

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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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	$\frac{(Hb_0 - Hb_i)/Hb_i}{(1 - \text{hematocrit})}$	$\frac{(v_1 - V_1)}{V_1}$
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	$k_r \times \text{dilution}$
Peripheral volume		
Volume change	infused volume – urine – central volume increase	$V_1 \times \text{dilution of } V_2$

Hb₀ = baseline hemoglobin concentration; Hb_i = hemoglobin concentration at time (i); PV = plasma volume; V₁ = central body fluid space; V₂ = central body fluid space; k_r = 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

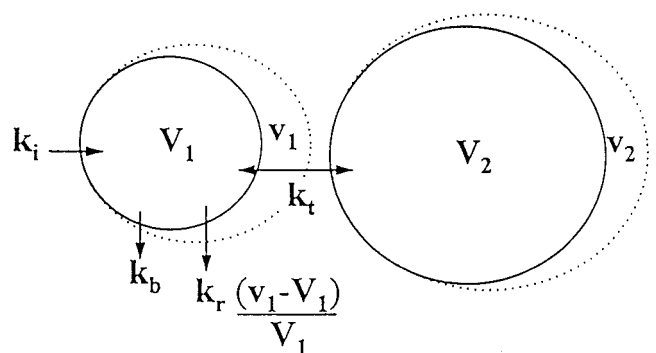


Fig. 1. The volume kinetic model used to analyze the data on the dilution of arterial blood during and after intravenous infusion of 0.9% NaCl in sheep. V₁ and V₂ = sizes of central and peripheral body fluid spaces, respectively; k_r = elimination rate constant; k_i = distribution rate constant; k_b = basal fluid losses.

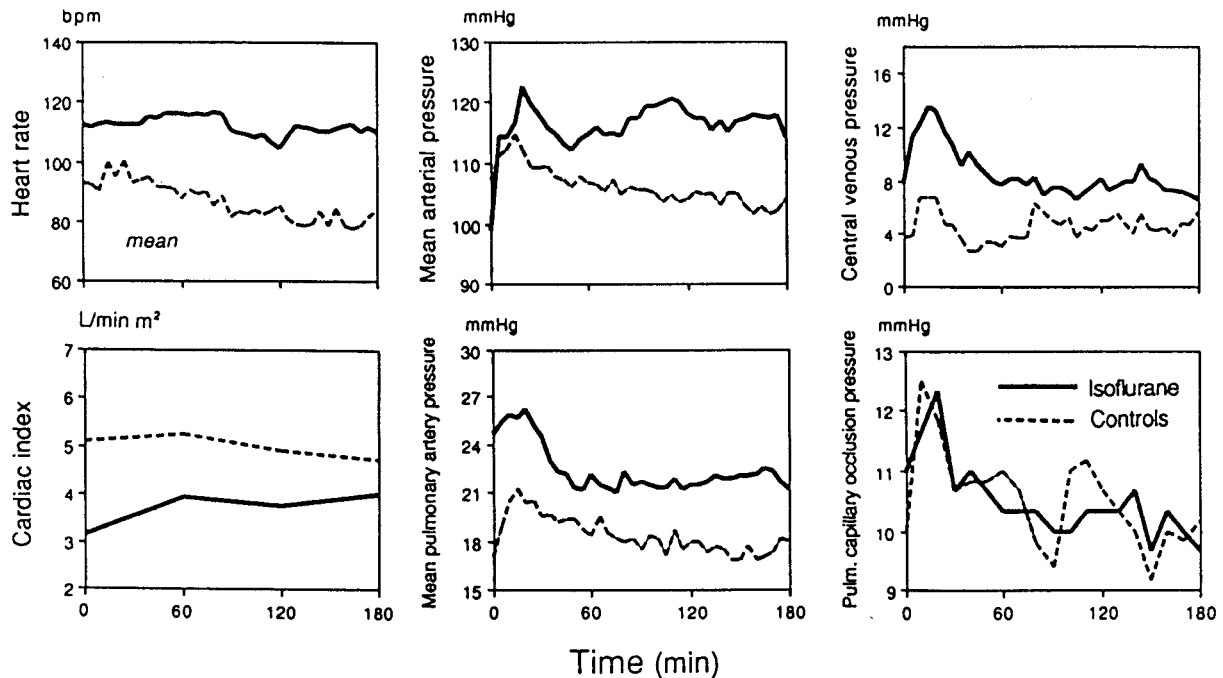


Fig. 2. Mean hemodynamic trends during and after infusion of 25 ml/kg of 0.9% saline over 20 min in sheep during isoflurane anesthesia (thick line) and while conscious (broken line).

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-

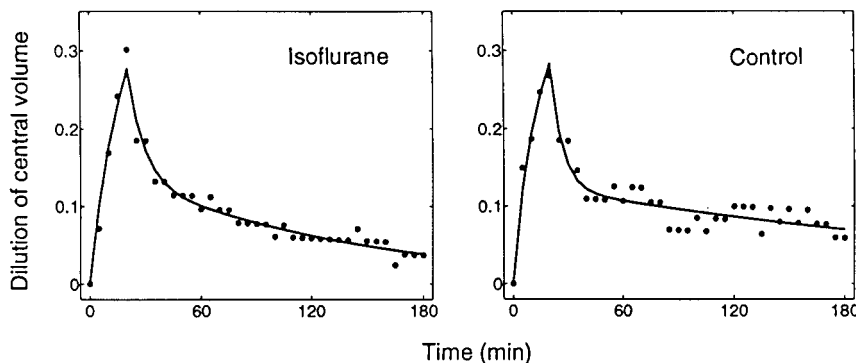
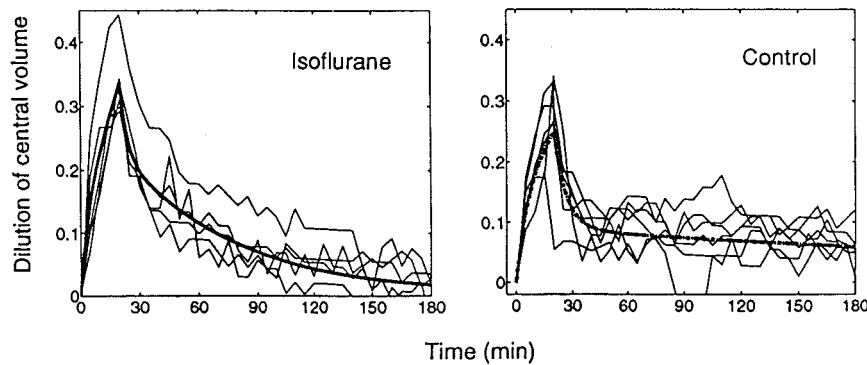


Fig. 3. Two representative experiments showing the optimal fit of individual data on the dilution of arterial plasma (as a decimal fraction) to the kinetic model shown in fig. 1. The solid line represents the modeled dilution of the central body fluid space, V_1 .

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_t) (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 by 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:

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

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

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 ± 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 ± SD).

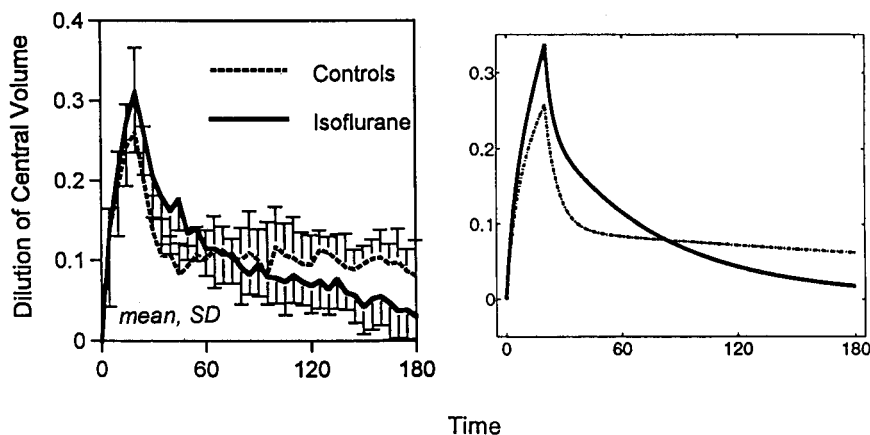


Fig. 5. The dilution of arterial plasma (as a decimal fraction) as indicated by changes in the hemoglobin concentration (*left*) and the modeled dilution of V_1 according to a volume kinetic analysis (*right*) during and after infusion of 0.9% NaCl in sheep.

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

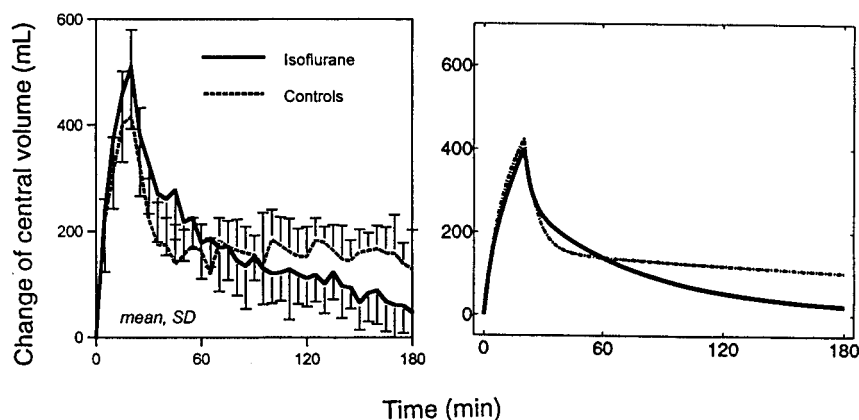
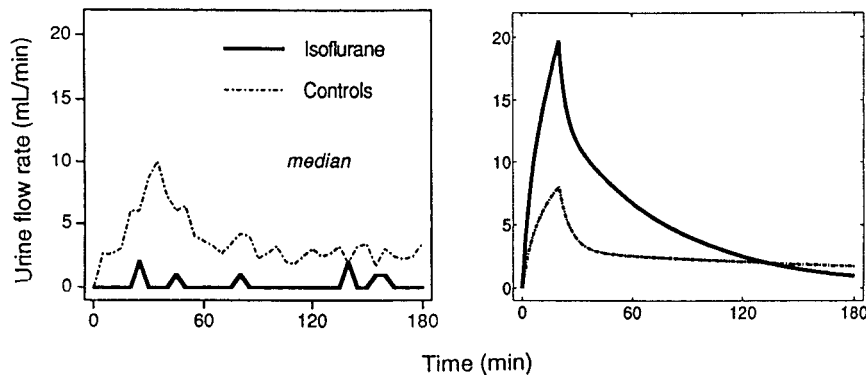


Fig. 6. The volume change of the central body fluid space as obtained by mass balance, using the plasma volume indicated by Evans blue as baseline (*left*) and volume kinetic analysis, using the size of V_1 as baseline (*right*), during and after infusion of 0.9% NaCl in sheep. The parameters shown in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

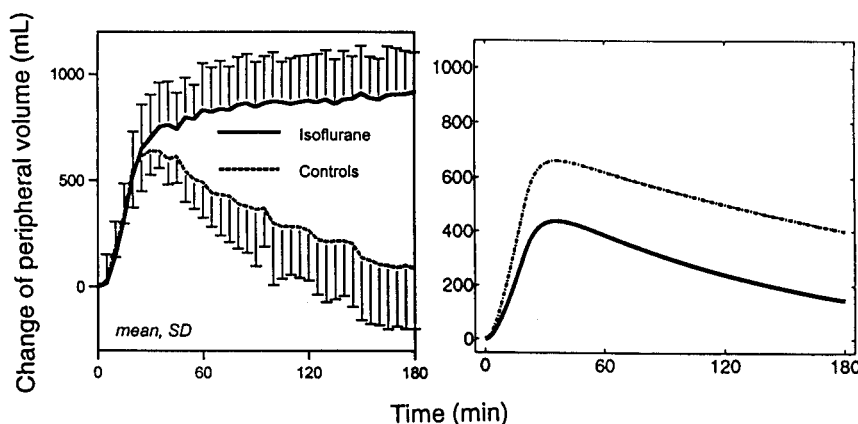
In previous studies, k_r , the elimination rate constant used in volume kinetics, correlated highly with urinary excretion, both in volunteers who received isotonic fluid^{4,6,16,17} and in conscious sheep in the current study. However, during continuous mechanical ventilation in the anesthetized state, 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 1.6 l. 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.



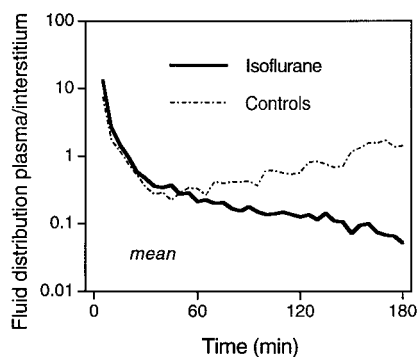


Fig. 9. Relation of fluid accumulation in plasma and interstitial tissue after infusion of normal saline in isoflurane-anesthetized and mechanically ventilated sheep. The figure is based on mean values for all six animals.

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.

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References

1. Prough DS, Mathru M: Acid-base, fluids, and electrolytes, Clinical Anesthesia, 3rd Edition. Edited by Barash PG, Cullen BF, Stoelting RK. Philadelphia, Lippincott-Raven, 1997, pp 157-87
2. Moore FD, Dagher FJ, Boyden CM, Lee CJ, Lyons JH: Hemorrhage in normal man: I. Distribution and dispersal of saline infusions following acute blood loss: Clinical kinetics of blood volume support. *Ann Surg* 1966; 163:485-504
3. Lamke LO, Liljedahl SO: Plasma volume changes after infusion of various plasma expanders. *Resuscitation* 1976; 5:93-102
4. Svensén C, Hahn RG: Volume kinetics of Ringer solution, dextran 70, and hypertonic saline in male volunteers. *ANESTHESIOLOGY* 1997; 87:204-12
5. Riddez L, Hahn RG, Brismar B, Strandberg A, Svensén C, Hedenstierna G: Central and regional hemodynamics during acute hypovolemia and volume substitution in volunteers. *Crit Care Med* 1997; 25:635-40
6. Drobin D, Hahn RG: Volume kinetics of Ringer's solution in hypovolemic volunteers. *ANESTHESIOLOGY* 1999; 90:81-91
7. Hahn RG, Resby M: Volume kinetics of Ringer's solution and dextran 3% during induction of spinal anaesthesia for caesarean section. *Can J Anaesth* 1998; 45:443-51
8. Ortiz RM, Wade CE: Water balance in rats exposed to chronic centrifugation. *J Appl Physiol* 2000; 89:56-60
9. Stoelting RK: Inhaled anesthetics, Pharmacology and Physiology in Anesthetic Practice, 3rd Edition. Edited by Stoelting RK. Philadelphia, Lippincott-Raven, 1999, pp 36-76
10. Bernards CM, Kern C, Cullen BF: Chronic cocaine administration reversibly increases isoflurane minimum alveolar concentration in sheep. *ANESTHESIOLOGY* 1996; 85:91-5
11. el Sayed H, Goodall SR, Hainsworth R: Re-evaluation of Evans blue dye dilution method of plasma volume measurement. *Clin Lab Haematol* 1995; 17:189-94
12. Boyd GW: The reproducibility and accuracy of plasma volume estimation in the sheep with both 131I gamma globulin and Evan's blue. *Aust J Exp Biol Med Sci* 1967; 45:51-75
13. Migita R, Gonzales A, Gonzales ML, Vandegriff KD, Winslow RM: Blood volume and cardiac index in rats after exchange transfusion with hemoglobin-based oxygen carriers. *J Appl Physiol* 1997; 82:1995-2002
14. Riesenfeld T, Hammarlund K, Norsted T, Sedin G: The temperature of inspired air influences respiratory water loss in young lambs. *Biol Neonate* 1994; 65:326-30
15. Cox P: Insensible water loss and its assessment in adult patients: A review. *Acta Anaesthesiol Scand* 1987; 31:771-6
16. Hahn RG, Drobin D: Urinary excretion as an input variable in volume kinetic analysis of Ringer's solution. *Br J Anaesth* 1998; 80:183-8
17. Hahn RG, Drobin D, Stahle L: Volume kinetics of Ringer's solution in female volunteers. *Br J Anaesth* 1997; 78:144-8
18. Hahn RG, Nilsson A, Stahle L: Distribution and elimination of the solute and water components of urological irrigating fluids. *Scand J Urol Nephrol* 1999; 33:35-41
19. Svensén C, Hjelmqvist H, Hahn RG: Volume kinetics of Ringer solution during endotoxaemia in conscious rabbits. *J Endotoxin Res* 1997; 4:425-30
20. Skillman JJ, Eltringham WK, Goldensen RH, Moore FD: Transcapillary refilling after hemorrhage in the splenectomized dog. *J Surg Res* 1968; 8:57-67
21. Hikasa Y, Okuyama K, Kakuta T, Takase K, Ogasawara S: Anesthetic potency and cardiopulmonary effects of sevoflurane in goats: Comparison with isoflurane and halothane. *Can J Vet Res* 1998; 62:299-306
22. McKinney MS, Fec JPH, Clarke RSJ: Cardiovascular effects of isoflurane and halothane in young and elderly adult patients. *Br J Anaesth* 1993; 71:696-701
23. Higuchi H, Sumita S, Wada H, Ura T, Ikemoto T, Nakai T, Kanno M, Satoh T: Effects of sevoflurane and isoflurane on renal functional and on possible markers of nephrotoxicity. *ANESTHESIOLOGY* 1998; 89:307-22
24. Kharasch ED, Yeo KT, Kenny MA, Buffington CW: Atrial natriuretic factor may mediate the renal effects of PEEP ventilation. *ANESTHESIOLOGY* 1988; 69:862-9
25. Pacher R, Frass M, Hartter E, Woloszczuk W, Leithner C: The role of α -atrial natriuretic peptide in fluid retention during mechanical ventilation with positive end-expiratory pressure. *Klinische Wochenschrift* 1986; 64:64-7
26. Venus B, Mathru M, Smith RA, Pham CG, Shirakawa Y, Sugiura A: Renal function during application of positive end-expiratory pressure in swine: Effects of hydration. *ANESTHESIOLOGY* 1985; 62:765-9

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_t (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.

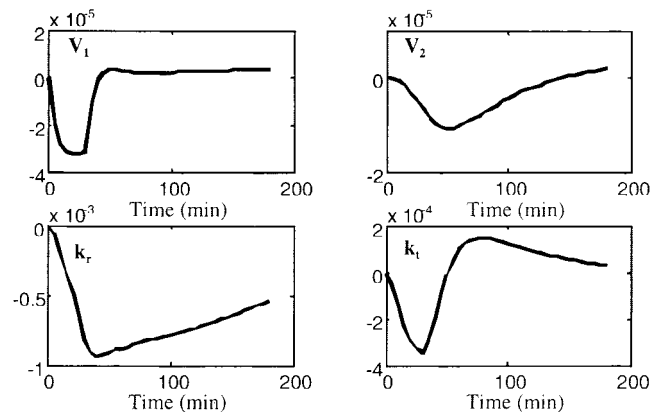


Fig. 10. Calculated partial derivatives.