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PRESENCE OF HUMAN REAGIN IN IMMUNOCHEMICALLY PURE IgG SERUM FRACTIONS

By

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Ishizaka et al. have accumulated evidence indicating that reagins are γ -E globulins (1-6). This concept has recently been supported by the discovery of a new human immunoglobulin having no antigenic determinant in common with α , δ , γ or μ -Ig polypeptide chains but showing antigenic relationship with γ -E globulin (7, 8). This IgND globulin is found in an increased amount in the serum of allergic patients (8) and is able to inhibit the P-K reaction in man at very low concentration (9).

Whether γ -E is the unique immunoglobulin carrier of the reagenic activity is still open to discussion. Heterogeneity of reagins has been suggested by several workers on the basis of the distribution of reagins following anion-exchange column chromatography and by the demonstration of skin sensitizing activity in chromatographic fractions containing very little or lacking γ -E (10-13). On the other hand, *Ishizaka* found a good correlation between the γ -E content of a serum fraction determined by radioimmunodiffusion and its reagenic activity estimated on a weight basis. This author showed by Diethylaminoethyl (DEAE)-cellulose chromatography of allergic sera that most of the reagins and γ -E were eluted with phosphate buffer of pH 8 and 0.02 M concentration.

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Since the presence of γ -E globulin in the eluate obtained at lower molarity was not reported by Ishizaka, it was of interest to study the reaginic activity of such a fraction. For this purpose, sera from 8 patients sensitive to pollens or house dust were fractionated on DEAE-cellulose by stepwise elution with 4 phosphate buffers of decreasing pH and increasing molarity. After concentration to the initial volume of serum applied to the column, the fractions were studied by immunoelectrophoresis and tested for P-K activity. Our results indicate that the fraction eluted with buffer 0.01 M, pH 7.8 had a definite reaginic activity and contained only IgG as detected by immunodiffusion.

MATERIALS AND METHODS

1) Allergic Sera

Eight human sera were employed in this study. Five were obtained from ragweed or grass pollen sensitive individuals (serum A.T., M.D., A.S., G.H. and J.H.) and three from house dust allergic patients (serum L.W., A.A., S.T.). A strong reaginic activity was demonstrated for each serum by P-K reaction with the corresponding allergen in normal individuals. All the sera were stored in small aliquots at -20° until used.

2) DEAE-Cellulose Chromatography

DEAE-cellulose chromatography was carried out essentially according to the method described by *Sober et al.* (14) and *Fahey et al.* (15). Commercially available DEAE-cellulose was purchased from Schleicher and Schull A.G. (Dassel, Germany) and from BIO-RAD Laboratories (Richmond, Calif.). Different batches of adsorbent, prepared in the same way, were used for the various sera. The cellulose was suspended in five times its volume of saline and after standing overnight, the cloudy supernatant liquid was decanted. The sediment was repeatedly washed with saline (6-8 times) by decantation until the final supernatant liquid became almost clear. The cellulose was then stirred for one hour in at least 2 liters of 0.5 M NaOH solution and afterwards washed by filtration on a Buchner funnel with about 3 liters of distilled water until the effluent was neutral. The adsorbent was then again stirred for 5 minutes in 0.5 M NaOH solution and further washed as previously described. The same sequence of washings was repeated once. Finally, the cellulose was thoroughly equilibrated with the starting buffer by successive washings, poured into columns compacted by application of hydrostatic pressure of about 50 cm. H_2O , transferred into a cold room and further equilibrated with the starting buffer.

Columns of two different sizes (1×30 cm and 2.5×40 cm) packed respectively with approximately 6 and 30 gr of cellulose were employed in this study. Small columns were used for all the sera except that of G.H. and J.H.

Before chromatography, the sera were dialysed against at least three changes of the starting buffer, 0.01 M sodium phosphate pH 7.8 at 4° C. After removal by centrifugation of a small amount of white flocculent precipitate occurring during the dialysis, the clear supernatant liquid was applied to the column and allowed to enter the adsorbent under flow conditions induced by gravity. According to their size, the columns were loaded with 5 or 25 ml of dialysed serum. Eight allergic sera were separately chromatographed by stepwise elution with the following phosphate buffers: I) Phosphate buffer 0.01 M, pH 7.8; II) Phosphate buffer 0.02 M, pH 6; III) Phosphate buffer 0.05 M, pH 4.5; IV) Phosphate buffer 0.05 M, pH 4.5 containing 1 M NaCl/L. Each buffer was run until no further protein was eluted with it. The flow rate was 2-3 ml/hour for the small columns and 15 ml for the larger ones and the eluates collected into 8 or 15 ml fractions. The protein concentration was estimated by measuring absorption at 280 m μ with a ZEISS PMQ 11 Spectrophotometer. For each serum, the eluates corresponding to the different buffers were pooled and the fractions labelled from I to IV increasing with the ionic strength of the buffers. As the elution volume of fractions II and III was large, each of them was divided into two approximately equal subfractions a and b. The fractions were concentrated to the initial volume of serum applied to the column by dialysis against concentrated solution of polyvinylpyrrolidone (PVP) or by ultrafiltration through DIAFLO membrane (Amicon Corp. Boston, Mass.) under positive nitrogen pressure (5 Kg/Cm ^{2}). After dialysis against buffered saline, the concentrated fractions were tested for P-K activity and studied by immunodiffusion.

3) Immunodiffusion

Microimmunoelectrophoresis was performed using the method of *Scheidegger* (16) and the AGAPHOR apparatus (Zurich, Switzerland). Double diffusion in agar was carried out by the method of *Ouchterlony* (17). After development of the precipitin bands, the slides were washed with saline for 8 hours and stained with amido-Schwarz 10B. Rabbit sera specific to human IgG, IgM or IgA were purchased from BEHRINGWERKE A.G. (Marburg/L, Germany). Horse anti-whole human serum was obtained from CENT. LAB. NETH. RED CROSS (Blood Transfusion Service, Amsterdam).

4) Assay of Reaginic Activity

The allergic sera and serum fractions were assayed for skin sensitizing activity by the Prausnitz-Kustner (P-K) test (18). Aliquots of 0.1 ml of the solutions were injected intradermally into sites on the back of non-atopic volunteers. Twenty four hours later, the sensitized sites were challenged with 0.03 ml of an aqueous extract of the corresponding allergen (Pollens or House Dust) containing

A.T. (GRASS POLLEN)
DEAE CELLULOSE (1 x 30)

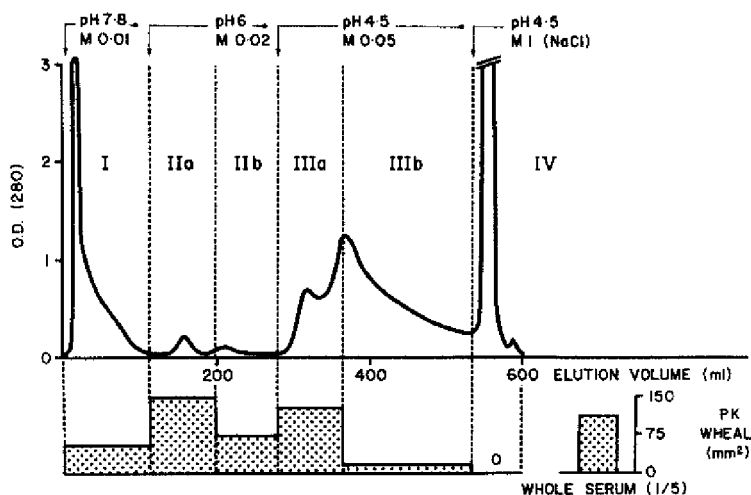


Fig. 1.

Chromatographic fractionation of serum A.T. on DEAE-cellulose and distribution of reaginic activity.

600 to 1000 PNU per ml. A positive reaction was characterized by erythema and wheal formation occurring 5 to 20 minutes after the injection of the allergen. The reaginic activity was estimated after 15 minutes by measuring the surface of the resultant wheal expressed in mm².

RESULTS

The distribution of skin sensitizing activity in different fractions obtained by DEAE-cellulose chromatography of sera A.T., S.T., J.H. and G.H., is illustrated in Figs. 1 to 4. The optical density curves of the eluates as well as the protein composition by immunoelectrophoresis indicated that these sera had been separated according to the elution pattern described by *Sober et al.* (14) and *Fahey et al.* (15). Similar results were observed with the other sera. Immunoelectrophoresis of the chromatographic fractions—using horse anti-

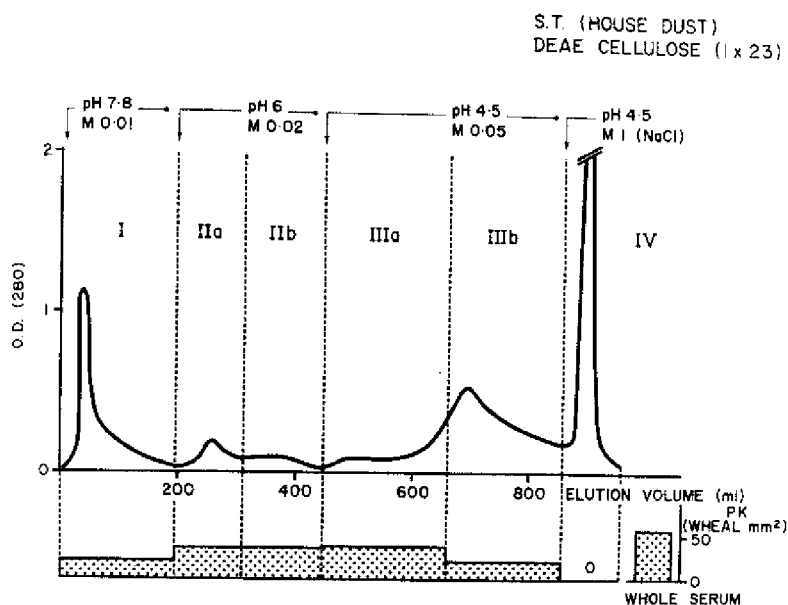


Fig. 2.

Chromatographic fractionation of serum S.T. on DEAE-cellulose and distribution of reaginic activity.

whole human serum—revealed that fraction I contained only IgG globulin; Fraction II: transferrin and a small amount of IgG; Fraction III: several components including the bulk of albumin and IgA; Fraction IV: many α globulins with some albumin and various immunoglobulins. By the method of Ouchterlony, it was confirmed that IgG was the only detectable immunoglobulin in fractions I and II. However, when large columns were used, traces of IgA could be found in fraction II b. Fig. 5 is a typical example of the immunoelectrophoretic pattern obtained when fraction I was tested against horse anti-whole human serum. In all the cases, a single component was detected in this fraction and the same precipitation line was observed when an antiserum specific to IgG was poured into the median trench.

The reaginic activity was usually spread over all the chro-

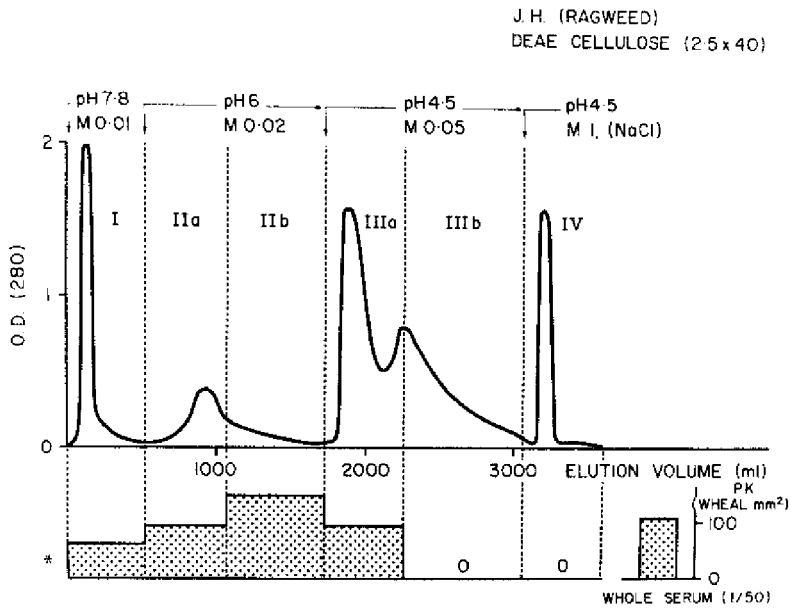


Fig. 3.

Chromatographic fractionation of serum J.H. on DEAE-cellulose and distribution of reagin activity. * After 1/10 dilution.

matogram and showed considerable variations with respect to the fractions. Only fractions IV and III b were occasionally devoid of any skin sensitizing activity. In all our sera, a definite reagin activity was associated with fraction I. However, the bulk of the skin sensitizing activity was consistently found in fractions II and III and was almost equally distributed between these two fractions. Nevertheless, it was obvious on a weight basis that fraction II was the most active.

DISCUSSION

By chromatography of 8 allergic sera on DEAE-cellulose, immunochemically pure IgG globulin was obtained when phosphate buffer 0.01 M, pH 7.8 was used for elution. For each serum chromatographed, we have found reagin activity as-

G.H. (RAGWEED)
DEAE CELLULOSE (2.5 x 40)

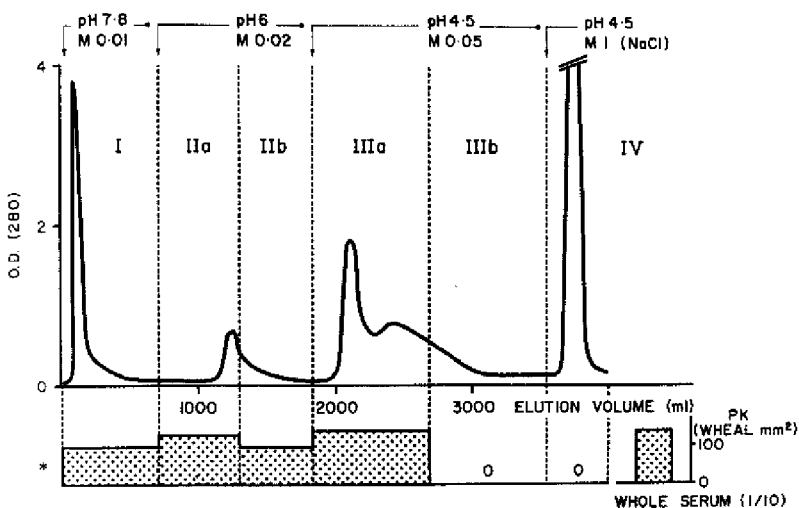


Fig. 4.

Chromatographic fractionation of serum G.H. on DEAE-cellulose and distribution of reaginic activity. * After 1/10 dilution.

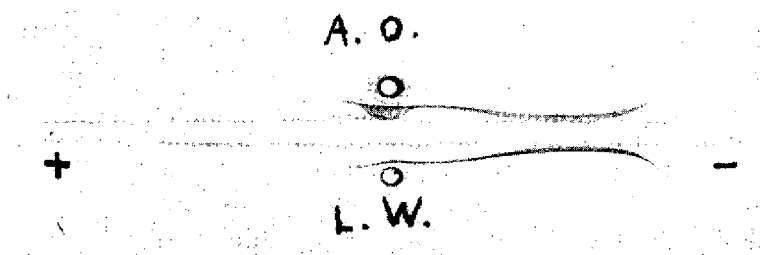


Fig. 5.

Immunoelectrophoresis of concentrated fractions I obtained by separate DEAE-cellulose chromatography of serum A.O. and L.W. (patients sensitive to house dust) using 0.01 M phosphate buffer at pH 7.8 for elution. Horse anti-whole human serum poured into the median trench reveals only IgG globulin.

sociated with this fraction. Our results confirm previous data by several workers (19-21) and suggest that some reagins belong to the IgG immunoglobulin class. However, it is noteworthy that the reaginic activity does not parallel the IgG concentration when serum or IgG fractions are fractionated by chromatography on an anion-exchanger or gel filtration. *Goodfriend et al.* (22) have compared skin sensitizing activity and protein concentration in pure IgG fractions obtained by chromatography on DEAE-Sephadex and found little activity in the first part of the eluate containing most of the IgG. The area following this first peak which had less protein exhibited stronger reaginic activity. Similar results were obtained when IgG globulin recovered by DEAE-cellulose chromatography was submitted to gel filtration on Sephadex G 200. After the gel filtration, we observed, as did *Ishizaka* (1), that the reaginic activity did not parallel the IgG peak but came off the column early in the 8 S region (23). These findings indicate that the reagins found in our IgG preparations belong either to some IgG molecules of different size and charge or to another protein present at too low a concentration to be detected by the usual immunodiffusion techniques. The latter possibility has been supported by *Ishizaka et al.* (1) and *Goodfriend et al.* (22) who have failed to precipitate the reagins from IgG fractions prepared in similar conditions with an antiserum specific to IgG. Furthermore, the former author has brought evidence indicating that a new immunoglobulin, called γE , was accounting for the whole of the reaginic activity of these fractions and sera. However, the demonstration of γE in IgG fractions obtained under the same experimental conditions as those used in the present study was not reported by *Ishizaka*. Therefore the question as to whether the reaginic activity found in our preparations is due to the IgG itself or contaminating γE is still uncertain. Experiments are currently in progress to get more information about the nature of the immunoglobulin accounting for the skin sensitizing activity of this fraction.

SUMMARY

Eight sera from patients sensitive to various pollens or house dust were separately chromatographed on DEAE-cellulose columns by stepwise elution with four phosphate buffers of decreasing pH and increasing molarity. Immunochemically pure IgG globulin was prepared for each serum using 0.01 M phosphate buffer at pH 7.8 for elution and equilibration of the cellulose and serum. Reaginic activity was consistently found in fraction I and the bulk of the activity of sera in fractions II and III. The presence of skin sensitizing activity in fraction I, which contains IgG free of other serum protein, seems to indicate that some reagins belong to the IgG class of immunoglobulin.

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