# Changes in Blood Volume and Hematocrit during Acute Preoperative Volume Loading with 5% Albumin or 6% Hetastarch Solutions in Patients before Radical Hysterectomy

Markus Rehm, M.D.,* Mathias Haller, M.D.,† Victoria Orth, M.D.,* Uwe Kreimeier, M.D.,† Mathias Jacob, M.D.,* Holger Dressel,‡ Sabine Mayer,‡ Heinz Brechtelsbauer,§ Udilo Finsterer, M.D.‖

*Background:* The impact of acute preoperative volume loading with colloids on blood volume has not been investigated sufficiently.

*Methods:* Before surgery, in 20 patients undergoing major gynecologic procedures, volume loading was performed during anesthesia by infusing approximately 20 ml/kg of colloid at a rate of 90 ml/min (group I: 5% albumin solution; group II: 6% hetastarch solution; n = 10 each). Plasma volume (indocyanine green dilution technique), erythrocyte volume (labeling erythrocytes with fluorescein), hematocrit, total protein, and hetastarch plasma concentrations (group II) were measured before and 30 min after the end of infusion.

*Results:* More than 1,350 ml of colloid (approximately 50% of the baseline plasma volume) were infused within 15 min. Thirty minutes after the infusion had been completed, blood volume was only 524 ± 328 ml (group I) and 603 ± 314 ml (group II) higher than before volume loading. The large vessel hematocrit (measured by centrifugation) dropped more than the whole body hematocrit, which was derived from double-label measurements of blood volume.

*Conclusions:* The double-label measurements of blood volume performed showed that 30 min after the infusion of approximately 20 ml/kg of 5% albumin or 6% hetastarch solution (within 15 min), only mean 38 ± 21% and 43 ± 26%, respectively, of the volume applied remained in the intravascular space. Different, *i.e.*, earlier or later, measuring points, different infusion volumes, infusion rates, plasma substitutes, or possibly different tracers for plasma volume measurement might lead to different results concerning the kinetics of fluid or colloid extravasation.

VOLUME loading (VL) with colloids is common clinical practice in the perioperative period, *e.g.*, before and during spinal or epidural anesthesia, before declamping the aorta during vascular surgery, or during preoperative acute hypervolemic hemodilution. However, the exact impact of VL with colloids on blood volume (BV), plasma volume (PV), and large vessel hematocrit ($Hct_{lv}$) have not been investigated sufficiently. Double-label measurements of BV can give information about fate, distribu-

tion, or redistribution of infused colloids. In this clinical study, to our knowledge, for the first time both compartments of BV were measured before and 30 min after an exactly defined VL with 5% albumin or 6% hetastarch solutions.

## Materials and Methods

The study was approved by the ethics committee at our institution, and all patients gave written informed consent. Twenty patients with a preoperative diagnosis of carcinoma of the cervix who were scheduled for radical hysterectomy were studied. All patients were American Society of Anesthesiologists physical status I–II without cardiovascular or pulmonary dysfunctions.

After arrival in the operating room, monitors were applied, and lumbar epidural catheters were placed in all patients. However, epidural anesthesia was not started until the 30 min post-VL measurements. After placement of the epidural catheter, general anesthesia was induced with fentanyl, thiopental, and *cis*-atracurium, and, after tracheal intubation, was maintained with 0.4–1.5 vol% isoflurane in a 50% oxygen–nitrous oxide mixture. Mechanical ventilation was performed to maintain arterial oxygen partial pressure at 200–250 mmHg and arterial carbon dioxide partial pressure at approximately 40 mmHg. Radial artery and central venous catheters were inserted. Cooling of the patients was prevented by means of a warming blanket. Before VL, no intravenous infusions were applied (except for very small amounts, which were necessary to inject the intravenous drugs). Perioperative monitoring included electrocardiogram, direct arterial blood pressure, central venous pressure, pulse oxymetry, repeated determinations of hemoglobin concentration (at least every 30 min; cyanhemoglobin method), and arterial blood gases. After a time interval of at least 20 min after induction of anesthesia, baseline measurements of PV (with indocyanine green [$PV_{ICG}$]), erythrocyte volume (EV), $Hct_{lv}$, and serum total protein concentration were performed during periods of stable anesthesia and hemodynamics.

### Determination of Plasma Volume with Indocyanine Green

Immediately before each dye injection, a calibration curve was constructed by measuring two times 10 ml of

the patient's blood having two known indocyanine green (ICG) concentrations (1.25 and 2.5 $\mu$g/ml of whole blood, respectively; ICG-Paesel, Frankfurt a. M., Germany). The light absorption of the blood was measured at 800 and 900 nm in a spectrophotometer that was developed by one of the authors (H. B.). After calibration, 0.25 mg/kg of ICG was injected into the central venous catheter as a bolus dose over 5 s (zero time = time of injection). For measuring ICG concentration, blood was continuously withdrawn (between the second and the fifth minute after injection) from the arterial catheter through a cuvette by means of a calibrated pump. The cuvette was attached to the spectrophotometer. For calculation of $PV_{ICG}$, see below (Calculations).

### Determination of Erythrocyte Volume

The method for measuring EV, using autologous erythrocytes stained with sodium fluorescein (SoF; Fluorescein-Lösung 10%, Alcon Pharma, Freiburg, Germany) and flow cytometry was developed in our laboratory and published in detail previously.[1,2] In brief, after arrival in the operating room, 40 ml of patient's blood was taken for labeling erythrocytes with SoF. In the laboratory, the blood was centrifuged, and thereafter, the erythrocyte suspension was incubated with 48 mg of SoF for 5 min. To prevent an excess of unbound fluorescein, the cells were washed twice using a solution containing calcium (Calcium Braun 10%, Braun Melsungen, Germany) and were resuspended to the volume of the initial blood sample (40 ml) using Ringer's lactate. For repeated measurements before and after VL, the cells were then divided in two aliquots for the two measurements.

Immediately before injection of ICG (see $PV_{ICG}$ measurements in Determination of Plasma Volume with Indocyanine Green), labeled erythrocytes were injected into the central venous catheter (20 ml per measurement). Four, 6, and 8 min after the injection of fluorescein-labeled erythrocytes, samples were drawn from the arterial catheter, stored on ice, and analyzed in the laboratory by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany; using an argon laser at 488 nm). For calculations of EV, see below (Calculations).

### Determination of Large Vessel Hematocrit and Total Protein Plasma Concentration

Large vessel hematocrit and total protein concentration were measured in arterial blood samples that were drawn approximately 1 min before the injection of ICG. $Hct_{lv}$ was measured in triplicate without correction for plasma trapping by centrifugation of the blood samples (12,000 rpm for 4 min). Total protein was determined using the Biuret method (variation coefficient < 2%).

### Volume Loading Procedure

After baseline measurements (of $PV_{ICG}$, EV, $Hct_{lv}$, and total protein concentration), VL was performed by infusing approximately 20 ml/kg of two different colloid solutions within 15 min at a rate of approximately 90 ml/min. Two groups of patients (n = 10 each) were investigated sequentially over a time span of 1.5 yr. In group I, 5% albumin solution (Centeon Pharma GmbH, Marburg, Germany) was used, and in group II, 6% hetastarch solution (molecular weight: 200,000 ± 25,000; degree of substitution: 0.5; Fresenius AG, Bad Homburg, Germany) was used for VL.

After completion of the infusion and a steady state interval of 30 min without any further infusions, all aforementioned measurements ($PV_{ICG}$, EV, $Hct_{lv}$, and total protein concentration) were taken again in identical sequence. At the measuring point after VL in group II, hetastarch plasma concentration was also measured according to the protocol presented below. Strictly after these measurements, epidural analgesia was started and 10 min after surgery began.

### Determination of Hetastarch Plasma Concentration

Measurement of hetastarch in plasma was conducted by a modified method described by Förster *et al.*[3] Plasma (0.5 ml) was transferred into a screw-topped tube containing 0.25 ml of 35% potassium hydroxide and placed into a boiling water bath for 45 min. After cooling and addition of 7.5 ml of ethanol (100%), the suspension was refrigerated at 4°C for 12 h. The samples were then centrifuged at 3,500 rpm at 0°C for 60 min. The supernatant was separated, and the remaining fluid was mixed with 2.5 ml of 2 M hydrochloric acid and then again placed in a boiling water bath for 120 min. After a second cooling procedure, the suspension was transferred into a 10-ml tube. Sodium hydroxide (2.5 ml) was added, and the suspension was filled with water up to the 10-ml mark of the tube. The same procedure was conducted with a hetastarch standard dilution sample (1.2 g/dl hetastarch in water). A total of 0.5 ml of the hydrolyzed sample or the hydrolyzed standard sample was transferred into a cuvette, 2 ml of gluco-quant suspension (hexokinase-glucose-6-phosphate-dehydrogenase; Boehringer, Mannheim, Germany) was added, and then the first light absorption (E1) was measured at 340 nm with a spectrophotometer (Cary 100 Bio, Varian, Melbourne, Australia). Afterward, 0.04 ml of gluco-quant was added, and after 10 min, the second light absorption (E2) was also determined at 340 nm. The difference (dE) between both values (E2 − E1) is proportional to the hetastarch concentration. The hetastarch concentration in the plasma sample (c) was derived by:

$$c = c\text{ standard} \times dE\text{ sample}/dE\text{ standard} \qquad (1)$$

where c standard = 1.2 g/dl.

Mean difference and SD of 200 *in vitro* measurements with different known hetastarch concentrations in plasma samples were −0.016 g/dl and ± 0.106 g/dl,

respectively, in comparison with the predicted (known) hetastarch concentrations.

### Calculations

Measured BV was derived by BV = $PV_{ICG}$ + EV, and whole body hematocrit ($Hct_{wb}$) was calculated as $Hct_{wb}$ = EV/BV.

**Calculations Concerning the Determination of Plasma Volume.** Indocyanine green concentration at injection time was derived by monoexponential extrapolation of the light absorption curve between minutes 2–5 back to zero time (using Excel for Windows, Microsoft, Redmond, Washington). If this value is put into the calibration curve, CBo, the theoretical whole blood concentration of the dye at injection time, is obtained. Theoretical plasma concentration of the dye at injection time (CPo) was calculated as:

$$CP_o = CB_o/(1 - Hct_{lv}).  \quad (2)$$

Measurements of $PV_{ICG}$ were calculated as:

$$PV_{ICG} = D/CPo,  \quad (3)$$

where D is the amount of dye injected.

**Calculations Concerning the Determination of Erythrocyte Volume.** Erythrocyte volume was calculated according to:

$$EV\ (ml) = (Ei \times Vi \times Hct_{lv})/(Ep \times FEf)  \quad (4)$$

where Ei = number of erythrocytes injected per milliliter of tagged cell suspension, Vi = volume of injected cell suspension in milliliters, $Hct_{lv}$ = large vessel hematocrit of the subject's arterial blood (measured in triplicate), Ep = number of erythrocytes per milliliter in the patient's arterial blood (measured in triplicate), and FEf = fraction of fluorescent erythrocytes determined by flow cytometry.

The fraction of fluorescent erythrocytes determined by flow cytometry was taken as the mean value from determinations of samples drawn at 4, 6, and 8 min after injection, counting in triplicate the fluorescent erythrocytes in 50,000 cells by means of the flow cytometer. Ei and Ep were obtained using a cell counter (530 nm; Coulter Electronics, Miami, FL).

### Statistical Analysis

As all measured and calculated data were distributed normally (assessed by Kolmogorov-Smirnov tests) and are presented as mean values with SDs. For demographic data, Student *t* tests for unpaired data were performed. A two-way analysis of variance for repeated measures was performed comparing intragroup and intergroup differences of measured and calculated variables. *Post hoc* testing was conducted using the Student-Newman-Keuls method for multiple comparisons. $P < 0.05$ was considered significant.

**Table 1. Patient Characteristics**

|  | Group I (n = 10) | Group II (n = 10) |
|---|---|---|
| Age | 39 ± 11 | 44 ± 12 |
| Height (cm) | 165 ± 5 | 168 ± 5 |
| Weight (kg) | 64 ± 13 | 65 ± 12 |
| Colloid infused (ml) | 1,379 ± 128 (Albumin) | 1,417 ± 209 (Hetastarch) |

Values are mean ± SD.

$P < 0.05$ difference between groups.

## Results

Demographic data of the patients in the two groups (table 1) did not show any significant intergroup difference. There was also no significant difference in the volumes of colloid infused for VL between the groups. Measured and calculated variables (before and after VL) in the two groups are shown in table 2. In group I, 30 min after infusion of 1,379 ± 128 ml (22.1 ± 3.6 ml/kg) of 5% albumin solution, $PV_{ICG}$ was only 507 ± 350 ml higher than before VL. As was to be expected, EV remained almost constant. $Hct_{lv}$ (measured by centrifugation) decreased more (−6.5 ± 1.4%) than $Hct_{wb}$ (−2.8 ± 2.7%; determined by double-label measurements of BV; see Calculations). Therefore, the ratio of $Hct_{wb}$ and $Hct_{lv}$ ($Hct_{wb}/Hct_{lv}$) increased significantly from 0.83 to 0.93. Thirty minutes after VL with 5% albumin solution, there was no significant change in total protein concentration in relation to the value before VL.

Patients in group II (see also tables 1 and 2) had a colloid infusion (6% hetastarch solution) of almost the same amount as patients in group I (22.1 ± 3.0 ml/kg). In comparison with group I, no significant differences were found in $PV_{ICG}$, EV, $Hct_{lv}$, $Hct_{wb}$, and the ratio of $Hct_{wb}/Hct_{lv}$ before and after VL. Thirty minutes after the infusion of 1,417 ± 209 ml of 6% hetastarch solution, $PV_{ICG}$ was only 597 ± 296 ml higher than before VL ($P > 0.05$ in relation to group I). Also in group II, $Hct_{lv}$ decreased more than $Hct_{wb}$, resulting in a significant increase in the ratio of $Hct_{wb}/Hct_{lv}$ from 0.84 to 0.95. VL with 6% hetastarch resulted in a significant total protein concentration decrease from 64 to 44 g/l. Mean hetastarch concentration in group II patients was 18 ± 1 g/l 30 min after completion of the infusion.

## Discussion

The most surprising results of the study were the small differences in BV and $PV_{ICG}$ before *versus* 30 min after the end of VL with the colloids used (table 2). Before our study, we expected (and we assume most anesthesiologists do) that 30 min after infusion, the nearly isooncotic colloids used would remain in blood to perhaps 80–90%. A volume effect around 90% of 5% albumin solution[4,5]

**Table 2. Measured and Calculated Variables before and after Volume Loading (VL) with 5% Albumin (Group I) or 6% Hetastarch Solution (Group II)**

| | Group I (n = 10) | | Group II (n = 10) | |
|---|---|---|---|---|
| | Before VL | After VL (Albumin) | Before VL | After VL (Hetastarch) |
| $PV_{ICG}$ (ml) | 3,014 ± 585 | 3,521 ± 673* | 2,984 ± 685 | 3,581 ± 706* |
| EV (ml) | 1,175 ± 223 | 1,192 ± 226 | 1,231 ± 171 | 1,238 ± 155 |
| BV (ml) | 4,189 ± 769 | 4,713 ± 868* | 4,215 ± 728 | 4,818 ± 721* |
| $Hct_{lv}$ (%) | 34.0 ± 2.9 | 27.5 ± 1.9* | 35.3 ± 4.5 | 27.3 ± 3.7* |
| $Hct_{wb}$ (%) | 28.2 ± 2.8 | 25.4 ± 2.2* | 29.7 ± 5.1 | 26.1 ± 4.6* |
| $Hct_{wb}/Hct_{lv}$ | 0.829 ± 0.050 | 0.925 ± 0.073* | 0.840 ± 0.078 | 0.953 ± 0.067* |
| Total protein concentration (g/l) | 60 ± 4 | 61 ± 3 | 64 ± 5 | 44 ± 5*† |
| Hetastarch plasma concentration (g/l) | — | — | — | 18 ± 1 |

Values are mean ± SD; for calculations see also Methods.

* $P < 0.05$ intragroup difference with respect to value before VL.   † $P < 0.05$ difference between groups.

$PV_{ICG}$ = plasma volume; EV = erythrocyte volume; BV = blood volume ($PV_{ICG}$ + RCV); $Hct_{lv}$ = large vessel hematocrit; $Hct_{wb}$ = whole body hemocrit; $Hct_{wb}/Hct_{lv}$ = ratio between whole body hemocrit and large vessel hemocrit.
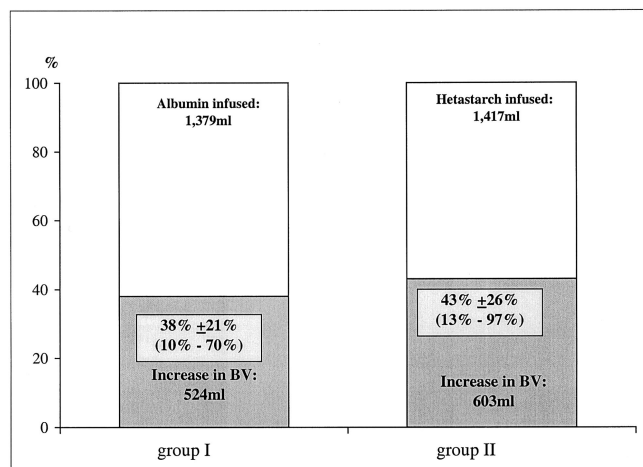
and 105% of 6% hetastarch solution (molecular weight: 200,000; degree of substitution: 0.62)[4] was already demonstrated during preoperative normovolemic hemodilution by means of the same PV measuring method as used in the current study. Figure 1 demonstrates, however, that in the current investigation only 38 ± 21% and 43 ± 26%, respectively, of the infused 5% albumin or 6% hetastarch solutions could be found in the intravascular space 30 min after VL. The large SDs and the respective minima and maxima (fig. 1) demonstrate a large interindividual variability. As there was only a weak correlation between BV before VL and the increase in BV 30 min after VL (r = 0.29; $P = 0.21$), from our data, we have no evidence that patients with low baseline BV values had a larger increase in BV than patients with a high baseline BV. After VL, $Hct_{lv}$ (measured by centrifugation of arterial blood samples) decreased more than $Hct_{wb}$ (derived by double-label measurements of BV; table 2), resulting in a significant increase in the ratio of $Hct_{wb}/Hct_{lv}$. Therefore, one might assume that $Hct_{lv}$ decreased dis-



**Fig. 1. Increase in blood volume (BV) 30 min after volume loading in relation to the amount of colloid infused (mean value, SD, and range).**

proportionally in relation to the increases in BV and $PV_{ICG}$. For assessing the possible physiological and clinical importance of our findings, first, one basic question should be asked: Are the small increases observed in BV and $PV_{ICG}$ simply a reflection of any measurement error or artifact? To answer this question, the three basic measuring methods ($Hct_{lv}$, EV, and $PV_{ICG}$) used in the current study are evaluated.

*Measurement of Large Vessel Hematocrit*

As previously mentioned, $Hct_{lv}$ measurements were taken in triplicate by centrifugation of arterial blood samples at 15,000g for 4 min without correction for plasma trapping. Two decimals were used, and the variation coefficient of repeated measurements was less than 2%. We did not correct for plasma trapping because previous work from our laboratory demonstrated that when the aforementioned centrifugation procedure was used, the effect of plasma trapping was minimal (< 1%).[6] The amount of trapped plasma depends, *e.g.*, on the centrifugal force and the length of centrifugation.[7,8] This amount is approximately 3%[8-10] or even lower, as a more recent investigation showed.[11] Assuming plasma trapping of 3%, we can simulate the respective changes in our results.

If the $Hct_{lv}$ values before and after VL are reduced by 3%, this will result in a reduction of EV in both groups before and after VL by exactly 3% (approximately 37 ml; see Calculations). In both groups, $PV_{ICG}$ will increase by a mean of 48 ml (1.6%) before VL and 40 ml (1.1%) after VL. As expected, a small decrease in EV combined with a small increase in $PV_{ICG}$ will result in only minimal changes in BV in relation to the values shown in table 2 (in both groups approximately +12 ml before and +4 ml after VL, respectively). In addition, the relation of $Hct_{wb}/Hct_{lv}$ before and after VL in both groups will be altered only minimally by a correction of $Hct_{lv}$ for plasma trapping as $Hct_{wb}$ will decrease to almost the same extent (approx-

imately 3%) as $Hct_{lv}$ decreases (3%). In conclusion, correcting the $Hct_{lv}$ values for plasma trapping of an unknown amount would not result in any fundamental change of our results.

### Erythrocyte Volume Measurement with Sodium Fluorescein

A close correlation between EV measurements using radiochromium and those using SoF-labeled erythrocytes was previously demonstrated by Lauermann *et al.*[12] In our laboratory, mean difference and variation coefficient for EV double measurements with SoF in healthy volunteers were 0.6 and 3.1%, respectively.[2] The results of an *in vivo* validation of our EV measuring method were published recently. After preoperative acute normovolemic hemodilution in 16 patients, the amount of erythrocytes removed ($399 \pm 81$ ml; calculated from volume and hematocrit of the whole blood in the hemodilution bags) could be determined precisely by means of EV measurements before and after acute normovolemic hemodilution. Mean difference between the amount of erythrocytes removed and the difference between EV before and after acute normovolemic hemodilution was $6 \pm 50$ ml ($\pm 4.2\%$ with respect to the EV before hemodilution measured with the SoF technique; n = 16).[1] After the labeling procedure (see Determination of Erythrocyte Volume), measuring samples of the injected cell suspension by flow cytometry showed that 100% of the injected erythrocytes were labeled with SoF. Therefore, we conclude that the EV measurement used in this study offered very precise data.

### Plasma Volume Measurement with Indocyanine Green

This method is referred to as the "whole blood method" for $PV_{ICG}$ determination, methodologic aspects of which were published previously.[13] Within 10 min, it gives reproducible results immediately in the operating room (mean difference and variation coefficient between double measurements: 0.3 and 6.2%, respectively).[14] In the current investigation, $PV_{ICG}$ measurements were taken almost simultaneously with EV measurements. However, any interference of the two tracers is very unlikely because of the different light absorption and fluorescence characteristics of ICG and SoF. In blood, ICG has its peak of light absorption at 805 nm and has no light absorption below 600 nm,[15] whereas SoF has an absorption maximum between 485 and 500 nm and does not absorb any light above 600 nm.[16] Maximum fluorescence emission for ICG and SoF appears at 835 and 520 nm, respectively, without any overlapping.[15,16]

### Critical View on the Blood Volume Measuring Method with Respect to Circulating and Noncirculating Compartments in the Intravascular Space

The precision of EV and $PV_{ICG}$ measurements used in this study have proven to be high. However, one might ask for the real distribution spaces of the tracers used within the time of our measurements (4–8 min after the injection of SoF-labeled erythrocytes and 2–5 min after the injection of ICG, respectively). To answer this question we must examine the distribution and the flow characteristics of erythrocytes and plasma in the vascular bed, including microcirculatory networks. Classic methods estimating microvascular hematocrit used the anatomic width of a microvessel and the number and mean corpuscular volume of erythrocytes on static pictures of microvessels.[17,18] Respective calculations led to the assumption that the hematocrit in microvessels is substantially lower than in large vessels.[18] This refers to the fact that in microvessels, erythrocytes typically travel near the central line of the blood stream and have a higher velocity than mean blood flow, a hypothesis brought forward by Fahraeus as early as 1928.[19] One important premise of the "classic model" is that the entire (total) PV is always circulating in macrovascular and microvascular beds.[18] An interpretation of our data in line with this classic model would mean that (1) our measured EV represents the circulating EV, (2) $PV_{ICG}$ should represent the total and circulating PV, and (3) the small increase in $PV_{ICG}$ in both groups 30 min after the completion of VL leads to the conclusion of a considerable extravasation of the administered fluids out of the intravascular space.

The results of several other recent studies using direct intravitalmicroscopy or other indirect estimations, however, questioned this classic model and the extent of Fahraeus' hypothesis.[20–22] These investigations have shown that there is a difference between the anatomic width of a microvessel and the width of the space available for circulating erythrocytes. An exclusion zone for erythrocytes adjacent the endothelial surface could be demonstrated. This zone was termed "plasma layer" or "endothelial surface layer" (ESL) and has a thickness of approximately 0.4–1.5 $\mu$m.[18,23–25] It contains fluid (without erythrocytes), is immobile or moves very slowly, and is in a dynamic equilibrium with the flowing plasma.[25] This new model implies that PV can be separated into two compartments: a circulating PV ($PV_{circ}$) and a resting, noncirculating PV in the ESL. In line with the new model, erythrocytes are circulating in the $PV_{circ}$, and the hematocrit in the central, circulating column of microvessels does not substantially differ from $Hct_{lv}$.[20–22] Moreover, the volume of ESL probably does not remain constant in case of replacing or diluting plasma with artificial fluids. Evidence was found that in such cases, a substantial part of the absorbed layer of plasma components (ESL) can be dissolved into the flow-

ing blood, thereby widening the circulating column and decreasing the thickness of the ESL.[25,26]

How can our data be interpreted in line with the new model? It is obvious that our measured EV should exclude the ESL and that it only concerns the circulating compartment of the intravascular space. However, because PV can be separated into a circulating compartment and a noncirculating compartment (the ESL), two different distribution spaces for ICG are conceivable. These are the $PV_{circ}$—if ICG does not enter the ESL within the time of our measurements— or total PV, which is the sum of $PV_{circ}$ and noncirculating PV, if ICG also enters the plasma of the ESL and equilibrates with it. Vink and Duling[27] were able to demonstrate that small anionic tracers (0.4–40 kd) entered the ESL with a half-time of 11–60 min, whereas small neutral tracers such as rhodamine entered the ESL within one transit time and equilibrated with the ESL within 1 min.[27] ICG is an anionic tracer (775 Da), but it combines with plasma proteins (mainly $\alpha$ lipoproteins) within a few seconds.[28,29] This dye was repeatedly used for infrared fluorescence videomicroscopy of skin capillaries.[30-33] These investigations showed that on the arteriolar or venular side of finger capillaries in normal subjects, the width of the circulating erythrocyte column averaged 68% in comparison with the width of the microvessels determined with ICG within 5 min after injection. As no leakage of the dye across the endothelial cells could be observed, the respective difference in diameters was attributed to the plasmatic zone between the erythrocyte column and the capillary wall (ESL). ICG did fill the total diameter of the capillaries (including the ESL) within 1 min.[30-33]

Because of the hypothesis (according to the new model) that the hematocrit in the circulating compartment in microvessels is not substantially different from $Hct_{lv}$, we should be able to calculate $PV_{circ}$ with the aid of our measured EV and $Hct_{lv}$ values and compare it with $PV_{ICG}$.

The $PV_{circ}$ can be calculated as:

$$PV_{circ} = (EV/Hct_{lv}) - EV \qquad (5)$$

If the distribution space for ICG represents total PV, the difference between $PV_{ICG}$ and $PV_{circ}$ would give an estimate of the volume of ESL:

$$\text{Estimated volume of ESL} = PV_{ICG} - PV_{circ}. \qquad (6)$$

Because of these assumptions, figures 2A and 2B would demonstrate the relation between $PV_{ICG}$, $PV_{circ}$, and the estimated volume of ESL before and after VL for both groups. At first glance, it can be seen that in both groups the proportions seem to be reasonable, as $PV_{ICG}$ is always higher than $PV_{circ}$. It was already demonstrated that 30 min after VL in groups I and II, $PV_{ICG}$ was only 507 ± 350 ml and 597 ± 296 ml higher than before VL, respectively (see also table 2). $PV_{circ}$, however, increased
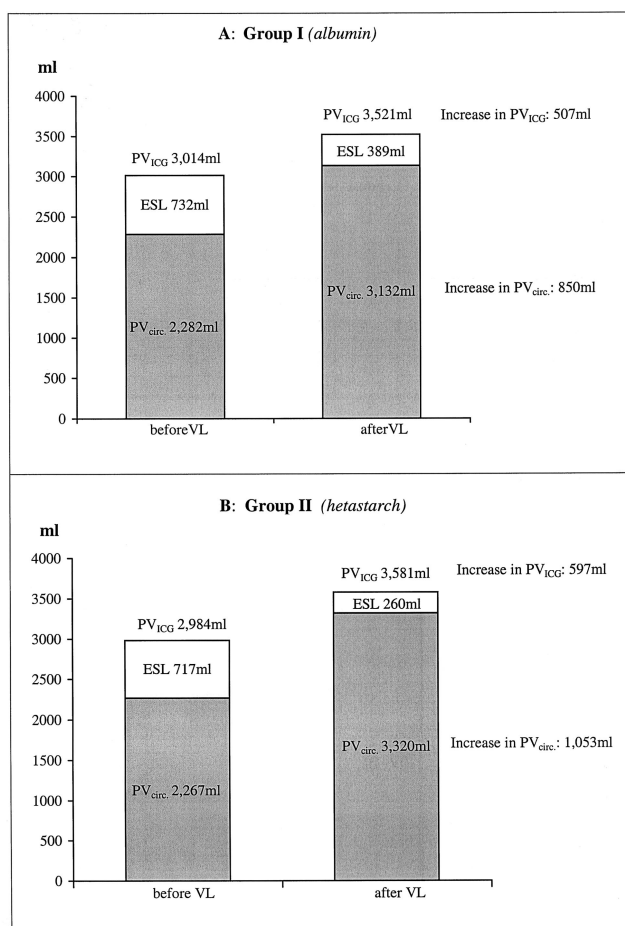


Fig. 2. (*A*) Relation of plasma volume measured with indocyanine green ($PV_{ICG}$), circulating plasma volume ($PV_{circ}$), and estimated volume of the endothelial surface layer (ESL) before and 30 min after volume loading (VL). Group I (albumin). (*B*) Relation of $PV_{ICG}$, $PV_{circ}$, and ESL before and 30 min after VL. Group II (hetastarch).

more, namely, 850 ± 134 ml in group I and 1,053 ± 199 ml in group II. This implies a decrease in the volume of ESL from approximately 700 to 400 ml (group I) and from approximately 700 to 300 ml (group II) 30 min after the completion of VL.

An interpretation in line with the new model could mean that there was only a small increase in total PV ($PV_{ICG}$) in relation to the amount of VL, that $PV_{circ}$ increased more than $PV_{ICG}$, and that the larger increase in $PV_{circ}$ in relation to the increase in $PV_{ICG}$ was caused by a considerable decrease ("washout") in the volume of ESL.

Up to now, the distribution space of ICG in the time of our measurements (2–5 min after injection) is not completely known. Consequently, we cannot finally decide which interpretation, the one according to the classic model or the other one according to the new model, is the right one. The aforementioned intravital videomicroscopic data, however, make us assume that in our experiment, the distribution space of ICG ($PV_{ICG}$) was more likely the total intravascular PV than the $PV_{circ}$ according to the new model. Regardless of whether

**Table 3. Balance of the Intravascular Protein and Hetastarch According to the Classic Model and the New Model**

| | Classic Model (Calculations Based on $PV_{ICG}$) | | New Model (Calculations Based on $PV_{circ}$) | |
| --- | --- | --- | --- | --- |
| | Group I (n = 10) | Group II (n = 10) | Group I (n = 10) | Group II (n = 10) |
| Albumin infusion (g) | 69 | — | 69 | — |
| Hetastarch infusion (g) | — | 85 | — | 85 |
| PV before VL ($PV_{ICG}$ or $PV_{circ}$) (ml) | 3,014 ($PV_{ICG}$) | 2,984 | 2,282 ($PV_{circ}$) | 2,267 |
| Total protein concentration before VL (g/l) | 60 | 64 | 60 | 64 |
| IVP before VL (g) | 181 | 191 | 137 | 145 |
| PV after VL ($PV_{ICG}$ or $PV_{circ}$) (ml) | 3,521 ($PV_{ICG}$) | 3,581 | 3,132 ($PV_{circ}$) | 3,320 |
| Total protein concentration after VL | 61 | 44 | 61 | 44 |
| IVP after VL (g) | 215 | 158 | 191 | 146 |
| Deficit in IVP after VL (g) | 35 | 33 | 15 | −1 |
| Hetastarch plasma concentration after VL | — | 18 | — | 18 |
| Intravascular hetastarch after VL (g) | — | 64 | — | 60 |
| Deficit in hetastarch after VL (g) | — | 21 | — | 25 |

Values are mean.

PV = plasma volume; VL = volume loading; IVP = intravascular protein.

$PV_{ICG}$ represents the sum of circulating and noncirculating PV (new model) or just the circulating (and entire) PV (classic model), the small increase in $PV_{ICG}$ 30 min after the completion of VL in any case leads to the conclusion of considerable extravasation of fluid out of the intravascular space. In other words, in line with both interpretations, the difference between the measured (small) increase in $PV_{ICG}$ 30 min after VL and the amount of volume infused should represent the total loss of fluid out of the intravascular space 30 min after the completion of VL (fig. 1). As long as the exact volume of ESL is unknown, as tracers that allow an accurate distinction between circulating and noncirculating plasma are not available, the generated data may be a step forward in the ongoing debate about the impact and amount of ESL.

### Considerations About the Fate of the Colloids Infused (Albumin or Hetastarch)

As may be taken from table 2, total protein concentration remained constant after VL with 5% albumin solution, whereas it significantly decreased after the infusion of 6% hetastarch solution. An estimate of the total amount of intravascular protein (IVP) before and 30 min after VL should result from the product of the respective total protein plasma concentration and PV:

$$\text{IVP}_{\text{before VL}} = \text{total protein concentration}_{\text{before VL}} \times \text{PV}_{\text{before VL}} \quad (7)$$

$$\text{IVP}_{\text{after VL}} = \text{total protein concentration}_{\text{after VL}} \times \text{PV}_{\text{after VL}} \quad (8)$$

The deficit in IVP after VL can be estimated by the difference between the increase in IVP and the amount of albumin infused:

$$\text{Deficit in IVP}_{\text{after VL}} = \text{albumin infusion} - (\text{IVP}_{\text{after VL}} - \text{IVP}_{\text{before VL}}) \quad (9)$$

In group II, the amount of hetastarch remaining in the intravascular space 30 min after VL can be calculated as:

$$\text{Intravascular hetastarch}_{\text{after VL}} = \text{hetastarch plasma concentration}_{\text{after VL}} \times \text{PV}_{\text{after VL}} \quad (10)$$

The deficit in intravascular hetastarch after VL can be estimated by the difference between the amount of hetastarch infused and the amount of intravascular hetastarch after VL:

$$\text{Deficit in intravascular hetastarch} = \text{hetastarch infusion} - \text{intravascular hetastarch}_{\text{after VL}} \quad (11)$$

At this point, the question of which PV is to be taken for such estimates arises. Because total protein as well as hetastarch plasma concentrations were determined from arterial blood samples, these should be concentrations with respect to the circulating intravascular compartment. According to the classic model, $PV_{ICG}$ should be used for PV, whereas according to the new model, $PV_{circ}$ (based on EV and $Hct_{lv}$ measurements) should be the right variable for such estimates. Table 3 shows the respective estimates according to the classic and new models.

Following the classic model, in group I, IVP increased 30 min after VL with 69 g of albumin from a mean of 181 g to only 215 g, so that 35 g of the protein infused (approximately 50%) did not remain in the intravascular space. In group II, there was a deficit in hetastarch of 21 g (64–85 g; table 3), which was accompanied by a surprising mean decrease (deficit) in IVP of 33 g.

With respect to $PV_{circ}$ according to the new model, however, there was a deficit in (circulating) IVP of only 15 g in group I and no decrease or deficit in (circulating) IVP in group II. In group II, the deficits in intravascular

hetastarch calculated by means of $PV_{ICG}$ or $PV_{circ}$ were not substantially different (table 3; classic model: 21 g *vs.* new model: 25 g). Unfortunately, it is unknown which and how many plasma proteins may constitute the ESL.[25] As a result, considerations about a "washout" of not only fluid but also of protein into the circulating compartment as a result of the decrease in the ESL after VL can only be speculative.

In conclusion, in line with both interpretations of our data, one according to the classic model and the other according to the new model, considerable deficits or losses of protein and hetastarch 30 min after VL could be observed. However, by means of the measuring methods used in this investigation, the fate of the colloids infused could not be cleared up completely.

In summary, in a special clinical setting, in 20 patients during anesthesia and before surgery, we measured BV by means of a double-label technique as the sum of $PV_{ICG}$ and EV before and 30 min after VL with two different, nearly isooncotic colloid solutions (20 ml/kg at a rate of 90 ml/min). Thirty minutes after the infusion of more than 1,350 ml of 5% albumin (group I) or 6% hetastarch solutions (group II), an increase in BV of only $524 \pm 328$ ml (group I) and of only $603 \pm 314$ ml (group II) could be measured. Different, *i.e.,* earlier or later, measuring points, different infusion volumes, infusion rates, plasma substitutes, and possibly different tracers for PV measurement may lead to different results concerning the kinetics of fluid or protein extravasations. Further investigations in this field are urgently needed.

# References

1. Orth V, Rehm M, Thiel M, Kreimeier U, Haller M, Brechtelsbauer H, Finsterer U: First clinical implications of perioperative red cell volume measurement with a nonradioactive marker (sodium fluorescein). Anesth Analg 1998; 87:1234–8

2. Haller M, Dressel H, Thiel M, Orth V, Brechtelsbauer H, Pfeiffer M, Finsterer U: Determination of red-cell volume with a nonradioactive marker (sodium fluorescein) in humans using flow cytometry. Infusionsther Transfusionsmed 1997; 24:132–7

3. Förster H, Wicarkzyk C, Duziak R: Bestimmung der plasmaelimination von hydroxyethylstärke und von dextran mittels verbesserter analytischer methode. Infusionstherapie 1981; 2:88–94

4. Haller M, Brechtelsbauer H, Akbulut C, Fett W, Briegel J, Finsterer U: Isovolemic hemodilution alters the ratio of whole-body to large- vessel hematocrit (F-cell ratio): A prospective, randomized study comparing the volume effects of hydroxyethyl starch 200,000/0.62 and albumin. Infusionsther Transfusionsmed 1995; 22:74–80

5. Rehm M, Orth V, Kreimeier U, Thiel M, Haller M, Brechtelsbauer H, Finsterer U: Changes in intravascular volume during acute normovolemic hemodilution and intraoperative retransfusion in patients with radical hysterectomy. ANESTHESIOLOGY 2000; 92:657–64

6. Finsterer U, Prucksunand P, Brechtelsbauer H: Critical evaluation of methods for determination of blood volume in the dog. Pflügers Arch 1973; 341:63–72

7. Chaplin H, Mollison PL: Correction for plasma trapped in the red cell column of the hematocrit. Blood 1952; 7:1227–37

8. Owen CA, Power MH: Intracellular plasma of centrifuged human erythrocytes as measured by means of iodo[131]-albumin. J Appl Physiol 1953; 5:323–9

9. England JM, Walford DM, Waters DAW: Re-assesment of the reliability of the hematocrit. Br J Haematol 1972; 23:247–56

10. Vazquez ON, Newerly K, Yalow RS, Berson SA: Determination of trapped plasma in the centrifuged erythrocyte volume of normal human blood with radioiodinated ($I^{131}$) human serum albumin and radiosodium ($Na^{24}$). J Lab Clin Med 1952; 39:595–604

11. Pearson TC, Guthrie DL: Trapped plasma in the microhematocrit. Am J Clin Pathol 1982; 78:770–2

12. Lauermann I, Wilhelm G, Kirchner E: Blood volume determination with sodium fluorescein and radioactive chromium: A clinical comparison of methods. Infusionsther Transfusionsmed 1994; 21:138–42

13. Haller M, Akbulut C, Brechtelsbauer H, Fett W, Briegel J, Finsterer U, Peter K: Determination of plasma volume with indocyanine green in man. Life Sci 1993; 53:1597–604

14. Rehm M, Haller M, Brechtelsbauer H, Akbulut C, Finsterer U: Changes in plasma volume in immediate pre- and postoperative periods in patients with major gynaecologic surgery. Infusionsther Transfusionsmed 1998; 25:222–8

15. Nielsen NC: Spectrophotometric determination of indocyanine green in plasma especially with a view to an improved correction for blank density. Scand J Clin Lab Invest 1963; 30:613–21

16. Jaanus SD: Dyes, Clinical Ocular Pharmacology, 2nd edition. Edited by Bartlett JD, Jaanus SD. Boston, Butterworths, 1989, pp 323–35

17. Barbee JH, Cokelet GR: Prediction of blood flow in tubes with diameters as small as 29 $\mu$. Microvasc Res 1971; 3:17–21

18. Klitzman B, Duling BR: Microvascular hematocrit and red cell flow in resting and contracting striated muscle. Am J Physiol 1979; 237:H481–90

19. Fahraeus R: Die strömungsverhältnisse und die verteilung der blutzellen im gefäßsystem. Klin Wochenschr 1928; 7:100–6

20. Desjardins C, Duling BR: Microvessel hematocrit: Measurement and implications for capillary oxygen transport. Am J Physiol 1987; 252:H494–503

21. Desjardins C, Duling BR: Heparinase treatment suggests a role for the endothelial cell glycocalyx in regulation of capillary hematocrit. Am J Physiol 1990; 258:H647–54

22. Keller MW, Damon DN, Duling BR: Determination of capillary tube hematocrit during arteriolar microperfusion. Am J Physiol 1994; 266:H2229–38

23. Vink H, Duling BR: Identification of distinct luminal domains for macromolecules, erythrocytes, and leucocytes within mammalian capillaries. Circ Res 1996; 79:581–9

24. Pries AR, Secomb TW, Sperandio M, Gaehtgens P: Blood flow resistance during hemodilution: Effect of plasma composition. Cardiovasc Res 1998; 37: 225–35

25. Pries AR, Secomb TW, Gaehtgens P: The endothelial surface layer. Pflügers Arch-Eur J Physiol 2000; 440:653–66

26. Pries AR, Fritzsche A, Ley K, Gaehtgens P: Redistribution of red blood cell flow in microcirculatory networks by hemodilution. Circ Res 1992; 70:1113–21

27. Vink H, Duling BR: Capillary endothelial surface layer selectively reduces plasma solute distribution volume. Am J Physiol Heart Circ Physiol 2000; 278: H285–9

28. Landsman MLJ, Kwant G, Mook GA, Zijlstra WG: Light-absorbing properties, stability, and spectral stabilization of indocyanine green. J Appl Physiol 1976; 40:575–83

29. Baker KL: Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma and $\alpha$1 lipoproteins.Proc Soc Biol Med 1966; 122:957–63

30. Montea G, Brülsauer M, Jäger K, Bollinger A: Infrared fluorescence videomicroscopy of skin capillaries with indocyanine green. Int J Microcirc Clin Exp 1987; 6:25–34

31. Brülsauer M, Bollinger A: Measurement of different human microvascular dimensions by combination of videomicroscopy with Na-fluorescein (NaF) and indocyanine green (ICG) in normals and patients with systemic sclerosis. Int J Microcirc Clin Exp 1991; 10:21–31

32. Bollinger A, Saesseli B, Hoffmann U, Franzeck UK: Intravital detection of skin capillary aneurysms by videomicroscopy with indocyanine green in patients with progressive systemic sclerosis and related disorders. Circulation 1991; 83:546–51

33. Zaugg-Vesti BR, Franzeck UK, von Ziegler C, Pfister G, Yanar A, Bollinger A: Skin capillary aneurysms detected by indocyanine green in type I diabetes with and without retinal microaneurysms. Int J Microcirc 1995; 15:193–8