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The Estimation of Precipitating Antibody Using a Turbidimetric Technique*

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Summary. The development of turbidity resulting from the precipitation between bovine serum albumin (BSA) and anti-BSA serum was studied in the spectrophotometer. Data obtained with the turbidimetric method correlated well with those obtained using the classical tube precipitin determination. The equivalence ratio and the concentration of precipitating antibody were determined turbidimetrically by a comparison of the data from both methods. Antibody concentration can be determined with this method in about 1 hour as compared with the 3 or more days required by the tube test. In addition, this technique requires substantially less reagent than other methods.

INTRODUCTION

The combination of antibody with a soluble antigen is often accompanied by visible turbidity. The extent of rate of turbidity development are usually functions of the relative concentrations of antigen and antibody. Martin (1942) and Boyden and DeFalco (1943) were among the first to apply turbidimetric measurements to the study of precipitation. The latter investigators speculated that such measurements would be ideally suited to extensive serological studies and would eliminate the use of tedious quantitative determinations. The use of turbidimetric measurements to study precipitation has resulted in the detection of small deviations not observable with other techniques. Gitlin and Edelhoch (1951), Junge, Junge and Krebs (1955) and Hawkins (1964) employed turbidimetric measurements for the study of antigen–antibody interactions in precipitation. Little attention, however, has been devoted to the employment of such techniques for the routine determination of precipitating antibody.

This report describes investigations that employ a spectrophotometer to measure the rate of turbidity development in the precipitin reaction and apply these data to the determination of antibody concentration.

MATERIALS AND METHODS

Antigens

Crystalline bovine serum albumin (BSA) was purchased from Pentex Corporation. For both immunization and testing, the antigen was suspended in 0·01 M phosphate buffer

* In conducting the research reported herein, the investigators adhered to Guide for Laboratory Animal Facilities and Care established by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, NAS-NRC.

(pH 6·8) prepared in 0·15 M NaCl. This buffered saline was employed for all dilutions and suspensions.

Antiserum

Antiserum against BSA was prepared in New Zealand White rabbits by intramuscular immunization with four to six weekly injections of 10 mg each of antigen emulsified in Freund's complete adjuvant.

Precipitin reactions

Equal volumes (1 ml) of serial dilutions of antigen and a constant concentration of antiserum were mixed and incubated at 37° for 2 hours. After refrigeration at 4° for 48–72 hours, the precipitates were collected by centrifugation, washed twice with 2-ml portions of cold buffered saline, and dissolved in 4 ml of 0·1 M NaOH. The protein concentration of the dissolved precipitates were determined using the Folin–Ciocalteau reagent (Lowry, Rosebrough, Farr and Randall, 1951).

With various modifications, usually in the method of protein or nitrogen determination, the quantitative tube precipitin test just described is the most commonly employed method for determining precipitating antibody titre.

Direct turbidimetric measurements were performed by rapidly mixing equal volumes (0·2 ml) of antigen and antibody and transferring the mixture to a cuvette with a 1-mm path length. The development of turbidity was recorded by readings at 15-second intervals at 350 mμ on a Beckman DK-2A spectrophotometer. A mixture of buffered saline and antisera in equal volumes was used in the reference cuvette to correct for absorbence by serum components such as haemoglobin. The temperature of the coil compartment remained relatively constant at about 27° throughout the experiments.

RESULTS

The precipitin curve shown in Fig. 1 was obtained by measuring the protein concentration of dissolved BSA–anti-BSA precipitates using the quantitative tube method. The rate of turbidity development using the same dilutions of reactants was measured turbidimetrically with results shown in Fig. 2. The highest rate of turbidity development was observed with an antigen–antibody ratio corresponding to the equivalence ratio as determined by the tube test.

After a relatively short lag period, the rate of turbidity development was essentially constant for about one minute after mixing. The rate in the linear region was measured and was expressed as the ΔOD/min. The rate observed with equivalent amounts of antigen and antibody was designated the $E\Delta$OD/min. The equivalence ratio, quantity of precipitated protein, and the rate of turbidity development were determined for a number of antigen and antibody dilutions using both the tube precipitin technique and the turbidimetric method, and the results were compared. When the $E\Delta$OD/min was plotted as a logarithmic function against antiserum dilutions, a linear relationship was obtained with the highest concentrations of antibody (Fig. 3). A plot of log $E\Delta$OD/min against precipitated protein, as shown in Fig. 4, revealed a linear relationship. In contrast, however, with the plot obtained with the antiserum dilutions, linearity in this case was lost with the higher concentrations of antibody.

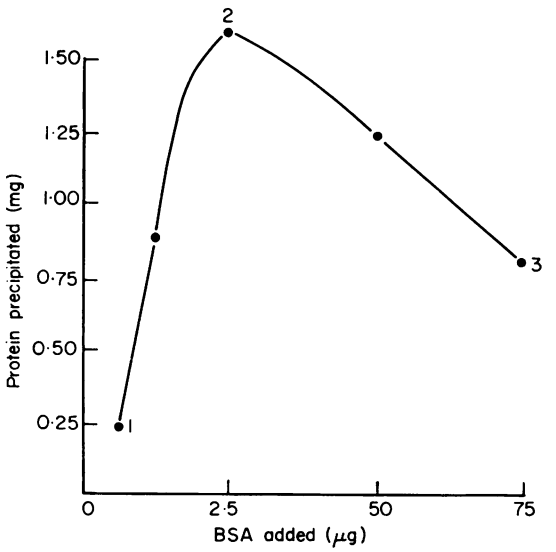

FIG. 1. Quantitative precipitation between BSA and anti-BSA serum measured by the tube precipitin technique. The figures by the points correspond to the curves in Fig. 2 (1, antibody excess; 2, equivalence; 3, antigen excess).

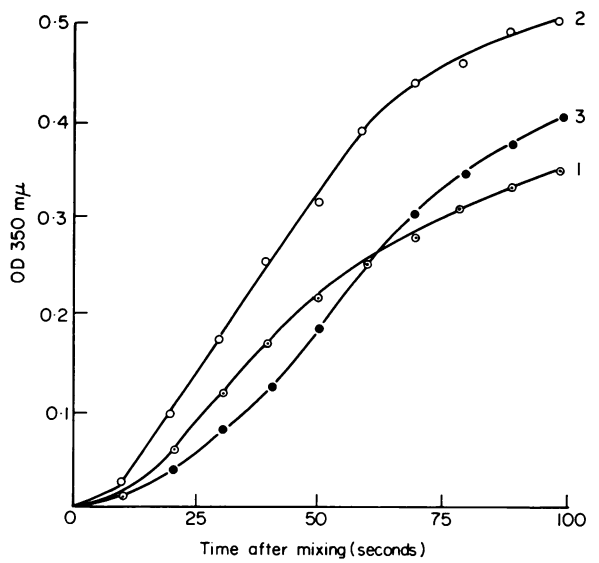

FIG. 2. Development of turbidity with various antigen to antibody ratios. The figures by each curve correspond to the points in Fig. 1 (1, antibody excess; 2, equivalence; 3, antigen excess).

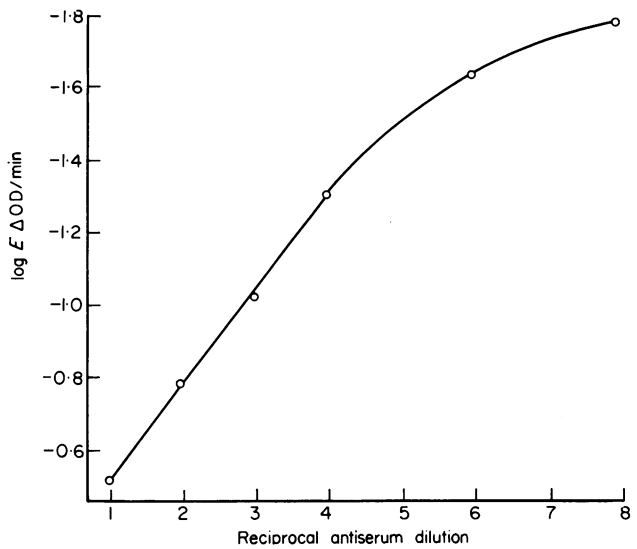

FIG. 3. The $E\Delta$OD/min values obtained using BSA as the antigen with various dilutions of anti-BSA serum.

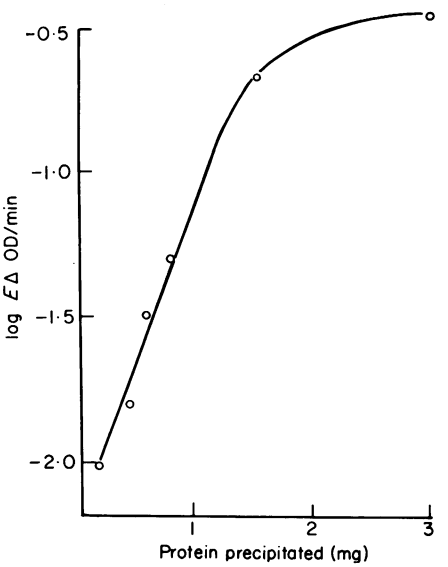

FIG. 4. Comparison of the values obtained with the quantitative tube precipitin test (protein precipitated) with those obtained with the turbidimetric technique (log $E\Delta$OD/min) in the BSA–anti-BSA system.

DISCUSSION

Hawkins (1964) noted that, in the equivalence and antigen excess zones, there is a considerable lag before the development of turbidity. He noted, as Eagle had earlier (1932), a significant increase in the velocity of the reaction when more concentrated

reactants were employed, presumably due to a decrease in the average inter-particle space and the subsequent increase in the number of total collisions. In the experiments reported here, a very short lag was observed because high concentrations of serum were employed. In most cases, the lag period under these conditions was short enough to be disregarded in calculations.

With the higher concentrations of antibody, the rate of turbidity in the equivalence zone appeared to follow almost a first-order kinetics reaction rather than the second- or higher-order reactions observed in zones of antigen or antibody excess. With more dilute serum samples, the rate of turbidity at equivalence began to deviate from the apparent first-order reaction observed with higher concentrations. As a result, there was a deviation from linearity when dilute antisera were employed in titre determinations. The opposite relationship was observed when the rates were plotted against protein precipitated for the same antiserum, that is, there was a deviation from linearity with the more concentrated serum samples.

Thus, linearity was observed in the sensitivity range of the spectrophotometer, except where macroscopic flocculation occurred in the cuvette at the time of measurement.

The experiments reported here indicate that the measurement of the rate of turbidity development can be used effectively to determine the antibody concentration of serum when high concentrations of reactants are used. The test can be performed on a large number of serum samples in a very short time because there is no necessity for collection and measurement of precipitates. Turbidimetric measurement as a means of titering antiserum employs far less reagent than the classical tube tests. The values obtained with multiple samples of the same dilution of antiserum or antigen were usually within 1 standard deviation. This is an important factor to be considered when the quantity of antiserum available for testing is limited.

Excellent correlations were obtained with the serological system described in the current study. The routine application of this procedure for precipitating systems should be seriously considered.

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