A Practical Scheme for the Estimation of Serum Total Protein and Albumin

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Summary

THE BROMOCRESOL GREEN (BCG) dye-binding method for serum albumin was found to be rapid and convenient. This method compares well with the electrophoresis method ($r = 0.91$) and the salt fractionation method ($r = 0.96$). A simplified scheme is proposed which allows for the rapid measurement of both albumin (BCG method) and total protein (Biuret method) together. This scheme is ideal for a busy clinical laboratory which has to cope with a large routine workload by manual methods.

Introduction

The measurement of serum total protein is usually carried out by the simple and popular Biuret method. Following this, serum albumin measurement is then undertaken by the salt fractionation method in which the globulins are precipitated by a sulphate-sulphite mixture and the albumin in solution quantitated by the Biuret method. This salt fractionation procedure for albumin is somewhat tedious and the method is prone to errors.

Recently Doumas *et al.* (1971) and Miyada *et al.* (1972) have perfected a specific dye binding technique for the quantitation of albumin using bromocresol green (BCG). We have evaluated the method of Doumas *et al.* and found it to be simple and reliable. We now propose a practical scheme whereby the new BCG method is combined with the Biuret method so that the measurement of serum albumin and total protein may be carried out together rapidly and accurately by the busy hospital laboratory.

Materials and Method

Re agents

1. *Biuret re agent*
   Dissolve 9 g of sodium potassium tartrate in 500 ml of 0.2 M sodium hydroxide. Add 3 g of copper sulphate ($CuSo_4.5H_2O$) and dissolve by stirring, then add 5 g of potassium iodide and make up the volume to 1 litre with 0.2 M sodium hydroxide.
2. *Succinate buffer, 0.1 M, pH 4.2*
   Dissolve 11.9 g of succinic acid in about 800 ml of water, adjust the pH to 4.2 with 2.0 M sodium hydroxide (about 35 ml) and dilute to 1 litre with water. Store at 4°C.
3. *Stock bromocresol green solution*
   Dissolve 419 mg of bromocresol green (from BDH, England or E. Merck, Germany) in 5 ml of 0.2 M sodium hydroxide in a 1 litre volumetric flask. Dilute to volume with water and store at 4°C.
4. *Working dye solution*
   Dilute 250 ml of the stock BCG solution with 750 ml of the 0.1 M succinate buffer. Add 4.0 ml of 30% Brij-35 (from Sigma Chemical Co. USA) and mix. Store at 4°C.
5. *Total protein and albumin standard.*
   The same standard is used for both total protein and albumin. The standard can be (1) commercial control serum, (2) commercial standard bovine serum albumin solution, (3) pooled serum, calibrated by the Micro-Kjeldahl method or against commercial control serum.

Method

Set up two test tubes, the first tube to be used for total protein and the second for albumin. Pipette 3.9 ml water into first tube, followed by 0.1 ml of of serum. Mix. Transfer 0.5 ml of the diluted serum into the second test tube. Also set up the standard and blank tubes similarly.

Total protein

Add 3.0 ml Biuret reagent to the first set of tubes. Mix and incubate at 37°C for 10 min. Read the absorbance on the EEL Colorimeter using the green filter (OGR 1) or on a Spectrophotometer at 540 nm, setting zero absorbance with the blank.

$$\text{Total protein (g/100 ml)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \text{Concentration of standard}$$

Albumin

Add 6.0 ml of working BCG dye solution to the second set of tubes. Mix, leave at room temperature for 10 min and then read the absorbance on the EEL Colorimeter with the red filter (Ilford 607) or at 628 nm, using the blank for zero absorbance.

$$\text{Albumin} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \text{Concentration of standard}$$

Results

An albumin standard curve was plotted to establish the linearity of the BCG-albumin complex with respect to albumin concentration (Fig. 1).

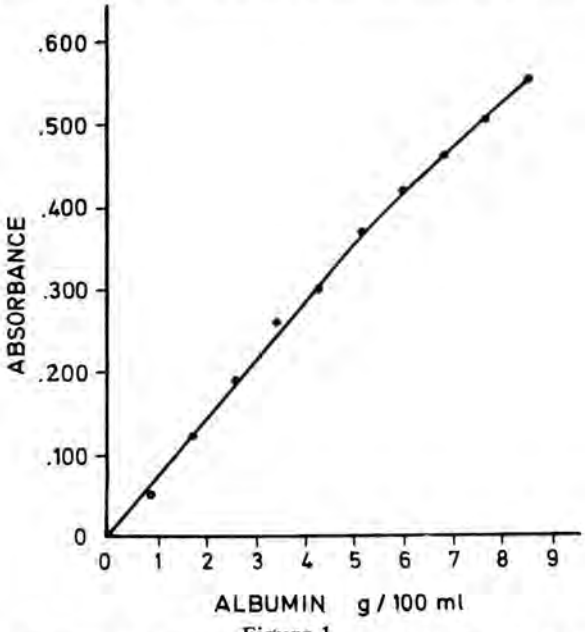

Figure 1
The absorbance-concentration relationship of the BCG method on the EEL Colorimeter.

Absorbance was read on the EEL Colorimeter and on the Coleman Junior IIA Spectrophotometer. On the EEL Colorimeter the Beer's Law holds good up to 5 g/100 ml, while the Coleman Junior gives linear absorbance up to 8 g/100 ml.

The albumin values obtained by the BCG method was compared with values obtained by electrophoresis of serum protein according to the Shandon System (Shandon Monograph on Electrophoresis using cellulose acetate membrane) and with values obtained by the sulphate-sulphite salt fractionation method (as described in Varley, 1967). The results are summarised in Table 1.

Table 1
Comparison of albumin values by 3 different methods.

	Albumin (g/100 ml)		
	BCG	Electro-phoresis	Sulphate-sulphite
Range found	1.1 – 4.5	0.8 – 4.4	0.8 – 4.2
Mean value	2.9	3.0	2.8
No. of samples	29	29	29

The correlation between the BCG and the electrophoresis method (Fig. 2) and between BCG and the sulphate-sulphite method (Fig. 3) is good.

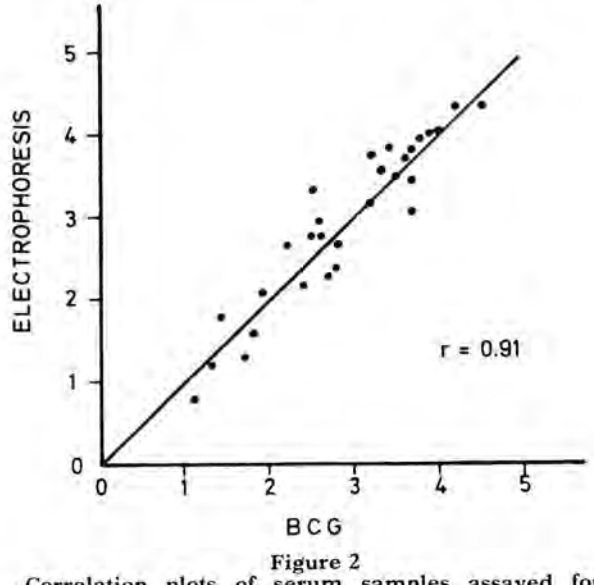

Figure 2
Correlation plots of serum samples assayed for albumin, in g/100 ml, by the Electrophoresis and and BCG methods.

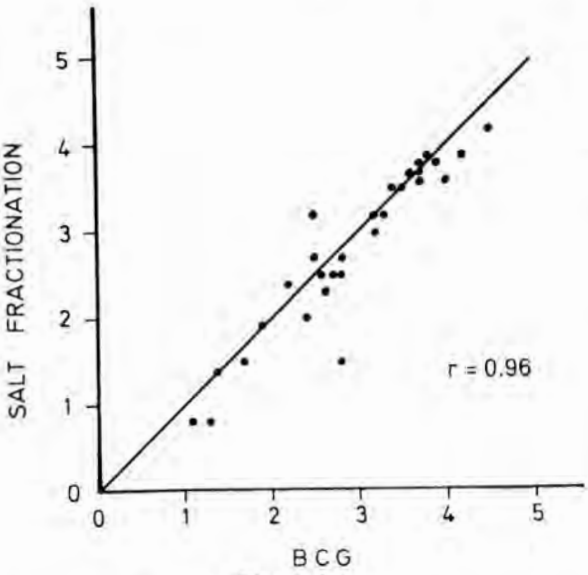

Figure 3
Correlation plots of serum samples assayed for albumin, in g/100 ml, by the Salt Fractionation and BCG methods.

The between-batch precision of the BCG method was established using a commercial control serum and a pooled serum as shown in Table 2.

Table 2
Precision studies on the BCG method.

	Hyland control serum (3.8 g/100 ml)[a]	Pooled serum
Mean value	3.6	3.7
Standard deviation	0.13	0.14
Coefficient of variation[b]	3.6 %	3.8 %
No. of determinations	20	20

[a] Value obtained by salt fractionation.
[b] Coefficient of variation, CV = SD/Mean

Discussion

Quality of BCG

Depending on the make of BCG, we encountered some problems with the dye-albumin complex. Initially we used BCG from May and Baker (supplied by the Government Medical Store) and found that the absorbance of the dye-albumin complex did not pass through the origin, although it gave a linear relationship with increasing albumin concentration. We subsequently used the BCG from BDH and Merck and found both suitable but Merck's was better.

Buffer pH

The recommended buffer pH is 4.2. We have tried out the dye solution buffered at 4.0 and 4.4 and found the results to be similar. The blank absorbance increases with the pH but the absorbance of the dye-albumin complex is highest at pH 4.2.

Albumin standard

The use of a commercially prepared control serum or a standard Bovine Serum Albumin solution (Metrix, Armour Pharmaceutical Co., Chicago, Illinois 60690) has been found to be convenient. To economise and facilitate measurement we recommend dilution of the reconstituted control serum or the standard BSA solution with 0.05 g% sodium azide solution. The control serum is diluted forty-times while the Metrix standard BSA solution (about 6.5 g/100 ml) is diluted eighty-times. Colour development is carried out with 0.5 ml and 3.5 ml solutions using the BCG and Biuret reagents respectively. Working standard solutions thus prepared should be stored in the refrigerator.

The working standard solution from the control serum will have both albumin and total protein values within the working ranges. The working BSA solution is suitable as an albumin standard, but low for total protein.

Crystalline BSA (obtained from Sigma Chemical Co.) may be used as standard but the powder must first be dried according to Doumas *et al.* (1971), otherwise the uncertain moisture in the powder gives an unreliable standard solution.

We have used the BCG manual method for albumin measurement in our laboratory satisfactorily. When establishing this test a standard curve should be done to show linear relationship between colour development and albumin concentration. This will serve to check the buffer, the BCG dye and the colorimeter.

Conclusion

We have found the BCG dye-binding method for serum albumin estimation to be simpler, faster and cheaper than the traditional methods of electrophoresis and salt fractionation, without any loss in accuracy. We have no doubt it will gradually replace the latter two methods. Our proposed scheme will help the busy clinical laboratory cope with the ever increasing requests for serum total protein and albumin/globulin ratio.

References

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