A Technique for Immunoassay of Human IgG[1] (34691)

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Several methods have been described in the last few years for the quantitative detection of immunoglobulins. The technique of radial diffusion (1, 2) in agar gel containing antibodies cannot detect immunoglobulins in concentrations below 1.25 μg/ml and it requires relatively large amounts of specific antisera. Moreover, the presence of smaller antigenic fragments or nonoptimal ratios between antigen and antibody can give erroneous results. Inhibition radioimmunoassay, using soluble antibody, incubation with labeled and unlabeled antigen and precipitation of complexes with an antigammaglobulin has been shown to be very sensitive, but too complicated for routine use (3, 4). More recently, inhibition radioimmunoassays, using antibodies conjugated physically or chemically to an insoluble or solid carrier have proven to be rapid, sensitive, inexpensive and reproducible for the detection of small amounts of antigens such as hormones (5–11) or immunoglobulins (12, 13). Previous work has utilized immunoabsorbents formed by diazotization of polyamino-polystyrene to a protein (PAS) (14–21). The present report describes a simple method for the detection of small amounts of IgG using an inhibition radioimmunoassay based on the conjugation of a specific antibody to polyamino-polystyrene.

Materials and Methods. 1. Polyamino-polystyrene (PAS) (obtained from Norsk-Hydroelestrik, Oslo, Norway), was suspended in water and centrifuged for 1 min at 1000 rpm at room temperature; the precipitate, containing most of the polyamino-polystyrene, was discarded; the supernatant was dried and preserved for subsequent use.

2. Antibody specific for human-IgG. Goat antihuman IgG was prepared on a G-200 Sephadex column in buffered isotonic saline (pH 7.4). The fractions representing the second peak were pooled and absorbed with three purified IgM paraproteins that collectively contained both kappa and lambda light chain determinants. The supernatant, rechromatographed on G-200 Sephadex, did not react in agar gel diffusion with IgM.

3. Isotopic labeling of IgG. Iodination with ^{125}I of DEAE-purified human IgG was performed with the technique of McFarlane (22).

4. Preparation of PAS–antibody (PAS-Ab) conjugates. 1.2 g of sodium nitrite was added slowly, with stirring, to 1.5 g of PAS suspended in 30 ml of HCl (1 *N*) at 0°. After 30 min of stirring, the sediment was washed successively with 100 ml of a 5% solution of sodium acetate, 100 ml of a 5% solution of urea, 200 ml of distilled water, 100 ml of buffered saline (pH 7.4), and finally suspended in 50 ml of buffered saline. To the diazotized PAS, 110 mg of goat antibody specific for human IgG was added and the mixture was stirred for 18 hr at 4°. The PAS sediment was washed with 100 ml of buffered saline. The amount of globulin fixed to PAS (1.5 g) was estimated to be approximately 70 mg by determining the amount of protein recovered in the washing. Unreacted azo-groups were then blocked by addition, with stirring, of 50 ml of glycine (0.2 *M*) for 18 hr at 4°. The sediment was washed twice with 100 ml of buffered saline and brought to a final volume of 140 ml with buffered saline. Merthiolate (1/10,000) was added and the suspension stored at 4°.

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5. Immunoassay for human IgG. One vol of PAS–antibody conjugate was washed with buffered saline and diluted with 40 vol of diluted (1:100) normal goat serum. After incubation for 30 min at 37°, 1-ml aliquots of this suspension were added to tubes containing 0.5 ml of solutions to be assayed and the tubes were incubated for 1 hr at 37° with occasional agitation. Standards, usually containing 0.01 to 10 μg of human IgG, were simultaneously run with each assay. Subsequently, 20 μl of ^{125}I IgG (usually containing 0.15 μg with approximately 3000 cpm) were added to each tube and the mixture was incubated for 1 hr at 37° with occasional agitation. After centrifugation at 2000 rpm for 30 min, the supernatant radioactivity was determined in a Packard Autogamma counter.

6. Zone centrifugation. Sucrose density gradient centrifugation was performed in a Spinco SW56 rotor using 0.8-ml layers of 45, 35, 25, and 15% sucrose dissolved in either buffered isotonic saline (pH 7.4) or glycine–HCl buffer (pH 3.2). Samples for analysis (usually 0.2 ml) were applied and the tubes were centrifuged at 40,000 rpm for 15 hr. Sequential fractions (0.2 ml) were collected from the bottom of tubes and analyzed for protein content (Folin-Ciocalteau) and IgG concentration.

Results and Discussion. Figure 1 shows the results obtained by using an amount of labeled antigen with approximately 3000 cpm (0.15 μg of IgG) and a dilution of PAS-Ab that bound between 85 and 90% of the antigen. These conditions were optimal for the detection of IgG in the range of 0.1 to 1.5 μg. The sensitivity could be increased by reducing the labeled antigen and using an amount of absorbant capable of binding 50% of the antigen. The reaction between the PAS-Ab and the antigen was complete after 1 hr of incubation at 37°. A very slight decrease in the speed of reaction was observed at room temperature, while at 4 and 56° it was markedly diminished. The results were not different if plastic tubes (Falcon Plastics, Los Angeles, Calif.) were substituted for glass tubes. The stability of PAS-Ab-Ag complexes is indicated in Fig. 1 where the radioactivity in the sediment, after several washings, decreased as the values of the supernatant increased. The PAS-Ab conjugates were stable; after 4 months of storage at 4°, no proteins were detectable in the supernatant of the suspension with the Folin-Ciocalteau technique and no decrease in the binding activity was found.

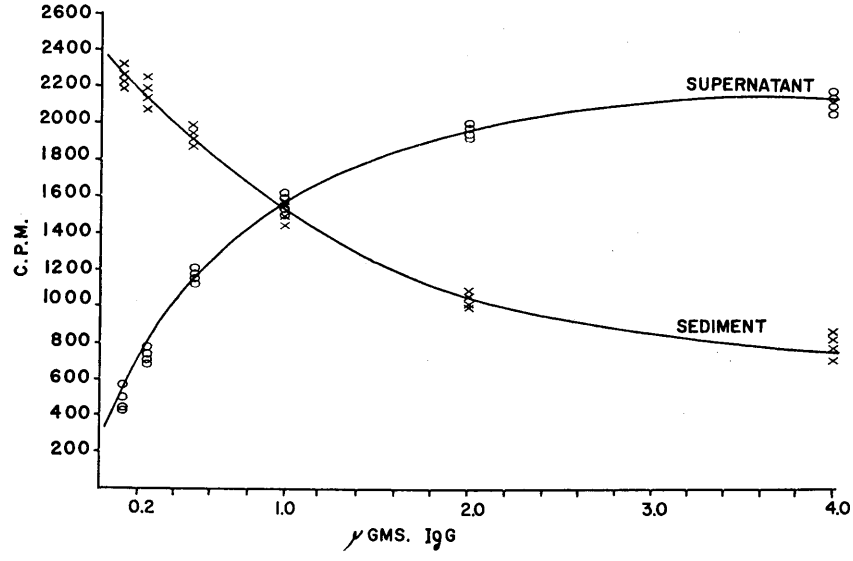

FIG. 1. Distribution of ^{125}I-labeled human IgG in supernatant and PAS-Ab sediment (after washing) with varying concentrations of nonradioactive IgG.

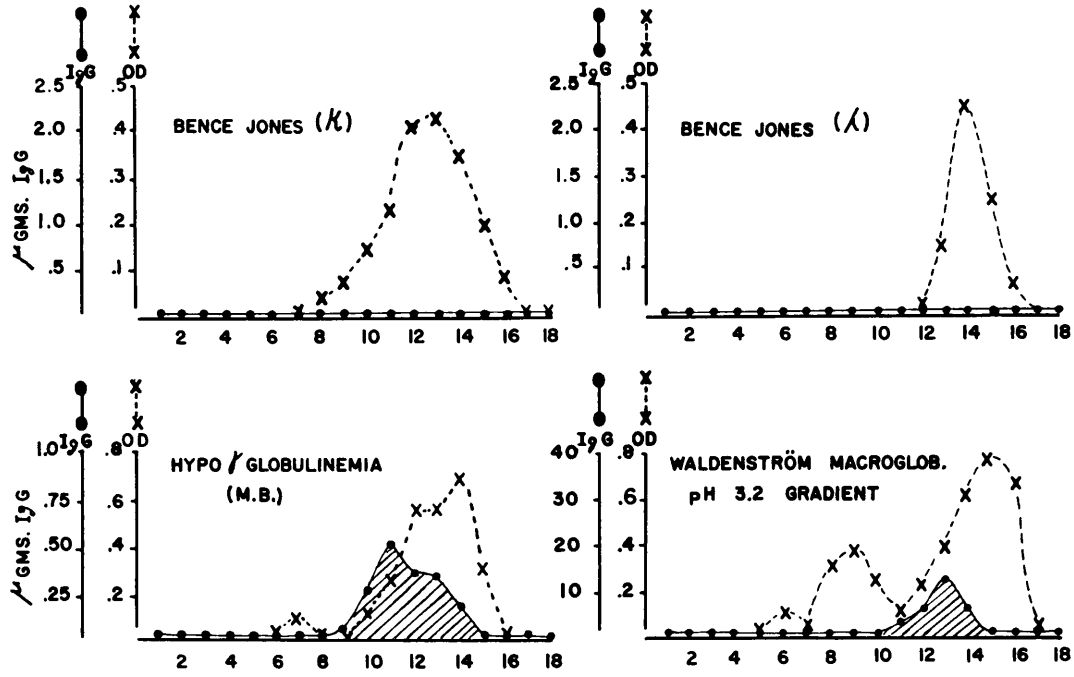

FIG. 2. Immunoassay of zone centrifugation fractions of human sera and purified Bence Jones proteins, demonstrating IgG specificity and the failure of IgM to react in the assay.

The specificity of the assay for IgG is indicated in Fig. 2 which illustrates zone centrifugation analysis of the two sera and two preparations of purified Bence-Jones proteins (kindly supplied by Dr. Elliott Osserman). The Bence Jones proteins did not react in the immunoassay and the distribution of IgG in the hypogammaglobulinemic and Waldenström macroglobulinemic sera was restricted to the zone of the gradient containing this protein. In order to prevent complexing of IgG with the paraprotein IgM, the Waldenström macroglobulinemic serum was separated in an acid (pH 3.2) gradient. This condition accounts for the slower sedimentation of IgG (peak in fraction 13 instead of fraction 11) in the analysis of this serum. (This effect of acid buffer has been observed in sedimentation velocity studies of IgG). There was no reaction of fractions containing IgM in the immunoassay.

The immunoassay for IgG was performed on human cerebrospinal fluids that had been subjected to quantitation of immunoglobulins by precipitin analysis in Dr. Elvin Kabat's laboratory. Figure 3 illustrates the close correspondence of values obtained with the two techniques.

Although the conditions of study allowed detection of IgG in the range of 0.1 to 1.5 μg, the system could be made more sensitive by decreasing the concentration of PAS-Ab and ^{125}I IgG so that its sensitivity was equivalent to that of other recently reported immunoassay techniques for immunoglobulin quantitation (12, 13). It is important to recognize that PAS, has, by itself, an affinity for proteins (18, 20). Incubation of PAS-Ab with diluted normal goat serum prevented any nonspecific interference of binding of ^{125}I-labeled antigen by proteins other than IgG. The method as described is limited to the quantitation of IgG but it is applicable to the assay of any antigen for which specific antisera can be obtained.

Summary. An immunoassay procedure based on the use of specific antibody coupled to polyaminostyrene is described. Quantitation of human IgG in the range of 0.1 to 1.5 μg was possible and by minor adjustments the sensitivity could be increased severalfold.

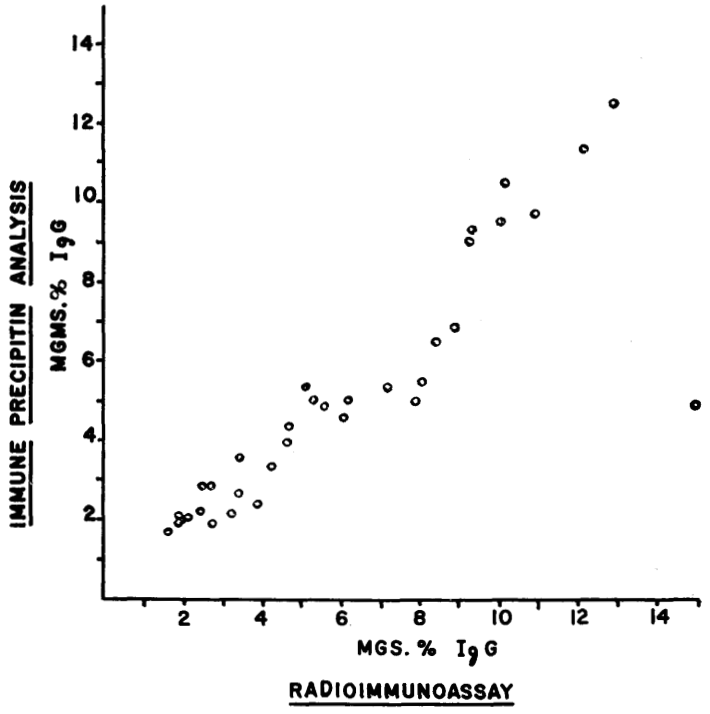

FIG. 3. Comparisons of the content of IgG in human cerebrospinal fluids using immunoassay and immune precipitin techniques.

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