

Aus der Klinik für Anästhesiologie
der Universität zu Lübeck

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***Plasma Volume Changes after 0.9% Saline Fluid Resuscitation in Sheep after
General Anesthesia, Mild Hemorrhage and Sepsis***

Inauguraldissertation

zur

Erlangung der Doktorwürde

der Universität zu Lübeck
-Aus der Sektion Medizin-



UNIVERSITÄT ZU LÜBECK

vorgelegt von
Kirk Brauer
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Lübeck 2011

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Tag der mündlichen Prüfung: 28.01.2013

Zum Druck genehmigt: Lübeck, den 28.01.2013

Plasma Volume Changes after 0.9% Saline Fluid Resuscitation in Sheep after General Anesthesia, Mild Hemorrhage and Sepsis

Introduction:

Perioperative fluid management is integral to the practice of anesthesiology. The goal of intravenous fluid administration is the maintenance of an adequate cardiac output to assure end organ perfusion and tissue oxygen delivery. The variety of clinical circumstances with which an anesthesiologist is confronted may demand a tailored approach to achieve said goal. The choice of volume substitute has historically centered on that between crystalloid and colloid volume expanders (when blood oxygen carrying capacity is adequate). While much has been debated on the suitability of one choice over the other for a variety of clinical circumstances, more questions than answers still remain. Our work focuses on the changes in plasma volume after infusion of a balanced crystalloid solution or 0.9% saline in sheep exposed to a variety of circumstances relevant to the clinical practice of anesthesiology: general anesthesia, hemorrhage and sepsis.

General Methodology:

To quantify the intravascular volume changes occurring after intravenous fluid administration, we employed an animal model of chronically instrumented sheep. Furthermore, we then applied both mass balance and volume kinetic approaches to the data obtained in sheep subjected to general anesthesia, hemorrhage as well as sepsis. All studies were approved by the Institutional Animal Care and Use Committee at the Texas Medical Branch (Galveston, TX).

Adult female merino sheep were chronically instrumented under halothane in oxygen anesthesia with a pulmonary artery catheter (Swan-Ganz; Baxter, Irvine, Ca) as well as femoral artery and femoral vein catheters (Intracath; Becton Dickinson, Sandy, UT). In addition, the sheep were splenectomized to prevent its contractile influence on blood hematocrit, as the spleen serves as a large, recruitable blood reservoir in these animals. After surgery, the sheep were allowed to recover for five days prior to experimentation, while given free access to food and water in metabolic cages and managed with an opioid for

pain control. The measurement catheters were connected to a continuous heparinized flush solution. Twenty four hours prior to each experimental protocol the animals were instrumented with a urinary bladder catheter (Sherwood Medical, St. Louis, MO) and withheld from access to food and water. During all protocols, the sheep were maintained in metabolic cages, therefore limiting their activity.

During experimental protocols, continuous hemodynamic variables were monitored via a four-channel system (Model 78304; Hewlett Packard, Santa Clara, Ca), including heart rate (HR), mean arterial pressure (MAP), pulmonary arterial pressure (PAP) and central venous pressure (CVP). Intermittent measurements of pulmonary arterial occlusion pressure (PAOP) were also performed. Blood temperature and cardiac output (CO) were monitored using a computer (9530 Baxter Edwards Critical Care). For all intravascular pressure measurements, the zero reference level was set at 12 cm above the sternal plane of each sheep. Urinary volumes were measured using a 250 ml graduated cylinder. Arterial blood gas and pH samples were withdrawn at intervals and measured (System 1302; Instrumentation Laboratory, Lexington, MA). Blood hemoglobin (Hb) and hematocrit (Hct) were measured from arterial blood samples (HemaVet; CDC Technologies, Oxford, CT). Total plasma protein (Prot) was analyzed via refractometer (Shuco; Tokyo, Japan) and plasma colloid osmotic pressure (COP) via oncometer (4100 Colloid Osmometer; Wescor, Logan, UT).

For each experimental protocol, the baseline plasma volume (PV) for each animal was measured using the Evans blue dye technique before volume loading. The calibration standard curves for this concentration analysis were determined for each animal before the dye infusion. Dye concentration was measured via a spectrophotometer (Model 1001, Spectronic; Milton Ray Company, Rochester, NY) at 620nm. Five minutes prior to each infusion measurement protocol, each animal received 3000 IU of heparin administered intravenously. All 0.9% saline infusions were administered intravenously over 20 min through the femoral vein catheter and consisted of 0.9% Saline (Baxter, Irvine, CA) kept in a temperature range of 39-40⁰C (normothermic for sheep): a

high-flow roller pump with warming coil and thermistor-regulated temperature-controlled bath was employed (Travenol Laboratories, Morton Grove, IL). The volume of 0.9% saline administered was 25 ml/kg body weight for each animal during each protocol. During the hemorrhage portion of the protocol, all animal were bled 300 ml over 5 min into a sterile blood donation bag (Teruflex Blood Bag Sytem, CPDA-1 Solution; Terumo Corporation, Tokyo, Japan). The preserved blood (at 20 ° C) was re-infused via the central venous catheter into the animal at the conclusion of the protocol.

Throughout each volume loading protocol, hematocrit (Hct) and hemoglobin (Hb) were measured and recorded three times during the 45 min baseline measurement period prior to infusion begin, and then every five minutes during the 180-min duration of each experimental protocol, with the 20 min volume infusion beginning at time 0 min. The urinary output was measured every five minutes as well. Intermittently measured hemodynamic parameters were recorded hourly (CO, PAOP) and dependent parameters calculated such as the systemic vascular resistance (SVR). Plasma volume was calculated at a time interval (t) from the following relation (Equation 1):

$$PV(t) = PV_0 \cdot [(Hb_0 - Hb_t) / Hb_t] / (1 - Hct) \quad (1)$$

The ensuing plasma volume expansion (PVE) after 0.9% saline infusion was corrected to account for each 2 ml sample of blood withdrawn throughout each experiment based on the assumption that, in sheep, the baseline blood volume (in L) was 6% of body weight (in Kg).

A two-volume kinetic model was found to fit our data better than a on-volume-of-fluid space model, i.e. a fitting of the data in the first instance resulted in a statistically lower squared difference between the theoretical and experimental data point. Essentially, fluid infused at a rate k_i is distributed in an expandable space with a volume (v_1) and communicating with a peripheral fluid space of another volume (v_2). The net rate of fluid exchange between these expandable volumes (v_1 and v_2) is proportional by a constant (k_t) to the relative difference in deviation from their baseline target volumes (V_1 and V_2).

Fluid elimination out of the volume v_1 occurs at a baseline rate k_b , as well as at a rate proportional by a constant (k_r) to the deviation from the target volume V_1 . Thus, the following differential equations describe the dilution changes at time (t) in v_1 and v_2 (Equations 2 and 3):

$$dv_1 / dt = k_i - k_b - k_r [(v_1 - V_1) / V_1] - k_t \{ [(v_1 - V_1) / V_1] - [(v_2 - V_2) / V_2] \} \quad (2)$$

$$dv_2 / dt = k_t \{ [(v_1 - V_1) / V_1] - [(v_2 - V_2) / V_2] \} \quad (3)$$

Matlab version 4.2 (Math Works Inc., Natick, MA) was used to model the kinetic equations, whereby a nonlinear, least-squares regression routine was repeated until no parameter changed by more than 0.1%. Since the plasma volume PV is assumed to constitute a portion of V_1 , the dilution of plasma volume at time (t) can be used to indicate $(v_1 - V_1) / V_1$ at time (t) such that (Equation 4):

$$(v_1 - V_1) / V_1 = [(Hb_0 - Hb_t) / Hb_t] / (1 - Hct) \quad (4)$$

Additional parameters reflecting bleed rate and renal clearance were introduced during the hemorrhage protocols, complicating somewhat these basic equations. The parameters V_1 , V_2 and k_t are given as best estimates with standard errors since they result from a nonlinear regression fitting of the data in one single analysis.

Data for these experiments are presented as mean \pm SD and statistical comparisons were made using repeated-measures analysis of variance. $P < 0.05$ was considered statistically significant. Data analysis was conducted using SAS[®], Release 8.2 (SAS, Cary, NC).

With the above description of our general experimental protocol and data analysis, we undertook the analysis of plasma volume changes in sheep after a 20 min, 25ml/kg, volume infusion of 0.9% saline under three different study conditions: (1) with general isoflurane anesthesia, (2) with mild (300 ml) controlled hemorrhage, and (3) with early and late sepsis induced via live *Pseudomonas aeruginosa* (*P. aeruginosa*) infusion (not shock).

Study (1):

Publication:

Volume Kinetic Analysis of the Distribution of 0.9% saline in Conscious versus Isoflurane-anesthetized Sheep, Kirk J. Brauer, MD., Christer Svensén, MD., Ph.D., Robert G. Hahn, MD., Ph.D., Lillian D. Traber, R.N., Donald S. Prough, MD. *Anesthesiology* 2002; 96: 442-9

Methods:

For the experimentation involving the volume infusion of 0.9% saline under general anesthesia, six sheep with a mean weight of 42 ± 5 Kg were subjected to two randomly ordered infusion protocols of 25 ml/Kg 0.9% saline over 20 min: one during 1.5% (minimum alveolar concentration for sheep = 1.53%) isoflurane in oxygen (Abbott Laboratories, Chicago, IL) with volume ventilation without positive end-expiratory pressure (Ohmeda, West Yorkshire, United Kingdom) and the other conscious with spontaneous ventilation (controls). Each protocol separated from the other by a least 24 h (Table 1). In addition, during general anesthesia, the ventilation was adjusted to maintain the hemoglobin oxygen saturation greater than 90% and end-tidal carbon dioxide at 30-32 mmHg.

Table 1:

	Protocol 1	----->	Protocol 2
n = 6 Sheep		24 h Recovery	
	Protocol 2	----->	Protocol 1

All animals tolerated the experimental procedures well, showing an increase in arterial and venous pressures during the infusion, which returned to baseline during the period thereafter. Also, during isoflurane anesthesia, sheep had a lower cardiac index (CI) and a higher HR, MAP and CVP ($P > 0.05$). A statistically significant decrease (albeit slight) in temperature was recorded during general anesthesia for the period beginning one hour after the infusion.

Results:

Simulation curves based on volume kinetic parameters agreed well with the dilution reflected by mass balance. Evans blue dye calculations revealed a baseline plasma volume of 1603 ± 91 ml and 1662 ± 122 ml for the isoflurane and control experiments respectively. An increased plasma volume, calculated by mass balance, of 510 ± 48 ml and 416 ± 67 ml was measured at the end of infusion for the isoflurane and control experiments respectively, corresponding to $50 \pm 6\%$ and $40 \pm 5\%$ of the infused volume ($P < 0.02$). In addition, during the last 60 min of the experiments, volume expansion was significantly less pronounced during isoflurane anesthesia compared to control experiments ($P < 0.03$). Moreover, a slightly smaller increase of the central volume (V_1) during isoflurane anesthesia was indicated by volume kinetic analysis when compared to mass balance calculations.

The measured urinary volume was significantly ($P < 0.03$) lower during isoflurane anesthesia (median of 9.0 ml and range: 4.0 - 150 ml) when compared to that in the controls (median 863 ml and range: 604 - 1122 ml). Moreover, while k_r (predicted urinary flow rate in the volume kinetic model) agreed well with the measured flow rate in the controls, it did not during isoflurane anesthesia: a theoretically calculated k_r using median urinary excretion approximated 35 ml/min in the controls (conscious) and 0.6 ml/min in the isoflurane anesthetized sheep.

Table 2:

Protocol	PV at Baseline (ml)	PVE after Infusion (%)	Median Urinary Volume (ml)
Control	1622 ± 122	40 ± 5	863
Isoflurane	1603 ± 91	50 ± 6	9
P-Value	> 0.05	< 0.02	< 0.03

Conclusion:

From these experiments comparing plasma volume changes after 0.9% saline infusion in conscious, normovolemic sheep to those in the same normovolemic sheep while anesthetized with isoflurane and mechanically ventilated, we have learned that a similar expansion of intravascular volume is achieved, despite a markedly different distribution. Indeed, in conscious, normovolemic sheep, the infused crystalloid volume is primarily lost in the urine; while in isoflurane-anesthetized, mechanically ventilated, normovolemic sheep, the infused volume is lost into the peripheral, interstitial volume. Therefore, these data suggest that isoflurane, mechanical ventilation, or both are associated with peripheral accumulation of infused 0.9% saline.

Study (2):

Publication:

Volume Turnover Kinetics of Fluid Shifts after Hemorrhage, Fluid Infusion, and the COmbination of Hemorrhage and Fluid Infusion in Sheep, Ake Norberg, MD., Ph.D., Kirk I. Brauer, MD., Donald S. Prough, MD., Johan Gabrielsson, Ph.D., Robert G. Hahn, MD., Ph.D., Tatsuo Uchida, M.S., Daniel L. Traber, Ph.D., Christer H. Svensén, MD., Ph.D. *Anesthesiology* 2005; 102: 985-94

Methods:

For the experimentation involving the volume infusion of 0.9% saline with mild hemorrhage, twelve sheep weighing 39.0 ± 5.9 kg were subjected to three experimental protocols in random order with a 48 h minimum recovery time between experiments: (a) in the first protocol (infusion only), the sheep received 25 ml/kg of 0.9% saline over 20 min, (b) in the second protocol (hemorrhage only), the sheep were bled 300 ml over 5 min, and (c) in the third protocol (hemorrhage-plus-infusion), the sheep were subjected to 300 ml blood loss over 5 min followed immediately by infusion of 25 ml/kg of 0.9% saline over 20 min (Table 3).

Table 3:

n = 12 Sheep:		Infusion Only	
		↓	
		48 h Recovery	
	↓		↓
	Hemorrhage only		Hemorrhage-plus-Infusion

All animals tolerated the three experimental procedures well. There were no significant differences in CO between the two hemorrhage protocols; whereas at 65 min after the start of the protocol, CO was significantly ($P < 0.05$) decreased in these two hemorrhage protocols compared to that in the infusion only protocol. In the hemorrhage only protocol, MAP was transiently decreased, while MAP was transiently increased in the infusion only protocol. In the hemorrhage-plus-infusion protocol, a significantly higher MAP was measured at the end of the fluid infusion compared with that in hemorrhage only.

Results:

Mass balance analysis showed significant differences in fractional changes of plasma volume between experimental protocols. Compared to the same baseline values (plasma volume = 1.61 ± 0.23 l), plasma volume dilution was significantly decreased immediately after hemorrhage and increased after infusion for the remainder of the protocol. Between hemorrhage only protocol and both infusion protocols, plasma volume dilution was significantly increased in the infusion protocols immediately after infusion and for 60 min thereafter. While, between hemorrhage only and infusion only protocols, plasma volume dilution was significantly decreased immediately after hemorrhage and for 60 min thereafter. Interestingly, when comparing infusion protocols, antecedent hemorrhage (hemorrhage-plus-infusion protocol) did not increase the magnitude of the plasma dilution, although the absolute dilution of hemoglobin

concentration was greater once hemorrhage was completed (Table 4). Mass balance analysis of transcapillary flow into the plasma volume during the 3-h procedure measured significant ($P < 0.05$) differences among the three protocols: for infusion only -114 ml / 180 min, for hemorrhage only 516 / 180 min, and for hemorrhage-plus-infusion -188 ml / 180 min (Table 5).

Table 4:

Significant (p<0.05)PVE compared to	Baseline	End-of-Infusion in Infusion-Only	End-of-Infusion in Hemorrhage-plus-Infusion	End-of-Hemorrhage in Hemorrhage-Only	End-of-Hemorrhage in Hemorrhage-plus-Infusion	60 min after Infusion in Infusion-Only	60 min after Infusion in Hemorrhage-plus-Infusion
Baseline	-	+	+	+	+	+	+
End-of-Infusion in Infusion-Only		-	-	+	-	-	-
End-of-Infusion in Hemorrhage-plus-Infusion			-	+	+	-	-
End-of-Hemorrhage in Hemorrhage-Only				-	-	+	+
End-of-Hemorrhage in Hemorrhage-plus-Infusion					-	+	+
60 min after Infusion in Infusion-Only						-	-
60 min after Infusion in Hemorrhage-plus-Infusion							-

Urinary output was significantly decreased by $70 \pm 20\%$ and $37 \pm 25\%$ for the hemorrhage only and hemorrhage-plus-infusion protocols when compared to the 0.9% saline infusion only protocol (Table 5). The 300 ml hemorrhage

volume constituted a fraction of 0.132 ± 0.019 of the blood volume, which significantly correlated with impairment of renal excretion ($r = 0.73$, $P < 0.01$).

Table 5:

Protocol	Transcapillary Flow (ml / 180 min)	Change in Urinary Output to Infusion-Only Protocol (%)
Infusion-Only	-114	----
Hemorrhage-Only	516	70 ± 20
Hemorrhage-plus-Infusion	-188	37 ± 25
P-Value	< 0.05	< 0.05

The consistency was good between observed and predicted data for the volume kinetic model amended for this set of protocols with two additional terms: one accounting for the recruitment of fluid into the central compartment after hemorrhage Cl_{bleed} (in ml / min; zero in the absence of bleeding) and the second, $b_{rate} (1-Hct)$, accounting for the loss plasma volume secondary to the bleed as follows (Equation 5):

$$dv_1 / dt = k_i - k_b - k_r [(v_1 - V_1) / V_1] - k_t \{ [(v_1 - V_1) / V_1] - [(v_2 - V_2) / V_2] \} - Cl_{bleed} - b_{rate} (1-Hct)$$

With b_{rate} the amount of bleeding divided by the bleeding time. In addition, since the urinary output is a very important factor in the analysis of these data, k_b was modeled as the product of Cl_R and $e^{(\alpha \cdot v_1)}$, where Cl_R is the baseline renal excretion at normal hydration, and alpha an exponent that describes the alteration of urinary output in response to changes of v_1 .

Each protocol resulted in a central volume dilution protocol characterized by depletion of volume at the end of hemorrhage and maximal dilution at the end of fluid infusion followed by stabilization of central volume at a level slightly above

baseline. The impairment of renal output related to the ratio between the amount of bleeding and the calculated blood volume. The applied volume turnover model was able to explain the dynamics of volume flow into V_1 .

In this study, hemorrhage in sheep caused a decrease of renal output, which strongly influenced volume kinetics, regardless of subsequent 0.9% saline fluid infusion. The application of volume turnover kinetics to this fluid shift protocol provides an important elaboration of the existing volume kinetic approaches: the current model could predict volume changes in a broader range of perturbations that more closely resemble clinically relevant scenarios.

Conclusion:

From these experiments comparing plasma volume dilution after 0.9% saline infusion in sheep with and without prior hemorrhage, as well as after hemorrhage alone, we have learned that, with antecedent hemorrhage, urinary output may not be a good endpoint to monitor as guide to hydration. Indeed, in this study there a marked impairment of urinary output was noted after hemorrhage, which caused an accumulation of the infused 0.9% saline mainly outside the central compartment. Therefore, patients undergoing surgery, who are concomitantly exposed to hemorrhage, may also be at risk of being overly hydrated, especially if urinary output is used as a monitor of hydration. Furthermore, according to this kinetic analysis, since the physiologic responses to hypovolemia reverse slowly, the main effect of 0.9% saline infusion during hemorrhage may be an undesired expansion of the peripheral interstitial compartment.

Study (3):

Publication:

Sepsis Produced by Pseudomonas Bacteremia Does Not Alter Plasma Volume Expansion After 0.9% Saline Infusion in Sheep, Christer H. Svensén, MD, PhD, Bryan Clifton, MD, Kirk I. Brauer, MD, Joel Olsson, MD, PhD, Tatsuo Uchida, MS, Lillian D. Traber, RN, Daniel L. Traber, PhD, and Donald S. Prough, MD, *Anesthesia and Analgesia* 2005; 101: 835-42

Methods:

For the experimental condition involving the volume infusion of 0.9% saline with early and late sepsis, six sheep weighing 42 ± 5 kg received an infusion of live *P. aeruginosa* ($6 \cdot 10^6$ colony-forming units \cdot kg⁻¹ \cdot h⁻¹) for the duration of the experiment and were subjected to three 25 ml / kg of 0.9% saline infusion protocols over 20 min at different time intervals from the initiation of the *P. aeruginosa* infusion: (a) the first infusion, twenty-four hours before initiation, controls, (b) the second infusion, four hours after initiation, during early sepsis, and (c) the third infusion, twenty-four hours after initiation, during late sepsis (Table 6).

Table 6:

Day 1	<i>No P. aeruginosa Infusion</i>	First Volume Infusion	Controls (n=6)
↓	24 hours later		
Day 2	<i>P. aeruginosa Infusion begins</i>		
↓	4 hours later	Second Volume Infusion	Early Sepsis (n=6)
	24 hours later		
Day 3	<i>P. aeruginosa Infusion continued</i>	Third Volume Infusion	Late Sepsis (n=5)

In order to avoid hypovolemia and hence septic shock during the bacterial infusion, the sheep received an empirically determined infusion of lactated Ringer's solution at a constant rate of $2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. The animals tolerated the experimental procedures well, with the exception of one, which died twenty hours after initiation of the *P. aeruginosa* infusion.

Results:

Compared to controls, at the end of the protocol (time = 180 min), HR and blood temperature were significantly higher in both early and late sepsis, while CO, PAP and CVP tended to be higher. SVR was significantly lower in the sepsis

protocols compared to controls, while PAOP tended to be lower. HR was significantly more rapid at the end of the measurement interval during early sepsis compared to late sepsis. Also at the end of the protocol, MAP was not statistically different among the three protocols. Neither did arterial blood gases demonstrate any differences among the three protocols (Table 7).

Table 7:

Significant difference compared to Parameter in Controls:	Early Sepsis	Late Sepsis
Heart Rate	+	+
Blood Temperature	+	+
Cardiac Output	-	-
Pulmonary Artery Pressure	-	-
Central Venous Pressure	-	-
Systemic Vascular Resistance	+	+
Pulmonary Artery Occlusion Pressure	-	-
Mean Arterial Pressure	-	-
Blood Gas Analysis	-	-
Total Plasma Protein	+	+
Colloid Oncotic Pressure	+	+

Compared to controls, at the end of the measurement interval, total plasma protein concentration was decreased in both sepsis protocols, with the concentration again significantly decreased in late versus early sepsis. The same decreased constellation for colloid oncotic pressure was measured as well, i.e significantly decreased in both sepsis protocols compared to controls, and again decreased in the late versus early sepsis protocols at the end of the measurement interval (Table 8).

Table 8:

Parameter	Significant Difference between Early and Late Sepsis
Heart Rate	+
Total Plasma Protein	+
Colloid Oncotic Pressure	+

At the beginning of the control, early sepsis and late sepsis, PV as measured by the Evan's blue dye method was 1750 ± 114 , 1678 ± 100 and 2128 ± 156 ml, respectively. For the protocols, PVE was 312 ± 50 , 386 ± 34 , and 400 ± 51 ml, respectively. Furthermore, PVE at the end of the measurement intervals was measured as 97 ± 21 ml for controls, 151 ± 40 ml in early and 102 ± 44 ml in late sepsis, respectively (Table 9).

Table 9:

Parameter	Controls	Early Sepsis	Late Sepsis
Plasma Volume (ml)	1750 ± 114	1678 ± 100	2128 ± 156
PVE at end of Infusion (ml)	312 ± 50	386 ± 34	400 ± 51
PVE 160 min after Infusion (ml)	97 ± 21	151 ± 40	102 ± 44

The volume kinetic analysis also showed similar results for the plasma volume dilution in the three protocols, again, with the two-volume model being statistically preferable to the one-volume model. The elimination rate constant k_r was not statistically different among the three protocols with a mean of 83 ± 16 , 109 ± 55 , and 41 ± 13 ml / mi for control, early and late sepsis respectively.

Conclusion:

From these experiments comparing plasma volume expansion after 0.9% saline infusion, we have learned that neither mass balance nor volume kinetic analysis showed any differences in crystalloid distribution or elimination between conscious, nonseptic sheep and conscious, normovolemic, hyperdynamic, septic sheep. Even with significantly altered reductions in total plasma protein and colloid oncotic pressure during sepsis, the gradient between intravascular and interstitial spaces was not sufficiently altered to change the distribution of infused 0.9% saline in relation to that in nonseptic control sheep.

Perioperative fluid management is integral to the practice of anesthesiology. The goal of intravenous fluid administration is the maintenance of an adequate cardiac output to assure end organ perfusion and tissue oxygen delivery. The variety of clinical circumstances with which an anesthesiologist is confronted may demand a tailored approach to achieve said goal. The choice of volume substitute has historically centered on that between crystalloid and colloid volume expanders. While much has been debated on the suitability of one choice over the other for a variety of clinical circumstances, more questions than answers still remain. Our work focuses on the changes in plasma volume after infusion of a balanced crystalloid solution or 0.9% saline in sheep exposed to a variety of circumstances relevant to the clinical practice of anesthesiology: general anesthesia, hemorrhage and sepsis.

In order to quantify the resultant plasma volume changes after infusion, we selected a chronically instrumented adult female merino sheep model. This model enabled the continuous measurement of hemodynamic data as well as the measurement of blood haemoglobin concentration every five minutes during the experimental protocols. With the determination of the baseline plasma volume through the Evan's blue dye technique, plasma volume changes could be ascertained every five minutes after a 20 min 0.9% saline infusion. In addition to the mass balance analysis, a two-volume kinetic analysis model was also employed to quantify volume shifts between intravascular and peripheral compartments after the saline infusion.

The experimental protocols undertaken under general anesthesia consisted in subjecting the sheep to 1.5% isoflurane in oxygen controlled ventilation anesthesia, and comparing the data to that in the conscious state. The experimental protocols undertaken under mild hemorrhage consisted in subjecting the sheep to a 300 ml bleed with and without immediate infusion, and comparing the data to that without bleeding. The experimental protocols undertaken during sepsis consisted in subjecting the sheep to a *Pseudomonas aeruginosa* bacteremia, and comparing the data during early and late stage sepsis to that in the nonseptic state.

From these experiments comparing plasma volume changes after 0.9% saline infusion in conscious, normovolemic sheep to those in the same normovolemic sheep while anesthetized with isoflurane and mechanically ventilated, we have learned that a similar expansion of intravascular volume is achieved, despite a markedly different distribution. Indeed, in conscious, normovolemic sheep, the infused crystalloid volume is primarily lost in the urine; while in isoflurane-anesthetized, mechanically ventilated, normovolemic sheep, the infused volume is lost into the peripheral, interstitial volume. Therefore, these data suggest that isoflurane, mechanical ventilation, or both are associated with peripheral accumulation of infused 0.9% saline.

From these experiments comparing plasma volume dilution after 0.9% saline infusion in sheep with and without prior hemorrhage, as well as after hemorrhage alone, we have learned that, with antecedent hemorrhage, urinary output may not be a good endpoint to monitor as guide to hydration. Indeed, in this study there a marked impairment of urinary output was noted after hemorrhage, which caused an accumulation of the infused 0.9% saline mainly outside the central compartment. Therefore, patients undergoing surgery, who are concomitantly exposed to hemorrhage, may also be at risk of being overly hydrated, especially if urinary output is used as a monitor of hydration. Furthermore, according to this kinetic analysis, since the physiologic responses to hypovolemia reverse slowly, the main effect of 0.9% saline infusion during hemorrhage may be an undesired expansion of the peripheral interstitial compartment.

From these experiments comparing plasma volume expansion after 0.9% saline infusion, we have learned that neither mass balance nor volume kinetic analysis showed any differences in crystalloid distribution or elimination between conscious, nonseptic sheep and conscious, normovolemic, hyperdynamic, septic sheep. Even with significantly altered reductions in total plasma protein and colloid oncotic pressure during sepsis, the gradient between intravascular and interstitial spaces was not sufficiently altered to change the distribution of infused 0.9% saline in relation to that in nonseptic control sheep.

Deutsche Zusammenfassung

Veränderungen des Plasmavolumens durch Infusion einer 0,9%-Kochsalzlösung beim Schaf nach Allgemeinanästhesie, mittlerem Blutverlust und Sepsis

Die Steuerung des Flüssigkeitshaushaltes ist integraler Bestandteil der Aufgaben des Anästhesisten. Ziel der intravenösen Infusion von Lösungen ist die Aufrechterhaltung eines adäquaten Herzzeitvolumens um die Durchblutung der Organe und die Sauerstoffversorgung des Gewebes zu gewährleisten. Da vom behandelnden Anästhesisten eine Reihe von klinischen Bedingungen beachtet werden müssen, ist ein rationaler Ansatz notwendig um die vorgegebenen Ziele zu erreichen. Für die Auswahl des Volumenersatzmittels stehen kolloidale und kristalloide Lösungen zur Verfügung. Die differenzierte Eignung der einzelnen Volumenersatzmethoden in den verschiedenen klinischen Situationen wird anhaltend diskutiert und ist noch nicht abschließend beantwortet. In unserer hier vorliegenden Arbeit wird die Veränderung des Plasmavolumens bei Infusion einer balancierten kristalloiden oder 0,9%iger Kochsalzlösung im Schafmodell in folgenden klinisch für die Narkoseführung relevanten Situationen untersucht: Allgemeinanästhesie, Blutverlust und Sepsis.

Es wurde ein tierexperimentelles Modell am chronisch instrumentierten erwachsenen weiblichen Merino-Schaf eingesetzt, um die Veränderungen des Plasmavolumens nach Infusionstherapie zu untersuchen. Dieses Modell ermöglicht während des Versuchsablaufs die kontinuierliche Messung hämodynamischer Daten sowie die fünfminütliche Messung der Hämoglobinkonzentration im Blut. Das Plasmavolumen wurde am Versuchsbeginn mit der Farbstoffverdünnungsmethode mit Evan's Blue bestimmt. Nach der Infusion der Kochsalzlösung über 20 Minuten wurde im Abstand von fünf Minuten das Plasmavolumen ermittelt. Zusätzlich zu der Analyse der Massenbilanz wurde mit einem kinetischen 2-Volumen-Modell die Volumenverschiebungen zwischen dem Intravasalraum und dem Interstitium gemessen.

Im experimentellen Protokoll für die Bedingung „Allgemeinanästhesie“ wurden die Schafe mit dem Inhalationsanästhetikum Isofluran in einer Konzentration von 1,5 % in Sauerstoff beatmet. Zur Kontrolle wurden die Werte von wachen Schafen herangezogen. Für die Bedingung „moderater Blutverlust“ wurde den Versuchstieren 300 ml Blut entnommen. Sofort danach wurde das Infusionsschema gestartet. Eine Gruppe erhielt keine Infusionstherapie. Als Kontrollen dienten Versuchstiere ohne Blutverlust. Für die Bedingung „Sepsis“ wurde bei den Schafen eine Bakteriämie mit *Pseudomonas aeruginosa* erzeugt. Die dann folgenden Phasen der frühen und späten Sepsis wurden mit Kontrolltieren ohne Sepsis verglichen.

Aus diesen Experimenten ergab sich, dass der intravasale Volumenerffekt nach Infusion einer physiologischen Kochsalzlösung bei wachen, normovolämischen Schafen gegenüber normovolämischen Schafen während Inhalationsanästhesie und mechanischer Beatmung ähnlich waren, obwohl sich die Verteilungswege deutlich unterschieden. In der wachen normovolämischen Gruppe wird die infundierte kristalloide Flüssigkeit hauptsächlich renal ausgeschieden, während bei beatmeten normovolämischen Tieren in Inhalationsanästhesie mit Isofluran das Volumen in das periphere Interstitium verschoben wird. Dies lässt die Schlussfolgerung zu, dass das Inhalationsanästhetikum Isofluran, die maschinelle Beatmung oder beides zu einer Ansammlung von infundierter Flüssigkeit im Gewebe führen.

Die Untersuchung der Veränderungen durch die Infusionstherapie mit und ohne vorherigen Blutverlust oder durch Blutverlust allein legen nahe, dass im Zustand nach Blutverlust die Diurese nicht zur Steuerung des Infusionsbedarfs herangezogen werden sollte. Blutverlust führte in unserer Studie zu einer deutlichen Verminderung der Diurese und einer extravasalen Akkumulation von NaCl 0,9% mit Flüssigkeitsansammlung im peripheren Gewebe. Daher besteht bei Patienten während eines operativen Eingriffs mit Blutverlust das Risiko einer überschießenden Infusionstherapie, wenn nur die Diurese als Parameter für die Volumentherapie herangezogen wird. Darüberhinaus lässt diese kinetische Analyse darauf schließen, dass sich die physiologische Antwort auf die Hypovolämie sich nur langsam zurückbildet und sich als Haupteffekt der

Infusion von 0,9% Kochsalzlösung im Rahmen eines Blutverlustes eine unerwünschte Expansion des peripheren, interstitiellen Kompartiments einstellt.

Bei dem Vergleich der Plasmavolumeneffekte nach Infusion von Kochsalzlösung ergibt sich, dass bei Vergleich wacher, nichtseptischer Schafe mit wachen normovolämischen septisch-hyperdynamen Schafen weder die Messung der Massenbilanz noch die kinetische Volumenanalyse Unterschiede in der Verteilung oder Elimination der infundierten kristalloiden Flüssigkeit zeigt. Sogar bei der signifikanten Verminderung in der Konzentration an Plasmaproteinen und an kolloid-osmotischem Druck bei der Sepsis ergab sich, dass sich der Gradient zwischen intravasalen und interstitiellen Räumen nicht ausreichend veränderte um die Verteilung der infundierten Lösung gegenüber den Kontrolltieren zu verschieben.

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5/2008 You're Great Award– Methodist Medical Center, Dallas: Excellence in patient care in severe emergency

2/2007 Letter of Gratitude– Methodist Medical Center, Dallas: For providing outstanding patient care and saving the Methodist solid organ transplant service (along with two colleagues)

6/2001-2002 Chief Resident Award

5/2002 1st Place Original Research Award from University of Texas Galveston, Gulf Atlantic Anesthesiology Resident Research Conference,

5/2001 1st Place Original Research Award from University of North Carolina Chappel Hill, Gulf Atlantic Anesthesiology Resident Research Conference,

5/2000 1st Place Original Research Award from University of South Florida College of Medicine, Gulf Atlantic Anesthesiology Resident Research Conference,

6/1990 Phi Beta Kappa

6/1990 Golden Key National Honor Society

6/1990 Outstanding Engineering Graduate Award, Revelle

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Sponsored National Lectures:

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Managing Postanesthetic Hypertension

Perioperative Hypertension

Diastolic Heart Failure

Intravenous Antihypertensive Agents

TEE and Hypertensive Heart Disease

Endocrine Management of Heart Failure

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Vasoactives in Trauma Resuscitation

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Fluid Resuscitation Techniques

Invited National/International Lectures:

Texas Society of Anesthesiologists Annual Meeting 2007: Regional Anesthesia Workshop, Brachial Plexus Blockade

Texas Society of Anesthesiologists Annual Meeting 2006: Transesophageal Echocardiography Workshop, The Basic intraoperative TEE exam on the cardiac patient

Texas Society of Anesthesiologists Annual Meeting 2005: Regional Anesthesia Workshop, Lumbar Plexus Blockade

Postgraduate continuing medical education course in Botucatu, Brazil 2004: Curso de Atualização em Anestesiologia Medicina Unesp/Botucatu 2004 Carreadores de oxigênio por hemoglobina modificada (Modified Hemoglobin Oxygen Carriers – Cardiopulmonary effects)

Acknowledgement :

This work would not have been possible without the generous support of my mentor Prof. Dr. Prough, MD.

In addition, it is my privilege to thank both Prof. Dr. Schmucker and Prof. Dr. Klotz for their patient assistance in bringing this process to fruition.

For his unceasing questions and kind guidance I should like to thank Dr. Traber, PhD and the fine staff at the Investigational Intensive Care Unit at UTMB, Galveston. Especially Lillian Traber, RN for her patience and guidance in the surgical preparation and care of our research animals.

Many an acute situation would not have found a quick and elegant solution without the help and assistance of Dr. Deyo.

For the new and exciting kinetic analysis approach I am most indebted to Drs. Hahn and Svensén.

For his patience and resolution of statistical problems I am thankful to Mr. Uchida, MS.

I have had also the pleasure of invigorating thought exchanges on hemodynamics and fluid resuscitation with Dr. Kramer.

The great staff and residents at the department of anesthesiology at UTMB for making it possible for the many away-presentations to be a success.

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1996 - 1998	Extern (AiP), Emergency Medicine University of California at San Diego
1990 - 1996	School of Medicine Medizinische Universität zu Lübeck, Germany
1986 - 1990	Summa cum Laude BA Bioengineering:premedical University of California, San Diego
1984 - 1986	Kreisgymnasium in Freiburg, Germany
1973 - 1984	Elementary and Highschool in Málaga, Spain
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Volume Kinetic Analysis of the Distribution of 0.9% Saline in Conscious versus Isoflurane-anesthetized Sheep

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Background: The distribution and elimination of 0.9% saline given by intravenous infusion has not been compared between the conscious state and during inhalational anesthesia.

Methods: Six adult sheep received an intravenous infusion of 25 ml/kg of 0.9% saline over 20 min in the conscious state and also during isoflurane anesthesia and mechanical ventilation. The distribution and elimination of infused fluid were studied by volume kinetics based on serial analysis of hemoglobin dilution in arterial blood and by mass balance that incorporated volume calculations derived from volume kinetic analysis and measurements of urinary volumes.

Results: The mass balance calculations indicated only minor differences in the time course of plasma volume expansion between the conscious and anesthetized states. However, isoflurane anesthesia markedly reduced urinary volume (median, 9 vs. 863 ml; $P < 0.03$). In conscious sheep, the central and peripheral volume expansion predicted by volume kinetics agreed well with the calculations based on mass balance. However, during isoflurane anesthesia and mechanical ventilation, calculation using volume kinetic analysis of the variable k_e , an elimination factor that, in conscious humans and sheep, is closely related to urinary excretion, represented both urinary excretion and peripheral accumulation of fluid. This suggests that the previous assumption that k_e approximates urinary excretion of infused fluid requires modification, *i.e.*, k_e simply reflects net fluid movement out of plasma.

Conclusions: In both conscious and anesthetized, mechanically ventilated sheep, infusion of 0.9% saline resulted in minimal expansion of plasma volume over a 3-h interval. In conscious sheep, infused 0.9% saline was rapidly eliminated from the plasma volume by urinary excretion; in contrast, the combination of isoflurane anesthesia and mechanical ventilation reduced urinary excretion and promoted peripheral accumulation of fluid.

INTRAVENOUS infusion of a balanced crystalloid solution or 0.9% saline is customarily used for volume replacement during surgery. Such fluid is believed to be evenly distributed throughout the extracellular fluid space. Because the extracellular fluid volume is 150–200 ml/kg, of which plasma volume represents 30–40 ml/kg, no more than one fifth of unexcreted fluid should remain within

the plasma volume after equilibration.¹ Experimental² and clinical data³ using isotopes allowed to equilibrate for more than 1 h confirm this concept. During or shortly after the infusion of fluid,⁴ however, volume expansion is more pronounced, which may explain the convention of replacing 1 ml of blood loss with only 3 ml of balanced salt solution or 0.9% saline. Moreover, rapid blood loss induces capillary refill from the interstitial fluid, which alters the kinetics of infused fluids and further reduces fluid requirements.^{5,6} Other factors that could also influence the volume effects of infused fluid include vasodilation, the magnitude of urinary excretion, pharmacologic effects of general anesthetics, and physiologic effects of adjunctive interventions such as mechanical ventilation.

To quantify the influence of isoflurane anesthesia on the kinetics of infused crystalloid, we administered 0.9% saline by intravenous infusion to sheep in the conscious state and during isoflurane anesthesia. Serial measurements of blood hemoglobin concentration were used to estimate the distribution of the infused fluid by two different approaches: volume kinetic analysis^{4,6,7} and mass balance. Based on previous data using volume kinetics to assess the effects of fluid infusion during spinal anesthesia,⁸ we hypothesized that the decreased sympathetic activity caused by isoflurane anesthesia⁹ would increase plasma expansion by the infused fluid as compared with the conscious state.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch (Galveston, TX). Six adult female merino sheep weighing between 35 and 52 kg (mean, 42 ± 5 kg) were studied. At least 48 h previously, each had been splenectomized during halothane anesthesia and had a pulmonary arterial catheter (Swan-Ganz; Baxter, Irvine, CA) and bilateral femoral arterial and venous catheters (Intracath; Becton Dickinson, Sandy, UT) inserted during sterile conditions. Each animal was subjected to two randomly ordered experiments that were separated by at least 24 h. In the first protocol, in which plasma volume expansion was studied in the conscious state, animals received 25 ml/kg of 0.9% saline over 20 min. In the second protocol, animals received an infusion of 25 ml/kg of 0.9% saline over 20 min during 1.5% (minimum alveolar concentration for sheep = 1.53%¹⁰) isoflurane (Abbott Laboratories, Chicago, IL) and positive pressure ventilation (Datex Engstrom, Helsinki, Finland).

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Received from the Department of Anesthesiology, The University of Texas Medical Branch, Galveston, Texas; and The Karolinska Institute, Stockholm, Sweden. Submitted for publication March 9, 2001. Accepted for publication September 12, 2001. Supported by departmental funds provided through the Rebecca Terry White Distinguished Chair in Anesthesiology, Sealy Smith Foundation, Galveston, Texas. Presented in part at the 73rd Clinical and Scientific Congress of the International Anesthesia Research Society, Los Angeles, California, March 12–16, 1999.

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Procedure

Twenty-four hours before the experimental procedure, the animals were instrumented with a urinary bladder catheter (Sherwood Medical, St. Louis, MO), and food and water were discontinued. Induction was accomplished with isoflurane, and animals were then intubated. After end-tidal carbon dioxide confirmed endotracheal tube placement, animals were given volume ventilation without positive end-expiratory pressure (Ohmeda, West Yorkshire, United Kingdom). Respiratory frequency and tidal volume were adjusted to maintain the hemoglobin oxygen saturation greater than 90% and end-tidal carbon dioxide at 30-32 mmHg. Inhalational anesthetic delivery was controlled to maintain an isoflurane concentration of 1.5%. A heating lamp was used to diminish temperature loss throughout the experiment.

Before fluid administration, animals were observed for 45 min, and baseline measurements were then taken. All animals were heparinized with 3,000 IU of intravenous heparin 5 min before the experiment started. All infusions consisted of intravenous administration of 0.9% saline (Baxter), kept in a temperature range of 39-40°C via a warming coil and a thermistor-regulated temperature-controlled bath, through a femoral venous catheter using a high-flow roller pump (Travenol Laboratories, Morton Grove, IL).

Hemodynamics

Hemodynamic variables, including heart rate, mean arterial pressure, pulmonary arterial pressure, and central venous pressure, were monitored continuously via a four-channel hemodynamic monitor (Model 78304; Hewlett Packard, Santa Clara, CA). Pulmonary arterial occlusion pressure was measured intermittently. In addition, blood temperature and cardiac output were monitored using a computer (9530 Baxter Edwards Critical Care). The zero reference level for all intravascular pressure measurements was set at 12 cm above the sternal plane. Temperature, heart rate, and intravascular pressures were recorded three times during baseline measurements and every 5 min during the experiment. The pulmonary arterial occlusion pressure was recorded three times during baseline measurements and every 10 min during the experiment. Cardiac output was measured using the cold thermodilution method in duplicate three times during baseline measurements and every hour during the experiment. Arterial blood gas and pH samples were analyzed (System 1302; Instrumentation Laboratory, Lexington, MA) and recorded three times during baseline measurements and every hour during the experiment. Urinary volumes were measured every 5 min using a 250-ml graduated cylinder.

Table 1. Summary of the Methods Used to Calculate the Dilution and the Volume Changes According to Mass Principles and Volume Kinetics

Kinetics	Mass Balance	Volume Kinetics
Central volume Dilution	$\frac{(Hb_0 - Hb_t)/Hb_t}{(1 - \text{hematocrit})}$	$\frac{(V_1 - V_i)}{V_1}$
Baseline volume	measured, PV_0	curve-fitting, V_1
Volume increase	$(PV_0 \times \text{dilution}) - PV_0$	$(V_1 \times \text{dilution}) - V_1$
Urine flow rate	measured urine	$k_e \times \text{dilution}$
Peripheral volume		
Volume change	infused volume - urine - central volume increase	$V_1 \times \text{dilution of } V_2$

Hb_0 = baseline hemoglobin concentration; Hb_t = hemoglobin concentration at time (t); PV = plasma volume; V_1 = central body fluid space; V_2 = central body fluid space; k_e = elimination rate constant.

Blood Chemistry

Blood hemoglobin and hematocrit were measured at baseline and every 5 min during the experiment via analysis of 1-ml arterial blood samples (HemaVet; CDC Technologies, Oxford, CT). Before sample withdrawal, 4-5 ml of blood was removed from the arterial catheter to avoid sample dilution. The withdrawn blood was reinfused through the central venous pressure catheter after sampling. The catheters were then flushed with 1-2 ml of heparinized saline. An additional 7 ml of blood was withdrawn every hour for analysis of parameters not reported here.

Plasma Volume

Baseline plasma volume was measured using the Evans blue dye technique^{11,12} at the beginning of each protocol (i.e., before saline infusion in the unanesthetized state and before saline infusion but after isoflurane anesthesia in the anesthetized state). After infusion of 4 ml Evans blue dye, 5-ml arterial blood samples were collected every 2 min for a total of four samples. Blood samples were centrifuged at 4,500 rpm for 7 min. Evans

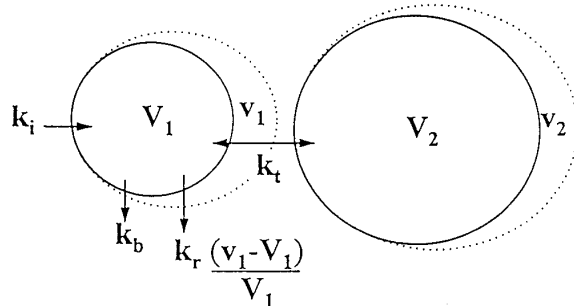


Fig. 1. The volume kinetic model used to analyze the data on the dilution of arterial blood during and after intravenous infusion of 0.9% NaCl in sheep. V_1 and V_2 = sizes of central and peripheral body fluid spaces, respectively; k_e = elimination rate constant; k_t = distribution rate constant; k_b = basal fluid losses.

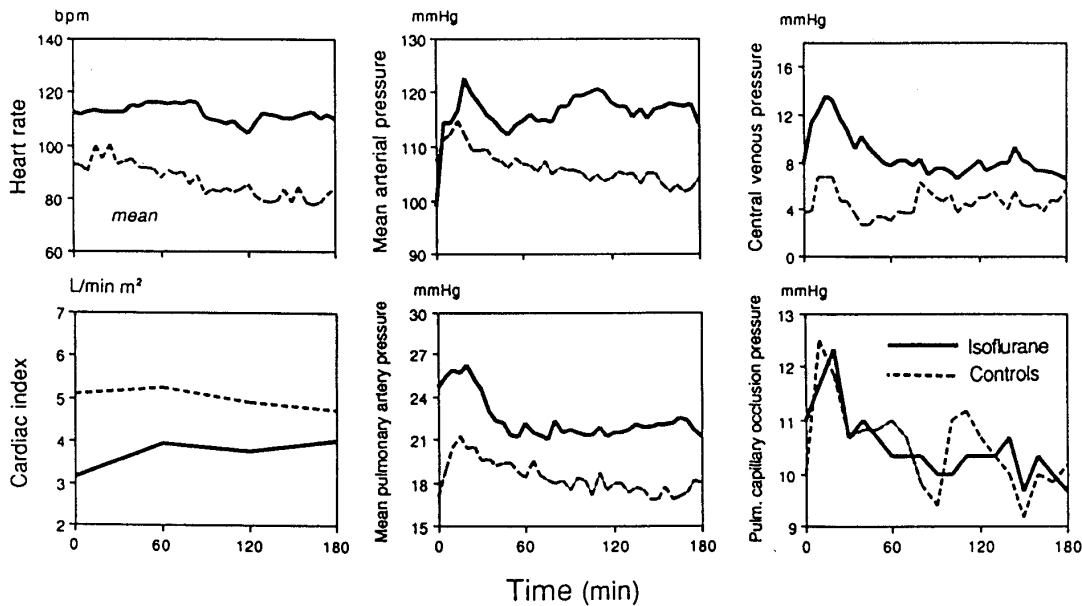


Fig. 2. Mean hemodynamic trends during and after infusion of 25 ml/kg of 0.9% saline over 20 min in sheep during isoflurane anesthesia (thick line) and while conscious (broken line).

blue concentration was measured in the plasma of these spun samples *via* a spectrophotometer (Model 1001, Spectronic; Milton Ray Company, Rochester, NY) at a wavelength of 620 nm. The obtained values were fit to a logarithmic decay curve of plasma dye concentration with respect to time using linear regression analysis. The concentration of the dye at time zero, representative of the plasma dye concentration at the time of infusion with instantaneous and complete mixing, was then extrapolated from the equation. Standard decay curves were constructed for each animal from the plasma collected before dye infusion.

Calculations

Mass Balance. The plasma volume at time n during the experiment was taken as the product of baseline plasma volume (as obtained by the dye technique) and the fractional change in hemoglobin concentration at

each 5-min time interval corrected for baseline hematocrit, using the following equation:

$$PV_n = PV_0 \times [(Hb_0 - Hb_n) / Hb_n] / (1 - \text{hematocrit}) \quad (1)$$

where PV_n and PV_0 represent plasma volume at time n and at baseline, respectively, and Hb_0 and Hb_n represent the total blood hemoglobin concentration at the beginning of the infusion (0) and at each 5-min time interval, respectively. A correction factor for blood removed and fluid used to flush the catheters was not used. Based on measurements performed previously in this laboratory, the Evans blue dye does not influence hemoglobin measurements.¹³

The peripheral accumulation of fluid was calculated by subtracting both the increase in plasma volume and the urinary excretion from the amount of infused fluid (table 1).

Volume Kinetics. The distribution of the fluid given by intravenous infusion was analyzed using a two-vol-

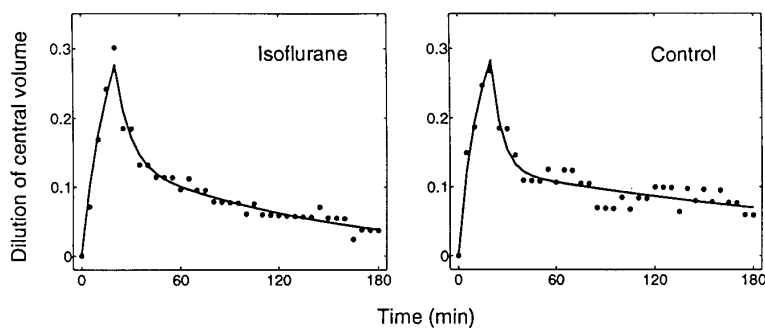


Fig. 3. Two representative experiments showing the optimal fit of individual data on the dilution of arterial plasma (as a decimal fraction) to the kinetic model shown in fig. 1. The solid line represents the modeled dilution of the central body fluid space, V_1 .

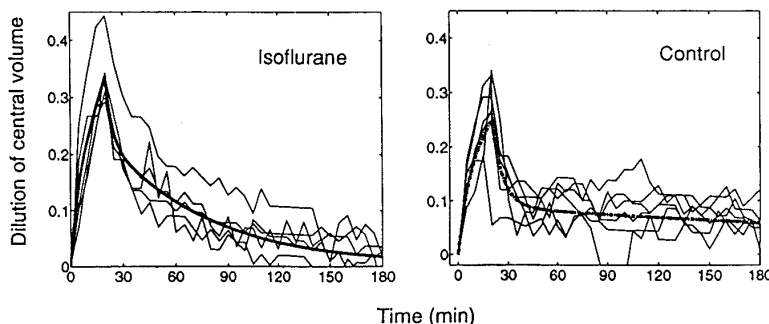


Fig. 4. Agreement between the dilution of arterial plasma (as a decimal fraction) over time for individual experiments (thin lines) and the modeled dilution of V_1 (based on the median values of the parameter estimates) from all six experiments (thick line) during and after infusion of 0.9% NaCl in sheep.

ume-of-fluid-space model. In this kinetic model, the fluid is given at a rate k_i and is distributed in an expandable space having a volume (v_1), communicating with a peripheral fluid space (v_2). The net rate of fluid exchange between v_1 and v_2 is proportional to the relative difference in deviation from their baseline values V_1 and V_2 by a constant (k_r) (fig. 1). Elimination occurs at a baseline rate k_b , which represents basal losses of fluid (assumed to be 0.3 ml/min [430 ml/24 h; personal verbal communication from George C. Kramer, Ph.D., Professor, Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX, November 2000^{14,15}] and further corrected to account for flushing the catheters) and at a rate proportional to a constant (k_r) to the deviation from the target volume, V_1 . The following differential equations describe the dilution changes in v_1 and v_2 , respectively:

$$\frac{dv_1}{dt} = k_i - k_b - k_r \frac{(v_1 - V_1)}{V_1} - k_t \left[\frac{(v_1 - V_1)}{V_1} - \frac{(v_2 - V_2)}{V_2} \right] \quad (2)$$

$$\frac{dv_2}{dt} = k_r \left[\frac{(v_1 - V_1)}{V_1} - \frac{(v_2 - V_2)}{V_2} \right] \quad (3)$$

As the plasma volume is a part of v , the dilution of the arterial plasma was used to indicate $(v_1 - V_1)/V_1$. Hence:

$$(v_1 - V_1)/V_1 = [(Hb_0 - Hb_n)/Hb_n]/(1 - \text{hematocrit}) \quad (4)$$

Table 2. Results of Volume Kinetic Analysis of 25 ml/kg of 0.9% NaCl Infused in 6 Sheep During Isoflurane Anesthesia and During Another Experiment When They Were Conscious Controls

	Isoflurane	Controls
V_1 (ml)	1180 (676)	1649 (502)
V_2 (ml)	1851 (620)	7534 (3198)
k_r (ml/min)	58.5 (5.3)	27.7 (19.1)
k_t (ml/min)	176 (149)	192 (46)
Mean square errors (MSQs)	0.3305	0.3716

The data are the best estimate of each parameter followed by, in parentheses, the standard deviations associated determining this parameter based on all animals in a single analysis.

V_1 and V_2 = central and peripheral body fluid space; k_r = elimination rate constant; k_t = distribution rate constant.

The best estimates of the model parameters V_1 , V_2 , k_r , and k_t and their associated SDs are obtained by fitting the mathematical solutions to equations 2 and 3, which have been presented previously,³ to the experimental data, first for each experiment individually and then for all animals in each group using a nonlinear least-squares regression routine programmed in Matlab version 4.2 (Math Works Inc., Natick, MA). In addition to the k_r estimated by this curve-fitting procedure, a theoretical k_r was calculated based on the urinary excretion divided by the area under the curve for the dilution-time profiles.¹⁶⁻¹⁸

The algorithms used to calculate the volume changes in V_1 and V_2 and the amount of eliminated fluid are shown in table 1.

Statistical Analysis

Data are presented as mean \pm SD, and statistical comparisons were made using repeated-measures analysis of variance. When there was a skewed distribution, the results were reported as the median and 25th and 75th percentiles, and the Wilcoxon matched-pair test was used for statistical comparisons. $P < 0.05$ was considered statistically significant.

Results

Hemodynamics

Infusion of 0.9% NaCl significantly increased the arterial and venous pressures, but they all returned to baseline during the postinfusion period (fig. 2). Sheep had a lower cardiac index and a higher heart rate, mean arterial pressure, and central venous pressure during isoflurane anesthesia compared with when they were conscious, although these differences were not statistically significant.

All animals tolerated the experimental procedures well. No group developed respiratory or metabolic acid-base disturbances. In the anesthetized state, body temperature was slightly but significantly decreased from 1 h after infusion and thereafter ($P < 0.05$). For example, the temperature at 2 h had decreased to $37.7 \pm 0.40^\circ\text{C}$ from the baseline of $38.2 \pm 0.25^\circ\text{C}$ (mean \pm SD).

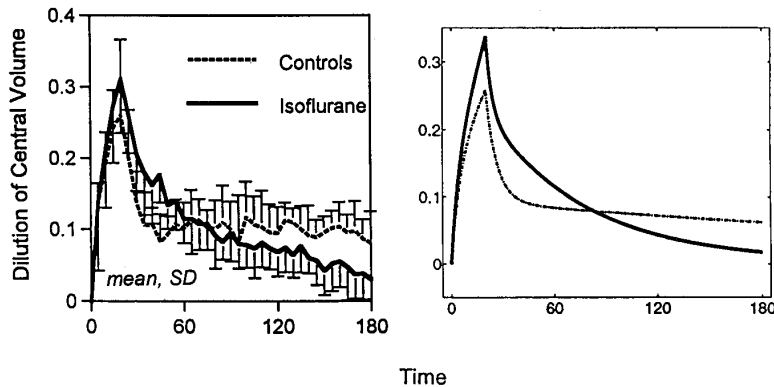


Fig. 5. The dilution of arterial plasma (as a decimal fraction) as indicated by changes in the hemoglobin concentration (*left*) and the modeled dilution of V_1 according to a volume kinetic analysis (*right*) during and after infusion of 0.9% NaCl in sheep.

Volume Kinetic Analyses

In 11 of the 12 experiments, the two-volume-of-fluid-space model fit the data better than the one-volume-of-fluid-space model, *i.e.*, fitting the two-volume-of-fluid-space kinetic model to the data resulted in a statistically lower squared difference between the theoretical and experimental data points (figs. 3 and 4).⁴ The kinetic parameters used for further comparison with mass balance calculations of the fluid distribution stem from one analysis of all data points in each group on a single occasion, without any use of the measured urinary excretion (table 2). These results show that the infused fluid expanded smaller body fluid spaces (V_1 and V_2) and that the elimination rate constant (k_r) was twice as high during isoflurane anesthesia as it was in the conscious state.

Volume Kinetics versus Mass Balance

Dilution. Simulation curves based on the volume kinetic parameters reported in table 2 agreed well with the dilution indicated by mass balance (fig. 5).

Central Volume. The baseline plasma volume, as indicated by Evans blue, was $1,603 \pm 91$ ml in the isoflurane experiments and $1,662 \pm 122$ ml in the controls. Mass balance indicated an increase in the plasma volume at the end of infusion of 510 ± 48 ml and 416 ± 67 ml, respectively, which corresponded to $50 \pm 6\%$ and $40 \pm$

5% of the infused volume ($P < 0.02$). Volume expansion was less pronounced in the isoflurane group than in the controls during the last 60 min of the experiments ($P < 0.03$; fig. 6, left).

Volume kinetic analysis indicated a slightly smaller increase of the central volume (V_1) during isoflurane anesthesia than did the mass balance calculations (fig. 6, right).

Urinary Excretion. Urinary volume was markedly lower during the isoflurane experiments, with a median of 9.0 ml (range, 4.0–150 ml), as compared with 863 ml (range, 604–1,122 ml) in the controls (Wilcoxon test, $P < 0.03$). The urinary flow rate predicted by the volume kinetic model, *i.e.*, the calculation of k_r , agreed well with the measured rate in the control experiments but not with those in the isoflurane experiments (fig. 7). A theoretical k_r , calculated using median urinary excretion, approximated 35 ml/min in conscious sheep and 0.6 ml/min in the presence of isoflurane; the latter differed greatly from the 58.5 ml/min obtained by curve-fitting (table 2).

Peripheral Volume. The volume change in the peripheral volume, V_2 , showed a similar profile when assessed by mass balance and volume kinetics in the control experiments; however, the assumption used in volume kinetics, that k_r primarily reflects urinary excretion, resulted in underestimation of peripheral

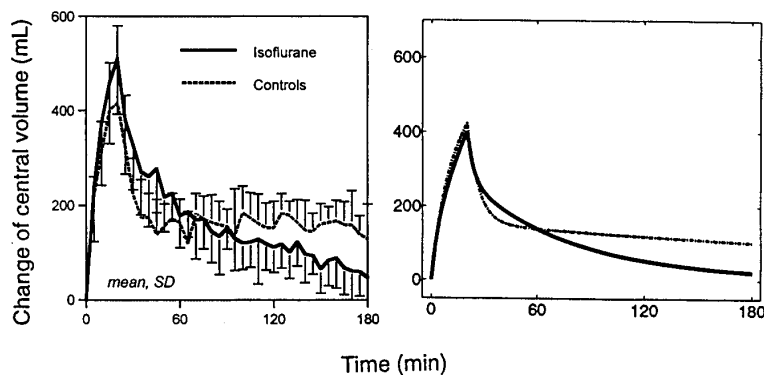
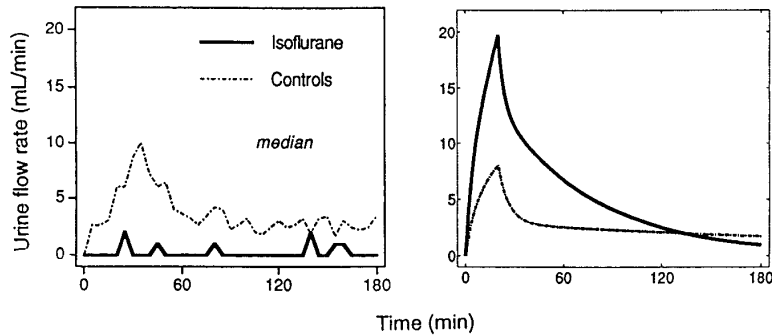


Fig. 6. The volume change of the central body fluid space as obtained by mass balance, using the plasma volume indicated by Evans blue as baseline (*left*) and volume kinetic analysis, using the size of V_1 as baseline (*right*), during and after infusion of 0.9% NaCl in sheep. The parameters shown in table 2 were used to create the volume kinetic curves.

Fig. 7. The measured urinary flow rate (left) and the elimination rate as indicated by volume kinetic analysis (right). Volume kinetic analysis suggests rapid elimination from plasma, which in conscious (control) sheep (and humans) is similar to urinary output. In isoflurane-anesthetized sheep, a small fraction of loss from plasma is accounted for by urinary output.



fluid accumulation during the isoflurane experiments (fig. 8).

Discussion

Infusion of 0.9% saline in normovolemic, conscious sheep and normovolemic, isoflurane-anesthetized, mechanically ventilated sheep results in similar expansion of intravascular volume, despite markedly different distribution of infused fluid. In normovolemic, conscious sheep, fluid is primarily excreted in the urine, whereas in normovolemic, isoflurane-anesthetized, mechanically ventilated sheep, fluid is lost into the interstitial volume to a much greater extent than would be predicted based on the ratio of plasma to interstitial volume. Had these experimental animals been traumatized or undergoing extensive surgery, one speculation would no doubt have been that they had accumulated edema in traumatized tissue. However, in the absence of surgical trauma, these data suggest that isoflurane, mechanical ventilation, or both are associated with peripheral accumulation of infused fluid.

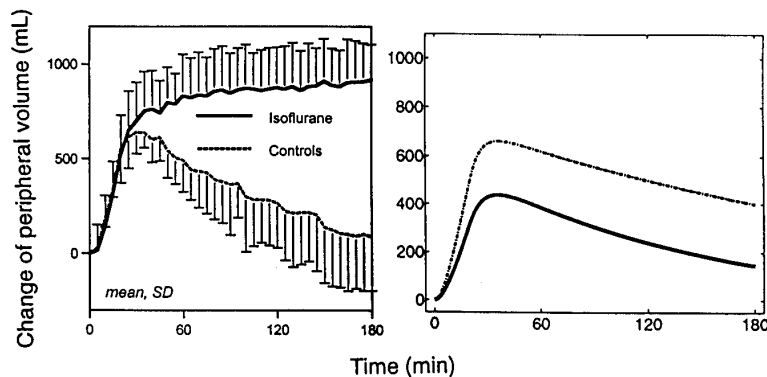
In previous studies, k_r , the elimination rate constant used in volume kinetics, correlated highly with urinary excretion, both in volunteers who received isotonic fluid^{4,6,16,17} and in conscious sheep in the current study. However, during continuous mechanical ventilation in the anesthetized state, k_r corresponded

to fluid losses consisting both of urinary excretion and peripheral accumulation. In volume kinetic terms, during mechanical ventilation and isoflurane anesthesia, fluid does not easily translocate from v_2 to v_1 in response to dilution of v_2 .

In the conscious state, the size of the central body fluid space, when estimated by either Evans blue dye or volume kinetics, was 1.6l. During isoflurane anesthesia, the smaller central body fluid space estimated by volume kinetics probably reflects vasodilation, similar to the reduction of V_1 described during spinal anesthesia and endotoxemia.^{7,19} The partial derivatives for the four parameters show that V_1 is primarily determined during infusion (see Appendix). The smaller V_1 in the isoflurane-anesthetized experiments reflects accumulation in V_2 even during infusion. The relatively horizontal dilution-time curve during the latter part of the control experiments created covariance between V_2 and k_r ($r^2 = -0.93$). This explains why the estimate of V_2 attained a larger value and a larger SD than would be expected, as well as why the model-predicted k_r was 20% lower than k_r based on the median urinary excretion. A covariance of that magnitude slightly somewhat distorts the estimates of V_2 and k_r . In previous studies, even greater covariance (*i.e.*, -0.98) required use of a fixed k_r based on urinary excretion.¹⁶

The described imbalance of the dilution between v_1 and v_2 during mechanical ventilation and isoflurane an-

Fig. 8. The volume change of the peripheral body fluid space as obtained by mass balance (left) and by volume kinetic analysis using V_2 as baseline (right), during and after infusion of 0.9% NaCl in sheep. Volume kinetic analysis suggests that peripheral fluid accumulation is modest and is rapidly eliminated in conscious (control) sheep. In contrast, in isoflurane-anesthetized sheep, mass balance demonstrates substantial peripheral accumulation that does not resolve over the course of the experiment.



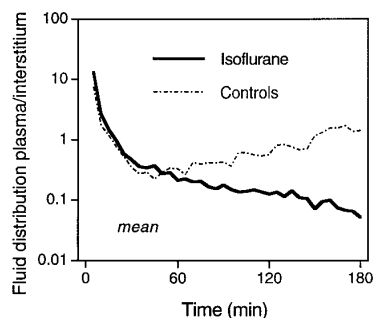


Fig. 9. Relation of fluid accumulation in plasma and interstitial tissue after infusion of normal saline in isoflurane-anesthetized and mechanically ventilated sheep. The figure is based on mean values for all six animals.

esthesia, however, can be adequately analyzed by volume kinetics only by splitting the constant for exchange of fluid between v_1 and v_2 into two microconstants, one for translocation of fluid from v_1 to v_2 and another for the reverse transport. Such a model should be used for anesthetized, mechanically ventilated subjects and could serve as a tool for quantifying the accumulation of peripheral edema. Although we cannot be certain that peripheral loss of fluid represents gastrointestinal accumulation, we could not explain the lack of such dysequilibrium in conscious sheep.

Mass balance, used traditionally to estimate fluid distribution, assumes conventional proportions of physiologic fluid spaces and assumes that hemoglobin is uniformly distributed throughout plasma volume. In the current mass balance calculations, plasma dilution differed very little between the two series of experiments. The only clear difference was more prolonged residual dilution in the controls. The altered mass balance of fluid during isoflurane anesthesia and mechanical ventilation is best reflected in the ratios of central to peripheral volume expansion. At the end of the infusions, the median ratio of the plasma/peripheral volume expansion was approximately 1.0 in both groups (*i.e.*, the fluid had been distributed evenly between these two fluid spaces). However, at 120 min, this ratio was 0.57 in the controls (*i.e.*, more than twice as much infused fluid was present in the periphery as in the plasma volume), compared with a ratio of only 0.12 in mechanically ventilated, anesthetized sheep (*i.e.*, 10 times more of the infused fluid was present in the periphery than in the plasma (fig. 9)). However, mass balance calculations do not permit analysis of the causes of changes in fluid distribution.

Volume kinetics, on the other hand, describe the fluid distribution between functional body fluid spaces over time using the assumptions (fig. 1) of free exchange of fluid between functional body fluid spaces. In contrast to mass balance, baseline plasma volume is not measured and urine is not collected (although the current experi-

ment illustrates that, during certain circumstances, collection of urine may be necessary). Although correction of the calculations for losses of hemoglobin are necessary, other experimental constraints have only a minor impact on the result of the calculations.⁶ When evaluating the comparisons between mass balance and volume kinetics, one must consider that the former method, by tradition, does not include corrections for blood sampling and baseline fluid losses. Removal of blood promotes a fluid shift from the periphery to the bloodstream and thus creates excess dilution.^{5,20} Volume kinetic parameters are influenced by large losses of hemoglobin; however, in the current study, the sampling volumes were small. Furthermore, estimates of volume kinetic parameters usually allow prediction of the distribution of fluid when given at other, theoretical rates.^{6,17}

The mechanism(s) of peripheral accumulation of fluid and lower urinary excretion during isoflurane anesthesia and mechanical ventilation require further study. The difference might be attributable to the renal effects of inhalational anesthesia, which reduces renal blood flow and glomerular filtration rate.^{21,22} Mechanical ventilation might also be responsible, because of inhibition of the release of atrial natriuretic peptide.^{23,24} Lower plasma concentrations of atrial natriuretic peptide are associated with a decline in cardiac index, creatinine clearance, urinary output, and urinary sodium concentration.²⁵ However, intravascular volume expansion usually increases the plasma atrial natriuretic peptide.²⁶

The primary limitations of the current study are the use of sheep as the experimental species and the necessity for combining mechanical ventilation with isoflurane anesthesia. The majority of published studies using volume kinetics involve humans^{4,6,7,16,17}; however, the close agreement between the analyses in conscious sheep and conscious volunteers suggests that data acquired in sheep can be extrapolated to humans. The combination of mechanical ventilation with isoflurane anesthesia precludes separation of the effects of the two interventions. Separation of those effects would require additional experiments in which conscious sheep were mechanically ventilated and in which anesthetized sheep were permitted to breathe spontaneously. Although potentially useful for interpretation of these data, such experiments potentially would be confounded if mechanical ventilation induced discomfort in conscious sheep or if isoflurane anesthesia decreased spontaneous minute ventilation and increased arterial carbon dioxide tension. Despite the inability of the current experiments to differentiate between the effects of mechanical ventilation and isoflurane anesthesia, these experiments suggest the necessity of making similar comparisons between conscious volunteers, in whom most volume kinetic data have been acquired, and anesthetized, mechanically ventilated patients.

In conclusion, these data suggest that crystalloid infusion during anesthesia and mechanical ventilation results in peripheral accumulation of fluid, at least in part because of reduced urinary excretion.

The authors thank Jordan Kicklighter, B.A. (Editor, editorial office of the Department of Anesthesiology, The University of Texas Medical Branch, Galveston, TX) for manuscript preparation and review.

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Appendix

Calculated partial derivatives, which illustrate the contributions of different portions of the plasma dilution curve to the estimate of the kinetic parameters V_1 (upper left), V_2 (upper right), k_r (lower left), and k_i (lower right). The x-axis denotes time (in minutes) after injection, and the y-axis represents the relative contribution of that time segment to the estimate of the kinetic parameters, i.e., the greater the distance of the line from zero, the greater the contribution of that portion of the plasma dilution curve to estimation of the parameter. These curves are determined for each experiment by the MatLab program.

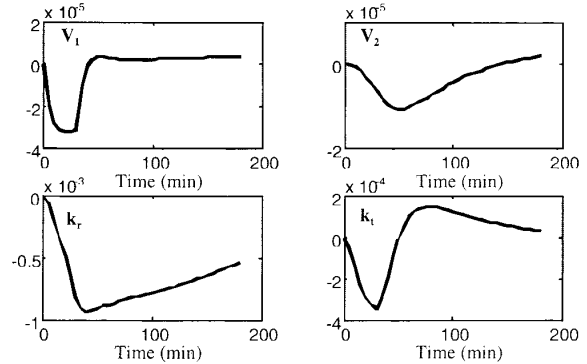


Fig. 10. Calculated partial derivatives.

Volume Turnover Kinetics of Fluid Shifts after Hemorrhage, Fluid Infusion, and the Combination of Hemorrhage and Fluid Infusion in Sheep

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Background: Hemorrhage is commonly treated with intravenous infusion of crystalloids. However, the dynamics of fluid shifts between body fluid spaces are not completely known, causing contradictory recommendations regarding timing and volume of fluid infusions. The authors have developed a turnover model that characterizes these fluid shifts.

Methods: Conscious, chronically instrumented sheep (n = 12) were randomly assigned to three protocol groups: infusion of 25 ml/kg of 0.9% saline over 20 min (infusion only), hemorrhage of 300 ml (7.8 ± 1.1 ml/kg) over 5 min (hemorrhage only), and hemorrhage of 300 ml over 5 min followed by infusion as noted above (hemorrhage plus infusion). A two-compartment volume turnover kinetic model containing seven model parameters was fitted to data obtained by repeated sampling of hemoglobin concentration and urinary excretion.

Results: The volume turnover model successfully predicted fluid shifts. Mean baseline volumes of the central and tissue compartments were 1799 ± 1276 ml and 7653 ± 5478 ml, respectively. Immediate fluid infusion failed to prevent hemorrhage-induced depression of cardiac output and diuresis. The model suggested that volume recruitment to the central compartment after hemorrhage was primarily achieved by mechanisms other than volume equilibration between the two model compartments.

Conclusion: Volume turnover kinetics is a promising tool for explaining fluid shifts between body compartments after perturbations such as hemorrhage and intravenous fluid infusions. The pronounced inhibition of renal output after hemorrhage prevailed regardless of fluid infusion and caused fluid retention, which expanded the tissue compartment.

IN the early 1960s Shires *et al.* suggested that perioperative fluid management should be more aggressive to restore intracellular and extracellular volume after hemorrhage and surgery.^{1,2} These guidelines were experi-

mentally successful³⁻⁵ and have provided guidance not only for treatment of hemorrhagic shock² but also for replacement of extracellular losses that are assumed to accompany elective surgical trauma.¹ These studies have been debated,⁶⁻⁸ and accumulating evidence suggests that these guidelines promote excessive fluid administration.^{9,10} Volume kinetic modeling,¹¹ similar to pharmacokinetic modeling, has been used to describe the distribution of different intravenous fluids and has effectively described changes of body fluid volumes after infusion of normal saline in sheep¹² and humans¹³ using a two-compartment model. Volume kinetic analysis has, however, been limited to situations in which fluid infusion increases plasma volume above a preinfusion baseline. Volume kinetic analysis could address a broader range of clinical situations if it were adapted to also assess responses to hemorrhage and intravascular retention of fluids after hemorrhage, which are clinical circumstances that initiate physiologic mechanisms that act to restore intravascular volume.

One such adaptation would be a turnover model in which intake plus physiologic production equals elimination. The concept of turnover implies a steady state and can be applied to many substances in the body, including water.^{14,15} The aim of the current study was to apply a turnover model to analyze data representing fluid shifts caused by both increases and decreases of intravascular volume. We fitted the model using the same set of parameters, including fluid volumes, to three experiments, each of which was performed in random order in each of 12 conscious sheep. The three experiments consisted of infusion only, hemorrhage only, or hemorrhage plus infusion. Additional goals were to determine whether the kinetics of the response to hemorrhage were modified by the fluid bolus and to characterize the sources and dynamics of the transcapillary refill occurring after hemorrhage.

Materials and Methods

Animal Preparation

The protocol for this study was approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch, Galveston, Texas, and conformed to guidelines for care and use of laboratory animals. Adult female merino sheep (n = 12) weighing 39.0 ± 5.9 kg were anesthetized with halothane in oxygen. A pulmonary arterial catheter (Swan-Ganz, Bax-

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Received from the Department of Anesthesiology and Intensive Care, Karolinska University Hospital, Huddinge, Stockholm, Sweden and the Department of Anesthesiology, University of Texas Medical Branch, Galveston, Texas. Submitted for publication June 2, 2004. Accepted for publication January 19, 2005. Support was provided solely from institutional and/or departmental sources. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Las Vegas, Nevada, October 23-27, 2004, and at the 13th Annual Meeting of the International Society for Anaesthetic Pharmacology, Las Vegas, Nevada, October 22, 2004.

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ter Edwards Critical Care, Irvine, CA) and bilateral femoral arterial and venous catheters (Intracath, Becton Dickinson, Sandy, UT) were inserted under sterile conditions. All animals underwent splenectomy through a left subcostal incision and the abdomen was closed using a three-layer closure. After surgery, catheters were connected to hemodynamic monitors *via* continuously flushed transducers. Analgesia consisted of buprenorphine administered intramuscularly. The sheep were maintained in metabolic cages with free access to food and water and allowed 5 days for postoperative recovery. Twenty-four hours before each experimental procedure was performed, each animal was instrumented with a urinary bladder catheter (Sherwood Medical, St. Louis, MO) and food and water were discontinued.

Experimental Procedures

Each animal was subjected to three experiments in random order with an interval of at least 48 h for recovery between experiments. At the beginning of each protocol, animals were observed without intervention for 45 min, during which time three sets of preprotocol measurements were taken. All animals were heparinized with 3000 U of heparin administered intravenously 5 min before each experiment. All infusions consisted of intravenous administration of 0.9% saline (Baxter, Irvine, CA) through a femoral venous catheter over 20 min using a high-flow roller pump (Travenol Laboratories, Morton Grove, IL).

In the first protocol (infusion only), after an initial resting period of 5 min, animals received 25 ml/kg of 0.9% saline over 20 min. In the second protocol (hemorrhage only), animals were bled 300 ml over 5 min. In the third protocol (hemorrhage plus infusion), animals were subjected to 300 ml blood loss over 5 min followed immediately by infusion of 25 ml/kg of 0.9% saline over 20 min.

Hemorrhage was accomplished over 5 min by connecting an arterial catheter to a sterile blood donation bag (Teruflex Blood Bag System, CPDA-1 Solution; Terumo Corporation, Tokyo, Japan). Accumulating blood was weighed on a balance scale (1 ml was assumed to weigh 1 g) to determine the endpoint of hemorrhage. The amount of hemorrhage (7.8 ± 1.1 ml/kg) was not adjusted to body size of the sheep. The rate of hemorrhage was controlled by regulating a pinch clamp. The laboratory environment was maintained at 20°C and physical activity of the sheep was limited by a cage.

Measurements and Mass Balance Analysis

Baseline plasma volume was measured using the Evans blue-dye technique¹⁶ at the beginning of each protocol. Standard curves for the Evans blue concentration analysis were determined for each animal from the plasma collected before dye infusion.

Hematocrit and hemoglobin concentration were mea-

sured and recorded three times before the protocol was started and every 5 min during each experiment using 1.0-ml arterial blood samples (HemaVet; CDC Technologies, Oxford, CT). All experiments lasted 3 h. Before sample withdrawal, 4 ml of blood was removed from the arterial catheter to avoid sample dilution. The withdrawn blood was reinfused through the femoral venous catheter after sampling. The catheters were then flushed with 1 to 2 ml of heparinized saline.

Cardiac output (CO) was measured using iced saline thermodilution (Cardiac Output Computer Model 9530; Baxter Edwards Critical Care, Irvine, CA) and recorded in duplicate three times before the start of the protocol, immediately after bleeding, and every hour during the experiment. Urinary volumes were measured every 5 min using a 250-ml graduated cylinder. Mass balance analysis was performed according to the equations in the appendix.

Developing the Turnover Model

Basic Turnover Concepts. The homeostasis of an endogenous compound, such as water, is maintained by the equilibrium between uptake, production, and loss. Turnover implies a steady state, and the most basic model contains the turnover rate (k_{in}), fractional turnover rate (k_{out}), and the amount of the compound in the body (A). It should be noted that k_{in} is often a zero-order process while k_{out} is a first-order process.^{14,17} The turnover of a system is mathematically described by:

$$\frac{dA}{dt} = k_{in} - k_{out} \cdot A. \quad (1)$$

At a steady state, $dA/dt = 0$. Then, the baseline value A_0 can be calculated under the assumption that k_{in} and k_{out} are time-independent parameters:

$$A_0 = \frac{k_{in}}{k_{out}} \quad (2)$$

If the subject of modeling is a fluid volume (fig. 1A), the basic turnover model can be written as

$$\frac{dV}{dt} = k_{in} - k_{out} \cdot V. \quad (3)$$

To explore that model and to estimate the turnover parameters, it is necessary to disturb the system by an exogenous supply of the compound under controlled conditions. In this study, the system was disturbed by introducing hemorrhage, infusing 0.9% saline, and combining hemorrhage and infusion.

Volume Turnover Analysis

Changes in plasma volume calculated from changes in hemoglobin concentration were taken as an index of the change in the volume of the central compartment, V_C (ml). This parameter should not be confused with total

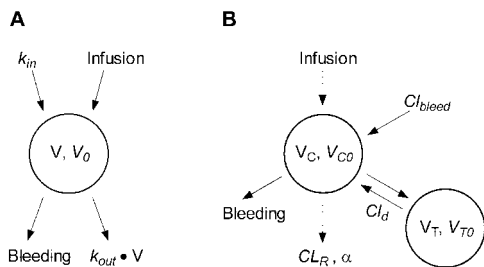


Fig. 1. (a) Basic single-compartment turnover model (Equation 3), which uses changes in the fluid volume (V) to estimate three model parameters: turnover rate k_{in} (equal to the baseline drinking rate of the sheep), the fractional turnover rate k_{out} , and the baseline fluid volume V_0 . (b) Applied two-compartment turnover model (Equations 4 through 8), which uses changes in the volume of the central compartment (V_C) and cumulative urinary output, respectively, to estimate six model parameters. Renal clearance is expressed as a combination of two parameters, CL_R and α ; V_{C0} and V_{T0} are the baseline volumes of the central and peripheral compartments, respectively; Cl_d is the intercompartmental distribution parameter; Cl_{bleed} is volume recruitment after hemorrhage from deeper compartments or from V_T by other mechanisms than equilibration of fractional volume changes.

plasma volume. V_C represents the sampling compartment and may include the plasma of the central blood volume and some part of a rapidly equilibrating subset of interstitial fluid in highly perfused regions. The cumulative urinary output (l) is measured as the main component of the total volume eliminated from the system. Those two sets of volume-time data were fitted to a two-compartment model that includes six model parameters (fig. 1B). V_T is the volume of the peripheral compartment (ml) and Cl_d is the intercompartmental distribution parameter (ml/min) that describes fractional volume changes between the two compartments. Cl_{bleed} (ml/min) is a distribution parameter related to the recruitment of fluid into the central compartment after bleeding, either from V_T by mechanisms not captured by Cl_d or from a deeper compartment that was not otherwise characterized in these experiments. Cl_{bleed} is triggered by compensatory circulatory changes after bleeding and is therefore zero in the absence of hemorrhage. Finally, renal elimination was modeled as an exponential function comprising two model parameters: CL_R is baseline renal excretion at normal hydration (ml/min) and α is an exponent that describes the alteration of urinary output in response to changes of V_C .

V_{C0} and V_{T0} are the baseline (preinfusion, prehemorrhage) volumes of the central and peripheral compartments (ml), respectively, thus making all three experimental protocols subject to simultaneous data analysis. This approach requires the assumption that each sheep returned to baseline volumes between the experimental sessions. CL_R was permitted to vary between nonbleeding (CL_{R1}) and bleeding experiments (CL_{R2}). All other parameters were assumed to be similar between the different sessions.

The k_{in} parameter, representing oral fluid intake gov-

erned by thirst, was set to zero because the animals were fasting throughout the experiments. Nonrenal routes of elimination and metabolic production of water were judged to be negligible. Because even small changes in V_C influence renal elimination, and in an effort to standardize between animals of different sizes, we chose to let the fractional volume changes of the two compartments govern both the renal excretion from V_C and the distribution between compartments. fV_C and fV_T are the fractional volume changes (unitless) of the central and peripheral compartments, respectively, and they were defined as:

$$fV_C = \frac{V_C - V_{C0}}{V_{C0}} \tag{4}$$

$$fV_T = \frac{V_T - V_{T0}}{V_{T0}} \tag{5}$$

The turnover of fluid volume in the central compartment was

$$\frac{dV_C}{dt} = Inf - b_{rate} \cdot (1 - bct_0) - Cl_d \cdot (fV_C - fV_T) + Cl_{bleed} - CL_R \cdot e^{\alpha fV_C} \tag{6}$$

Inf is the infusion rate of 0.9% saline. Bleeding rate, b_{rate} , is the amount of bleeding divided by bleeding time. b_{rate} is corrected by baseline hematocrit, bct_0 , to give the volume loss of the central compartment (i.e., plasma loss). This term becomes zero once bleeding stops. Note that Cl_{bleed} is zero in the absence of hemorrhage. The corresponding turnover of the peripheral compartment was

$$\frac{dV_T}{dt} = Cl_d \cdot (fV_C - fV_T) \tag{7}$$

Finally, the accumulated volume of renal excretion (A_e) is increased according to

$$\frac{dA_e}{dt} = CL_R \cdot e^{\alpha fV_C} \tag{8}$$

Weighting according to a constant absolute error was applied. For each sheep all six data sets, consisting of hemoglobin dilution and renal output data, respectively, from each of the three protocols, were analyzed simultaneously by a system of nine differential equations (Equations 6 through 8 for each experimental session). This regression analysis was performed using WinNonlin Professional 4.0.1 software (Pharsight, Cary, NC). To check parameter identifiability, we did a systematic reduction of one model parameter at a time and compared the change in the objective function value as expressed by the total sum of squared residuals with the full seven-parameter model. Special emphasis was placed on assessment of the correlation matrix (parameter correlation) and parameter precision (CV%).

Transcapillary Refill

Transcapillary influx and efflux were calculated using mass balance analysis as the sum of changed plasma volume, plasma loss during hemorrhage, and accumulated urinary output minus infused volume of crystalloid. The corresponding, although not equal, total flow into V_C was predicted from the pharmacokinetic analysis as the sum of flow between V_T and V_C added to the volume recruitment characterized by Cl_{bleed} . To determine whether a prompt infusion of 0.9% saline could prevent some of the physiologic effects of hemorrhage, we performed a second kinetic analysis where Cl_{bleed} was allowed to vary between the two hemorrhage protocols. This volume turnover kinetic analysis contained eight parameters.

Statistical Analysis

Data are presented as mean \pm SD or as median and range if significant according to the Shapiro-Wilk W test of normality. The three protocols (infusion only, hemorrhage only, hemorrhage plus infusion) were compared for transcapillary flow using the Wilcoxon signed ranks test. Cardiac output, mean arterial pressure, and plasma volume were expressed as fractional changes from baseline and were analyzed using analysis of variance for a two-factor experiment with repeated measures on significance. All effects and interactions were assessed at the 0.05 level of significance. The three protocols were compared at end of hemorrhage (5 min), the end of infusion (25 min), and at 65, 125, and 185 min after the beginning of the protocol. The outcomes at those five time points were compared with the baseline (*i.e.*, 1.0) for each protocol. Fisher's least significant difference procedure was used for multiple comparisons of least squares means with 0.005 as the comparison-wise error rate to minimize type II errors. Data analysis was conducted using PROC MIXED with LSMEANS options in SAS®, Release 8.2 (SAS Institute, Cary, NC).¹⁸

Results

Hemodynamic Effects

Baseline values were as follows: blood volume was 2.32 ± 0.33 l, plasma volume was 1.61 ± 0.23 l, CO was 4.3 ± 1.1 l/min, baseline hematocrit was 0.301 ± 0.037 , and baseline hemoglobin concentration was 10.3 ± 1.6 g/dl. All animals tolerated the three experimental procedures well. The circulatory effects of the three protocols are summarized in figure 2, A and B. At 65 min after the start of the protocol, CO was significantly decreased in the two hemorrhage protocols compared with the infusion-only protocol. There were no significant differences in CO between the two hemorrhage protocols. Mean arterial blood pressure was transiently decreased by hemorrhage only and increased by infusion

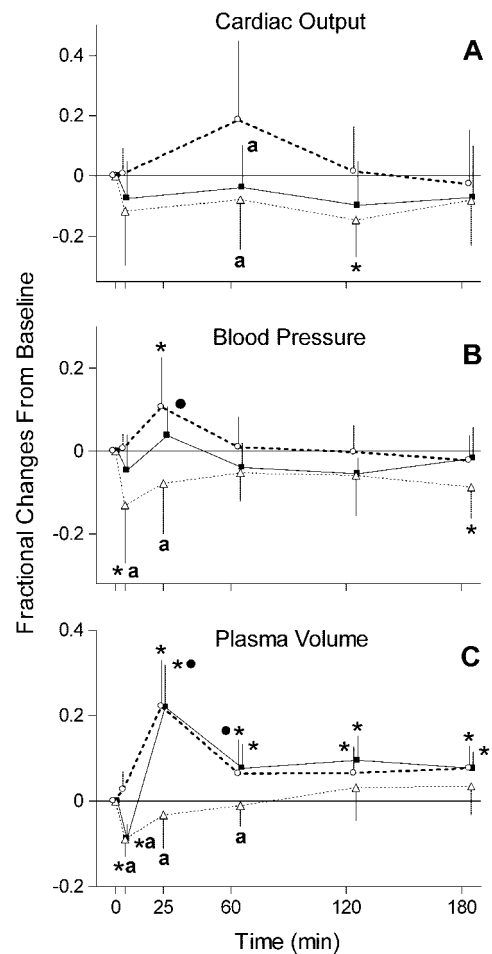


Fig. 2. Relative changes in cardiac output (a), mean arterial blood pressure (b), and plasma volume (c) in sheep ($n = 12$) after infusion of 0.9% saline (open circles, bold broken line), hemorrhage 300 ml (open triangles, dotted line), or hemorrhage followed by fluid infusion (filled squares, black line). Hemorrhage 300 ml ended at 5 min and fluid was infused between 5 and 25 min. Plasma volume was used as an index for the central compartment in the volume turnover kinetic analysis. *Significant changes from baseline for $P < 0.05$; aSignificant changes from the mean of the infusion-only protocol at a specific time point for $P < 0.05$; ● Significant changes between the hemorrhage-plus infusion and the hemorrhage-only protocols for $P < 0.05$. Values are mean \pm SD, and the baseline is represented as a dotted line.

only. In the hemorrhage-plus-infusion experiment, a short-lasting effect of the infusion was seen, causing a significantly higher blood pressure at the end of fluid infusion compared with hemorrhage only.

Mass Balance Analysis

Significant differences in fractional changes of plasma volume between the three experimental protocols are displayed in figure 2, C. Thus, antecedent hemorrhage did not increase the magnitude of the plasma dilution produced by crystalloid fluid infusion when the same preinfusion prehemorrhage baseline was used for each sheep in all three experimental sessions, although absolute dilution of hemoglobin concentration was greater in

Table 1. Volume Shifts by Mass Balance Analysis and Turnover Modeling During the 3 Hour Observational Range

Volume Shift (mL/3 hrs)	Experimental session		
	Infusion only	Hemorrhage only	Hemorrhage + infusion
Transcapillary flux by mass balance analysis of plasma dilution	-114 (-269; 713)	516 (285; 756) ^a	-188 (-384; 559) ^{ab}
Cl _d -related changes in V _C by turnover modeling	-120 ± 268	-80 ± 381	-663 ± 410
Cl _{bleed} -related changes in V _C (seven-parameter model)	—	554 ± 414	554 ± 414
Cl _{bleed1} - and Cl _{bleed2} -related changes in V _C , respectively (eight-parameter model)	—	608 ± 455	364 ± 268

Note that the central compartment (V_C) is not equal to plasma volume. Data are presented as mean ± SD if normally distributed or else as median (range). A positive value implies influx of fluid to the plasma or V_C, respectively, whereas a negative value should be interpreted as efflux from plasma or V_C.

^a Significantly different from the infusion-only protocol at 3 hrs for $P < 0.05$; ^b Significantly different between the hemorrhage-plus infusion and the hemorrhage-only protocols for $P < 0.05$ by Wilcoxon signed-ranks test.

Cl_{bleed} = volume recruitment into the central compartment by other mechanisms than equilibration of fluid volumes explained by Cl_d; Cl_d = intercompartmental distribution parameter; Subscriptions ₁ and ₂ refer to the hemorrhage only and combined protocols, respectively.

the hemorrhage-plus-infusion experiment once hemorrhage was completed. Plasma dilution at 185 min was similar between protocols. Mass balance analysis of transcapillary flow into the plasma volume during the 3-h procedure is presented in Table 1.

Urinary Output

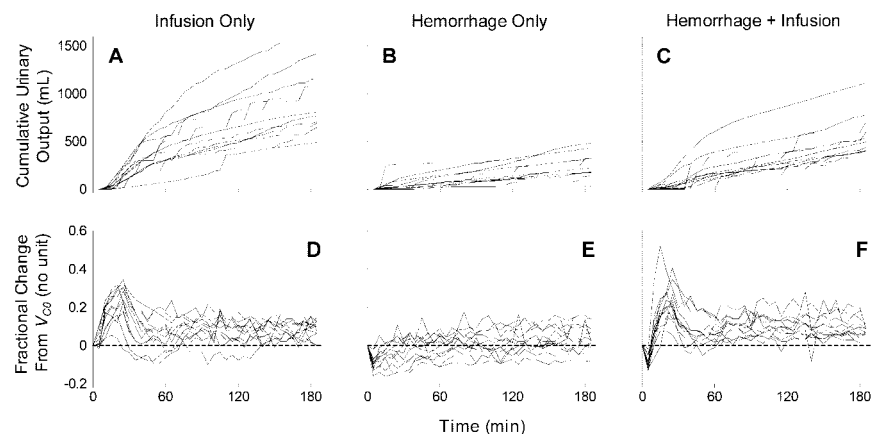
Cumulative urinary output was 924 ± 371 ml, 255 ± 135 ml, and 537 ± 233 ml at 180 min in the infusion-only, hemorrhage-only, and hemorrhage-plus-infusion protocols, respectively (fig. 3, A, B, C). Hemorrhage significantly decreased urinary output by $70 \pm 20\%$ and $37 \pm 25\%$, respectively, in the two bleeding experiments compared with the infusion-only procedure. The 300-ml bleeding constituted a fraction of 0.132 ± 0.019 of the blood volume, and this fraction significantly correlated with impairment of renal excretion in the third protocol, where bleeding was followed by crystalloid infusion, ($r = 0.73$, $P < 0.01$).

Volume Turnover Analysis

Each protocol resulted in a distinctly different pattern of central volume dilution profiles with depletion of volume at the end of bleeding and maximal dilution at the end of fluid infusion followed by stabilization of

central volume at a level slightly above the baseline (fig. 3 D, E, F). All six data sets (time-central compartment dilution and time-cumulative urinary output, respectively, from each of the three protocols) were analyzed simultaneously for each animal. The consistency was good between observed and predicted data for the proposed model (fig. 4). The model contained seven parameters because CL_R was permitted to vary between bleeding (CL_{R2}) and nonbleeding experiments (CL_{R1}). The parameter estimates for each animal are presented in Table 2. Mean V_{CO} was 1.8 l—slightly more than the mean plasma volume measured by Evans blue; and mean V_{T0} (≈ 7.6 l) was about four times greater than mean V_{CO} . The mean correlation between the parameters in the regression analysis was high for V_{CO} and Cl_d (-0.75 ± 0.18), CL_{R2} and α (-0.66 ± 0.27), CL_{R1} and α (-0.64 ± 0.27), and between CL_{R1} and CL_{R2} (0.62 ± 0.28). All other correlations averaged less than 0.54. In addition to the moderate covariance between parameters, model identifiability was tested by elimination of the parameter Cl_{bleed} , which resulted in a mean increase in total sum of squared residuals from 0.44 to 0.67 (+51%) for the 12 sheep (Table 3). Renal output impairment, as predicted by the applied model, related to the ratio between the amount of hemorrhage and calculated

Fig. 3. a–c, Plots of cumulative urinary output versus time in sheep after infusion of 25 ml/kg 0.9% saline in 20 min (infusion only), bleeding of 300 ml in 5 min (hemorrhage only), and the combination of both, with bleeding immediately preceding the infusion (hemorrhage plus infusion). d–f, Plots of fractional changes from the baseline volume of the central compartment (V_{CO}), obtained from changes in hemoglobin concentrations versus time for the corresponding experimental sessions.



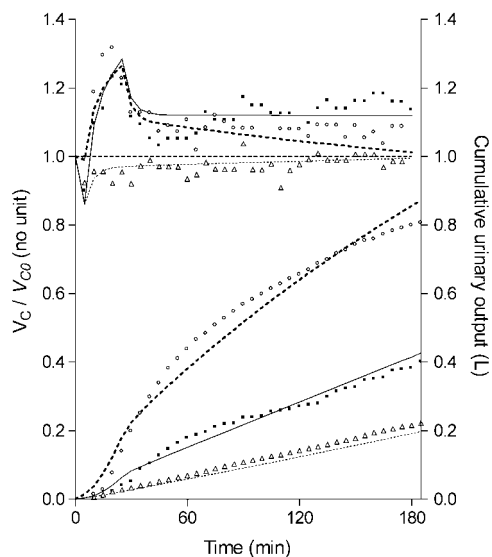


Fig. 4. Measured (symbols) and predicted (lines) data for a single animal (#152). All data were analyzed simultaneously. The three upper datasets refer to dilution of the central compartment measured by dilution of plasma (left axis). The three lower datasets refer to urinary output (right axis). Three experimental sessions are depicted: infusion of 0.9% saline 25 ml/kg in 20 min (open circles, bold broken lines), hemorrhage of 300 ml (7.5 ml/kg) in 5 min (open triangles, dotted lines), and the combined experiment with hemorrhage 300 ml followed by infusion (filled squares, black lines).

blood volume (fig. 5). The median ratio $CL_{R2}:CL_{R1}$ was 0.42 (0.12–0.87).

The applied volume turnover model was able to explain the dynamics of volume flow into V_C (fig. 6). In the hemorrhage-only protocol, endogenous volume recruitment into V_C was most rapid during the first 15 min after the end of bleeding, and likewise, in the two infusion experiments, the rapid dynamics of flow between compartments was finalized within 15 to 30 min after cessa-

tion of fluid infusion. The model also permitted a partitioning of volume flows between V_C and V_T related to the parameter Cl_d and endogenous volume recruitment after hemorrhage represented by the parameter Cl_{bleed} (Table 1). However, results were inconsistent between subjects. Three sheep experienced dehydration of V_T in the fluid-only protocol, and in three cases, recruitment by dilution gradients dominated over Cl_{bleed} flow in the hemorrhage-plus-infusion experiment.

In the second analysis, Cl_{bleed} was allowed to vary between the hemorrhage-only (Cl_{bleed1}) and hemorrhage-plus-infusion (Cl_{bleed2}) protocols (Table 1). The model thus contained eight parameters, which decreased mean total sum of squared residuals by 9% (Table 3). No Cl_{bleed} parameter had a mean correlation to any other parameter exceeding 0.39. For 12 sheep the within-subject difference between Cl_{bleed1} and Cl_{bleed2} , 1.4 ± 2.7 ml/min, did not reach significance ($P = 0.11$).

Discussion

Volume Turnover Concept

The applied turnover approach has not been used previously in fluid shift experiments; it provides an important elaboration of existing volume kinetics. In this sheep study, hemorrhage caused an inhibition of renal output, which strongly influenced volume kinetics, regardless of subsequent fluid infusion.

In comparison with studies performed with the original volume kinetic model, the current model could predict volume changes in a broader range of perturbations that more closely resemble clinically relevant scenarios. The congruence between systemic physiology and the turnover model, in which physiologic mechanisms mediate a return to baseline volumes, is appealing. The

Table 2. Values and Coefficient of Variation for Seven Model Parameters After Simultaneous Analysis of Three Experimental Sessions

Sheep	V_{C0} (mL)	V_{T0} (mL)	Cl_d (mL/min)	Cl_{bleed} (mL/min)	CL_{R1} (mL/min)	CL_{R2} (mL/min)	α
152	1058 (17)	5059 (6)	201 (12)	2.1 (11)	3.1 (4)	1.2 (4)	5.1 (7)
153	2539 (18)	5942 (134)	1102 (292)	0.5 (71)	9.6 (2)	1.1 (5)	7.5 (11)
161	1461 (13)	6842 (9)	209 (14)	2.6 (12)	4.8 (4)	1.3 (6)	8.7 (8)
164	392 (17)	15741 (66)	745 (131)	0.8 (84)	4.3 (3)	2.3 (2)	0.0 (>999)
169	1227 (23)	11716 (11)	408 (18)	7.8 (10)	1.2 (9)	0.5 (15)	16.0 (10)
171	4772 (9)	6840 (21)	429 (52)	1.7 (22)	0.9 (22)	0.3 (26)	30.6 (15)
186	443 (14)	4527 (6)	203 (13)	4.3 (7)	1.3 (12)	0.5 (15)	7.0 (10)
190	1007 (13)	3667 (7)	149 (16)	3.5 (7)	2.4 (7)	0.8 (8)	4.3 (11)
199	2415 (34)	2374 (55)	186 (126)	4.0 (9)	5.4 (4)	3.2 (7)	8.4 (14)
207	3362 (16)	20272 (29)	156 (18)	3.5 (25)	4.8 (5)	2.8 (6)	5.1 (15)
217	1600 (14)	5794 (9)	145 (17)	6.1 (8)	1.5 (10)	1.2 (12)	4.4 (16)
225	1308 (15)	3067 (12)	182 (31)	0.0 (>999)	3.1 (5)	2.7 (4)	4.8 (7)
Mean \pm SD	1799 \pm 1276	7653 \pm 5478	343 \pm 297	3.1 \pm 2.3	3.5 \pm 2.5	1.5 \pm 1.0	8.5 \pm 7.9

Data expressed as value (coefficient of variation). Coefficient of variation is calculated as $(100 \times \text{SD}/\text{estimate})$. Sheep were bled 300 mL in 5 min, received infusion of 0.9% saline 25 mL/kg in 20 min or received the combination with bleeding immediately followed by infusion.

α = an exponent explaining the response in renal output to changes in the central volume; Cl_{bleed} = volume recruitment into the central compartment after hemorrhage by other mechanisms than equilibration of fluid volumes explained by Cl_d ; Cl_d = the intercompartmental distribution parameter; CL_{R1} = the baseline renal output in normal conditions; CL_{R2} = the baseline renal output after hemorrhage; V_{C0} = baseline volume of central compartment; V_{T0} = baseline volume of peripheral compartment.

Table 3. Testing of Model Identifiability by Changes in the Objective Function Value as Expressed by the Total Sum of Squared Residuals and Akaike Information Criterion

Model	Parameters different from selected model	Mean AIC	Mean TSSR	%TSSR of selected model
(5)-parameter	- Cl_{bleed} - CL_{R2}	132.5	2.616	592
(6a)-parameter	- CL_{R2}	29.9	1.702	385
(6b)-parameter	- α	-36.8	1.030	233
(6c)-parameter	- Cl_{bleed}	-115.4	0.669	151
(7)-parameter		-196.0	0.442	100
(8)-parameter	+ Cl_{bleed2}	-216.2	0.406	92

The selected model was the seven-parameter model containing the parameters V_{C0} , V_{T0} , Cl_d , Cl_{bleed} , CL_{R1} , CL_{R2} , and α . Removing parameters strongly impaired model fit. Adding an extra parameter only marginally improved model fit and the number of parameter estimates with poor precision increased.

α = an exponent explaining the response in renal output to changes in the central volume; AIC = Akaike Information Criterion; Cl_{bleed} = volume recruitment into the central compartment after hemorrhage by other mechanisms than equilibration of fluid volumes explained by Cl_d ; Cl_{bleed2} appears when Cl_{bleed} is permitted to vary between the two hemorrhage protocols; Cl_d = the intercompartmental distribution parameter; CL_{R1} = the baseline renal output in normal conditions; CL_{R2} = the baseline renal output in after hemorrhage; TSSR = total sum of squared residuals; V_{C0} = baseline volume of central compartment; V_{T0} = baseline volume of peripheral compartment.

turnover model can also incorporate explanatory response models to describe how the body achieves homeostasis. One important objective in turnover modeling is the determination of an appropriate baseline, which is essential for estimating other parameters correctly. Keeping the baseline (V_{C0} and V_{T0}) constant between all three protocols permits a joint analysis of all experimental data for each subject. Thus, a certain combination of model parameters can be determined with good precision, even if they could never simultaneously be estimated by means of a single experimental data set. For example, if the hemorrhage-only protocol is analyzed without reference to the other protocols, there is little information regarding the shift between V_C and V_T . However, that does not mean that the body in this particular situation behaves as a one compartment but rather that the data from this isolated protocol are insufficient to discriminate between V_C and V_T . This ap-

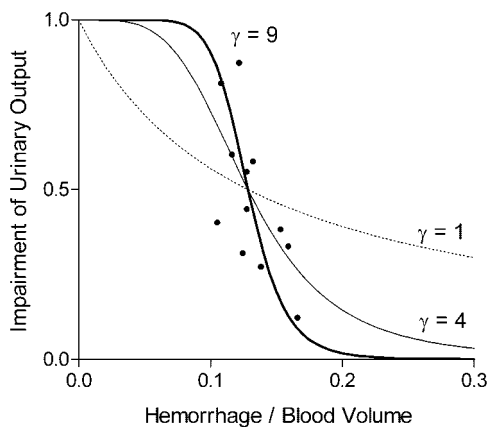


Fig. 5. Impairment of urinary excretion rate related to the magnitude of hemorrhage. Filled symbols show the ratio between CL_{R2} from the two hemorrhage sessions and CL_{R1} from the fluid infusion-only experiment for each animal related to bleeding as a fraction of blood volume. The relationship can be described as an inhibitory I_{max} -function. The steepness of the curve is related to the exponential parameter (γ). Different γ values are displayed around the mean bleeding fraction that reduced renal excretion by 50%.

proach of joint analysis suggests that the apparently greater plasma dilution effect of crystalloids after hemorrhage, reported in volunteers,¹³ is primarily attributable to the comparison of volumes during the unstable posthemorrhagic period.

The turnover rate of water in a temperate environment is approximately 12% per day in sheep,¹⁹ as compared with 7% per day in man.^{20,21} Normally, most of the water is lost by renal excretion and respiratory losses, whereas losses to transdermal evaporation, sweat, and feces are of minor importance.^{21,22} The impact of fasting on the state of hydration was unclear in this study because baseline data for renal excretion were not determined. Therefore, the state of hydration could vary both between and within subjects at the beginning of the three different experiments and contribute to the variations in response to fluid infusions or hemorrhage. This is, how-

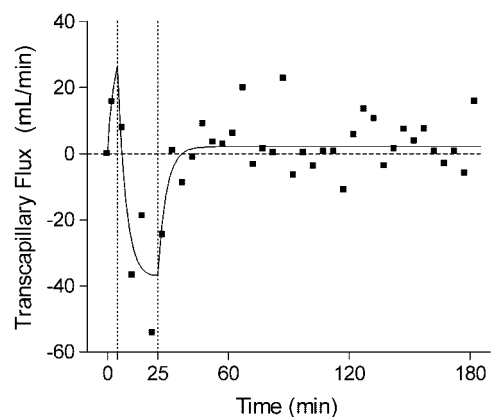


Fig. 6. Transcapillary flux in one sheep during the protocol with hemorrhage 0–5 min 300 ml followed by infusion of 25 ml/kg of 0.9% saline between 5 and 25 min (between dotted lines). Filled squares represent the mass balance calculation of influx to the plasma (positive values) and efflux from the plasma (negative values), respectively. The line represents the flux of the central compartment V_C predicted by the volume turnover model as the sum of volume change equilibration between V_C and V_T and by other mechanisms represented by Cl_{bleed} . Note that V_C is not equal to plasma volume regardless of the strong correlation between the flux over the two volumes.

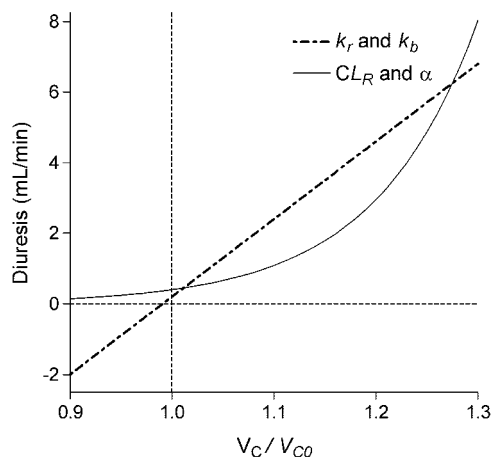


Fig. 7. Simulation of urinary output rate related to dilution of the central compartment (V_c/V_{co}), expressed as the ratio between the central volume (V_c) to the baseline value at steady state (V_{co}). The urinary excretion rate increases by a factor of 20 to 40 from a 30% dilution of the central compartment. The dot-dashed line is based on previous volume kinetic models where urinary output is expressed as a combination of k_r , fractional dilution and some arbitrary part of k_b .¹¹ The solid line is based on Equation 8, where CL_R determines the size and α determines the bending of the curve.

ever, less likely because we provided *ad libitum* water until a determined time before each experiment. In addition, the order of the experiments was randomized and at least 48 h elapsed between each experiment.

Circulatory Effects

In this study, a moderate hemorrhage (13% of blood volume) at a rate of 60 ml/min caused a 14% decrease in mean arterial pressure. This can be compared with a 23% hemorrhage at a rate of 21 ml/min needed by other investigators to achieve a 25% decrease in blood pressure in sheep.²³ Interindividual variation in the changes of CO and blood pressure was considerably greater than time-equivalent changes in plasma volume, possibly because of multifactorial control of blood pressure changes and variability in determining CO by thermodilution.

Renal Output

In contrast with previous experiments in volunteers in whom intermittent voluntary voiding was used to quantify urinary output,^{11,13} urinary bladder catheterization and direct measurement of urinary output provided a data set that could be fitted to the general model for direct calculations of urinary output dynamics. In healthy subjects the renal excretion rate of water can increase 20-fold or more from baseline after rapid fluid infusions even if dilution of plasma is moderate.¹¹ By modeling urinary output as an exponential function (fig. 7), we solved the problem of negative diuresis that appears in hypovolemia if a zero-order process is applied to the hemorrhage-only experiment as in previous volume kinetics.¹³ Therefore, we predicted continued but

reduced urinary output despite the volume deficit, and explained the effect of hemorrhage on urinary output by a single modified parameter, CL_R .

We speculate that the relationship between hemorrhage and impairment of urinary output could be described as an inhibitory I_{max} function (fig. 5). Consequently, CL_R would have an identical expression between protocols permitting it to be incorporated into Equations 6 and 8:

$$CL_R = CL_{R0} \cdot \frac{fB_{50}^\gamma}{fB_{50}^\gamma + fB^\gamma}, \quad (9)$$

where CL_{R0} is the baseline urinary output at V_{co} , fB is the actual bleeding as a fraction of blood volume, fB_{50} is the fractional bleeding that causes a 50% decrease in urinary output, and γ is an exponent that describes the steepness of the response. However, this speculation requires validation by performing repeated experiments in which the amount of bleeding is varied in the same subject.

Volume Effects

Perioperative measurement of blood pressure and urinary output are commonly used endpoints for the administration of intravenous fluids. In this study, there was a marked impairment of diuresis after hemorrhage that caused an accumulation of infused crystalloids, mainly outside V_c , in the combined protocol. This highlights the difficulty of determining optimal blood volume substitution during surgery and hemorrhage and supports the suggestion that overhydration might be a common feature⁹ especially if urinary output is used as a monitor of hydration.

Conventional prediction of plasma volume expansion after fluid infusion is based on the assumption that retained fluid is distributed across anatomic and physiologic body fluid spaces.²² According to this, crystalloid solutions that contain sodium concentrations similar to that of normal serum, such as 0.9% saline and lactated Ringer's solution, would be distributed proportionately throughout the extracellular fluid space expanding plasma volume and the interstitial fluid space in a ratio of approximately 1 to 4. However, this theoretical model is less informative than examining kinetic profiles of infused fluid and applying them to functional volumes of distribution. Kinetic analysis based on dilution of plasma, as was used in this study, displays the time-dependent nature of the volume effect of an infused crystalloid solution. Kinetic profiles reveal that plasma expansion is more pronounced at the end of an infusion while rapidly decreasing to a level less than conventionally predicted.¹¹

Differences in perfusion and compliance between various organs and tissues will contribute to the discrepancy between physiologic fluid spaces and model param-

eters. Even V_C is likely to be influenced by fluid spaces other than plasma volume because equilibration of infused fluid is much more rapid with extracellular water in highly perfused visceral organs than with the blood in low-flow organs such as resting muscles. Thus, all model parameters are strictly kinetic and should not be interpreted as representing physiologic fluid spaces, although these parameters could still be useful in describing and predicting changes in different situations of fluid balance disturbance.

Volume Exchange Between V_C and Other Fluid Compartments

Considerable amounts of extravascular fluid can be mobilized into the circulation after hemorrhage to compensate for lost blood volume.²⁴⁻²⁶ Conventionally, this has been called transcapillary refill, and the contributing mechanisms include constriction of arterioli that decrease capillary hydrostatic pressure,²⁵ enhancement of lymphatic flow,^{27,28} and osmotic attraction of fluid from the interstitium to the vascular tree because of hyperglycemia.²⁹ Furthermore, the antidiuretic effect of hemorrhage has been reported repeatedly.^{30,31} The current study demonstrates the net effects of these multiple mechanisms as a slow increase of V_C over time. This analysis further showed that the Cl_{bleed} -related flow into V_C dominated over the expansion of V_C caused by Cl_d -related flow from V_T in most cases (Table 1). This suggests that equilibration of relative volume changes only played a minor role in total recruitment into the central volume. The eight-parameter analysis showed no significant blunting of volume recruitment to V_C by the mechanisms explained by Cl_{bleed} . However, statistical power was 36%, and a total of 32 animals would have been necessary with the current study design to reach 80% power. The strength of the Cl_{bleed} parameter is interesting. It may be that the body strives not only to restore the lost fluid volume in V_C but also the lost erythrocyte mass. However, incorporating this concept into the kinetic model failed to improve the overall fit. There is a parallel in the mass balance analysis in that the body strives to restore not only the plasma volume but also the blood volume. Hemorrhage also appears to translocate protein to the plasma volume in sheep³² and humans.³³ According to this kinetic analysis, physiologic responses to hypovolemia reverse slowly. Therefore, the major effect of crystalloid infusion during hemorrhage seems to be the unwanted expansion of V_T . Because the infused fluid is not eliminated as urine, it is located peripherally rather than in the central volume as intended.

Clinical Implications

The most important clinical implication of these experimental studies relate to physiologic responses to volume expansion after hemorrhage. If, as suggested by

these studies in sheep, urinary output is suppressed during and after hemorrhage and this cannot be affected by fluid infusion, urinary output may be a flawed monitor of the adequacy of volume reconstitution for a considerable interval after plasma volume is restored to normal or even above normal. If persistent low urinary output is interpreted as continued hypovolemia, fluid treatment may not be beneficial and may only add to increased interstitial accumulation.

Points to Consider in Future Designs

The current modeling analysis raises a number of design issues that may help to improve the physiologic value of model parameters in future study protocols. First, accurate and precise turnover model parameters could be obtained by measuring intake and loss of fluid during a preexperimental observation period. This baseline analysis will capture volume turnover kinetics under unperturbed conditions. Then, the natural turnover rate k_{in} could be assessed. The state of dehydration caused by fasting could also be incorporated into the model. Second, time-dependent turnover model parameters are physiologically attractive but require a proper sampling design. The mechanisms of renal output regulation and transcapillary refill are multifactorial, and identification and measurement of such factors would be useful.^{34,35} Third, experiments with different volumes of bleeding are necessary to fully quantify the relationship between bleeding fraction, renal excretion, and volume recruitment (Cl_{bleed}) in the model. Fourth, the influence of anesthesia on simple fluid infusions has been previously described by volume kinetics.^{12,36} It would be of great clinical interest to assess the performance of the new volume turnover model during anesthesia and the combined experimental design of hypovolemia and hypervolemia. Finally, a mixed-effects modeling approach will make it possible to cross-validate the model and predict the outcome of future experiments.

Conclusions

In summary, we envision that the turnover concept presented improves the prediction over previous models of volume kinetics. Prediction and partitioning of the sources of fluid recruitment are possible with the dynamic approach of turnover volume kinetic modeling. Further elaboration of this concept will enhance our knowledge on the relative impact of different factors in the regulation of fluid shifts in hypovolemia and hypervolemia.

The pronounced effects on circulation, volume recruitment, and renal output during and after hemorrhage were mainly unaffected by the immediate infusion of a threefold volume of crystalloid within the observational range of 3 h. Thus, the main clinical effect of infused

0.9% saline was the undesired expansion of the peripheral compartment.

The authors thank Lillian Traber, R.N. (Laboratory Supervisor, Anesthesia Investigational Intensive Care Unit), and Jordan Kicklighter, B.A. (Editor, Department of Anesthesiology, University of Texas Medical Branch, Galveston, Texas).

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Appendix

Treatment of Data in the Bleeding Experiments

Plasma dilution data were obtained using the formula

$$(PV_n - PV_0)/PV_0 = [(Hb_0 - Hb_n)/Hb_n]/(1 - bct_0), \quad (\text{A1})$$

where PV is plasma volume and Hb is measured blood hemoglobin. The subscript 0 represents the baseline value and n represents the n^{th} data point. The presence of bleeding and excessive blood sampling confuses the measured plasma dilution data and calls for a correction by mass balance calculations:

$$BV_0 = (1 - bct_0)/PV_0 \quad (\text{A2})$$

$$MHb_0 = BV_0 \cdot Hb_0, \quad (\text{A3})$$

where BV is blood volume and MHb is total body mass of hemoglobin. BV_0 is calculated from PV_0 when PV_0 is determined by dilution of Evans Blue but can also be approximated as a set fraction of body weight. For each new point in time, denoted $n + 1$, the plasma volume can be calculated using Equations A4-A6:

$$MHb_{n+1} = MHb_n - \text{incremental blood loss} \cdot Hb_n \quad (\text{A4})$$

$$BV_{n+1} = MHb_{n+1}/Hb_{n+1} \quad (\text{A5})$$

$$PV_{n+1} = BV_{n+1} \cdot (1 - bct_0 \cdot Hb_{n+1}/Hb_0). \quad (\text{A6})$$

Sepsis Produced by *Pseudomonas* Bacteremia Does Not Alter Plasma Volume Expansion After 0.9% Saline Infusion in Sheep

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Clinicians generally consider sepsis to be a state in which fluid is poorly retained within the vasculature and accumulates within the interstitium. We hypothesized that infusion of 0.9% saline in conscious, chronically instrumented sheep with hyperdynamic bacteremic sepsis would be associated with less plasma volume expansion (PVE) and greater interstitial fluid volume expansion than in conscious, nonseptic sheep. Six conscious adult sheep received an IV infusion of 25 mL/kg of 0.9% saline over 20 min ($1.25 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in a control nonseptic state and during early and late sepsis (4 and 24 h, respectively, after initiation of a standard infusion of live *Pseudomonas aeruginosa*). The distribution and elimination of infused fluid were studied by mass balance (after measurement of plasma volume using Evans

blue dye) and volume kinetic analysis. Mass balance demonstrated no significant differences in the time-course of PVE between control, early sepsis, and late sepsis. At the end of the infusions, which averaged $1050 \pm 125 \text{ mL}$ in sheep weighing an average of $42 \pm 5 \text{ kg}$, calculated PVE was $312 \pm 50 \text{ mL}$, $386 \pm 34 \text{ mL}$, and 400 ± 51 , respectively. Volume kinetic analysis was similar in all three protocols. In both nonseptic and septic sheep, infusion of 0.9% saline resulted in similar peak PVE and resolution of PVE over a 3-h interval and similar kinetic parameters. Contrary to clinical impressions and to our hypothesis, the distribution of 0.9% saline in this animal model was not changed by bacteremia produced by infusion of *Pseudomonas aeruginosa*.

(Anesth Analg 2005;101:835–42)

The conventional view is that fluid requirements are greatly increased in septic patients because of increased vascular permeability and more rapid loss of fluid from the intravascular space (1). No studies have quantified changes in the retention of crystalloid fluids within the vascular space in clinically relevant experimental models of sepsis.

In our laboratories we have developed a hyperdynamic model of sepsis in conscious, chronically instrumented sheep that replicates many of the hemodynamic features of clinical sepsis (2–4). We used the

criteria for sepsis as defined by the Consensus Conference Committees of the Society of Critical Medicine, the European Society of Intensive Care Medicine, the American College of Chest Physicians, the American Thoracic Society, and the Surgical Infection Society (5,6). In this experimental model, sheep receive an infusion of live *Pseudomonas aeruginosa* (*P. aeruginosa*), which is associated with increased body temperature, increased heart rate (HR), and increased neutrophils in the setting of an infusion of live bacteria (7). These features meet the criteria for sepsis. The model does not produce septic shock, which would include evidence of tissue hypoperfusion such as lactic acidosis, although sepsis in this model has been associated with organ dysfunction, including renal failure (8), increased pulmonary transvascular fluid flux (9), and loss of hypoxic pulmonary vasoconstriction (10).

Clinical sepsis is typically hyperdynamic if adequate IV fluids have been provided for resuscitation (11). Hyperdynamic sepsis is characterized by increased core temperature, increased energy expenditure, peripheral vasodilation, increased cardiac output (CO) and decreased systemic vascular resistance

Accepted for publication February 15, 2005.

Presented, in part, at the 73rd Clinical and Scientific Congress, International Anesthesia Research Society, Los Angeles, California, March 12–16, 1999.

Supported, in part, by departmental funds provided through the Rebecca Terry White Distinguished Chair in Anesthesiology (Sealy Smith Foundation).

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DOI: 10.1213/01.ane.0000166763.24782.26

(SVR) (11,12). In the experimental model of *Pseudomonas* sepsis used by our laboratory, CO increased and SVR decreased (13).

To quantify the influence of sepsis on the kinetics of infused crystalloid, we administered 0.9% saline by IV infusion to conscious sheep in which hyperdynamic sepsis had been induced by continuous infusion of live *P. aeruginosa*, as described previously (2,4). Serial measurements of blood hemoglobin concentration ([Hb]) were used to calculate plasma dilution, and from that we calculated the distribution of the infused fluid by two different approaches—mass balance analysis (14) and volume kinetic analysis (15–17). We hypothesized that infusion of 0.9% saline in conscious, chronically instrumented sheep with hyperdynamic, bacteremic sepsis would be associated with less plasma volume expansion (PVE) and greater interstitial fluid volume expansion than in conscious, nonseptic sheep.

Methods

The protocol for this study was approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Six adult female merino sheep were anesthetized with halothane in oxygen. A pulmonary arterial catheter (Swan-Ganz, Baxter, Irvine, CA) and bilateral femoral arterial and venous catheters (Intracath, Becton Dickinson, Sandy, UT) were inserted under sterile conditions. To eliminate the influence of a contractile spleen on [Hb], all animals underwent splenectomy through a left subcostal incision and the abdomen was closed in three layers. The catheters were connected to continuously flushed transducers. After emergence from anesthesia, analgesia consisted of buprenorphine 0.3 mg bid IM. The sheep were maintained in metabolic cages with free access to food and water for a minimum of 5 days of postoperative recovery. Twenty-four hours before the three experimental protocols, a urinary bladder catheter (Sherwood Medical, St. Louis, MO) was inserted and food and water were withheld.

All animals were heparinized with 3000 IU of heparin IV 5 min before each protocol. Conscious, chronically instrumented sheep weighing 42 ± 5 kg received 25 mL/kg of 0.9% saline over 20 min and blood was sampled every 5 min for a total of 180 min (“control”) (see Fig. 1 for a flowchart of the protocol). The 0.9% saline infusion (Baxter, Irvine, CA) was kept in a temperature range of 39°C to 40°C (via a warming coil and thermistor-regulated, temperature-controlled bath) and given through a femoral venous catheter over 20 min using a high-flow roller pump (Travenol Laboratories, Morton Grove, IL). After an interval of at least 24 h, an infusion of live *P. aeruginosa* ($6 \cdot 10^6$ colony-forming units \cdot kg⁻¹ \cdot h⁻¹) was initiated and continued for the duration of the experiment. Four hours (“early sepsis”) and 24 h (“late sepsis”) after the *Pseudomonas* infusion was begun, sheep received another infusion of 25 mL/kg of 0.9% saline over 20 min and [Hb] was measured every 5 min for 180 min. Before each fluid infusion started, an Evans blue calculation of plasma volume (PV) was performed. Lactated Ringer’s solution was infused during the bacterial infusion at a rate of 2 mL \cdot kg⁻¹ \cdot h⁻¹, a rate of infusion that we have used empirically in this model to provide maintenance fluids after the onset of sepsis.

Hemodynamic variables, including HR and mean arterial blood pressure (MAP), were monitored continuously via a 4-channel hemodynamic monitor (Model 78304; Hewlett Packard, Santa Clara, CA). CO, pulmonary arterial pressure (PAP), central venous pressure (CVP), and pulmonary arterial occlusion pressure (PAOP) were measured using a thermistor-tipped pulmonary arterial catheter and a computer (9530; Baxter Edwards Critical Care, Irvine, CA). CO was measured hourly. The zero reference level for all hemodynamic data was set at 12 cm above the standing animal’s sternal plane. SVR was calculated hourly using the standard formula. Urinary output was measured every 5 min using a 250-mL graduated cylinder.

Hematocrit ([Hct]) and [Hb] were measured and recorded 3 times during baseline measurements and every 5 min during the 180-min duration of each experiment via analysis of 1 mL arterial blood samples (HemaVet; CDC Technologies, Oxford, CT). At the beginning of each protocol and at 60, 120, and 180 min

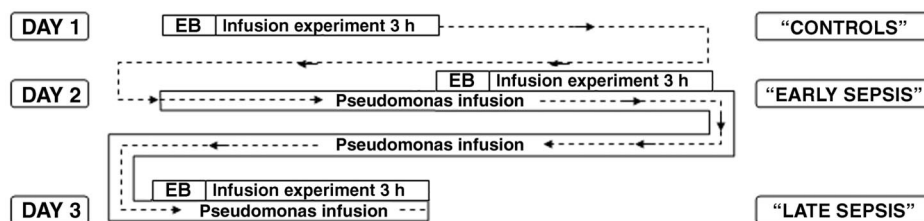


Figure 1. Flow chart of experimental design. Every protocol starts with a plasma volume measurement (EB, Evans Blue) and lasts a total of 3 h. In each protocol a saline infusion is given over 20 min after completion of plasma volume determination. Blood to measure hemoglobin concentration is sampled every 5 min for 180 min during the experiments.

after beginning the protocol, total plasma protein ([Prot]) was analyzed via a refractometer (Shuco; Tokyo, Japan) and plasma colloid osmotic pressure (COP) was analyzed using an oncometer (4100 Colloid Osmometer; Wescor, Logan, UT). Arterial blood gas and pH samples were withdrawn, analyzed (System 1302; Instrumentation Laboratory, Lexington, MA), and recorded three times during baseline measurements and hourly during the experiment.

PV was measured for each sheep before each protocol using Evans blue dye (14,18). An initial 45-mL arterial blood sample was taken before the first measurement as a standard. After rapid injection of 4 mL of Evans blue, 5-mL arterial blood samples were collected every 2 min for a total of 4 samples. Blood samples were centrifuged at 4500 rpm for 10 min and the plasma dye concentration was measured in a spectrophotometer at a wavelength of 805 nm (Spectronic, Milton Ray Company, Rochester, NY). The obtained values were fitted using least-squares regression to a logarithmic time decay curve of plasma dye concentration. Calibration standards were constructed for each animal from the plasma collected before dye infusion.

The dilution of arterial plasma was used to quantify PVE. For this purpose, repeated measurements of [Hb] in arterial blood were performed, as described above, at 5-min intervals for a duration of 180 min in each experiment, on a bedside [Hb] analyzer (HemaVet). Calculated expansion was corrected to account for the loss of 2 mL of blood for each sample withdrawn throughout the experiments, based on the assumption that baseline blood volume in sheep was 6% of body weight. The calculated PV at time (t) was as follows:

$$PV(t) = PV_0 \times [([Hb]_0) - [Hb]_t] / [Hb]_t / (1 - Hct) \quad (1)$$

where $[Hb]_0$ and $[Hb]_t$ represent [Hb] at the beginning of the infusion (0) and at each 5-min time interval, respectively. From the calculation of PVE at each time-point, individual PVE curves were constructed for the entire 180 min of each experiment.

The distribution of the fluid given by IV infusion was analyzed using a two-volume kinetic model. In this model (15), fluid infused at a rate k_i is distributed in an expandable space having a volume (v_1) and communicating with a peripheral fluid space (v_2). The net rate of fluid exchange between v_1 and v_2 is proportional to the relative difference in deviation from their baseline target volumes, V_1 and V_2 , by a constant, k_r . Elimination occurs at a baseline rate k_b , which represents basal losses of fluid, and at a rate proportional by a constant (k_r) to the deviation from the target volume, V_1 . k_b was initially assumed to be 0.3 mL/min (19,20), but was further corrected, using a value of (-) 0.1 mL/min to account for flushing the catheters. The kinetic curves were modeled using Matlab version 4.2 (Math Works Inc., Natick, MA), whereby a nonlinear,

least-squares regression routine is repeated until no parameter changes by more than 0.001 (0.1%) in subsequent iterations. The output of the kinetic analysis consists of the lowest possible squared average difference between the data points as predicted by the model and the data points actually measured, as well as the corresponding best estimate and the standard error (SE) for each parameter in the model. The following differential equations describe the dilution changes in v_1 and v_2 , respectively (Equations 2 and 3):

$$\frac{dy_1}{dt} = k_i - k_b - k_r \frac{(v_1 - V_1)}{V_1} - k_t \left[\frac{v_1 - V_1}{V_1} - \frac{(v_2 - V_2)}{V_2} \right] \quad (2)$$

$$\frac{d_{v_2}}{dt} = k_t \left[\frac{(v_1 - V_1)}{V_1} - \frac{(v_2 - V_2)}{V_2} \right] \quad (3)$$

Because PV is assumed to constitute part of V_1 , PV and v are closely related and the dilution of arterial plasma can be used to indicate $(v_1 - V_1)/V_1$:

$$(v_1 - V_1)/V_1 = [([Hb]_0 - [Hb]_t)/[Hb]_t]/(1 - Hct) \quad (4)$$

Kinetic analysis was performed on *all* experiments within each protocol as a group (i.e., data from all six animals were analyzed together). The best estimates of the model parameters V_1 , V_2 , and k_i and their associated standard errors were obtained by fitting the mathematical solutions to Equations 2 and 3. To reduce the number of unknown parameters, the mean renal clearance (urinary excretion divided by the area under the dilution-time curve) for infused fluid was taken to represent k_r , used in each of the three group analyses. Hence, the analysis was made more robust by assuming that elimination occurred solely by renal excretion.

The three conditions (control, early sepsis, and late sepsis) were assessed for HR, MAP, CO, SVR, plasma dilution, PVE, cumulative urine, and calculated elimination rate constant (k_r) at 180 min. Because of the uncertainty of homogeneity of variance and normality of random error terms, those measurements were analyzed using the Friedman test. The condition was assessed at the 0.05 level of significance. Data analysis was conducted using SAS[®], Release 8.2 (SAS, Cary, NC).

The kinetic analysis is presented as a result of a nonlinear regression fitting of data in one single analysis and the parameters (V_1 , V_2 , and k_i) are therefore given as best estimates with the corresponding standard errors of the estimates.

Results

The animals tolerated all experimental procedures well, with the exception of one animal that died 20 h after induction of sepsis.

Table 1. Physiologic Variables

Variable	Group	Time (min)				
		0	20	60	120	180
Heart rate (bpm)	Control	92.2 ± 6.8	96.5 ± 7.6	102.7 ± 12.2	92.3 ± 10.4	89.3 ± 6.6
	Early	151.3 ± 13.1	136.7 ± 17.3	143.2 ± 15.8	142.3 ± 11.3	156.2 ± 10.6*†
	Late	142.6 ± 9.6	150.6 ± 7	147 ± 7.7	141 ± 112.2	136.6 ± 11.8*
Mean arterial pressure (mm Hg)	Control	105 ± 6.2	109.3 ± 5.6	104.3 ± 5.2	104.2 ± 6.3	102.3 ± 7.1
	Early	101.2 ± 7.1	111.3 ± 9	104 ± 8.6	100.2 ± 8.9	98.2 ± 6.5
	Late	102.8 ± 9.6	106.4 ± 10.8	94.6 ± 12	96.4 ± 12.8	101.2 ± 11.4
Cardiac output (L/min)	Control	4.88 ± 0.29	—	5.8 ± 0.21	5.11 ± 0.25	5.12 ± 0.29
	Early	5.71 ± 0.41	—	6.02 ± 0.53	5.52 ± 0.39	7.05 ± 0.82
	Late	7.41 ± 0.95	—	7.94 ± 1.12	8.05 ± 1.35	7.55 ± 1.12
Systemic vascular resistance (dynes·s·cm ⁻⁵)	Control	1677 ± 166	—	1386 ± 103	1585 ± 135	1567 ± 144
	Early	1295 ± 92	—	1421 ± 213	1305 ± 169	1140 ± 158*
	Late	1090 ± 155	—	936 ± 136	973 ± 133	1076 ± 154*
Plasma dilution (%)	Control	0	19 ± 3.3	7.5 ± 1.2	7 ± 1.1	5.7 ± 1
	Early	0	20.5 ± 1.5	6.5 ± 1.9	7.5 ± 2.3	9.2 ± 2.3
	Late	0	19 ± 2.9	6.8 ± 2.1	5.2 ± 1.5	5.8 ± 1.9
Hematocrit (%)	Control	28.6 ± 2.2	24.7 ± 1.8	28.4 ± 2.3	27.5 ± 1.9	27.2 ± 1.9
	Early	25.8 ± 3.5	23 ± 3.9	24.4 ± 3.8	24.5 ± 3.8	24.6 ± 3.8
	Late	17.5 ± 1.8	14.9 ± 1.6	17.2 ± 2	16.5 ± 1.7	16.9 ± 2.1
Temperature (°C)	Control	39.1 ± 0.1	39.1 ± 0.1	39.4 ± 0.4	39.3 ± 0.3	39.3 ± 0.3
	Early	41.3 ± 0.1	41.3 ± 0.1	41.7 ± 0.1	41.7 ± 0.2	41.8 ± 0.1*
	Late	41.4 ± 0.1	41.3 ± 0.1	41.4 ± 0.2	41.4 ± 0.2	41.4 ± 0.2*
Pulmonary arterial pressure (mm Hg)	Control	17.2 ± 0.7	20.5 ± 1.4	18 ± 1.2	18.8 ± 1.1	18 ± 1.3
	Early	23.2 ± 2.5	25.7 ± 3.6	22.2 ± 2.4	24.3 ± 2.7	25.2 ± 2.3
	Late	25 ± 4.1	29.4 ± 3	24.6 ± 3.2	23.6 ± 2.7	24.2 ± 3.3
Central venous pressure (mm Hg)	Control	4.3 ± 1.8	8.5 ± 1.7	4.5 ± 1.5	4.3 ± 1.2	3.5 ± 1.2
	Early	2.8 ± 1.2	10.5 ± 1.4	2.7 ± 1	2.8 ± 0.6	3.2 ± 1.3
	Late	8.3 ± 2	10.8 ± 3.1	8.4 ± 0.9	6.2 ± 0.7	6.2 ± 1.4
Pulmonary arterial occlusion pressure (mm Hg)	Control	10.8 ± 0.5	13.7 ± 0.8	10.5 ± 1	11.3 ± 0.8	11 ± 0.4
	Early	10.3 ± 1.5	14 ± 2.9	11.2 ± 2.1	12.2 ± 2.4	10.7 ± 1.5
	Late	10.6 ± 2.4	15.4 ± 1.7	9.4 ± 2.3	8.3 ± 1.9	8.3 ± 2.5
pH	Control	7.46 ± 0.01	—	7.42 ± 0.01	7.46 ± 0.01	7.45 ± 0.01
	Early	7.47 ± 0.02	—	7.46 ± 0.02	7.48 ± 0.02	7.46 ± 0.02
	Late	7.45 ± 0.03	—	7.42 ± 0.02	7.42 ± 0.03	7.41 ± 0.04
Pco ₂ (mm Hg)	Control	38.3 ± 1.1	—	38 ± 0.6	36.4 ± 0.5	37.7 ± 0.6
	Early	39.7 ± 2.1	—	37.6 ± 1.7	36.7 ± 1.3	38.4 ± 1.5
	Late	37.3 ± 3	—	37.9 ± 1.1	37.4 ± 1.3	39.2 ± 2.3
Arterial O ₂ saturation (%)	Control	91.2 ± 0.7	—	91.3 ± 0.9	92.6 ± 0.4	92.3 ± 0.5
	Early	90.1 ± 0.4	—	91 ± 1	90.6 ± 0.7	90.3 ± 0.6
	Late	88.9 ± 0.8	—	88 ± 1.5	88.7 ± 1.8	87.4 ± 4.2
Total protein concentration (g/dL)	Control	5.87 ± 0.15	—	5.47 ± 0.17	5.55 ± 0.15	5.6 ± 0.15
	Early	4.83 ± 0.21*	—	4.6 ± 0.19	4.73 ± 0.21	4.63 ± 0.2*
	Late	4 ± 0.3*†	—	3.52 ± 0.33	3.64 ± 0.33	3.8 ± 0.49*†

Data presented as means SEM. Control: n = 6; early sepsis (4 hr): n = 6; late sepsis: n = 5.

*Mean of the variable at 180 min is significantly different from the mean of the control group at 180 min.

† Indicates that the late septic mean is significantly different from the early septic mean.

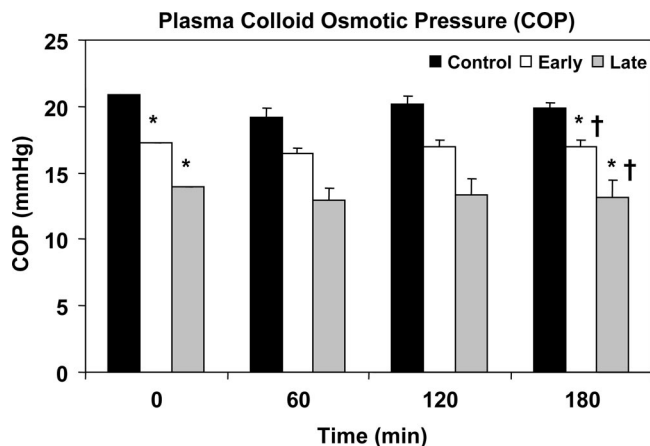


Figure 2. Plasma colloid osmotic pressures (COP, in mm Hg) at the beginning and end of early sepsis and at the beginning and end of late sepsis were significantly lower than at the beginning and end of the control protocol. Similarly, COP at the beginning and end of both early and late sepsis was significantly lower than at the beginning and end of the control protocol. COP at the beginning and end of late sepsis was significantly lower than at the beginning and the end of early sepsis. * $P < 0.05$ versus control; † $P < 0.05$ versus early sepsis (statistical comparisons were not performed at the 60-min and 120-min intervals).

HR at the end of analysis intervals (at 180 min after beginning the infusions) in early and late sepsis was significantly more rapid than at 180 min in the control protocol (Table 1 displays all hemodynamic data). HR at the end of early sepsis was significantly more rapid than at the end of late sepsis. The three conditions were not significantly different at 180 min for MAP. CO at 180 min tended to be higher in the sepsis protocols, although the changes were not statistically significant. SVR at 180 min during early and late sepsis was significantly lower than at the end of the control interval. Temperatures at 180 min were significantly higher in early as well as late sepsis compared with controls. Although PAP and CVP tended to be higher and PAOP lower in the sepsis protocols, there were no significant differences at 180 min.

Arterial blood gases did not show any differences among the three protocols (Table 1). In both the early and late sepsis protocols at 180 min, [Prot] decreased significantly in comparison to the control [Prot]. Protein concentration was also significantly decreased at 180 min during late versus early sepsis. Plasma COP at the beginning and the end of the early septic interval was significantly less than at the beginning and the end of the control interval, respectively. Similarly, plasma COP at the beginning and the end of the late septic interval was significantly less than at the beginning and the end of the early septic interval, respectively (Fig. 2).

PV estimated by the Evans blue method were 1750 ± 114 , 1678 ± 100 and 2128 ± 156 mL, respectively, at the start of the control, early sepsis, and late sepsis protocols. PV changes are shown both as dilution in

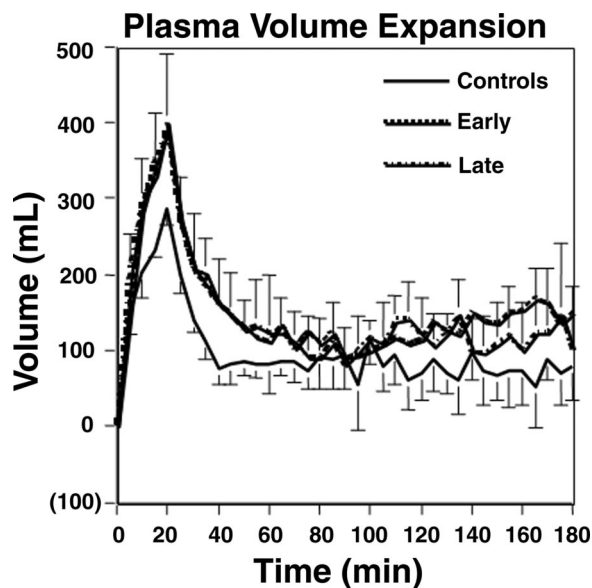


Figure 3. Mass balance analysis: Plasma volume expansion in mL in the three protocols. The error bars represent SEM values.

percentage (Table 1) and PVE (by mass balance analysis) in mL (Fig. 3). At the end of the infusions (20 min into the protocols), PV dilution was $19\% \pm 3\%$ in the control protocol, $21\% \pm 2\%$ in the early sepsis protocol and $19\% \pm 3\%$ in the late sepsis protocol. The corresponding PVE was 312 ± 50 mL, 386 ± 34 mL, and 400 ± 51 mL in the control, early sepsis, and late sepsis protocols, respectively (Fig. 3). Three hours after initiating the infusions, PV dilution was $6\% \pm 1\%$, $9\% \pm 2\%$, and $6\% \pm 2\%$. PVE at the end of the experiments was 97 ± 21 , 151 ± 40 and 102 ± 44 mL, respectively (Fig. 3).

The volume kinetic analysis showed similar results at all three intervals (Table 2, Fig. 4 a-c). In each individual experiment, the two-volume model was statistically preferable to the one-volume model. The elimination rate constant k_r did not show statistical differences, with a mean of 83 ± 16 mL/min in the control protocol, 109 ± 55 mL/min in the early sepsis protocol and 41 ± 13 mL/min in the late sepsis protocol. If one high outlier in the early sepsis group had been excluded, the mean would have been 55 ± 19 mL/min in that protocol.

Data are shown as best estimates and standard errors of the estimates as the result of a single nonlinear regression analysis of all experiments. Data marked by * were not results of the curve fitting but rather were calculated as mean renal clearances (urinary excretion divided by the area under the dilution-time curve) for infused fluid. One animal had an extremely high k_r value at 180 min in the early sepsis group. Excluding this observation would have resulted in a value of 55 ± 19 in that group. The k_r values of this animal in the

Table 2. Results of Volume Kinetic Analysis of 25 mL/kg of 0.9% Saline Infused in Sheep During Control and Under the Influence of Sepsis

	Control	Early sepsis	Late sepsis
V_1 (mL)	1567 ± 469	1325 ± 593	1673 ± 478
V_2 (mL)	5830 ± 2594	4429 ± 2047	6775 ± 2908
k_t (mL/min)	192 ± 16	192 ± 17	201 ± 19
Cumulative urinary output (mL)	892 ± 135	964 ± 215	772 ± 291
k_r (mL/min)*	83 ± 16	109 ± 55	41 ± 13

Data are shown as best estimates and standard errors of the estimates as the result of a single nonlinear regression analysis of all experiments. Data marked by * were not results of the curve fitting but rather were calculated as mean renal clearances (urinary excretion divided by the area under the dilution-time curve) for infused fluid. One animal had an extremely high k_r value at 180 min in the early sepsis group. Excluding this observation would have resulted in a value of 55 ± 19 in that group. The k_r values of this animal in the control and the late sepsis states were normal. V_1 and V_2 , central and peripheral body fluid space; k_t , distribution rate constant; k_r , elimination rate constant.

control and the late sepsis states were normal. V_1 and V_2 , central and peripheral body fluid space; k_t , distribution rate constant; k_r , elimination rate constant.

Discussion

Neither mass balance nor volume kinetic analysis showed any differences in crystalloid distribution or elimination between conscious, nonseptic sheep and conscious, normovolemic, hyperdynamic, septic sheep. Theoretically, changes in capillary permeability during sepsis should reduce intravascular retention of a colloid bolus, whereas the influence of a bolus of crystalloid fluid is more difficult to predict. Again theoretically, decreases in COP could result in less retention of crystalloid fluid in the PV and greater loss into the interstitium. Crystalloid fluid is transported readily between the PV and interstitial fluid space in the normal, nonseptic state. Even if plasma proteins are extravasated more rapidly during sepsis, as the reductions in COP and [Prot] (Table 1 and Fig. 2) suggest happened in this experiment, the COP gradient between the intravascular and interstitial space apparently did not change sufficiently to alter the distribution of the crystalloid bolus.

The relevance of these observations to human sepsis requires discussion of the characteristics of this experimental model of sepsis. Models of experimental sepsis have become progressively more sophisticated and specific over the past two decades. Early experimental models of sepsis typically consisted of administration, usually to anesthetized animals, of large doses of endotoxin that produced circulatory shock. Models of progressive sepsis progressing to septic shock are also produced by cecal ligation and puncture, usually in rodents (21,22). Recently, our laboratory has preferred the experimental model of hyperdynamic sepsis produced by a continuous infusion of live *Pseudomonas aeruginosa* in conscious sheep. Both continuous endotoxin infusion and continuous *Pseudomonas aeruginosa* infusion in conscious sheep produce highly similar

hemodynamic changes that reflect those in septic patients (but not patients in septic shock); in fact, responses to hemodynamic interventions in endotoxin-infused and *Pseudomonas*-infused conscious sheep have closely resembled responses in septic humans (23). The hemodynamic responses in the present study showed that these animals were hyperdynamic because HR and temperature were significantly higher and SVR was significantly lower in the early and late sepsis protocols compared with controls (Table 1).

Our failure to confirm our hypothesis with regard to fluids is surprising because the data seem contrary to clinical experience with fluid management in sepsis. Moreover, the data do not agree with the only previous study of volume kinetics in experimental sepsis (24). In the previous study, in which *hypodynamic* sepsis was induced in rabbits by a bolus of endotoxin, Svensen et al. (24) found that the central fluid space V_1 was significantly smaller and that the apparent central retention of infused crystalloid solution was increased during sepsis. V_1 , which should be considered a functional rather than a physiologic or anatomic volume, is probably influenced both by the magnitude of PV and by the fraction of well-perfused tissues. In the previous study by Svensen et al. (24), the authors speculated that the forces acting to preserve central volume outweighed those that promoted diffusion of fluid to more peripheral fluid spaces. In this respect, the volume kinetics of crystalloid infusion in rabbits with endotoxic shock resembled the kinetics of crystalloid infusion in mildly and moderately hemorrhaged volunteers (16), in whom antecedent hemorrhage enhanced plasma dilution produced by a given volume of fluid. We speculate that the primary difference between the previous study in rabbits and the present study in sheep is that the model in rabbits was hypodynamic and produced relative hypovolemia.

These studies in septic sheep again illustrate the principle that IV-infused crystalloid fluids do not produce static responses. Rather, they produce an acute increase in intravascular volume that rapidly diminishes as fluid is redistributed and excreted. In addition, acute changes in intravascular volume alter the

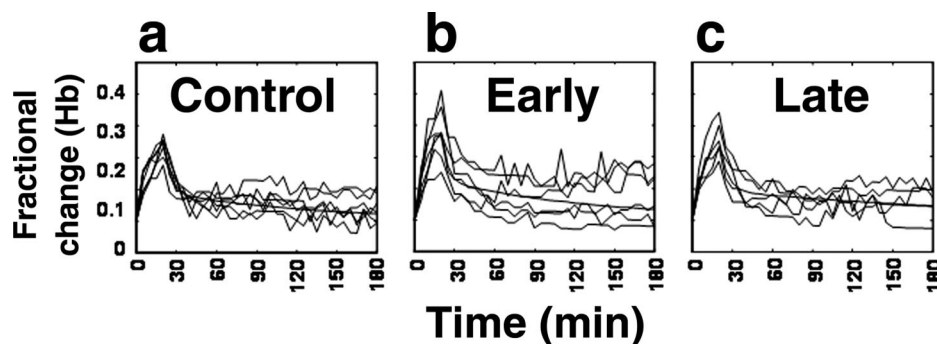


Figure 4. The volume kinetic model used to analyze the data on the dilution of arterial plasma during and after IV infusion of 0.9% saline in sheep at the 3 intervals (control [A], early sepsis [B], and late sepsis [C]). Thin lines are individual dilution-time curves for the primary fluid space during IV infusion of 0.9% saline for 20 min. The thick line in each graph is the model-predicted curve for these experiments, based on the mean values for the predominant kinetic model—the two-volume model in these experiments. All data were corrected for the influence of blood sampling.

physiologic state and may secondarily generate compensatory responses that may be superimposed on underlying acute or chronic physiologic disturbances. Consequently, kinetic analysis of changes in PVE in response to fluid infusion under a variety of physiologic circumstances should enhance the clinical process of estimating fluid requirements.

The mass balance technique in this study was used to complement volume kinetic analysis by quantifying changes in PV. For this purpose, Evans blue dye was used to measure initial PV. The distribution of a dye or a tracer may be influenced by changes in capillary permeability. Presumably, errors induced by increased capillary permeability in estimation of PV using Evans blue dye, which is heavily protein bound, would result in measurements of PV that are artifactually low. Preservation in this model of PV during early and late sepsis argues against errors induced by increased capillary permeability to protein.

In contrast to mass balance analysis, volume kinetic analysis does not require determination of PV. Volume kinetics assesses the distribution of fluid based on the dilution of plasma. In this model, the body's tendency to excrete infused fluid can be described as the renal clearance, k_r . Because of inter-animal variations in k_r , calculated from the individual plasma dilution curves, we calculated a fixed k_r for each protocol, based on actual urinary output (25). The fixed k_r was reduced by late sepsis, although the change was not statistically significant; however, the magnitude of change was considerably less than the previously reported influence of isoflurane anesthesia on k_r (26).

One important limitation of this study is the small number of animals, one of which died 4 hours before the late sepsis infusion. However, because the differences in kinetic and mass balance variables between protocols were small, even markedly larger groups would be unlikely to alter the conclusions from this study. Thus, in sheep, the distribution and elimination

of an infused crystalloid is apparently not changed by an IV infusion of bacteria that induces systemic changes that fulfill the criteria for sepsis. Further investigation is required to determine whether this finding is clinically relevant in humans. Future experimental studies should extend these observations to severe sepsis and septic shock and should include analyses of the kinetics of colloid solutions.

In summary, it is apparent from this study that hyperdynamic, septic sheep were readily able to eliminate infused fluid while maintaining similar levels of PVE in response to fluid infusion. The elimination pattern of crystalloids was similar to that of nonseptic animals.

The authors wish to thank Jordan Kicklighter, BA, in the editorial office of the Department of Anesthesiology, The University of Texas Medical Branch, for manuscript preparation and review.

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