Immunochemical Determination of Serum Albumin with a Centrifugal Analyzer

Mogens Blom and Niels Hjørne

The turbidity resulting from the reaction between albumin and specific anti-human serum was measured with a high precision by using a GEMSAEC centrifugal analyzer. The reaction was enhanced by polyethylene glycol to shorten the reaction time (5 min) and to displace the point of equivalence between antigen and antibody to an albumin concentration unlikely to occur in human sera (about 100 g/liter). An additional program for the computer was necessary to fit the absorbance readings of individual sera to the nonlinear standard curve. Serum albumin values obtained by the described method correlated well with values obtained by the electro-immuno-technique. About 100 samples could be analyzed per hour, 500 µl of 100-fold diluted antiserum being used per specimen.

A centrifugal analyzer combines a high capacity for producing analytical results with a high degree of precision in photometric assays. When in addition only small volumes of sample and reagent are required, this type of analytical instrument seems suitable for immunochemical analyses of specific proteins in series. However, it can be presumed that bidimetric measurements of the opacity, produced by the antigen-antibody reactions in a "discrete" system like this will not detect samples representing "antigen in excess," as is the case in continuous-flow systems (1). Because of this, we chose determination of serum albumin as a model; its variation in concentration under either normal or pathological conditions is relatively narrow. Another reason has been the need for an automated specific method for quantitating serum albumin, as the specificity of the bromcresol green method has recently been questioned (2, 3).

Centrifugal analyzers used in daily routine work demand short reaction times for the analyses concerned. Albumin determination can be shortened by taking advantage of the enhancing effect of polymers on antigen-antibody reactions (4). Moreover, use of a polymer such as polyethylene glycol in antiserum dilutions will increase the turbidity and decrease consumption of antibody.

Materials and Methods

Apparatus

We used the GEMSAEC centrifugal analyzer (Electro-Nucleonics Inc., Caldwell, N. J. 07006, Model 400535-3).

The original FOCAL programs, which are parts of the DT-4 system, were used for the analysis. An additional program was prepared in order to fit the absorbance readings from the individual samples to a nonlinear standard curve (precipitation curve) obtained from four albumin standards, with increasing concentrations included in each run. This additional program splits the slightly bent curve into three intervals and assumes each of these parts of the curve to be linear before calculations. The program furthermore calls attention to values outside the range of the calibration curve.

A printout of the special program is shown in Figure 1.

Reagents

Antiserum. Anti-human albumin antiserum was obtained commercially (Technicon Instruments Corp., Tarrytown, N. Y.; cat. No. T21-451-51). This antiserum was diluted 100-fold with a solution of sodium chloride (9 g/liter) and polyethylene glycol (Polyethylene glycol, mol wt 4000, pure, cat. No. 4807 h; Koch-Light Laboratories Ltd., Colnbrook, En-
Fig. 1. Printout of the additional program, which serves to fit absorbance readings of individual samples to the nonlinear standard curve

gland) in different concentrations. Before use, the solutions were clarified by passage through a 0.22 μm (av pore size) filter (Millipore Corp., Bedford, Mass.; cat. No. GSWP 02500).

Standards and human sera. Reference serum (Behringwerke, Marburg, Germany ORDT 03-02) was used for calibration. Human sera from hospital patients were manually prediluted 400-fold with saline (9 g/liter). Saline used for this purpose as well as for flush in the pipetting unit (“Rotoloder”) was filtered through a sintered-glass filter (No. 4; Soverel, Paris 844.44) before use.

Procedure

The transfer disc is loaded with the Rotolader as follows: sample volume 10 μl, flush volume 40 μl, and reagent volume 500 μl. The sample is placed in well B and the antibody solution in well C. The 16 positions of the transfer disc are loaded according to the following arrangement of the sample cups: a water blank in position 1, standards with increasing concentrations (15.06, 30.10, 45.75, 60.09 g/liter) in positions 2–5, samples in positions 6–16. If other standard values are wanted, the new values must be incorporated in the additional program.

The following GEMSAEC settings are used in the analysis:

Filter position
430–560 nm
Wavelength
440 nm
Reaction temperature
37 °C
Reaction mode
End point
Running mode
Auto
Initial reading
(RI) 300 s
Reading interval
(RI) 60 s
No. of readings
(NR) 01

The header information necessary for the program is the following:

For absorbance–time curves, the system was operated manually.

For blank determinations, antibody was omitted from the saline–polyethylene glycol solution.

For comparison studies, electroimmunoassays were carried out according to Laurell (5).
Results

Concentration of polyethylene glycol and reaction time. Operating the instrument manually, we took absorbance readings every 30 s for 570 s for different concentrations of polyethylene glycol in antibody solutions. The amounts of albumin in the reaction mixture corresponded to standards of concentration 30.10 and 75.06 g/liter, respectively. The time courses are shown in Figures 2 and 3. Polyethylene glycol in a concentration of 50 g/liter gave for the lower albumin concentration the highest turbidities after 150 s, and for the higher one turbidities between those obtained by use of 80 and 110 g/liter of polyethylene glycol. Accordingly, we chose to use polyethylene glycol in a concentration of 50 g/liter. An additional reason was problems with the loading of the disc because of air bubbles in the pipettor when higher concentrations of polyethylene glycol were used.

The time for endpoint reading was set to 300 s.

Figure 4 shows absorbance values given by different albumin concentrations in a standard run. The point of equivalence between antigen and antibody was situated between 90 and 105 g/liter. Higher concentrations gave decreasing turbidities, but such amounts are unlikely to occur in human sera.

Blank values. No blank values higher than 0.001 were detected, even for obviously turbid sera, as might be expected with such a high final sample dilution (21 600-fold).

Precision. Intra-run precision was estimated by running three samples with a low, medium, and high concentration of albumin 132 times, distributed over 12 discs for each concentration. The coefficients of variation are given in Table 1, and Table 2 represents the day-to-day precision for the same three controls analyzed daily for 21 days.

Comparison with electroimmunoassays. Sera from 35 hospitalized patients were estimated by both the electroimmunoassay and the described method, with use of the same reference serum. Figure 5 shows a comparison of results by the two methods.

Discussion

Our immunochemical method for albumin determination is acceptably precise, and the results correlate well with those obtained by electroimmunoassays.
Consumption of antiserum is comparable to the consumption in continuous-flow systems, when polyethylene glycol enhancement is used. About 100 specimens can be analyzed per hour with the GEMSAEC system, and the results are printed out. The method thus represents an attractive alternative for albumin determinations by users of centrifugal analyzers.

The possibility of similarly quantitating other serum proteins will be restricted by the fact that “antigen in excess” cannot be recognized, as it can in continuous-flow systems. This implies that all possible values for a given protein must be included in the ascending part of the precipitin curve, which to a certain extent can be prolonged by the use of polymer enhancement and higher concentration of antibody. Furthermore, separate blank runs may be necessary for proteins that are present in much lower concentrations than albumin.

References