Automated Albumin Method Underestimates Pharmaceutical-Grade Albumin in Vivo, Ole P. Børmer,1 Lise Marit Amlie,1 Elisabeth Paus,1 and Ulf Kongsgård2 (1 Central Laboratory and 2 Department of Anesthesiology, Norwegian Radium Hospital, N-0310 Oslo, Norway; 2 author for correspondence: fax 47 2273 0725, e-mail ole.borner@labmed.uio.no)

Modern transfusion medicine relies on the use of components separated from whole blood. For volume substitution, albumin that has been purified from donor plasma and heat treated to reduce possible infectivity is the routine product. Such albumin for infusion is expensive compared with the synthetic or semisynthetic alternatives; therefore, it should be used only when strictly indicated, and its use should be guided by the patient’s serum albumin concentration. This requires the purified albumin to be measured correctly by the assay method used. When we checked the accuracy of the Vitros “dry chemistry” bromcresol green (BCG) albumin method (Ortho-Clinical Diagnostics), the albumin assay used at our hospital, by analyzing our routine blood bank albumin solution (Octapharma), we found only 70% of the expected value. Measurement of two other albumin preparations for infusion (Baxter and Pharmacia & Upjohn) and a purified human albumin calibrator (cat. no. A-1533; Sigma) consistently gave similar low values with the Vitros analyzer. In contrast, a conventional BCG albumin assay gave the expected results with all of these preparations.

If purified albumin is also underestimated after infusion into the patient, this could have serious consequences both medically and economically. The present study was performed to determine whether this was the case. In addition, a gel filtration experiment was performed to see if the underestimation was caused by the presence of albumin polymers in the purified and heat-treated product, which might interfere with sample diffusion in the Vitros slide.

Serum samples from 17 postoperative and intensive care patients were used. The patients had received substantial amounts (40–600 g; median, 180 g) of albumin by infusion of a 200 g/L solution up to 72 h prior to the assay. For comparison, serum samples from 22 patients, covering a wide range of albumin values, were obtained from the routine laboratory. These patients were verified not to have received albumin infusions. All sera were kept at −20 °C until analysis.

Serum albumin was measured with a Vitros 950 analyzer, using ALB slides (lot no. 0911-0157-6785) and with a manual BCG method performed according to the method described in the Tietz Textbook of Clinical Chemistry (1). To minimize differences caused by standardization, the Vitros Chemistry Calibrator Kit 4 (lot no. 0426) and its primary assay values for albumin were used in calibrating the manual assay. These primary assay values were the albumin values found when the calibrators were measured by Ortho-Clinical Diagnostics with a conventional BCG method on a Cobas analyzer (2). The serum albumin was also quantified by serum protein electrophoresis (SPE; Beckman Paragon SPE System; Beckman Instruments), using densitometry and the total serum protein value measured by the Vitros 950 analyzer in calculations. For comparison with a fundamentally different method, albumin was also measured immunonephelometrically on a Beckman Immage Immunochemistry System (Beckman Instruments).

The relationships obtained by linear regression analysis of the albumin results obtained with the color-binding assays for the two patient populations are shown in Fig. 1, A and B. Fig. 1A demonstrates some calibration difference but reasonable correlations between the Vitros and manual BCG methods within each patient group: for the transfused patients, Vitros = 0.76(manual BCG) − 0.72 g/L; r = 0.93; for the nontransfused patients, Vitros = 0.77(manual BCG) + 5.27 g/L; r = 0.98. However, the Vitros analyzer significantly underestimated albumin concentrations (compared with the manual BCG method) in our transfused patients, as indicated by the difference in the y-intercepts of the two regression lines; the mean difference (vertical distance between regression lines) was 6 g/L over the measured albumin range. Comparison of the Vitros and Immage results (not shown) revealed significant discrepancies at low albumin concentrations, as described earlier (3). In this comparison, the Vitros assay also underestimated the transfused patients by a mean of 6 g/L, as in the comparison with the manual BCG method. In contrast, Fig. 1B demonstrates the similarity of results in the two patient populations when albumin values from the manual BCG method are compared with the SPE results: for transfused patients, SPE = 0.79(manual BCG) + 4.5 g/L; r = 0.97; for the nontransfused patients, SPE = 0.86(manual BCG) + 3.2 g/L; r = 0.99.

The bias of the Vitros results in the transfused patients depended on the amounts of albumin given. Compared with the manual BCG values, the 7 patients receiving up to 100 g of albumin (i.e., less than the albumin content in a normal plasma volume) had significantly lower bias than the remaining 10 patients, who had received 160 g or more (bias, 5.7 and 9.1 g/L, respectively; P = 0.0009; Student two-sided t-test). These bias values include both the population difference described above and the calibration difference between methods shown by the slope of the regression formula.

It had been shown previously that highly purified commercial albumin preparations for laboratory use may contain significant amounts of albumin polymers (4, 5). The presence of such polymers in the pharmaceutical-grade preparation could possibly explain the lower values measured with the Vitros assay because they could retard transport and color development in the dry chemistry slide. To investigate this possibility, we performed a gel chromatography experiment with the purified albumin preparation on a Superdex 200 column (conditions described in legend to Fig. 1C). To ensure albumin concentrations within the working ranges of the Vitros and Immage assays in the most important fractions, we overloaded the column with material, causing a broadening of...
the albumin monomer peak. Nevertheless, only negligible amounts of dimers and polymers were seen (Fig. 1C). The absorbance values at 280 nm (not shown) closely followed these albumin values, and polyacrylamide gel electrophoresis of the starting material confirmed the low concentration of dimers or polymers (not shown). Notably, the results obtained with the Vitros assay were much lower than those obtained with the manual BCG and Immage methods in all fractions of the albumin monomer peak that were within the working range. The ratio between the Vitros method and the two other methods seemed to change over these fractions, which we assume is related to linearity problems at these very low albumin concentrations in a nonphysiological matrix. Thus, the conclusion is that the aberrant albumin results apparently are not caused by the presence of polymers, but by a change in the albumin monomer itself.

Our experiments demonstrated the problem to be associated with all albumin preparations tested, from several suppliers. All were made from blood donor plasma by modifications of the Cohn serum protein fractionation process, followed by further purification, addition of stabilizers (N-acetyltryptophan and sodium caprylate, problems. The first relates to the fact that the Vitros assay cannot be used reliably to monitor serum albumin concentrations in patients receiving albumin infusions. Such use of albumin is in itself questionable in most cases (6) or even harmful (7), and studies indicate that albumin infusions usually are prompted by low albumin values and not by clinical judgment of the patients’ needs (8). In cases of uncorrected hypoalbuminemia, the Vitros albumin assay often gives falsely high values because of the low albumin/globulin ratio (3). Thus, in hospitals using the Vitros assay, the practical impact of a falsely low albumin value may be especially strong.

Our findings raise several important questions and problems. The first relates to the fact that the Vitros assay cannot be used reliably to monitor serum albumin concentrations in patients receiving albumin infusions. Such use of albumin is in itself questionable in most cases (6) or even harmful (7), and studies indicate that albumin infusions usually are prompted by low albumin values and not by clinical judgment of the patients’ needs (8). In cases of uncorrected hypoalbuminemia, the Vitros albumin assay often gives falsely high values because of the low albumin/globulin ratio (3). Thus, in hospitals using the Vitros assay, the practical impact of a falsely low albumin value may be especially strong.

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**Fig. 1.** Correlation between albumin values obtained with the manual BCG albumin method and the Vitros assay (A) or SPE (B), and albumin concentration in fractions from gel filtration experiment (C).

(A and B), albumin (g/L) was measured in serum samples from patients receiving (●) or not receiving (○) albumin infusions. Linear regression lines (——–) and their 95% confidence limits (— — —) are shown, demonstrating the highly significant difference between patient populations in A. See text for mathematical expressions. (C), a 2-mL sample of 200 g/L albumin for infusion (Octapharma) was applied to a 2.6 × 60 cm Superdex 200 column (Pharmacia) and eluted with phosphate-buffered saline, pH 7.4, at 2.5 mL/min. Fractions of 2.5 mL were collected. The albumin concentration was measured with the manual BCG method (●) and with the Vitros (●) and Immage (○) assays. Only monomer peak concentrations were within the working range of the assays. The void volume (Vv) and the elution of mouse monoclonal IgG (molecular mass, 160 kDa) are shown as markers.
both at 0.08 mmol/g albumin in the preparation used here), and heat treatment. It is possible that this introduces changes that affect BCG binding by the albumin, causing a shift in spectral band in the Vitros slide but not affecting the same process in the manual BCG assay used in this study. It should be noted that in the Vitros slide, the albumin concentration remains essentially unchanged in the 10-μL serum sample during color development, whereas in the manual BCG method, the serum sample is diluted 1:250 with color reagent.

If a change in the BCG-binding properties of serum albumin is the explanation, it raises the possibility that in vivo transport functions also are altered in the purified albumin preparations. To our knowledge, this important question has not been investigated to any extent, and certainly deserves further investigations.

References

Thiols as a Measure of Plasma Redox Status in Healthy Subjects and in Patients with Renal or Liver Failure, Anders Andersson,1* Arne Lindgren,2 Margret Arnadottir,3 Hanne Prutz,4 and Björn Hultberg1 (Departments of 1 Clinical Chemistry, 2 Neurology, 3 Nephrology, and 4 Internal Medicine, University Hospital, S-221 85 Lund, Sweden; * author for correspondence: fax 46-46189114, e-mail anders.andersson@klinikum.lu.se)

Plasma thiols have been the object of growing interest because numerous studies have indicated that even a mild degree of hyperhomocysteinemia is associated with an increased risk of developing occlusive vascular diseases (1–5). However, the mechanism behind the vascular injuries is still unknown. Studies of the possible pathogenetic mechanism of increased plasma homocysteine concentrations are difficult because little is known about the mechanism for the formation of different homocysteine species in vivo.

We recently published a method that measures reduced and total fractions of homocysteine, cysteine, glutathione, and cysteinylglycine (6). In a preliminary study, we found that patients suffering from stroke have hyperhomocys-teinemia, whereas their reduced homocysteine was within the health-related reference interval (7). We hypothesized that the increased concentrations of the oxidized forms of homocysteine in plasma were attributable to a hyperoxidative state in the plasma. We also observed (8) that patients suffering from renal failure had concentrations of reduced homocysteine within the reference interval despite increased total homocysteine. The redox state of homocysteine in plasma may be influenced by other thiols (9), such as glutathione, which is involved in maintaining the intracellular thiols in reduced form.

In the present study, we therefore investigated the relationships between homocysteine, cysteine, and glutathione in 29 healthy subjects and 15 patients with renal or liver failure. We used a newly developed preparation procedure, especially designed to minimize several known pitfalls that frequently influence plasma glutathione determinations. Increased hemolysis during sample collection causes falsely increased plasma glutathione measurements because of the high glutathione contents in the blood cells. Plasma glutathione also decreases with time because of the activity of γ-glutamyltransferase in plasma. Furthermore, reduced glutathione disappears within minutes in cell-free plasma because of oxidation (7, 10–12). In the present study, we also determined thiols in whole blood and hemoglobin in plasma.

Specimens were collected from nine anuric hemodialysis patients (seven men and two women; mean age, 76 years; range, 55–79 years). The renal diagnoses were polycystic kidney disease (n = 2), diabetic nephropathy (n = 2), glomerulonephritis (n = 2), nephrosclerosis (n = 1), bilateral nephrectomy attributable to cancer (n = 1), and light chain nephropathy (n = 1). No patient had clinical signs of heart failure or respiratory insufficiency, and there was no laboratory evidence of liver dysfunction in any of the cases. The subjects received daily supplementation with 5 mg of folic acid and 5 mg of pyridoxine.

Specimens were also collected from six male patients with liver disease (mean age, 56 years; range, 28–74 years). All of the patients had advanced liver disease with cirrhosis, one because of autoimmune hepatitis, and the others because of alcohol. None of the patients had pulmonary diseases or impaired renal function.

After the exclusion of two individuals because of increased sample collection hemolysis (353 and 2670 mg hemoglobin/L plasma), 29 apparently healthy individuals (14 men and 15 women; mean age, 64 years; range, 41–87 years) participated in the study. Their ages did not differ significantly from the age of the patients with renal or liver failure. Eight apparently healthy individuals participated in the comparison of the present method with the previously described method (6).

Blood was drawn (with a tourniquet applied) into an EDTA-Vacutainer Tube® that had been prechilled in ice water. Immediately (within 10 s) after sample collection, the tube was placed in ice water and chilled for 6 min. During the cooling period, 50 μL of blood was mixed with 450 μL of 33 g/L sulfosalicylic acid and placed at −70 °C for later analysis of reduced thiols in whole blood. After