CLIN. CHEM. 23/4, 663–665 (1977)

The Immediate Reaction between Bromcresol Green and Serum as a Measure of Albumin Content

David Webster

I investigated the immediate (<30s) reaction between bromcresol green and serum, as a measure of albumin content. Fifty sera were analyzed in duplicate for albumin by electrophoresis on cellulose acetate by a procedure previously shown to give results in good agreement with two immunoprecipitation techniques, and by a bromcresol green method with the absorbance being read as soon as possible after mixing. Results by the dye method were 3.0 g/liter higher than those by electrophoresis; this difference was independent of the albumin content of the serum. Slight turbidity, jaundice, or added hemoglobin did not significantly interfere with albumin determination. Apparently the immediate reaction between serum and the dye reagent provides a simple, reliable measure of albumin content when 3.0 g/liter is subtracted from the result obtained.

There has been criticism of the use of bromcresol green (BCG) for determination of albumin in serum (*1–3*), because proteins other than albumin also react with the BCG reagent under the conditions of most assays, causing considerable overestimation, particularly at low albumin concentrations (*4*). Gustafsson (*5*) postulated that BCG reacts with serum proteins in two steps, the first being an immediate reaction (<1 min), which is chiefly attributable to albumin, and a slower reaction, which is a measure of "acute phase reactants."

I have attempted to assess the suitability of the immediate reaction between serum and BCG for the measurement of albumin content and to compare the results with those by electrophoresis on cellulose acetate, which gives results previously shown to be in excellent agreement with the more specific methods of immunoprecipitation (*1*).

Materials and Methods

Sera

Fifty sera, many with low albumin concentrations, were assayed in duplicate by the electrophoretic method and by the BCG method described below. I also studied the rate of increase in absorbance for both normal and abnormal sera with the BCG reagent, to assess the magnitude of the error caused by nonalbumin reacting material.

The results for albumin concentrations of 1000 sera consecutively received by our laboratory for routine assay, analyzed by the BCG immediate reaction method, have been compared with those for a similar number of routine specimens analyzed by the electrophoretic method a year earlier.

Cellulose Acetate Electrophoresis

This was performed exactly as described previously (*1*).

Bromcresol Green Method

The BCG reagent used was that described by Doumas et al. (*6*), except that the dye concentration was slightly less, 86 mg/liter, and the concentration of all constituents in the final solution was less than in that method, because I included an initial dilution of the serum with isotonic saline before color development.

Bromcresol green reagent: Dissolve 40 g of succinic acid in about 3 liters of distilled water. Add 385 mg of BCG previously dissolved in 10 ml of 0.1 mol/liter NaOH. The BCG I used was from Hopkin and Williams Ltd., Romford, Essex. Now add 18 ml of Brij-35 surfactant solution (300 g/liter) and mix. Adjust the pH to 4.2 with 10 mol/liter NaOH solution; about 18 ml will be required. Dilute to 4.5 liters with distilled water and store at 4 °C.

Sodium chloride solution: Dissolve 9 g of NaCl in 1 liter of distilled water.

Human albumin standard: Dissolve about 1.0 g of human serum albumin (Hoechst Pharmaceuticals, Hounslow, Middlesex) in 23 ml of distilled water and standardize the solution by the biuret method described by Doumas (*7*).

Procedure

The protein solution, 20 μl, was dispensed into 1.0 ml

Biochemistry Department, Ronkswood Hospital, Worcester WR5 1 HN, U.K.

Received Dec. 9, 1976; accepted Jan. 28, 1977.

of saline solution in an 8-ml test tube. In this study this was done manually with a 20-μl "Microcap" capillary (Drummond Scientific Co.), but for routine use an automatic diluter is more suitable.

BCG reagent, 4.0 ml, was then added to the tube, which was stoppered and the contents rapidly mixed by inversion.

The absorbance was measured as quickly as possible in a spectrophotometer set at 632 nm, vs. a reagent blank. The time between BCG reagent addition and absorbance measurement was about 20 s—always less than 30 s.

The albumin concentration of the sera was calculated by comparison with a human serum albumin standard similarly treated.

A single albumin standard of about 40 g/liter is adequate, because good linearity is obtained up to 60 g/liter. Duplicates of this standard albumin solution were read at the beginning, middle, and end of each assay batch, and the mean absorbance of all six readings was used in the calculation.

In routine use, 3.0 g/liter was subtracted from the results obtained by the procedure described above to give albumin concentrations that were in excellent agreement with those obtained by the electrophoretic method (see *Results*). In the comparison of 50 sera assayed by both techniques, all absorbances were recorded in a Pye-Unicam SP1800 spectrophotometer. In the comparison of 1000 consecutively received sera for albumin determination by both methods, the absorbances were recorded in a Vitatron colorimeter with a flow-through cuvette and with use of 620- and 520-nm filters, respectively.

Results

The means for the duplicate albumin concentrations of the 50 sera analyzed by both the electrophoretic and BCG techniques agreed well. The regression equation was y (BCG) = 1.008 x (electrophoresis) + 2.817, the correlation coefficient was 0.995. The mean for results by the BCG method was 3.022 g/liter greater than the electrophoretic method mean on the same specimens. When 3.0 g/liter was subtracted from each BCG result, half of the sera gave the same result by both methods, 17 (34%) were within 1 g/liter, seven (14%) were within 2 g/liter, and only one sample differed by 3 g/liter. No sample differed by more than 3 g/liter.

The 1000 consecutively received routine sera analyzed for albumin content during October 1975 by the electrophoretic method and compared with a similar number of sera analyzed by the BCG method during October 1976 (Table 1) indicate no significant difference in the spread of results obtained by the two techniques.

I tested the linearity of the relation between concentration and absorbance obtained with the BCG reagent and a series of human serum albumin standards over the concentration range 10 to 80 g/liter; it was linear to an albumin concentration of 60 g/liter. A serum albumin concentration of 40 g/liter gives an absorbance of about 0.45 at 632 nm.

Table 1. Comparison of the Distribution of 1000 Consecutive Routine Serum Albumin Concentrations, as Determined by Cellulose Acetate Electrophoresis and by the BCG Method (20-s Reaction Time)

Serum albumin concn Range, g/liter	Percentage of results within the range: BCG method[a]	Electrophoretic method[b]
<20	4	5
20–24	8	7
25–29	17	14
30–34	24	28
35–39	29	30
>39	18	16
	100	100

[a] Analyses performed in October 1976.
[b] Analyses performed in October 1975.

When hemoglobin (erythrocyte hemolysate) was added to a pooled human serum to give concentrations ranging from 0.5 to 4.0 g/liter, the albumin concentrations obtained by the BCG method were not in any case significantly altered. Ten sera from jaundiced patients, with bilirubin concentrations ranging from 60 to 460 μmol/liter, were analyzed by both the BCG and electrophoretic methods; the albumin concentrations were not significantly different. Four sera were 1 g/liter less by the BCG method and four were 1 g/liter higher, the remaining two sera gave the same result by both methods. When seven sera with various degrees of turbidity, none grossly lipemic, were analyzed for albumin by both methods, the results were not significantly different.

A batch of 12 sera varying in albumin content from 13–40 g/liter was investigated with respect to timing of the increase in absorbance after addition of the BCG reagent. After rapidly mixing the saline–serum dilution with the BCG reagent, we measured the absorbances at 632 nm vs. a reagent blank at 20 and 40 s and 1, 2, 3, 4, 5, 10, 15, 20, 30, and 60 min. The results were plotted and the zero-time absorbance for each serum was obtained by extrapolation. The absorbance at each of the above time intervals after mixing was then calculated as a percentage increase over the zero-time value. Table 2 summarizes the results obtained after subdividing the sera into two groups based on albumin concentrations greater or less than 30 g/liter. These results are typical of the increases and are not intended to represent the minimum and maximum values that may be encountered. Values in excess of 66% at 5 min have been obtained in sera not included in the above table.

Discussion

It would appear that the immediate (<30 s) reaction between serum and the BCG reagent, used under the

Table 2. Mean Percentage Increases in Absorbance for Two Groups of Six Sera at Various Times after Mixing with the BCG Reagent[a]

Serum albumin concn, g/liter	Mean % increase over zero-time absorbance after 2 min	5 min	10 min	20 min	30 min
>30	4	6	8	10	12
(31–44)	(2–5)	(4–9)	(6–12)	(8–14)	(9–16)
<30	7	13	17	23	26
(13–29)	(3–11)	(7–21)	(12–30)	(17–36)	(19–39)

Range shown in parentheses.

[a] The zero-time absorbance was obtained by extrapolation of the 20, 40 and 60 s values.

conditions described above, gives a reliable measure of the albumin content of the specimen. The mean and fairly constant difference of 3.0 g/liter between the results by the electrophoretic and the BCG methods appears to be independent of the albumin content of the serum and several factors may contribute to it. Human serum may contain low concentrations of certain non-albumin material that react almost instantaneously with the BCG reagent, and the fact that the absorbance is recorded 20–30 s after mixing and not at zero-time will also produce a small positive difference.

The regression equation obtained in this study for the comparison of the 50 sera is similar to that obtained by Gustafsson (*5*), which related to the BCG result at 0 min: y (BCG, 0 min) = $0.98x + 1.83$ (n = 90, $r = 0.95$). Most of the albumin concentrations in the sera compared in his study exceeded 30 g/liter; in this study 36 of the 50 sera had values <30 g/liter. Also, in this study I used the means of duplicates to calculate the regression equation and correlation coefficient. If one considers these differences, the results of the two studies agree well.

The electrophoretic method I used for comparison has been shown (*1*) to give results in good agreement with both the radial immunodiffusion and the immunoelectrophoresis "rocket" techniques for the determination of serum albumin. Since changing from the electrophoretic method to the BCG procedure described above under *Materials and Methods,* results obtained in my laboratory for routine serum albumin measurements do not appear to have altered noticeably. The percentage of results in each concentration range are remarkably similar (Table 1). Besides the 50 sera compared above, many hundred routine specimens were analyzed for albumin concentration by the two methods during the initial comparison studies, and the same satisfactory agreement was found. The largest discrepancy so far seen between results by the two methods for the same specimen is 6 g/liter, and this was for a serum with a peculiar electrophoretic pattern. The albumin and α-globulins did not show up as discrete bands but as a diffuse, continuously stained area.

The fact that slightly turbid, grossly hemolyzed, and markedly jaundiced sera can be analyzed reliably with this BCG reagent without any significant interference is a decided advantage of this simple technique for albumin determination. Ferreria and Price (*2*) reported that increased bilirubin in serum interfered with the binding of BCG and albumin, but it seems more likely, in view of the present findings, that the interference is between BCG and non-albumin-reacting material. The results they obtained for jaundiced sera were 3 g/liter higher with BCG than with a manual immunoprecipitation technique, whereas results for non-jaundiced sera were on average 5 g/liter higher.

In view of the continuous increase in absorbance that occurs when the BCG reagent is added to serum it is indeed surprising that more caution has not been exercised in its use in both discrete and continuous-flow systems for the determination of serum albumin. Automated continuous-flow systems with various combinations of mixing coils will give erroneously high results the magnitude of which depends on the length of time between mixing the sample with the BCG reagent and measurement of the absorbance. This probably explains why comparisons between the BCG method and more acceptable methods for albumin determination show considerable variation in the slope of the regression lines obtained in similar studies (1–3, 5.) The equations obtained by Gustafsson (*5*) for the BCG method at 0 min ($y = 0.98x + 1.83$) and 60 min ($y = 0.62x + 23.56$) giving some idea of the magnitude of the variation possible. Manual BCG methods that include a color-development period will also show considerable variation of results depending upon the duration of the delay before the color is measured. It would seem advisable that all users of the BCG method for albumin determination should reduce the time interval between color development and measurement to the absolute minimum to ensure results that more nearly represent the true albumin concentrations, as determined by the more specific technique of immunoprecipitation.

References

1. Webster, D., Bignell, A. H. C., and Attwood, E. C., An assessment of the suitability of bromocresol green for the determination of serum albumin. *Clin. Chim. Acta* **53,** 101 (1974).

2. Ferreria, P., and Price, C. P., A comparison of bromocresol green and immunoprecipitation methods for the determination of serum albumin. *Clin. Chim. Acta* **55,** 259 (1974).

3. Slater, L., Carter, P. M., and Hobbs, J. R., Measurement of albumin in the sera of patients. *Ann. Clin. Biochem.* **12,** 33 (1975).

4. Webster, D., A study of the interaction of bromocresol green with isolated serum globulin fractions. *Clin. Chim. Acta* **53,** 109 (1974).

5. Gustafsson, J. E. C., Improved specificity of serum albumin determination and estimation of "acute phase reactants" by use of the bromcresol green reaction. *Clin. Chem.* **22,** 616 (1976).

6. Doumas, B. T., Watson, W. A., and Biggs, H. G., Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta* **31,** 87 (1971).

7. Doumas, B. T., Standards for total serum protein assays—a collaborative study. *Clin. Chem.* **21,** 1159 (1975).