Effects of Dextran on Five Biuret-Based Procedures for Total Protein in Serum

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We evaluated the effect of dextran on values for total protein in serum as measured by the biuret method with five widely used automated instruments: the American Monitor Parallel; the Du Pont aca II; the Roche Cobas-Bio; the Kodak Ektachem 400; and the Beckman Astra 8. Dextran concentrations as great as 25 or 30 g/L had relatively little or no influence on total protein measurements by the latter three instruments. Dextran concentrations exceeding 6 g/L caused falsely low results with the aca, whereas the Parallel gave falsely high results when the dextran concentration exceeded 2 g/L. The aca total protein procedure could be protected from the interference by dextran concentrations up to 30 g/L by injecting 0.4–0.8 mL of ethylene glycol directly into the reagent pack before sampling. However, we could not eliminate the interference with the Parallel procedure by any simple means; we thus recommend that it not be used for measuring total protein in serum samples from patients who are being treated with dextran.

Additional Keyphrases: analytical error · discrete analysis · centrifugal analyzer · multilayer film analysis

Low-molecular-mass dextran (dextran 40) expands plasma volume and is commonly used in maintaining blood pressure or relieving vasospasm in hemodynamically unstable patients undergoing neurosurgery. It also markedly decreases the coagulant activity of factor VIII (1). At a concentration frequently achieved during dextran therapy, dextran reportedly causes positive analytical interference with serum total protein measurements by the biuret procedure, the extent of interference reportedly varying with the various biuret reagents (2–7). However, we recently noticed that, whereas the value for total protein in a serum sample from a patient receiving dextran was misleadingly high (184 g/L) as measured with the American Monitor Parallel, it was only 10 g/L with the Du Pont aca. This led us to assess systematically the influence of dextran on five commonly used biuret-based procedures for determination of total protein in plasma or serum.

Materials and Methods

The patient, a 22-year-old woman, was admitted for evaluation of the cause of recent onset of severe headaches. Her cerebrospinal fluid was grossly bloody, and angiography revealed an aneurysm of the middle cerebral artery. Intravenous dextran 40 (50 mL/h), was immediately begun, to maintain blood pressure and prevent vasospasm, and the aneurysm was surgically repaired. During surgery, cryoprecipitate (a plasma-derived preparation of the cold-precipitable proteins that includes factor VIII) was infused because her factor VIII coagulant activity was only 16% of normal. She had no history of clotting abnormalities, so this deficiency was ascribed to the therapy with dextran. Postoperatively, her concentrations of total protein in serum were high, which we investigated. After dextran therapy was stopped, values for factor VIII coagulant activity and total protein returned to normal.

Serum sampled from the patient at various times during her hospitalization was stored at −20 °C until analyzed. To prepare samples containing concentrations of dextran 40 ranging from 0 to 30 g/L, we mixed different volumes of a 100 g/L solution of "Rheomacrodex" (Pharmacia, Piscataway, NJ 08854) with a constant volume of a pooled specimen of patients' serum. Isotonic saline (NaCl, 9 g/L) made up the difference in volume, ensuring that the total protein concentration of all the samples was the same.

We used the following instruments to measure total protein in serum in duplicate: the "Astra 8" (Beckman Instruments, Inc., Clinical Instruments Division, Brea, CA 92621), involving a biuret rate-reaction method; the "Parallel" (American Monitor Corp., PO Box 88605, Indianapolis, IN 46288), in which the biuret reaction is used in a direct measurement; the "aco II" (Du Pont Co. Clinical Systems, Wilmington, DE 19898), in which the copper–protein complex formed in the biuret reaction is measured bichromatically at 540 and 510 nm; the "Ektachem 400" (Eastman Kodak Co. Clinical Division, Rochester, NY 14650), in which the biuret reagents are layered as a dry film on slides and the end-point absorption at 540 nm is determined by reflectance spectroscopy; and the "Cobas-Bio" centrifugal analyzer (Roche Analytical Instruments, Inc., Nutley, NJ), in which we measured total protein by using a reagent kit from Data Medical Associates, Arlington, TX 76011.

Results

Figure 1 illustrates the influence of dextran on total-protein measurements by the biuret method as performed with these five instruments. Dextran in concentrations up to 30 g/L had no appreciable effect on values for total protein obtained with the Ektachem 400 or Astra 8. The Cobas-Bio method was not appreciably affected until the dextran concentration exceeded 25 g/L (at 30 g/L, dextran increased the values for apparent total protein by 35%). The Parallel also showed falsely increased results: at a dextran concentration as low as 2 g/L, total protein values were 13% greater than those in the control; at 10 g/L, the value for apparent total protein was 138% of the control value. In contrast, dextran had a negative interference in the aca, but this effect did not become noticeable until the dextran concentration exceeded 5 g/L. With the aca, dextran at 10 g/L lowered the results for total protein by 16% in comparison with the control; at 30 g/L the decrease was about 78%.

We retrospectively measured total protein in many of the serum samples collected during the course of the patient's

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hospitalization, while she was receiving intravenous infusions of dextran. Figure 2 confirms the variations in values for total protein as determined with the five instruments. Values for all samples analyzed on the Astra 8, Cobas-Bio, and Ektachem 400 agreed within 15 g/L. The results obtained with the Parallel for samples, diluted when necessary, were as much as 80 g/L more than those obtained with the Astra 8, whereas samples analyzed with the acu were 20 g/L lower than results with the Astra 8. Not until four to five days after therapy with dextran was discontinued did the values for total protein obtained with the Parallel or the acu agree closely with those from the other instruments.

Adding polyethylene glycol to biuret reagent has been proposed as a way of eliminating the interference by dextran, by preventing the formation of a turbid dextran-copper complex (3). We found that adding 0.4 to 0.8 mL of polyethylene glycol directly to the acu pack before the instrument began sampling successfully eliminated interference by dextran concentrations as great as 30 g/L. Less than 0.4 mL of polyethylene glycol was ineffective; volumes greater than 0.4 mL had a slight dilutional effect.

Discussion

In the Parallel, acu, Astra 8, Ektachem 400, and Cobas-Bio, total protein is measured by the biuret reaction. However, the composition of the reagents, the modes of measurement, and the response to dextran vary significantly with each procedure. The Cobas-Bio procedure includes a reagent containing 9 g of tartrate per liter. Although Flack and Woollen (7) report that, to avoid interference by dextran, tartrate concentration should be maintained at either <5 or >15 g/L, we found no appreciable effect of dextran on the assay until the dextran concentration exceeded 25 g/L—a concentration likely to be encountered during therapy (5). Our findings contrast with those of Magid and Rensbo (5), who demonstrated that a method involving a reagent with similar composition was affected by dextran at concentrations as low as 4.6 g/L. We ascribe this lack of substantial interference with the Cobas-Bio procedure to the centrifugation of the reaction mixtures during analysis, which probably removes the interfering dextran precipitate. The manufacturer of the Astra 8 procedure would not reveal the concentration of tartrate in the total protein reagent; we speculate that the lack of interference by dextran in this procedure is due to either the presence of a proper concentration of tartrate (<5 or >15 g/L) or the measurement of the rate of change in absorbance at 545 nm. In the Ektachem 400, protein in the sample migrates into a dye-mordant layer, where it reacts with copper tartrate in a highly alkaline environment (maintained by lithium hydroxide) to produce a violet complex. The lack of interference by dextran with this procedure probably is ascribable to the very high amount of tartrate (3500 μg, to be hydrated by 10 μL of serum).

Apparently, the bichromatic procedure used in the acu cannot correct for the negative interference when dextran exceeds 5 g/L. The positive interference that dextran has on the Parallel procedure is more disturbing, because misleadingly high values for total protein could potentially influence diagnostic and perhaps costly therapeutic decisions. Indeed, in the patient we studied, diagnostic procedures to rule out multiple myeloma were underway before we recognized that the total-protein values for previous specimens from this patient were falsely increased. Our experience to date with the Parallel procedure suggests that the most feasible approach to eliminate the dextran interference with this method would be to alter the composition of the reagent by including the proper amount of tartrate. Without data to document the accuracy of an improved formulation, the Parallel procedure should not be used for analysis of total protein in patients receiving dextran.

References