

# Acid-Base Changes Caused by 5% Albumin versus 6% Hydroxyethyl Starch Solution in Patients Undergoing Acute Normovolemic Hemodilution

## A Randomized Prospective Study

Markus Rehm, M.D.,\* Victoria Orth, M.D.,\* Stefan Scheingraber, M.D.,\* Uwe Kreimeier, M.D.,† Heinz Brechtelsbauer,‡ Udilo Finsterer, M.D.§

**Background:** Preoperative acute normovolemic hemodilution (ANH) is an excellent model for evaluating the effects of different colloid solutions that are free of bicarbonate but have large chloride concentrations on acid-base equilibrium.

**Methods:** In 20 patients undergoing gynecologic surgery, ANH to a hematocrit of 22% was performed. Two groups of 10 patients each were randomly assigned to receive either 5% albumin or 6% hydroxyethyl starch solutions containing chloride concentrations of 150 and 154 mm, respectively, during ANH. Blood volume (double label measurement of plasma and red cell volumes), pH,  $P_{aCO_2}$ , and serum concentrations of sodium, potassium, chloride, lactate, ionized calcium, phosphate, albumin, and total protein were measured before and 20 min after completion of ANH. Strong ion difference was calculated as serum sodium plus serum potassium minus serum chloride minus serum lactate. The amount of weak plasma acid was calculated using a computer program.

**Results:** After ANH, blood volume was well maintained in both groups. ANH caused slight metabolic acidosis with hyperchloremia and a concomitant decrease in strong ion difference. Plasma albumin concentration decreased after hemodilution with 6% hydroxyethyl starch solution and increased after hemodilution with 5% albumin solution. Despite a three-times larger decrease in strong ion difference after ANH with 6% hydroxyethyl starch solution, the decrease in pH was nearly the same in both groups.

**Conclusions:** ANH with 5% albumin or 6% hydroxyethyl starch solutions led to metabolic acidosis. A dilution of extracellular bicarbonate or changes in strong ion difference and albumin concentration offer explanations for this type of acidosis. (Key words: Acid-base balance; colloid infusion; hyperchloremia; Stewart approach.)

DESPITE the frequent practice of preoperative acute normovolemic hemodilution (ANH), to our knowledge,

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\* Staff Anesthesiologist, † Assistant Professor, ‡ Medical Engineer, § Professor of Anesthesiology.

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Address reprint requests to Dr. Rehm: Klinik für Anaesthesiologie, Ludwig-Maximilians-Universität, Marchioninistrasse 15, D-81377 Munich, Germany. Address electronic mail to: jfreeden@ana.med.uni-muenchen.de. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

there are no systematic investigations describing acid-base changes in the course of this procedure. This is quite surprising, because ANH is a good model for investigating and quantifying the effects of different intravenous infusions on acid-base equilibrium. Therefore, we evaluated acid-base changes in the course of ANH in a prospective, randomized, clinical study with all relevant variables being measured. To document exact normovolemia after ANH, simultaneous double label measurements of blood volume were performed. By withdrawing blood in the course of ANH, bicarbonate is removed from plasma. By simultaneously infusing colloids that have large chloride concentrations but are free of bicarbonate, changes in the respective plasma concentration may be expected. According to the Stewart model,<sup>1</sup> two components determine acid-base changes in the course of ANH: changes in cations and anions, the distribution space for which is the extracellular volume (ECV), and changes in the concentration of plasma proteins. Consequently, if colloid solutions are used for ANH, two different compartments, namely ECV (*i.e.*, the distribution space of small ions) and plasma volume (PV; *i.e.*, the distribution space of colloids), must be considered separately. Infusion solutions that are free of bicarbonate but contain large chloride concentrations may lead to dilution of bicarbonate in the extracellular space.<sup>2</sup> Additionally, using two different colloid solutions—one containing albumin in a supraphysiologic concentration and one free of albumin—produces opposite changes in serum albumin concentration. Both the dilution of ECV and changes in plasma albumin concentration may influence acid-base equilibrium. Taking into account that removing plasma and replacing it with intravenous colloid solutions alters ECV and the amount of extracellular bicarbonate and chloride, we established a balance for both anions and predicted their theoretical concentrations in ECV after ANH. Based on this balance, a comparison was made between the respective predicted electrolyte concentrations after ANH and our measured values. Concurrently, the Stewart approach<sup>1</sup> was used to evaluate the effect of changing serum albumin concentration on the acid-base changes observed. The predicted effect of changes in serum albumin concentration on pH, calculated using a mathematical model by Figge *et al.*,<sup>3</sup> was compared with our measured changes in pH.

## Materials and Methods

We studied 20 female patients without apparent cardiac, pulmonary, or renal disease (American Society of Anesthesiologists class I or II) scheduled for radical hysterectomy because of cancer of the cervix. An expected mean surgical blood loss of about 1,500 ml (about 35% of the patient's blood volume) and a reported transfusion rate between 22 and 92% were the reasons for using preoperative ANH as a blood-saving method.<sup>4-6</sup> Before surgery, written informed consent was obtained from each patient, and the protocol was approved by the ethics committee of our institution. The patients were randomly assigned to receive either 6% hydroxyethyl starch solution (HES group:  $n = 10$ ) or 5% albumin solution (HA group:  $n = 10$ ). The HES solution contained 154 mM of sodium and 154 mM of chloride (pH 5.44; Fresenius AG, Bad Homburg, Germany), and the HA solution contained 158 mM of sodium, 150 mM of chloride, 4 mM of octanoate, and 4 mM of N<sup>1</sup>-acetyltryptophanate (pH 6.86; Centeon Pharma GmbH, Marburg, Germany). Neither solution contained bicarbonate or citrate. These solutions are commonly used in Europe; however, their electrolyte compositions may differ from those of colloids used in the United States.

### Experimental Procedure

General anesthesia was induced using intravenous thiopental, sufentanil, and cisatracurium and was maintained with isoflurane 0.4-1.5 vol% in an oxygen-nitrous oxide mixture at a 1:1 ratio; additional doses of sufentanil and cisatracurium were administered as appropriate. Radial arterial and central venous catheters were inserted. Mechanical ventilation was performed to maintain arterial oxygen tension ( $P_{aO_2}$ ) at 250-300 mmHg and arterial carbon dioxide tension ( $P_{aCO_2}$ ) as close as possible to 40 mmHg. Intraoperative monitoring consisted of end-tidal  $P_{aCO_2}$ , electrocardiography, central venous pressure, arterial blood pressure, pulse oximetry, and esophageal temperature. During hemodilution and surgery, the patient's temperature was kept constant by warming infusion fluids and using a warming blanket.

All measurements were performed before starting surgery during periods of stable anesthesia and hemodynamics. Before ANH, no intravenous infusions were given, and during ANH, no crystalloids were administered. Simultaneous measurements of hematocrit (centrifugation [12,000/min; 4 min] without correction for plasma trapping), PV, and red cell volume were carried out (see Determination of Plasma Volume and Determination of Red Cell Volume). At the same time, arterial blood samples were analyzed for  $P_{aO_2}$ , pH, and  $P_{aCO_2}$  using standard electrodes; concentrations of serum so-

dium ( $Na^+$ ), potassium ( $K^+$ ), chloride ( $Cl^-$ ), and ionized calcium ( $Ca^{2+}$ ) using ion-selective electrodes; and lactate ( $Lac^-$ ) using an enzymatic method (quantification of  $H_2O_2$ ), all integrated in a Radiometer analyzer (Radiometer ABL 620 GL, Radiometer Co., Copenhagen, Denmark). Additionally, serum phosphate ( $PO_4$ ), determined by ultraviolet photometry of a phosphomolybdate complex, serum total protein concentration, determined using the Biuret method, and albumin concentration, determined using colorimetry of bromocresol complex, were measured from the same blood samples. The amount of weak plasma acid was calculated with the aid of a computer program developed by Figge *et al.*<sup>3</sup> and was termed [Prot<sup>-</sup>]. Standard serum bicarbonate concentration [Bic] and standard base excess [BE] were determined with a blood gas analyzer that uses the Henderson-Hasselbalch equation and the Siggaard-Andersen nomogram.<sup>7</sup>

After baseline measurements, blood was removed at a rate of about 60 ml/min (following gravity) and was simultaneously replaced with HA or HES at nearly the same rate. A previous study in our laboratory had shown normovolemia after ANH with a 15% surplus of HA in relation to the amount of blood removed.<sup>8</sup> Consequently, we planned to infuse 15% more colloid in relation to blood removed. Initially, approximately 500 ml/m<sup>2</sup> of blood were removed. The hemodilution bags were weighed on a precision scale so that the volume of blood withdrawn could be evaluated immediately. For fine tuning, frequent determinations of hematocrit were performed at the end of ANH to reach a target value of 22%. The hemodilution procedure took about 20 min. After completion of ANH and a steady-state interval of 20 min without any further infusions and before starting surgery, measurements of all variables mentioned previously were performed in the same manner as before ANH.

### Determination of Plasma Volume

Immediately before each dye injection, a calibration curve was constructed using the patient's blood with two known indocyanine green concentrations (Paesel Frankfurt, Germany; 1.25 and 2.5  $\mu$ g/ml of whole blood). At the same time, blood samples were taken to determine hematocrit (centrifugation of blood samples without correction for plasma trapping; variation coefficient < 2%) and serum total protein (Biuret method; variation coefficient < 2%). Next, 0.25 mg/kg of body weight indocyanine green was injected into the central venous catheter as a bolus (within 5 s). From 2-5 min after injection, blood was continuously withdrawn from the arterial catheter at a rate of 15 ml/min (consequently, without a relevant impact on PV) through a cuvette attached to a densitometer by means of a calibrated pump in a closed system. The pump, the cuvettes, and

the densitometer were constructed by one of the authors (H.B.). Optical density of the blood (corrected for blank) was read in the densitometer at 800 and 900 nm. The density of blood at injection time was derived by monoexponential extrapolation of the density curve between 2–5 min back to zero time (the time of injection). This value, when put in the calibration curve, gives CBo, the theoretical whole-blood concentration of the dye at injection time, which is the initial volume of distribution.

The theoretical plasma concentration of the dye at injection time (CPo) was calculated as:  $CPo = CBo / (1 - Hct)$ . PV was calculated as:  $PV = D / CPo$ , where D is the amount of dye injected.

This method is referred to as the “whole-blood method” for PV determination, methodologic aspects of which have been published previously.<sup>9</sup> The whole-blood method provides reproducible results in the operating room within 10 min (mean difference and variation coefficient between double measurements: 0.3 and 6.2%, respectively).<sup>10</sup>

#### Determination of Red Cell Volume

This method, using autologous red cells stained with sodium fluorescein and determination by flow cytometry, was developed in our laboratory and has been published in detail elsewhere.<sup>11,12</sup> Briefly, per measurement, 20 ml of the patient’s blood were labeled with 50 mg of sodium fluorescein and injected into a central vein. After injection, samples were drawn from the arterial catheter at 4, 6, and 8 min, stored on ice, and analyzed by flow cytometry (Becton Dickinson, Heidelberg, Germany).

Red cell volume (in milliliters) was calculated according to the following equation:  $RCV = (RCi \times Vi \times Hctp) / (RCp \times FRCf)$ , where RCi indicates number of injected red cells per milliliter of tagged cell suspension, Vi indicates volume of injected cell suspension in milliliters, Hctp indicates hematocrit of the patient’s blood, RCp indicates number of red cells per milliliter in the patient’s blood, and FRCf indicates fraction of fluorescent red cells determined by flow cytometry.

FRCf was taken as the mean value of determinations of samples drawn at 4, 6, and 8 min after injection, counting in triplicate the number of fluorescent red cells per 50,000 cells with the aid of the flow cytometer. RCi and RCp were obtained using a cell counter (Coulter Electronics, Miami, FL). In our laboratory, mean difference and variation coefficient for red cell volume double measurements with sodium fluorescein were 0.6 and 3.1%, respectively.<sup>11</sup>

#### Calculations

Before and after ANH, for each sample, a “bedside” strong ion difference (SID) was calculated as follows:

$$SID = [Na^+] + [K^+] - [Cl^-] - [Lac^-] \quad (1)$$

The anion gap was calculated as follows:

$$\text{anion gap} = [Na^+] + [K^+] - [Cl^-] - [Bic] \quad (2)$$

The ECV before ANH was calculated as follows:

$$ECV_{\text{before ANH}} = \text{body weight (kg)} \times 0.2 \quad (3)$$

ECV after ANH was derived as follows:

$$ECV_{\text{after ANH}} = ECV_{\text{before ANH}} + \text{colloid supply} - \text{plasma removed} \quad (4)$$

The amount of plasma removed was determined by weighing the empty and full hemodilution bags and measuring hematocrit of blood contained in the respective bags:

Plasma content of ANH bags

$$= [(weight\ of\ full\ ANH\ bags - 110g) / 1.05g/ml] \times (1 - Hct_{bag}) \quad (5)$$

where 110 g is the sum of weight (40 g) and of CPDA fluid content (70 g) of empty ANH bags. The specific gravity of blood was taken to be 1.05.<sup>13</sup>

The calculated amount of bicarbonate or chloride in ECV before ANH was obtained as follows:

$$\begin{aligned} &\text{calculated amount of bicarbonate} \\ &\text{or chloride in } ECV_{\text{before ANH}} \\ &= [Bic\ or\ Cl^-]_{\text{before ANH}} \times ECV_{\text{before ANH}} \quad (6) \end{aligned}$$

The amount of bicarbonate or chloride removed during ANH was derived as follows:

$$\begin{aligned} &\text{amount of (bicarbonate or chloride) removed} \\ &= ([Bic\ or\ Cl^-]_{\text{before ANH}} + [Bic\ or\ Cl^-]_{\text{after ANH}}) / 2 \\ &\quad \times \text{plasma removed} \quad (7) \end{aligned}$$

This equation involves the premise that the concentration of the respective electrolyte in plasma removed was the mean of the respective electrolyte plasma concentration before and after ANH.

The amount of chloride supplied was calculated as follows:

$$\begin{aligned} &\text{amount of chloride supply} = \text{colloid supply} \\ &\quad \times [Cl^-] \text{ of the respective colloid} \quad (8) \end{aligned}$$

Because both colloids used were free from bicarbonate, no bicarbonate was supplied during ANH.

The predicted amount of bicarbonate in ECV after ANH was calculated as follows:

$$\begin{aligned} &\text{predicted amount of bicarbonate in ECV}_{\text{after ANH}} \\ &= \text{calculated amount of bicarbonate in ECV}_{\text{before ANH}} \\ &\quad - \text{bicarbonate removed} \quad (9) \end{aligned}$$

The predicted amount of chloride in ECV after ANH was calculated as follows:

$$\begin{aligned} &\text{predicted amount of chloride in ECV}_{\text{after ANH}} \\ &= \text{calculated amount of chloride in ECV}_{\text{before ANH}} \\ &\quad - \text{chloride removed} + \text{chloride supplied} \quad (10) \end{aligned}$$

The predicted concentration of bicarbonate or chloride in ECV after ANH was calculated as follows:

$$\begin{aligned} &\text{predicted [Bic or Cl}^{-}\text{]}_{\text{after ANH}} \\ &= \text{predicted amount of (bicarbonate or chloride)} \\ &\quad \text{in ECV}_{\text{after ANH}} / \text{ECV}_{\text{after ANH}} \quad (11) \end{aligned}$$

The calculated amount of bicarbonate or chloride in ECV after ANH was derived as follows:

$$\begin{aligned} &\text{calculated amount of (bicarbonate or chloride) in} \\ &\text{ECV}_{\text{after ANH}} = [\text{Bic or Cl}^{-}]_{\text{after ANH}} \\ &\quad \times \text{ECV}_{\text{after ANH}} \quad (12) \end{aligned}$$

The predicted change in [Bic] during ANH according to the Stewart approach<sup>1</sup> was calculated as follows:

$$\begin{aligned} &\text{predicted change in [Bic]} = \text{change in SID} \\ &\quad - \text{change in [Prot}^{-}\text{]} \quad (13) \end{aligned}$$

This equation demands that Paco<sub>2</sub> remains constant.

*Statistical Analysis*

As all measured and calculated data described previously were normally distributed (tested by Kolmogorov-Smirnov tests), these are presented as mean values with standard deviations. For demographic data, Student *t* tests for unpaired data were performed. Student *t* tests for paired or unpaired samples were performed as appropriate to compare intragroup and intergroup differences before and after ANH. A value of *P* < 0.05 for intergroup differences before ANH, a value of *P* < 0.002 for intergroup differences after ANH, and intragroup differences ( $\alpha$ -adjustment according to Bonferroni: 0.05/25) concerning acid-base variables were considered to be statistically significant.

**Results**

Patient characteristics are presented in table 1. There were no significant differences in weight, height, body

**Table 1. Patients' Characteristics, Amount of Blood and Plasma Removed, and Colloid Supply during ANH**

	HES Group (n = 10)	HA Group (n = 10)
Age	39 ± 8	46 ± 8
Height (cm)	166 ± 7	165 ± 3
Weight (kg)	62 ± 13	72 ± 17
Body surface area (m <sup>2</sup> )	1.70 ± 0.18	1.82 ± 0.21
Blood removed (ml)	1,269 ± 217	1,576 ± 227*
Plasma removed (ml)	863 ± 158	1,050 ± 173*
Colloid infused (ml)	1,469 ± 246	1,831 ± 271*

Mean ± SD. Intergroup differences are indicated (\* *P* < 0.05).

ANH = acute normovolemic hemodilution; HES = hydroxyethyl starch; HA = albumin.

surface area, and the time of infusion. Because of slightly higher preoperative body surface area and hematocrit values in the HA group, the amount of blood removed and amount of colloid infused was significantly higher than in the HES group. As shown in table 2, there were no differences in blood volume, PV, red cell volume, or hematocrit before and after ANH between both groups. Total protein and plasma albumin concentration decreased during ANH with HES, whereas total protein remained unchanged and plasma albumin concentration significantly increased during ANH with HA. The only intergroup difference was in serum albumin concentration; the mean value after ANH in the HES group was exactly half that in the HA group.

In table 3, pH, Paco<sub>2</sub>, [Bic], [BE], [Lac<sup>-</sup>], [Na<sup>+</sup>], [Cl<sup>-</sup>], [K<sup>+</sup>], SID, [Prot<sup>-</sup>], [PO<sub>4</sub>], [Ca<sup>2+</sup>], and the anion gap at the measuring points before and after ANH are shown. At baseline, no intergroup differences in these variables were found. Both groups had a significant decrease in pH from about 7.39 to 7.34, and Paco<sub>2</sub> did not show

**Table 2. Plasma Volume (PV), Red Cell Volume (RCV), Blood Volume (BV), Hematocrit (Hct), Serum Total Protein, and Serum Albumin Concentration**

	Before ANH	After ANH
HES group		
PV (ml)	2,906 ± 436	3,325 ± 361*
RCV (ml)	1,221 ± 179	836 ± 168*
BV (ml)	4,126 ± 494	4,161 ± 460
Hct (%)	34.1 ± 4.1	21.9 ± 2.9*
Total protein (g/l)	6.2 ± 0.4	3.7 ± 0.5*
Albumin (g/l)	4.2 ± 0.5	2.4 ± 0.3*
HA group		
PV (ml)	3,075 ± 515	3,536 ± 552*
RCV (ml)	1,345 ± 131	855 ± 133*
BV (ml)	4,421 ± 612	4,391 ± 660
Hct (%)	36.1 ± 2.6	22.5 ± 1.8*
Total protein (g/l)	6.1 ± 0.4	5.8 ± 0.3
Albumin (g/l)	4.2 ± 0.4	4.8 ± 0.4*†

Mean ± SD.

\* *P* < 0.05 intragroup difference, with respect to the respective value before acute normovolemic hemodilution (ANH). † *P* < 0.05 difference between groups.

HES = hydroxyethyl starch; HA = albumin.

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**Table 3. Measured and Calculated Values of Acid–Base States**

	Before ANH	After ANH
<b>HES group</b>		
pH	7.384 ± 0.027	7.335 ± 0.028*
Paco <sub>2</sub> (mmHg)	40.1 ± 2.8	41.7 ± 2.2
[Bic] (mM)	23.6 ± 1.1	21.6 ± 1.1*
[BE] (mM)	-0.9 ± 1.3	-3.4 ± 1.4*
[Lac <sup>-</sup> ] (mM)	0.6 ± 0.1	0.5 ± 0.1
[Na <sup>+</sup> ] (mM)	140 ± 1.5	140 ± 1.5
[Cl <sup>-</sup> ] (mM)	105 ± 1.7	111 ± 2.3*
[K <sup>+</sup> ] (mM)	3.4 ± 0.2	3.4 ± 0.2
SID (mM)	37.0 ± 1.2	31.7 ± 1.8*
[Prot <sup>-</sup> ] (mM)	11.5 ± 1.2	6.5 ± 0.7*
[PO <sub>4</sub> ] (mM)	1.2 ± 0.2	1.2 ± 0.2
[Ca <sup>2+</sup> ] (mM)	1.1 ± 0.1	1.0 ± 0.1
Anion gap (mM)	13.9 ± 1.0	10.4 ± 1.3*
<b>HA group</b>		
pH	7.394 ± 0.028	7.336 ± 0.032*
Paco <sub>2</sub> (mmHg)	38.6 ± 3.0	40.2 ± 3.2
[Bic] (mM)	23.6 ± 1.1	21.0 ± 0.9*
[BE] (mM)	-1.1 ± 1.4	-4.0 ± 1.0*
[Lac <sup>-</sup> ] (mM)	0.8 ± 0.2	0.6 ± 0.2
[Na <sup>+</sup> ] (mM)	141 ± 1.5	142 ± 1.5
[Cl <sup>-</sup> ] (mM)	106 ± 2.9	109 ± 2.9*
[K <sup>+</sup> ] (mM)	3.4 ± 0.3	3.3 ± 0.2
SID (mM)	37.3 ± 2.2	35.7 ± 1.7†
[Prot <sup>-</sup> ] (mM)	11.5 ± 1.1	12.8 ± 0.9*†
[PO <sub>4</sub> ] (mM)	1.1 ± 0.1	1.2 ± 0.1
[Ca <sup>2+</sup> ] (mM)	1.0 ± 0.1	1.0 ± 0.1
Anion gap (mM)	15.0 ± 1.6	15.6 ± 1.9†

Mean ± SD.

\*  $P < 0.002$  intragroup difference, with respect to the respective value before ANH. †  $P < 0.002$  difference between groups after ANH.

ANH = acute normovolemic hemodilution; HES = hydroxyethyl starch; Paco<sub>2</sub> = arterial carbon dioxide tension; [Bic] = standard bicarbonate; [BE] = standard base excess; [Lac<sup>-</sup>] = lactate; [Na<sup>+</sup>] = sodium; [Cl<sup>-</sup>] = chloride; [K<sup>+</sup>] = potassium; SID = strong ion difference; [Prot<sup>-</sup>] = weak plasma acid; [PO<sub>4</sub>] = phosphate; [Ca<sup>2+</sup>] = calcium; HA = albumin.

major deviations from 40 mmHg in either group. In the HES group, [Bic] and [BE] significantly decreased from 23.6 to 21.6 mM and -0.9 mM to -3.4 mM, respectively, after ANH. In the HA group, [Bic] significantly decreased from 23.6 to 21.0 mM and [BE] significantly decreased from -1.1 mM to -4.0 mM after ANH. [Lac<sup>-</sup>] did not change in either groups. There were no relevant changes in [Na<sup>+</sup>] and [K<sup>+</sup>]; however, [Cl<sup>-</sup>] significantly increased in both groups during ANH. This increase was larger after application of HES (+6 mM) than after HA (+3 mM). SID decreased by a mean of 5.3 mM in the HES group and did not change significantly in the HA group (-1.6 mM). As a result of dilution with an albumin-free solution, [Prot<sup>-</sup>] decreased by about 5 mM in the HES group in contrast to the HA group, in which [Prot<sup>-</sup>] increased slightly but significantly during ANH. [Ca<sup>2+</sup>] and [PO<sub>4</sub>] levels remained fairly constant and did not show any relevant difference within or between groups. The anion gap significantly decreased in the HES group, whereas it did not significantly change in the HA group. After ANH, significant intergroup differences were found for SID, [Prot<sup>-</sup>], and the anion gap.

Table 4 shows the bicarbonate and chloride balance

for ECV, provided that ECV is the distribution space of the respective electrolytes; calculated and predicted values in table 4 are based on mean values in table 1 and 3; for calculation, see Methods, equations 3–12. Table 4 shows that in both groups the predicted mean amounts of bicarbonate and chloride in ECV after ANH (based on a balance of removed and supplied electrolytes) differed only slightly from the respective calculated mean values (based on measured [Bic] and [Cl<sup>-</sup>] after ANH). Additionally, in both groups the mean values of predicted [Bic] and [Cl<sup>-</sup>] after ANH were only slightly different from the respective measured mean values (table 3). This may indicate that the measured changes in [Bic] and pH in both groups are simply caused by the dilution of ECV with bicarbonate-free solutions. Table 5 presents a comparison between measured changes in [Bic] and predicted changes in [Bic], which were derived from the changes in SID and [Prot<sup>-</sup>] during ANH according to the Stewart approach (see equation 13).<sup>1</sup> The measured decrease in [Bic] during ANH was well explained in the HA group, whereas the Stewart equations<sup>1</sup> did not explain the measured decrease in [Bic] in the HES group.

## Discussion

The main finding of this study was a slight but significant metabolic acidosis after ANH with both HA and

**Table 4. Balance of Bicarbonate and Chloride for the Extracellular Volume (ECV)**

	HES Group	HA Group
ECV before ANH (l)	12.460	14.450
ECV after ANH (l)	13.066	15.230
Calculated amount of bicarbonate in ECV before ANH (mmol)	294	341
Bicarbonate removed (mmol)	20	23
Predicted amount of bicarbonate in ECV after ANH (mmol)	274	318
Predicted [Bic] after ANH (mM)	21.0	20.9
Calculated amount of bicarbonate in ECV after ANH (mmol)	282	319
Measured [Bic] after ANH (mM) (table 3)	21.6	21.0
Calculated amount of chloride in ECV before ANH (mmol)	1,308	1,532
Chloride removed (mmol)	93	113
Chloride supply (mmol)	226	275
Predicted amount of chloride in ECV after ANH (mmol)	1,441	1,694
Predicted [Cl <sup>-</sup> ] after ANH (mM)	110	111
Calculated amount of chloride in ECV after ANH (mmol)	1,450	1,660
Measured [Cl <sup>-</sup> ] after ANH (mM) (table 3)	111	109

For calculation of predicted and calculated values, mean values in tables 1 and 3 were used; for calculation of the different variables, see Methods equations 3–12.

HES = hydroxyethyl starch; HA = albumin; ANH = acute normovolemic hemodilution; [Bic] = standard bicarbonate concentration; [Cl<sup>-</sup>] = chloride concentration.

**Table 5. Measured Change in Standard Bicarbonate [Bic], Standard Base Excess [BE], Strong Ion Difference (SID), and Plasma-weak Acid [Prot<sup>-</sup>] during ANH, and Predicted Change in [Bic] According to the Stewart Approach**

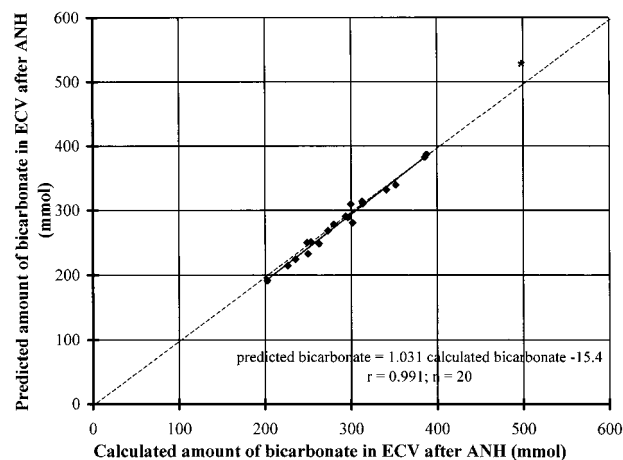
(mm)	HES Group	HA Group
Change in [Bic]	-2.1	-2.5
Change in [BE]	-2.5	-2.9
Change in SID	-5.3	-1.6
Change in [Prot <sup>-</sup> ]	-5.0	1.4
Predicted change in [Bic]	-0.3	-3.0

For calculation of predicted change in [Bic] according to the Stewart approach, see equation 13.

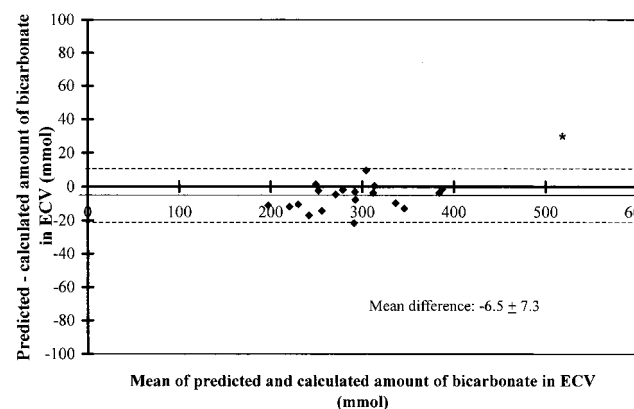
ANH = acute normovolemic hemodilution.

HES solutions. In the current investigation, the double label measurements of blood volume showed that ANH was isovolemic in both groups. The acidosis in both groups seems to be without major clinical relevance. However, the generated data could constitute a further step toward a better understanding of acid-base equilibrium. What are the mechanisms behind this acidosis? The acidosis definitely was of metabolic origin, because  $P_{aCO_2}$  was kept almost constant and [Bic] and [BE] decreased significantly, but it was not a lactic acidosis. The term "dilutional acidosis" might be appropriate, because in both groups, about 35% of blood volume was replaced by intravenous infusions free of bicarbonate and ECV increased by about 600–800 ml as a result of ANH. Originally proposed by Peters and van Slyke,<sup>2</sup> this term was generally used in some older publications,<sup>14–16</sup> recent case reports,<sup>17–20</sup> and several letters to the editor<sup>21–25</sup> in the context of diluting not only PV but the entire ECV with different electrolyte solutions that were free of bicarbonate. This would result in a dilution of serum bicarbonate, with resulting metabolic acidosis. Table 4 shows a balance for extracellular bicarbonate that was based on this "dilution hypothesis." In both groups, about 20 mmol of bicarbonate were removed without substitution (see equation 7). This should result in a predicted decrease in the amount of extracellular bicarbonate (equation 9). The mean calculated amount of extracellular bicarbonate after ANH in both groups, which was based on measured [Bic] after ANH (equation 12), was only slightly different from the respective predicted value, which was based on balance calculations (equation 9). Figure 1 shows this comparison by means of a regression analysis and a Bland-Altman plot. One patient in the HA group was marked separately with an asterisk, because with 110 kg of body weight, the respective ECV could not be quantified with sufficient precision. This patient was not included in the calculations of linear regression and mean difference. The correlation coefficient of 0.99 and the slope of 1.03 indicate the excellent correlation between predicted and calculated amount of bicarbonate in ECV after ANH. Figure 2 presents a comparison between predicted [Bic] after

ANH (see equation 10) and the respective measured [Bic]. In this case also, there is good correlation and a regression slope close to 1. The fact that, according to figures 1 and 2, this simple mathematical balance of extracellular bicarbonate gives such precise bicarbonate concentrations after ANH almost in every patient in each group leads to the conclusion that changes in bicarbonate in the course of ANH are caused by the dilution of ECV with bicarbonate-free solutions. The balance for chloride of ECV, which is also presented in table 2, shows an almost similar result. As a result of the large chloride concentrations in the colloid solutions used, the net chloride balance during ANH in both groups was more than 100 mmol (equations 7 and 8). The predicted mean increase in the amount of extracellular chloride (equation 10) as well as the predicted mean increase in  $[Cl^-]$  (equation 11) were also in close agreement with the respective mean calculated or measured values. These results support the conclusion that changes in serum anions and cations in the course of ANH are

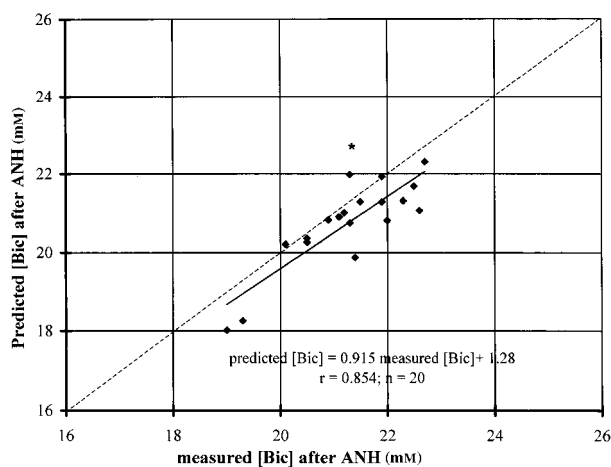


**Bland Altman Plot**

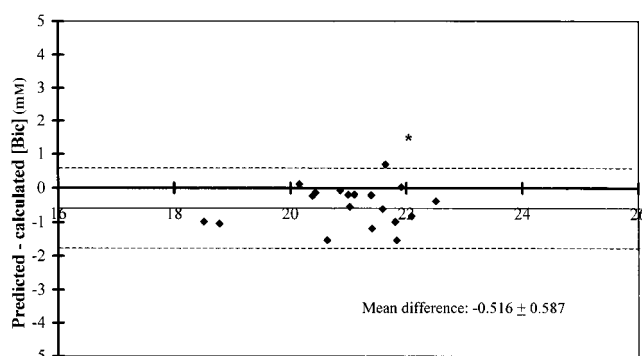


95 % Confidence interval [ - - ]; mean difference (predicted - calculated) [ — ]

**Fig. 1. Comparison of calculated and predicted amount of bicarbonate in extracellular volume (ECV) after acute normovolemic hemodilution (ANH) according to the "dilution hypothesis"; linear regression analysis and Bland-Altman plot.**



Bland Altman Plot



Mean of predicted and calculated [Bic] (mM)

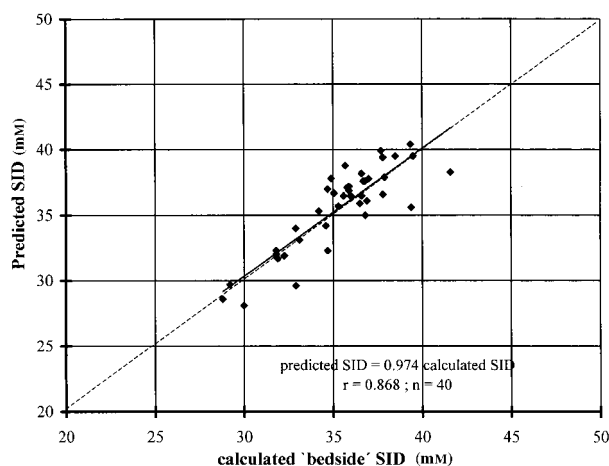
95 % Confidence interval [ - - ]; mean difference (predicted - calculated) [ — ]

**Fig. 2.** Comparison of measured bicarbonate concentration ([Bic]) and predicted [Bic] after acute normovolemic hemodilution (ANH) according to the “dilution hypothesis”; linear regression analysis and Bland-Altman plot.

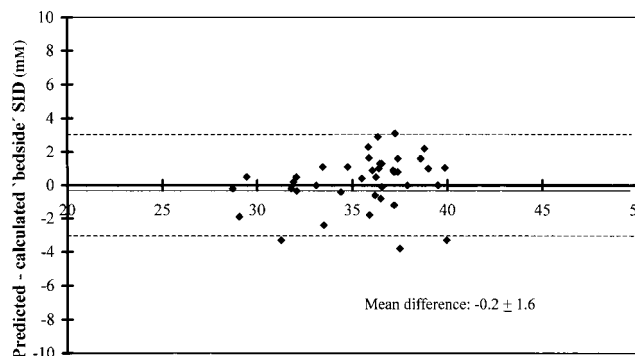
simply caused by the dilution of ECV. The “dilution hypothesis” takes electrolyte concentrations in PV and ECV (which should be similar), such as bicarbonate or chloride, and the amount of water in ECV into account. However, the effect of changes in plasma albumin concentration on changes in acid-base equilibrium is not represented in this theory.

There is an alternative to explain the metabolic acidosis observed. The Stewart approach, which is discussed in detail elsewhere,<sup>26</sup> defines  $P_{aCO_2}$ , SID, and the sum of all anionic charges of weak plasma acids (called  $A_{tot}$  by Stewart and calculated as the product of total protein and the empirically derived factor 2.43 according to van Slyke *et al.*<sup>27</sup>) as independent pH-regulating variables, whereas pH and [Bic] are dependent variables.<sup>1</sup> Apart from a few studies in exercise medicine,<sup>28,29</sup> experimental medicine,<sup>30</sup> and critical care medicine,<sup>31–35</sup> The Stewart approach has not been commonly applied in the past decade. The Stewart approach postulates that a decrease in SID or an increase in  $[Prot^-]$  will result in a decrease

in pH on condition of constant  $P_{aCO_2}$  (and vice versa; see equation 13).<sup>1</sup> By defining [Bic] as being exclusively dependent on SID and  $[Prot^-]$  during metabolic acid-base changes, the traditional “dilution hypothesis” is poorly considered by the Stewart approach.<sup>1</sup> A previous investigation by our laboratory demonstrated an ongoing hyperchloremic metabolic acidosis after rapid intraoperative infusion of 0.9% saline in contrast to an infusion of lactated Ringer’s solution in patients undergoing gynecologic surgery.<sup>36</sup> In both groups, the different changes in SID and  $[Prot^-]$  were in good agreement with the predicted changes in [Bic] according to the Stewart approach.<sup>1</sup> However, in this study, each infusion regimen involved a similar intraoperative decrease in total protein, so that the impact of hypoproteinemia on acid-base balance could not be quantified adequately.<sup>37</sup> Recent investigations gave a new quantification of the negative charges of serum proteins and therefore their quality as anions.<sup>3,38,39</sup> Figge *et al.*<sup>3</sup> demonstrated that nonalbumin proteins may be neglected, because they do not bear negative charges and consequently do not act as



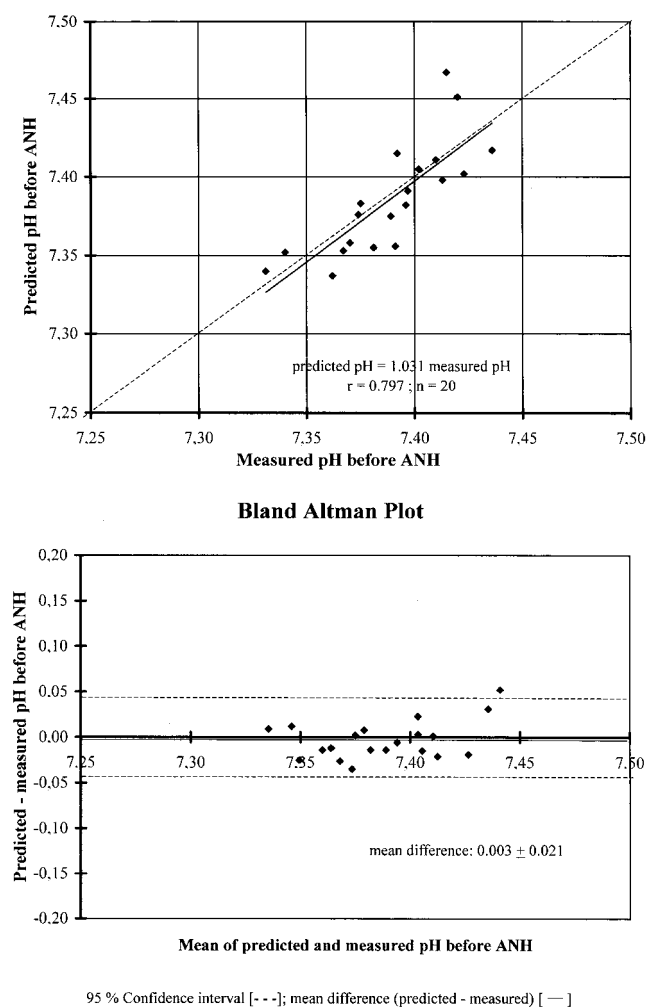
Bland Altman Plot



Mean of predicted and calculated bedside SID (mM)

95 % Confidence interval [ - - ]; mean difference (calculated - measured) [ — ]

**Fig. 3.** Comparison of calculated “bedside” strong ion difference (SID) and predicted SID according to the computer program by Figge *et al.*;<sup>3</sup> linear regression analysis and Bland-Altman plot.

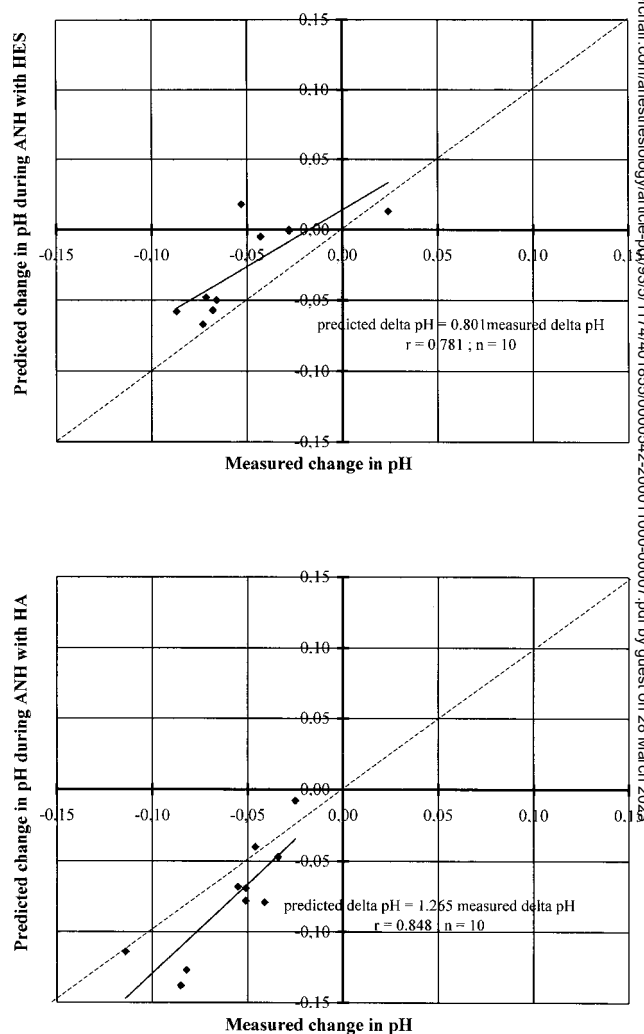


**Fig. 4.** Comparison of measured pH and predicted pH before acute normovolemic hemodilution (ANH) according to the computer program by Figge *et al.*;<sup>3</sup> linear regression analysis and Bland-Altman plot.

anions.<sup>39</sup> Calculations of  $[A_{\text{tot}}]$  or  $[\text{Prot}^-]$  using the van Slyke factor therefore will result in an overestimation of the negative charges of plasma proteins. In the current study,  $[\text{Prot}^-]$  was calculated using a computer program developed by Figge *et al.*<sup>3</sup>

Table 5 illustrates changes in  $[\text{Bic}]$ , SID, and  $[\text{Prot}^-]$  during ANH in both groups. In the HA group,  $[\text{Prot}^-]$  increased and SID decreased slightly by about 1.5 mM each, so that the measured decrease in  $[\text{Bic}]$  by a mean of 2.5 mM was in good agreement with the predicted decrease in  $[\text{Bic}]$  according to the Stewart equations.<sup>1</sup> In contrast, both SID and  $[\text{Prot}^-]$  decreased by about 5 mM in the HES group, so that the measured decrease in  $[\text{Bic}]$  by a mean of 2.5 mM could not be explained adequately by the model of Figge *et al.*<sup>3</sup> This observation seems to be important, and the reasons behind it should be clarified. The mathematical model by Figge *et al.*<sup>3</sup> is based on electrical neutrality, the dissociation equilibrium for water and the carbonic acid system, and the dissociation equilibrium for all ionizable groups on the albumin mol-

ecule. It solves  $[\text{Prot}^-]$  as a function of SID,  $\text{Paco}_2$ , plasma albumin concentration, and  $[\text{PO}_4]$ . In this study, the latter three values are measured variables; however, the calculation of SID was different from that done by Figge *et al.*<sup>3</sup> In the current study, SID was calculated as:  $\text{SID} = [\text{Na}^+] + [\text{K}^+] - [\text{Cl}^-] - [\text{Lac}^-]$  (see equation 1). All components of this equation are variables measured by the Radiometer analyzer; thus, SID can be calculated quickly in the operating room. Figge *et al.*<sup>3</sup> calculated an apparent SID using the following equation:  $\text{SIDapp} = [\text{Na}^+] + [\text{K}^+] + [\text{Mg}^{2+}] + [\text{Ca}^{2+}] - [\text{Cl}^-]$ . They demonstrated an excellent correlation between SIDapp and an effective SID, which was predicted by pH,  $\text{Paco}_2$ , plasma albumin concentration, and  $[\text{PO}_4]$  according to the Stewart approach.<sup>1</sup> Figure 3 shows the comparison between our "bedside" SID and the effective SID predicted by the computer program. Because there is a good correlation between these two SID values, we



**Fig. 5.** Comparison of measured changes in pH and predicted changes in pH during acute normovolemic hemodilution (ANH) (separately for the 6% hydroxyethyl starch solution [HES] and 5% albumin solution [HA] groups) according to the computer program by Figge *et al.*;<sup>3</sup> linear regression analysis.



conclude that the apparent SID by Figge *et al.*<sup>3</sup> should also be in good correlation with our "bedside" SID. The computer program by Figge *et al.*<sup>3</sup> also gives a predicted pH as a function of SID,  $P_{aCO_2}$ , plasma albumin concentration, and  $[PO_4]$ . Figure 4 illustrates that the preoperatively measured pH values were in good agreement with the predicted pH values obtained using the Figge *et al.*<sup>3</sup> model (slope 1.03). However, in both groups, the changes in pH during ANH were not predicted precisely, as shown in figure 5 by the respective slopes of the regression lines of 0.80 in the HES group and 1.27 in the HA group. This indicates that the computer program did not predict the pH values after ANH with satisfying precision. The reason for this observation is unclear. One explanation could be that after ANH,  $[Prot^-]$  was not estimated correctly by the computer program of Figge *et al.*<sup>3</sup> Especially concerning the HA group, the dissociation of some amino acids of the albumin molecule, and therefore anionic charges of albumin, possibly could have been altered after ANH as a result of a disturbed chemical equilibrium in serum.<sup>3,38,39</sup> Some mechanisms could be the presence of different anionic charges on the synthetic albumin molecule or changes in binding ions. However, the discrepancy between the predicted constancy in  $[Bic]$  during ANH in the HES group according to the Stewart approach<sup>1</sup> and the measured decrease in  $[Bic]$  (table 5) leads to the assumption that, in this group, the Stewart approach fails.

The main link between the Stewart approach<sup>1</sup> and the "dilution hypothesis" is found in the changes in SID. SID depends on the dilution of the respective cations and anions in ECV. However, if hydroxyethyl starch is used as a volume substitute, a second compartment, namely PV, must be considered. The large decrease in  $[Prot^-]$  after having infused an artificial colloid that is free of albumin should be independent of and disproportional to the dilution of ECV. This is different from *in vitro* experiments with plasma samples. In this setting, only one distribution space (the plasma sample *in vitro*), which determines the concentrations of both the proteins and the electrolytes, must be considered. It has already been demonstrated that the Stewart equations work well under these conditions.<sup>3,38,39</sup> However, it seems possible that the different distribution spaces of colloids and electrolytes *in vivo* are not considered sufficiently by the Stewart approach.<sup>1</sup> Further systematic analysis of this topic will be valuable.

After having replaced 35% of blood volume during ANH with HA or HES solutions, a slight metabolic acidosis was observed. The reason for this acidosis in both groups was the dilution of bicarbonate in ECV and a concomitant hyperchloremia. Some indications were found that the impact of the artificial decrease in  $[Prot^-]$  on pH due to the infusion of an albumin-free colloid could not be quantified adequately by the Stewart equations<sup>1</sup> and the Figge *et al.*<sup>3</sup> model. Acidosis in the course

of ANH possibly could be avoided by a more "physiologic" composition of electrolytes in colloid solutions, such as that in lactate-buffered solutions, which unfortunately are not yet available in Europe.<sup>40</sup> Further investigations, perhaps with these "new" colloid solutions, could provide more information regarding the impact of isolated hypoproteinemia on acid-base equilibrium.

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