, and 4% gelatin was larger 3 hrs after the start of infusion when given with a slow infusion rate than when given with a fast infusion rate. This difference was more pronounced with albumin than with the other colloids. Given in equal volumes, the PV-expanding effect 3 hrs after start of the infusion was better for 5% albumin than for 6% HES and 4% gelatin. The plasma-expanding effect of 0.9% NaCl was not affected by the infusion rate, and 0.9% NaCl was not more effective than any of the colloids, even though it was given in a 4 times larger volume. If these results can be transferred to clinical practice, the total volume needed of colloids to maintain normovolemia in patients with sepsis/SIRS would be significantly reduced if given at a slow instead of a fast infusion rate.

ACKNOWLEDGMENT

We thank Helene Axelberg for skilled technical assistance.

REFERENCES

1. Fleck A, Raines G, Hawker F, et al: Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1985; 1:781–784
2. Groeneveld AB, Teule GJ, Bronsveld W, et al: Increased systemic microvascular albumin flux in septic shock. *Intensive Care Med* 1987; 13:140–142

3. Haskell A, Nadel ER, Stachenfeld NS, et al: Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J Appl Physiol* 1997; 83:407–413
4. Fishel RS, Are C, Barbul A: Vessel injury and capillary leak. *Crit Care Med* 2003; 31(8 Suppl):S502–S511
5. Huxley VH, Scallan J: Lymphatic fluid: exchange mechanisms and regulation. *J Physiol (Lond)* 2011; 589(Pt 12):2935–2943
6. Rippe B, Haraldsson B: Transport of macromolecules across microvascular walls: the two-pore theory. *Physiol Rev* 1994; 74:163–219
7. Mellander S, Johansson B: Control of resistance, exchange, and capacitance functions in the peripheral circulation. *Pharmacol Rev* 1968; 20:117–196
8. Dubniks M, Persson J, Grände PO: Effect of blood pressure on plasma volume loss in the rat under increased permeability. *Intensive Care Med* 2007; 33:2192–2198
9. Nygren A, Redfors B, Thorén A, et al: Norepinephrine causes a pressure-dependent plasma volume decrease in clinical vasodilatory shock. *Acta Anaesthesiol Scand* 2010; 54:814–820
10. Valeri CR, Donahue K, Feingold HM, et al: Increase in plasma volume after the transfusion of washed erythrocytes. *Surg Gynecol Obstet* 1986; 162:30–36
11. Persson J, Grände PO: Volume expansion of albumin, gelatin, hydroxyethyl starch, saline and erythrocytes after haemorrhage in the rat. *Intensive Care Med* 2005; 31:296–301
12. Woodard GE, Rosado JA: Natriuretic peptides in vascular physiology and pathology. *Int Rev Cell Mol Biol* 2008; 268:59–93
13. Huxley VH, Tucker VL, Verburg KM, et al: Increased capillary hydraulic conductivity induced by atrial natriuretic peptide. *Circ Res* 1987; 60:304–307
14. Imm A, Carlson RW: Fluid resuscitation in circulatory shock. *Crit Care Clin* 1993; 9:313–333
15. Grocott MP, Mythen M: Fluid therapy. *In:* Baillière's Clinical Anaesthesiology. Harcourt Publishers Ltd, 1999, pp 363–381
16. Persson J, Grände PO: Plasma volume expansion and transcapillary fluid exchange in skeletal muscle of albumin, dextran, gelatin, hydroxyethyl starch, and saline after trauma in the cat. *Crit Care Med* 2006; 34: 2456–2462
17. Mao EQ, Tang YQ, Fei J, et al: Fluid therapy for severe acute pancreatitis in acute response stage. *Chin Med J* 2009; 122:169–173
18. Maitland K, Kiguli S, Opoka RO, et al; FEAST Trial Group: Mortality after fluid bolus in African children with severe infection. *N Engl J Med* 2011; 364:2483–2495
19. Hubbard WJ, Choudhry M, Schwacha MG, et al: Cecal ligation and puncture. *Shock* 2005; 24 Suppl 1:52–57
20. Scheiermann P, Hoegl S, Revermann M, et al: Cecal ligation and incision: an acute onset model of severe sepsis in rats. *J Surg Res* 2009; 151:132–137
21. Margarson MP, Soni NC: Plasma volume measurement in septic patients using an albumin dilution technique: comparison with the standard radio-labelled albumin method. *Intensive Care Med* 2005; 31: 289–295
22. Dubniks M, Persson J, Grände PO: Plasma volume expansion of 5% albumin, 4% gelatin, 6% HES 130/0.4, and normal saline under increased microvascular permeability in the rat. *Intensive Care Med* 2007; 33: 293–299
23. Valeri CR, Cooper AG, Pivacek LE: Limitations of measuring blood volume with iodinated I 125 serum albumin. *Arch Intern Med* 1973; 132:534–538
24. Waitzinger J, Bepperling F, Pabst G, et al: Pharmacokinetics and Tolerability of a New Hydroxyethyl Starch (HES) Specification [HES (130/0.4)] after Single-Dose Infusion of 6% or 10% Solutions in Healthy Volunteers. *Clin Drug Investig* 1998; 16:151–160
25. Tuba J, Wiberg GS: On rat serum amylase. I. Studies in the normal and the alloxan diabetic animal. *Can J Med Sci* 1953; 31: 377–386
26. Gandhi RR, Bell DR: Influence of saline infusions on transvascular protein transport. *Am J Physiol* 1992; 262(2 Pt 2):H443–H450
27. Rippe B, Haraldsson B: Fluid and protein fluxes across small and large pores in the microvasculature. Application of two-pore equations. *Acta Physiol Scand* 1987; 131:411–428