Detection of circulating IgG aggregates and immune complexes using ^{125}I protein A from *Staphylococcus aureus*

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Hällgren, R., and Wide, L. (1976). *Annals of the Rheumatic Diseases*, 35, 306–313. **Detection of circulating IgG aggregates and immune complexes using ^{125}I protein A from *Staphylococcus aureus*.** A method for detection of circulating immune complexes by the use of ^{125}I labelled staphylococcal protein A is described. In a polyethylene glycol solution as little as 1–2 mg/l of soluble heat aggregated human IgG could be quantitated. Variables which might influence the assay were examined. Separation of immune complexes in serum from monomeric IgG was essential and achieved by gel chromatography on Sephadex G200. This assay may be suitable for clinical routine for detection and quantitation of immune complexes. A preliminary study on the clinical application of the method is presented. 58 % of patients with systemic lupus erythematosus and 42 % of patients with rheumatoid arthritis had increased levels of immune aggregates in serum compared to a group of healthy individuals.

Circulating immune complexes are known to play a role in a variety of immunopathological processes in man. Animal experiments have contributed to the understanding of the elimination of immune complexes and of the pathogenesis of glomerular and vascular lesions induced by tissue deposition of such complexes (Cochrane and Koffler, 1973). The presence of circulating immune aggregates depends on the balance between rate of formation and removal of complexes by the reticuloendothelial system. The size of the complexes is important in this context. For example, immune complexes greater than 19S are quickly removed from the circulation of rabbits, mainly by the liver, while smaller antigen-antibody complexes remain longer in the circulation (Cochrane and Hawkins, 1968). Several approaches have been used to show immune complexes in sera and tissues.

Immune aggregates in sera from patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis have been detected by analytical ultracentrifugation (Kunkel and others, 1961) and biological techniques such as histamine release (Baumal and Broder, 1968) and platelet aggregation (Wager and others, 1973). Circulating immune aggregates have been found in sera from patients

with SLE and other hypocomplementaemic states (Agnello and others, 1971), dermatitis herpetiformis (Mowbray and others, 1973), leprosy (Rojas-Espinosa, Mendez-Navarrate, and Estrada-Parra, 1972), and polyarteritis nodosa (Gocke and others, 1971) by agar gel precipitation with Clq. Isolated rheumatoid factors have also been used to detect soluble complexes (Winchester, Kunkel, and Agnello, 1971). A simple, sensitive, and specific method is not currently available for detecting immune complexes.

This paper describes in detail a new method for the detection of circulating IgG aggregates and immune complexes based on the large molecular size of immune complexes and the binding properties of protein A of *Staphylococcus aureus* (SpA) to the Fc-part of IgG (Forsgren and Sjöquist, 1966). Variables which might influence the assay procedure have been examined. A preliminary study on the clinical application of the method is also presented.

Materials

Assay buffer was made up of 500 ml 0.1 mol/l phosphate buffer pH 7.5, 500 ml 0.9 % (w/v) NaCl, 10 ml 5 % (w/v) NaN_3 , 5 ml tween-20 and 5 ml 20 % human serum albumin (Kabi, Sweden). Solutions of 50 g/l polyethylene glycol 6000 (PEG) were prepared by dissolving

polyethylene glycol of molecular weight 6000 (Union Carbide Corp., USA) by heating and mixing in assay buffer. SpA was kindly supplied by Prof. J. Sjöquist, Department of Medical and Physiological Chemistry, University of Uppsala, Sweden (Sjöquist, Meloun, and Hjelm, 1972). Human IgG was supplied by AB Kabi. Heat aggregation was performed for 20 min at 63°C in a 2% IgG solution.

The aggregates (agg IgG) were separated from the monomeric IgG (mon IgG) by gel filtration on a 90 × 1.5 cm column of Sephadex G200 (Pharmacia AB, Uppsala, Sweden) equilibrated with 0.1 mol/l TRIS-HCl, pH 8.0, containing 0.5 mol/l NaCl. Concentrations of agg IgG were determined spectrophotometrically at 280 nm.

Clq was prepared from fresh human serum according to the method of Shelton, Yonemasu, and Stroud (1972). Purity was shown by immunoelectrophoresis using anti-human total serum and anti-Clq serum (Behringwerke AB, W. Germany). Concentrations of Clq were determined spectrophotometrically using the extinction coefficient of 6.8 at 278 nm (Yonemasu and others, 1971).

Tetanus toxoid fluid, 500 Lf units/ml was kindly supplied by SBL, Sweden. A healthy volunteer was immunized by repeated injections of tetanus toxoid. 10 Lf units of tetanus toxoid was incubated at 37°C for 1 hour and at room temperature for 12 hours with 1.0 ml of the immune serum to produce tetanus-antitetanus complexes.

Methods

LABELLING OF PROTEINS

Agg IgG and SpA were trace labelled with ^{125}I (Radiochemical Centre, Amersham, England) by the chloramine T method (Hunter and Greenwood, 1962) to specific activities of 25 μCi and 50 $\mu\text{Ci}/\mu\text{g}$ of agg IgG and SpA respectively.

GEL CHROMATOGRAPHY

A Fractionation of 3 ml serum samples was performed by gel chromatography on a 90 × 1.5 cm column of Sephadex G200 at 20°C in phosphate buffered saline (PBS) pH 7.4. Flow rate was 10 ml/h. Collected fractions of 5 ml were further analysed for ^{125}I SpA precipitation ability. *B* Fractionation of 0.2 ml serum samples was performed on 0.9 × 30 cm columns of Sephadex G200 at 20°C in PBS pH 7.4. Flow rate was 7 ml/h controlled by a peristaltic pump. Effluent was collected in fractions of 0.3 ml. Columns with almost identical elution patterns of ^{125}I agg IgG and of ^{125}I mon IgG were used. 20 μl Blue Dextran (Pharmacia, Sweden) was applied for each separation. 1.5 ml of the first peak identified by Blue Dextran was used for analyses in SpA precipitation test. This volume includes approximately 75% of applied ^{125}I agg IgG and only trace amounts of ^{125}I mon IgG.

COLLECTION OF BLOOD SAMPLES

Serum samples from 18 healthy blood donors were used for comparison with the clinical material consisting of 62 consecutive patients referred to the Department of Internal Medicine, University Hospital, Uppsala. Pathological sera were obtained from 24 patients with rheumatoid arthritis, 17 with SLE, 8 with terminal ileitis, and 4 with scleroderma. 9 patients with metastatic tumours of

different kinds were also investigated (fibro 1, retro-peritoneal 1, and lymphosarcoma 2, thyroid carcinoma 1, ovary 2, and breast adenocarcinoma 2). Blood was drawn aseptically into plain tubes, allowed to clot at room temperature, and centrifuged at 4000 rpm. Sera were stored at room temperature and fractionated within 8 hours. After gel filtration the fractions to be used for analysis were stored at +4°C.

^{125}I SpA PRECIPITATION TEST PROCEDURE

(^{125}I SpA-PEG PROCEDURE)

200 μl of the sample to be tested + 200 μl of the PEG-assay buffer + 100 μl of ^{125}I SpA (40 000 cpm) diluted in the assay buffer were put into disposable plastic test tubes which were then sealed with a plastic cap and placed in racks attached to a Heto Rotamix. They were rotated for 16 hours at 4°C, after which all tubes were removed from the rack and centrifuged at 3500 g for 5 minutes. The plastic plugs were carefully removed. 1 ml of a saline solution with 0.5% tween-20 was added to each tube with an automatic pipetting machine and the tubes were again centrifuged at 3500 g for 5 minutes. The supernatant was removed by suction using a modified syringe needle attached to a plastic plate to prevent the needle from removing more than the desired amount of the supernatant. The washing cycle was repeated three times more. The tubes were sealed with plastic caps and placed in the automatic gamma counter.

^{125}I AGG IgG PRECIPITATION TEST PROCEDURE

(^{125}I AGG IgG-PEG PROCEDURE)

This procedure was, except for the labelled protein, identical with the above procedure. 200 μl of the sample, 200 μl of the PEG-assay buffer, and 100 μl of ^{125}I agg IgG (40 000 cpm) were added to plastic test tubes, incubated, washed, and counted.

IMMUNE COMPLEX ASSAY PROCEDURE FOR CLINICAL ROUTINE

To test the amount of immune complexes in serum, mon IgG was separated from the macromolecular IgG aggregates by gel filtration. A number of small columns were operated in parallel, as described above (gel chromatography *B*) giving a high capacity of the gel filtration procedure. The void volume containing immune aggregates was heat-inactivated at 56°C for 30 minutes before assayed according to the ^{125}I SpA-PEG procedure. All samples were run in duplicate. As a laboratory standard for calibration, different amounts of agg IgG were mixed with the heat inactivated first peak (diluted to give an OD 280 of 0.3) of pooled normal sera fractionated on a G200 to give a standardized protein solution. Agg IgG and the standard protein solution were frozen in small samples at -20°C and thawed on the day to be used. The degree of ^{125}I SpA precipitation activity in the samples investigated are expressed in corresponding agg IgG equivalents, $\mu\text{g}/\text{ml}$.

Results

INFLUENCE OF PEG ON PRECIPITATION REACTION BETWEEN ^{125}I SpA AND AGG IgG

Different amounts of agg IgG and mon IgG were incubated with a constant amount of ^{125}I SpA

(40 000 cpm) in PBS and in the presence of PEG. The appropriate concentration of PEG was determined which gave maximal precipitation of ^{125}I SpA-agg IgG complexes without simultaneous precipitation of free agg IgG. The solubility of trace labelled agg IgG was studied at final PEG concentrations ranging from 0.5–5%. Agg IgG remained soluble at a final PEG concentration of 2%, but at higher PEG concentrations agg IgG was in part precipitable.

FIG. 1 Influence of PEG on the precipitation reaction between SpA and mon IgG and agg IgG respectively. Different amounts of agg IgG and mon IgG were incubated with ^{125}I SpA (40 000 cpm) in PBS and in a 2% PEG solution. After standing at 4°C for 16 hours the mixture was centrifuged and the precipitate washed four times. ^{125}I SpA precipitation activity by agg IgG (▲) and by mon IgG (△) in PBS, and by agg IgG (●) and mon IgG (○) in PEG solution is shown

Mon IgG as well as SpA remained soluble at higher PEG concentrations. Therefore, a final PEG concentration of 2% was chosen for the study of the precipitation reaction between ^{125}I SpA and IgG. As shown in Fig. 1, precipitation of ^{125}I SpA was enhanced by small amounts of agg IgG in a PEG solution. Amounts as small as 1–2 mg agg IgG/l in the test solution could be detected. With increasing concentrations of agg IgG, an increased percentage of ^{125}I SpA was precipitated. Maximal precipitation was obtained for 20 mg agg IgG/l corresponding to approximately 70% of added ^{125}I SpA. No precipitation was obtained using mon IgG even in a PEG solution.

INFLUENCE OF SERUM ON PRECIPITATION REACTION BETWEEN ^{125}I PROTEIN A AND AGG IgG

The SpA-PEG technique used for detection of agg IgG in buffer solutions was tried for the detection of circulating IgG aggregates and immune complexes in sera of patients. From a theoretical point of view, mon IgG in serum could influence the precipitation

reaction between immune aggregates and ^{125}I SpA by competition giving a false negative reaction. On the other hand, anti-IgG immunoglobulins and other coprecipitating agents present in serum might give a false-positive reaction by precipitation of ^{125}I SpA-mon IgG complexes.

In order to study these influences a normal serum without rheumatoid factor activity was investigated in the following manner. Serial dilutions of the serum were tested according to the ^{125}I SpA-PEG procedure. There was no ^{125}I SpA precipitation activity in any serum dilution as shown in Fig. 2. By addition of agg IgG to a final concentration of 20 mg/l to each serum dilution no precipitation was obtained in undiluted serum. The suppression of ^{125}I SpA precipitation by serum was significant even at a serum dilution of 1/125 (Fig. 2).

FIG. 2 Precipitation reaction between ^{125}I SpA (40 000 cpm) with different dilutions of a normal serum in a 2% PEG solution (○). By addition of agg IgG (20 mg/l) to each serum dilution an increase of ^{125}I SpA precipitation activity (●) proportional to the degree of the serum dilutions is shown. Activities were estimated according to the ^{125}I SpA-PEG procedure

In order to determine which serum component had inhibited the agg IgG- ^{125}I SpA precipitation the serum was chromatographed on a 1.5 × 90 cm column of Sephadex G200. Each fraction was analysed according to the ^{125}I SpA-PEG procedure with and without addition of agg IgG at a final concentration of 5 mg/l. The results are shown in Fig. 3. It was apparent that the inhibitory effect of serum on the ^{125}I SpA-agg IgG precipitation was localized to the second peak of the chromatogram where IgG is found. This may imply that the inhibitory effect is due to a competition between IgG and agg IgG.

FIG. 3 Fractionation pattern with OD measurement at 280 nm (solid line) of serum from a healthy individual separated on Sephadex G200. Each fraction was analysed according to the ^{125}I SpA-PEG procedure with (●) and without (o) addition of agg IgG at a final concentration of 5 mg/l

By using a new technique (to be published) an SLE patient was found to have rheumatoid factor activity. This was measured by using the ability of heat inactivated sera to precipitate soluble ^{125}I agg IgG in a final 2% PEG solution. The total serum of the patient investigated precipitated ^{125}I agg IgG as well as ^{125}I SpA. After separation of the serum on a 1.5×90 cm column of G200 and analysis of each fraction in the ^{125}I SpA-PEG and ^{125}I agg IgG-PEG tests the precipitating pattern of Fig. 4 was obtained, indicating that the rheumatoid factor activity was located in the second peak. ^{125}I SpA precipitating activity was similarly located. If the ^{125}I SpA precipitation was due to the same factor that precipitated ^{125}I agg IgG, a competitive inhibition of the ^{125}I SpA precipitation by addition of a certain

FIG. 4 Fractionation pattern with OD measurement at 280 nm (solid line) of serum from an SLE patient. Each fraction was analysed for the ability to precipitate soluble ^{125}I agg IgG (o) and ^{125}I SpA (●) in a 2% PEG solution

amount of agg IgG might be expressed.

In order to test this concept the second peak was pooled and different amounts of agg IgG were added to the pooled fractions before being assayed in the ^{125}I SpA-PEG test. By adding large amounts of agg IgG (200 μg) an increased precipitation of ^{125}I SpA was obtained compared to the precipitating activity of the pooled fractions *per se*. An inhibitory effect on ^{125}I SpA precipitation was obtained when small amounts of agg IgG (2 μg) were added (Fig. 5). These findings support the concept that the same factor that reacts with agg IgG also binds to IgG after reaction with ^{125}I SpA.

FIG. 5 Influence on ^{125}I SpA precipitating activity by addition of different amounts of agg IgG to 0.5 ml of the pooled second peak (corresponding to effluent volumes 170–210 ml) from the Sephadex G200 fractionated SLE serum in Fig. 4. Activity was assayed according to the ^{125}I SpA-PEG procedure

FIG. 6 Fractionation pattern with OD measurement at 280 nm of serum from a patient with active SLE separated on Sephadex G200 (solid line). Each fraction was assayed according to the ^{125}I SpA-PEG procedure (●). The effect of preincubation of each fraction with Clq (10 mg/l) for one hour on the precipitation pattern of ^{125}I SpA (o) is also illustrated

The detection of circulating immune complexes by the use of ^{125}I SpA was limited by different serum factors, and the need to separate the macromolecular immune aggregates from mon IgG to obtain adequate results was evident. Sera from 5 patients with active SLE and 5 healthy volunteers were separated on a Sephadex G200 (1.5×90 cm) column. Each fraction was analysed according to the ^{125}I SpA-PEG procedure. Quite a different precipitating pattern was obtained in these two groups. The serum from 3 of the 5 SLE patients produced a heavy precipitation of ^{125}I SpA in the macromolecular region (Fig. 6). This precipitation profile is not obtained with sera from normal healthy individuals. The amount of ^{125}I SpA precipitated in Fig. 6 described a double-peak in the macromolecular region which could reflect at least two types of immune complexes. The first peak could be complexes of a size corresponding to $\geq 19\text{S}$ and the second peak of a size between 7S and 19S .

In order to study whether the precipitation of ^{125}I SpA in the macromolecular region was due to immune complexes the influence of highly purified Clq was investigated. $5\text{ }\mu\text{g}$ Clq was added to $500\text{ }\mu\text{l}$ of each fraction of the SLE patient serum investigated above (Fig. 6) and incubated for 1 hour at room temperature. The degree of precipitation of added ^{125}I SpA was then studied. As shown in Fig. 6 there was a distinct inhibition of ^{125}I SpA precipitation in the presence of Clq. These findings might suggest that Clq reacts with present immune complexes and probably by steric hindrance prevents the binding of ^{125}I SpA.

Further support for the concept that the ^{125}I SpA precipitating activity in the macromolecular region corresponds to immune complexes formed *in vitro* was provided by testing tetanus-antitetanus complexes. There was no significant precipitation of ^{125}I SpA in the macromolecular fractions of an antitetanus serum separated according to the gel chromatography procedure B. A significant increase of ^{125}I SpA precipitation was obtained in the corresponding fractions of the same serum after addition of tetanus according to the procedure described above.

^{125}I SpA PRECIPITATING ACTIVITY IN SLE AND OTHER PATHOLOGICAL SERA

In order to see if the adoptive immune complex assay could be of clinical use, screening was done on patients with different disorders, selected for the high probability of raised immune complex levels in their sera. The precipitating activity in the assay is expressed in corresponding agg IgG equivalent, mg/l. In Fig. 7 a representative calibration curve in the standardized macromolecular solution is presented.

The upper limit of precipitating activity for 18

normal sera was less than 4 mg/l and results were only considered abnormal and positive if above this value. Fig. 8 shows the distribution of various amounts of immune aggregates in sera of healthy subjects and of various patients. 14 of 24 sera from patients with rheumatoid arthritis and 10 out of 17 sera of patients with SLE gave positive reactions. The highest amount of immune complex, 80 mg/l , was found in a patient with hypocomplementaemic SLE nephritis. The level of immune aggregates in this small group of patients was generally correlated to the activity of the disease.

FIG. 7 Calibration curve for various concentrations of agg IgG in a macromolecular solution. Different amounts of agg IgG were mixed with the heat inactivated first peak (diluted to give an OD of 0.3) of pooled normal sera fractionated on a G200. The ^{125}I SpA precipitating activity was estimated according to the ^{125}I SpA-PEG procedure

FIG. 8 ^{125}I SpA precipitating activity with various human sera analysed according to the adoptive immune complex assay and expressed in corresponding agg IgG equivalent (mg/l). Diagnosis: A rheumatoid arthritis; B SLE; C scleroderma; D metastatic tumour; E terminal ileitis; F control group

In a study of sera from 9 patients with metastatic tumours of different forms, only one with breast adenocarcinoma together with liver and pulmonary metastases had detectable immune aggregates, 27 mg/l. No patient with terminal ileitis was positive in the immune complex assay while one out of 4 patients with scleroderma was.

Discussion

Circulating immune complexes have been detected by a variety of methods. These methods have taken advantage of the fact that immune complexes are macromolecular substances which can be separated from other serum proteins. Both chromatographic separation (Soothill and Hendrickse, 1967) and ultracentrifugation in sucrose density gradients (Kunkel and others, 1961) are based on that principle. A more widely used indirect method is based on the anticomplementary activity of immune complexes (Kohler and Bensen, 1969). More specific methods use the property of direct interaction with complexed antibody. The Clq component of complement, which is known to precipitate soluble immune complexes and agg IgG, have been used in a gel diffusion to detect circulating immune complexes in the sera of patients with SLE and other hypocomplementary states. This method is rather insensitive and besides low molecular weight substances of unknown nature exist which also react with Clq giving false-positive results (Agnello, Winchester, and Kunkel, 1970). By radiolabelling of Clq and studying the precipitation of immune complexes in PEG solution, as little as 50 mg agg IgG/l serum could be detected (Nydegger and others, 1974). The sensitivity of this method is high but the accuracy may be inadequate since it is necessary to heat inactive sera before assaying. This procedure leads to a considerable degree of aggregation of both IgG and IgM and as Clq reacts with both, false-positive results proportional to the immunoglobulin level may be suspected (Johnson, Mowbray, and Porter, 1975). The relative instability of purified, radiolabelled Clq may also be a problem.

These experiments were done to explore the possibility of increasing the sensitivity of SpA-IgG reactions by polymer enhancement. SpA, which is a cell wall constituent of *Staph. aureus*, reacts with the Fc part of human IgG. We have shown that agg IgG, as well as mon IgG, in low concentrations does not precipitate ^{125}I SpA in buffer solution. There is, however, in a certain polymeric solution a pronounced precipitation of agg IgG but not of mon IgG. By a polyethylene glycol concentration of 2%, as little as 1–2 mg agg IgG/l test solution could be detected. Neutral polysaccharides increase the precipitation of antigen-antibody complexes (Helsing, 1966). The same effect was obtained with ^{125}I SpA-agg IgG complexes. This enhancement of the

precipitation by polymers has been explained as a steric exclusion of the complexes from the domain of the polysaccharides (Laurent, 1963).

The ability to detect small amounts of agg IgG by ^{125}I SpA and the differences in the precipitation reaction between ^{125}I SpA and mon IgG, ^{125}I SpA and agg IgG, respectively, in a polymeric solution seemed applicable to detection of immune complexes in serum. At least three factors in sera might, however, influence the ^{125}I SpA-agg IgG reaction and therefore the detection of immune aggregates. Mon IgG did act competitively and prevented precipitation of agg IgG with ^{125}I SpA. Rheumatoid factors, i.e. anti-IgG immunoglobulins of IgG, IgA, and IgM nature, bind to a precipitate agg IgG and IgG in complex with antigen (Heimer and Levin, 1966). Results of the present investigation show that rheumatoid factors also may react with ^{125}I SpA-IgG complexes and this probably explains why some sera with rheumatoid factor activity gave a ^{125}I SpA precipitation in spite of the competition with mon IgG. Clq binds to the Fc part of IgA and was found to interfere with the ^{125}I SpA-agg IgG reaction, probably due to steric hindrance.

Considering the interference of these serum factors, it was necessary to accurately measure the immune complex level in serum to separate mon IgG from immune aggregates and to eliminate Clq. Sera were fractionated by gel chromatography (G200) and the void volumes were heat inactivated at 56°C for 30 minutes to destroy Clq and assayed for the contents of immune complexes according to the ^{125}I SpA-PEG procedure. In order to have a test suitable for practical clinical use it was essential to speed the fractionating rate up as it was most of the time consuming part of the assay. This was achieved by running small columns (0.9 × 30 cm) of G200. The chromatography procedure will be a minor problem when automatic chromatography apparatus is used to a greater extent.

The immune complex assay was applied to the study of fresh sera from patients with SLE and rheumatoid arthritis. The results indicated a high frequency of immune complexes in these disorders. 58% of the SLE patients had a precipitating activity in serum ranging from 12–80 mg/l, and 42% of the patients with rheumatoid arthritis with a range of 5–50 mg/l. The results are consistent with the observations of other investigators, who have also observed increased frequency of immune complexes in sera of patients with these diseases. However, depending on the method used there is a wide variation in the frequency observed and it is difficult to make a proper comparative analysis (Agnello and others, 1971; Nydegger and others, 1974; Norberg, 1974). The nature of IgG complexes found in rheumatoid arthritis sera have been discussed and there

is support that the dominant constituents are IgG-rheumatoid factor complexes (Schrohenloher, 1966). It is likely that DNA-anti-DNA complexes are involved in the pathogenesis of SLE (Harbeck and others, 1973). None of the normal individuals had immune aggregates estimated as precipitating activity exceeding 4 mg/ml. It is not known if the small amounts of IgG complexes detectable in sera of healthy individuals reflect a true normal level of immune complexes.

In our study 8 patients with terminal ileitis did not have increased levels of IgG complexes compared to the reference group. These results are at variance with those of other investigators (Doe, Booth, and Brown, 1973) but there are several possible explanations for these differences. The nature of immune complexes in terminal ileitis have not been identified but alimentary proteins as an exogenous source of antigens have been suggested. Both the time relation of blood sampling to food intake and the condition of the small intestine may influence the frequency of positive reactions.

The possible presence of immune complexes in sera of patients with scleroderma does not seem to have been investigated previously. The serum from one of the patients with this disease was positive in our immune complex assay and it cannot be excluded that immune complexes may be involved in the pathogenesis of this disorder.

Considerable evidence indicates that human tumours are antigenic for their hosts and are eliciting humoral immune response (Hellström, Hellström, and Pierce, 1968). Circulating antibody can be shown when the antigenic mass is small but during periods of rapid growth and in the presence of a large mass of tumour tissues no free antibody is detectable (Ambrose, Andersson, and Coggin,

1971). When sera from 9 patients with different forms of cancer were investigated one patient with a metastatic breast adenocarcinoma had an increased amount of ^{125}I SpA-precipitable material in the macromolecular region. Only a few investigators have, in selected and isolated cases with metastasing tumours, directly shown immune complexes in sera (Ludwig and Cusumano, 1974). These results indicate a low frequency of detectable immune aggregates in sera of cancer patients.

The immune complex assay using ^{125}I SpA is not capable of detecting all forms of immune complexes in serum. SpA reacts with the Fc part of human IgG subclasses 1, 2, and 4 (Kronwall and Williams, 1969), giving the method a specificity for immune aggregates composed of these subclasses only. The degree of ^{125}I SpA precipitation activity of the macromolecular fractions of sera investigated are expressed in corresponding agg IgG equivalent mg/l. This translation may give a false accuracy to the test based on the assumption that differently composed IgG complexes give the same precipitation as agg IgG. Larger complexes may be overestimated and smaller complexes underestimated. These circumstances make the ^{125}I SpA-PEG procedure only a semiquantitative method for quantitation of immune complexes. In spite of these limitations, this technique seems to be a sensitive and reproducible method suitable for clinical routine for detection of small amounts of immune complexes in serum.

The technical assistance of Mrs. Margit Tjernberg and Mr. Christer Bengtsson is greatly appreciated. This work was supported by the Medical Faculty of the University of Uppsala and the Swedish Medical Research Council (16-637).

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