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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 experimentally successful[3–5] and have provided guidance not only for treatment of hemorrhagic shock[2] 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 sheep[12] and humans[13] 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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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 technique[16] 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 measured 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

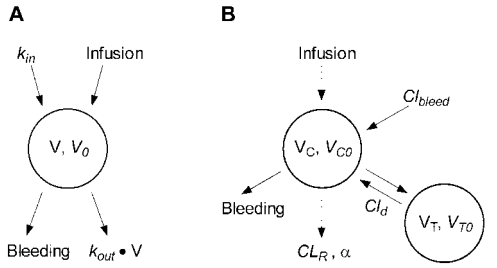

Fig. 1. (*a*) Basic single-compartment turnover model (Equation 3), which uses changes in the fluid volume (V) to estimate three model parameters: turnover rate k_{in} (equal to the baseline drinking rate of the sheep), the fractional turnover rate k_{out}, and the baseline fluid volume V_0. (*b*) Applied 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 governed 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:

$$fV_C = \frac{V_C - V_{C0}}{V_{C0}} \quad (4)$$

$$fV_T = \frac{V_T - V_{T0}}{V_{T0}}. \quad (5)$$

The turnover of fluid volume in the central compartment was

$$\frac{dV_C}{dt} = Inf - b_{rate} \cdot (1 - hct_0) - Cl_d \cdot (fV_C - fV_T) + Cl_{bleed} - CL_R \cdot e^{\alpha fV_C}. \quad (6)$$

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, hct_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

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

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

$$\frac{dA_e}{dt} = CL_R \cdot e^{\alpha fV_C}. \quad (8)$$

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 ± 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]

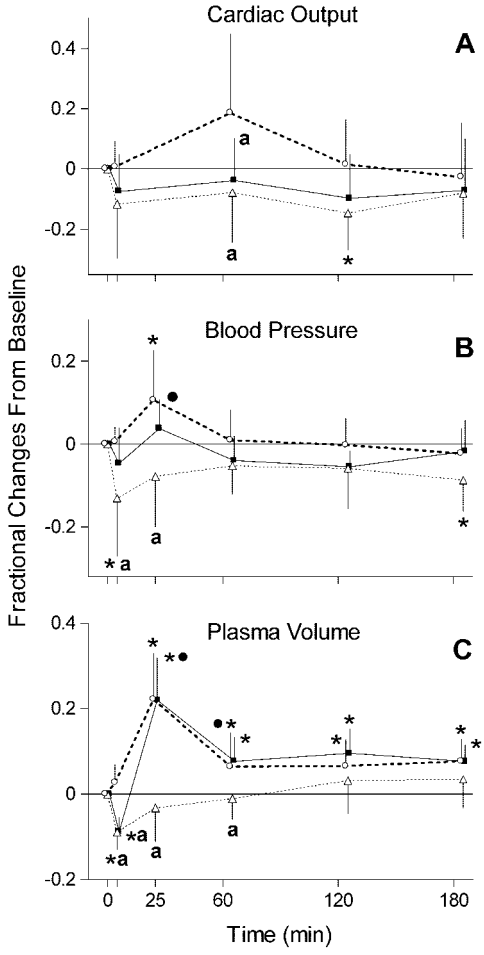

Fig. 2. Relative changes in cardiac output (*a*), mean arterial blood pressure (*b*), and plasma volume (*c*) in sheep (n = 12) after infusion of 0.9% saline (*open circles, bold broken line*), hemorrhage 300 ml (*open triangles, dotted line*), or hemorrhage followed by fluid infusion (*filled squares, 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 $P < 0.05$; [a]Significant 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 ± SD, and the baseline is represented as a *dotted line*.

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 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 then 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 ± 20% and 37 ± 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_{C0} 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_{C0}. The mean correlation between the parameters in the regression analysis was high for V_{C0} 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

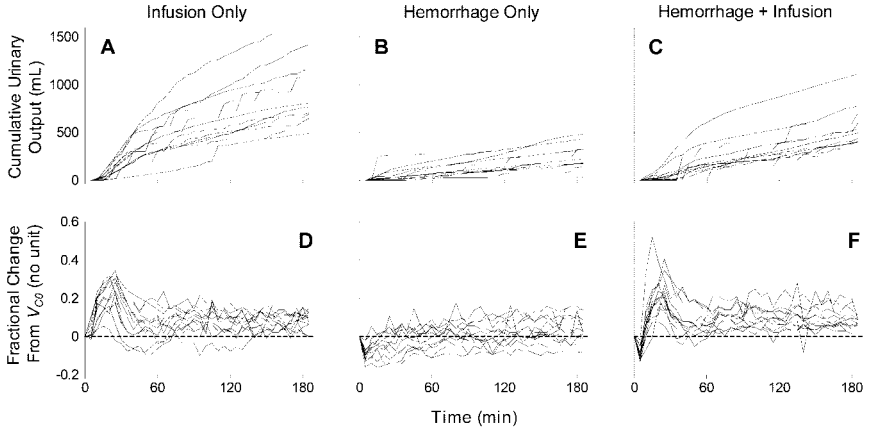

Fig. 3. *a–c*, Plots of cumulative urinary output *versus* time in sheep after infusion of 25 ml/kg 0.9% saline in 20 min (infusion only), bleeding of 300 ml in 5 min (hemorrhage only), and the combination of both, with bleeding immediately preceding the infusion (hemorrhage plus infusion). *d–f*, Plots of fractional changes from the baseline volume of the central compartment (V_{C0}), obtained from changes in hemoglobin concentrations *versus* time for the corresponding experimental sessions.

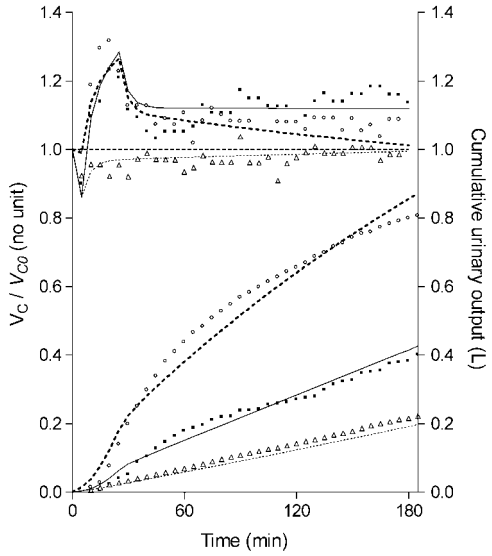

Fig. 4. Measured (*symbols*) and predicted (*lines*) data for a single animal (#152). All data were analyzed simultaneously. The three upper datasets refer to dilution of the central compartment measured by dilution of plasma (*left axis*). The three lower datasets refer to urinary output (*right axis*). 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 cessation 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 ± SD	1799 ± 1276	7653 ± 5478	343 ± 297	3.1 ± 2.3	3.5 ± 2.5	1.5 ± 1.0	8.5 ± 7.9

Data expressed as value (coefficient of variation). Coefficient of variation is calculated as (100 × SD/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	$-\alpha$	−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 approach 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-

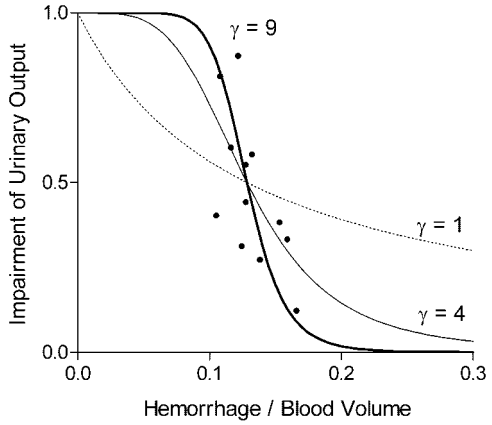

Fig. 5. Impairment of urinary excretion rate related to the magnitude of hemorrhage. *Filled symbols* show the ratio between CL_{R2} from the two hemorrhage sessions and CL_{R1} from the fluid infusion-only experiment for each animal related to bleeding as a fraction of blood volume. The relationship 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%.

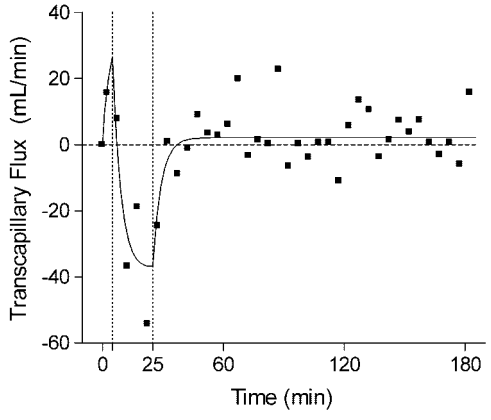

Fig. 6. Transcapillary flux in one sheep during the protocol with hemorrhage 0–5 min 300 ml followed by infusion of 25 ml/kg of 0.9% saline between 5 and 25 min (*between dotted lines*). *Filled squares* represent the mass balance calculation of influx to the plasma (positive values) and efflux from 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.

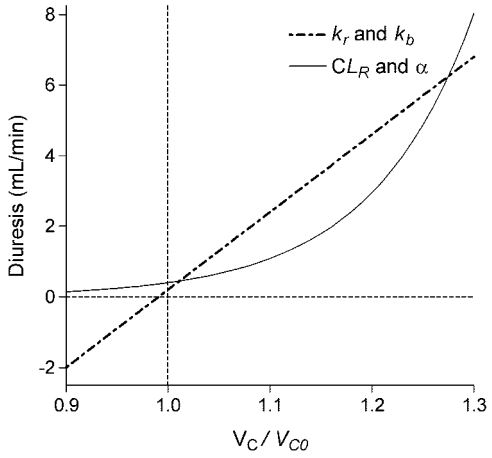

Fig. 7. Simulation of urinary output rate related to dilution of the central compartment (V_C/V_{C0}), expressed as the ratio between the central volume (V_C) to the baseline value at steady state (V_{C0}). The urinary excretion rate increases by a factor of 20 to 40 from a 30% dilution of the central compartment. The *dot-dashed line* is based on previous volume kinetic models where urinary output is expressed as a combination of $k_r \cdot$ 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 multifactoral 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_{C0}, 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 feature[9] 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 sheep[32] 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 multifactoral, 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.

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References

1. Shires GT, Williams J, Brown F: Acute changes in extracellular fluid associated with major surgical procedures. Ann Surg 1961; 154:803–10
2. Shires GT, Coln D, Carrico J, Lightfoot S: Fluid therapy in hemorrhagic shock. Arch Surg 1964; 88:688–93
3. Wiggers C: Physiology of Shock. New York: Commonwealth Fund, 1950, pp 121–46
4. Shires T, Williams J, Brown FT: A method for the simultaneous measurement of plasma volume, red blood cell mass and extracellular fluid space in man using radioactive ^{131}I, ^{35}S labeled sulphate, and ^{51}Cr. J Lab Clin Med 1960; 55:776–83
5. Shires GT, Brown FT, Canizaro PC, Somerville N: Distributional changes in extracellular fluid during acute hemorrhagic shock. Surg Forum 1960; 11:115–7
6. Anderson RW, James PM, Bredenberg CE, Collins JA, Levitsky S, Hardaway RM: Extracellular fluid and plasma volume studies on casualties in the Republic of Viet Nam. Surg Forum 1967; 18:29–30
7. Gutelius JR, Shizgal HM, Lopez G: The effect of trauma on extracellular water volume. Arch Surg 1968; 97:206–14
8. Reid DJ: Intracellular and extracellular fluid volume during surgery. Br J Surg 1968; 55:594–6
9. Holte K, Sharrock NE, Kehlet H: Pathophysiology and clinical implications of perioperative fluid excess. Br J Anaest 2002; 89:622–32
10. Brandstrup B, Tønnesen H, Beier-Holgersen R, Hjortsø E, Ørding H, Lindorff-Larsen K, Rasmussen M, Lanng C, Wallin L, and The Danish Study Group on Intraoperative Fluid Therapy (Iversen L, Gramkow C, Okholm M, Blemmer T, Svendsen P-E, Rottensten H, Thage B, Riis J, Jeppesen I, Teilum D, Christensen A, Graungaard B, Pott F): Effects of intravenous fluid restriction on postoperative complications: Comparison of two perioperative fluid regimens. Ann Surg 2003; 238:641–8
11. Svensen C, Hahn RG: Volume kinetics of Ringer solution, dextran 70, and hypertonic saline in male volunteers. ANESTHESIOLOGY 1997; 87:204–12
12. Brauer KI, Svensen C, Hahn RG, Traber LD, Prough DS: Volume kinetic analysis of the distribution of 0.9% saline in conscious versus isoflurane-anesthetized sheep. ANESTHESIOLOGY 2002; 96:442–9
13. Drobin D, Hahn RG: Volume kinetics of Ringer's solution in hypovolemic volunteers. ANESTHESIOLOGY 1999; 90:81–91
14. Rescigno A, Segre G: Drug and Tracer Kinetics. Waltham, MA, Blaisdell, 1966, pp 138–9
15. Lassen NA, Perl W: Tracer kinetic methods in medical physiology. New York, Raven Press, 1979, pp 102–12
16. Boyd GW: The reproducibility and accuracy of plasma volume estimation in the sheep with both ^{131}I gamma globulin and Evan's blue. Aust J Exp Biol Med Sci 1967; 45:51–75
17. Gabrielsson J, Weiner D: Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, 3rd edition. Stockholm, Swedish Pharmaceutical Press, 2000, pp 109–21
18. SAS Users Guide, 8th edition. Cary, NC, SAS Institute, Inc., 1999, pp 2083–226
19. Midwood AJ, Haggarty P, McGaw BA, Mollison GS, Milne E, Duncan GJ: Validation in sheep of the doubly labeled water method for estimating CO_2 production. Am J Physiol 1994; 266:R169–79
20. Southgate DA: Body content and distribution of water in healthy individuals. Bibl Nutr Dieta 1987; 40:108–16
21. Schoeller DA: Hydrometry, Human Body Composition. Edited by Roche AF, Heymsfield SB, Lohman TG. Champaign, IL, Human Kinetics, 1996, pp 25–44
22. Guyton AC, Hall JE: Textbook of Medical Physiology, 10th Edition. Philadelphia, WB Saunders, 2000, pp 264–94
23. Jonasson H, Hjelmqvist H, Rundgren M: Repeated hypotension induced by nitroprusside and haemorrhage in sheep: Effects on vasopressin release and recovery of arterial blood pressure. Acta Physiol Scand 1989; 137:427–36
24. Pruitt BA, Moncrief JA, Mason AD: Efficacy of buffered saline as the sole replacement fluid following acute measured hemorrhage in man. J Trauma 1967; 7:767–82
25. Lundvall J, Hillman J: Fluid transfer from skeletal muscle to blood during hemorrhage: Importance of beta adrenergic vascular mechanisms. Acta Physiol Scand 1978; 102:450–8
26. Riddez L, Hahn RG, Brismar B, Strandberg A, Svensen C, Hedenstierna G: Central and regional hemodynamics during acute hypovolemia and volume substitution in volunteers. Crit Care Med 1997; 25:635–40
27. Mellander S: On the control of capillary fluid transfer by precapillary and postcapillary vascular adjustments: A brief review with special emphasis on myogenic mechanisms. Microvasc Res 1978; 15:319–30
28. Drucker WR, Chadwick CDJ, Gann DS: Transcapillary refill in hemorrhage and shock. Arch Surg 1981; 116:1344–53
29. Järhult J, Holmberg J, Lundvall J, Mellander S: Hyperglycemic and hyperosmolar responses to graded hemorrhage. Acta Physiol Scand 1976; 97:470–5
30. Atkins EL, Pearce JW: Mechanisms of the renal response to plasma volume expansion. Can J Biochem Physiol 1959; 37:91–102
31. Johnson JA, Zehr JE, Moore WW: Effects of separate and concurrent osmotic and volume stimuli on plasma ADH in sheep. Am J Physiol 1970; 218:1273–80
32. Grimes JM, Buss LA, Brace RA: Blood volume restitution after hemorrhage in adult sheep. Am J Physiol 1987; 253:R541–4
33. Skillman JJ, Awwad HK, Moore FD: Plasma protein kinetics of the early transcapillary refill after hemorrhage in man. Surg Gynecol Obstet 1967; 125: 983–96
34. Mardel SN, Simpson SH, Kelly S, Wytch R, Beattie TF, Menezes G: Validation of a computer model of haemorrhage and transcapillary refill. Med Eng Phys 1995; 17:215–8
35. Simpson SH, Menezes G, Mardel SN, Kelly S, White R, Beattie T: A computer model of major haemorrhage and resuscitation. Med Eng Phys 1996; 18:339–43
36. Connolly CM, Kramer GC, Hahn RG, Chaisson NF, Kirschner RA, Svensén C, Hastings DA, Chinkes D, Prough DS: Isoflurane but not mechanical ventilation promotes third-space fluid losses during crystalloid volume loading. ANESTHESIOLOGY 2003; 98:670–81

Appendix

Treatment of Data in the Bleeding Experiments

Plasma dilution data were obtained using the formula

$$(PV_n - PV_0)/PV_0 = [(Hb_0 - Hb_n)/Hb_n]/(1-btc_0), \quad \text{(A1)}$$

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:

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

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

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