

Human reaginic antibodies and immunoglobulin E

Kimishige Ishizaka and Teruko Ishizaka, Denver, Colo.

EDITOR'S NOTE: The Editorial Board has solicited the following review, since the topic is one of great importance and current interest to allergists.

The term "reagin" is applied to the spontaneously produced skin-sensitizing antibodies found in allergic human sera. The presence of this substance in the sera of atopic patients was first demonstrated by Prausnitz who had passively sensitized his forearm to fish by intracutaneous injection of serum from his allergic patient, Küstner. The skin site receiving the serum gave an erythema-wheal reaction on challenge with the specific antigen to which the donor was sensitive.¹ Subsequently, the passive transfer of immediate hypersensitivity to other allergens, such as horse serum and grass pollen, was observed in a similar manner. These observations definitely showed that atopic individuals have antibodies responsible for the erythema-wheal reactions. Since then, the nature of reaginic antibodies has been studied by many investigators in numerous laboratories. Whenever new methods for fractionation of serum proteins were developed, the methods were applied to study physicochemical properties of reaginic antibodies. Despite many painstaking efforts, however, the exact nature of the antibodies remained unknown for 40 years.

Recent improvement in this field is based on progress in the structure and classification of immunoglobulins and on the development of sensitive immunochemical methods. It has been established that immunoglobulins are composed of 2 kinds of polypeptide chains, i.e., heavy and light chains, and that the structure of heavy chains differs from the class and subclasses of the immunoglobulins, whereas light chains are common for all classes and subclasses.^{2, 3} It also

Children's Asthma Research Institute and Hospital.

This work was supported by Research Grant AI-04985 from the United States Public Health Service and Grant GB-4646 from the National Science Foundation.

Received for publication Aug. 14, 1968.

became clear that the immunological properties of antibodies differ depending on the class to which the antibodies belong.^{4, 5} Evidence has been accumulated that the immunological properties of antibodies, such as their capacity to induce anaphylactic reactions in guinea pigs and to fix complement (C'), are based on the partial structure of the heavy chains in the immunoglobulins.^{6, 7} The characteristic immunological properties of reaginic antibodies strongly suggested to us the possibility that these antibodies may have peculiar heavy-chain structures and therefore that they may belong to a distinct immunoglobulin class. Studies in this direction lead to identification of a unique immunoglobulin, IgE (γ E), as a carrier of reaginic activity.⁸ In this review, our investigations on this approach, together with the work of others, will be summarized following the order of logical argument.

HISTORICAL BACKGROUND

Earlier studies by Scherrer⁹ and Stull and associates¹⁰ have shown that reaginic antibodies were present in the pseudoglobulin fraction rather than the cuglobulin fraction when the patients' sera were fractionated by precipitation with either ammonium sulfate or sodium sulfate. On the other hand, the Cohn low-temperature ethanol-fractionation procedure concentrated reaginic activity in fraction III which is predominantly composed of β -globulin.¹¹ Electrophoretic characteristics of reaginic antibodies have been studied by various methods such as free-boundary electrophoresis,¹² electrophoresis convection,¹³ and zone-separation procedures.¹⁴ The results were divergent; however, reaginic antibodies were not detected in the slow-moving, γ_2 -globulin in most cases. Maximum reaginic activity was found in the β - to γ_1 -globulin region,^{14, 15} especially by zone electrophoresis. When reaginic sera were fractionated by chromatography on a diethylaminoethyl (DEAE)-cellulose column, the first fraction, which was eluted with low ionic strength buffer and composed of the major portion of the γ G-immunoglobulin, usually lacked reaginic activity.^{16, 17} The sedimentation coefficient of the antibodies was also studied. The activity was found between the 7S and 19S fractions.¹⁸ With the use of sucrose-density gradient ultracentrifugation, Anderson and Vannier¹⁹ found the average sedimentation coefficient of reaginic antibodies to be 7.8S. All of this information strongly suggests that reaginic antibodies are different from IgG (γ G) and IgM (γ M) with respect to their physicochemical properties.

After the third major immunoglobulin, IgA (γ A), was isolated and characterized,²⁰ the relationship between γ A and reaginic antibodies was studied in many laboratories. This approach was quite reasonable because γ A-immunoglobulin is a pseudoglobulin and its electrophoretic mobility is in the range of β_2 to γ_1 . Furthermore γ A-immunoglobulin consisted of monomer (7.0S) and polymer forms (10.5S, 13S, 17S), which may account for the apparently divergent claims as to the molecular size of reagin. Since 1962, evidence accumulated to support the concept that the antibodies were associated with γ A. Heremans and Vaerman²¹ as well as others^{22, 23} reported that the γ A-globulin fraction of atopic patients' sera had skin-sensitizing activity. Fireman, Vannier, and Goodman²⁴ incubated patients' sera with a sheep antiserum against A-myeloma protein and

found that reaginic activity in the sera was lost by precipitation of γ A. Third, we have demonstrated that the γ A-globulin fraction of normal serum, but neither γ G nor γ M, blocked passive sensitization of human skin with reagin.²⁵ In passive-cutaneous anaphylaxis (PCA) in the guinea pig, it has been established that the immunoglobulin to which the skin-sensitizing antibodies belong nonspecifically blocks passive sensitization, whereas the other serum proteins failed to do so.²⁶ The findings on the blocking of Prausnitz-Küstner (P-K) reactions therefore supported the concept that reaginic antibodies belong to γ A. Finally, Yagi and associates²⁷ detected γ A antibody against ragweed allergen in patients' sera by radioimmuno-electrophoresis. This observation does not necessarily indicate a relationship between γ A and reaginic activity. However, the information previously described in combination strongly supported the concept that reaginic activity is associated with γ A. However, Loveless²⁸ demonstrated reaginic activity in a serum sample which lacked a detectable amount of γ A. Furthermore, our studies on human anti-A isoagglutinins⁷ showed that none of the γ G, and γ A, and γ M antibodies sensitized human skin even with one microgram of these antibodies, indicating that γ A antibodies are not necessarily skin sensitizing.

GAMMA E-IMMUNOGLOBULIN AS A CARRIER OF REAGINIC ACTIVITY

Reviewing the previous findings which supported the relation between γ A and reaginic antibody, one might notice 2 assumptions for the conclusion. First, the concept for purity of γ A preparation was based on conventional immunochemical methods which are generally more sensitive than chemical methods for detecting impurities. However, one has to consider the limitation of the methods: Impurities cannot be detected when the antibodies against the impurities are lacking. In a strict sense, the demonstration of a single precipitin band between a "pure" γ A preparation and antihuman serum merely indicates the presence of γ A in the preparation but does not exclude the presence of impurities. Second, immunologists had a general feeling that the sensitivity of P-K reactions in human beings might be comparable to that of PCA reactions in the guinea pig in which 0.03 to 0.1 μ gN per milliliter of antibody was required for a positive reaction.²⁹ If this is the case, the only protein which accounts for the high P-K titer of γ A preparations of reaginic sera should be γ A-immunoglobulin itself. However, the assumption is not correct, because the P-K titer of some reaginic sera is in the range of 1:10,000 and the γ A concentration in the serum is on the order of 0.3 mg.N per milliliter. This fact cannot be explained unless all γ A in the patient's serum is the antibody against the single allergen to which the donor is sensitive. If the P-K reaction is much more sensitive than PCA reactions in the guinea pig, it is still possible that reaginic antibodies in the γ A fraction may belong to "impurities" which were not detected by the usual immunochemical methods.

With these considerations in mind, we have repeated our own previous experiments as well as those performed by others. When reaginic sera from ragweed-sensitive patients were fractionated by DEAE-cellulose column chromatography and gel filtration through Sephadex G-200 columns, the reaginic activity in the fractions did not parallel either γ G or γ A concentrations.³⁰ Although the

reagin-rich fractions contained both γ G and γ A, reaginic activity in the fractions was not precipitated by rabbit antisera specific for either γ G or γ A. The results were different from those reported by Fireman and associates.²⁴ In order to confirm our findings, γ A isoagglutinin was added to a reagin-rich fraction as a marker, and γ A in the mixture was precipitated. The results showed that γ A isoagglutinin was completely precipitated by anti- γ A, whereas essentially all reaginic activity remained in the supernatant fluid.³¹ The physicochemical properties of reaginic antibodies were also different from those of γ A isoagglutinin. When the mixture of reagin-rich fraction with γ A isoagglutinin was analyzed by sucrose-density gradient ultracentrifugation, the sedimentation velocity of reaginic antibody was intermediate between those of polymer and monomer forms of γ A isoagglutinins, with a sedimentation coefficient of approximately 8S.³¹ As reported by Anderson and Vannier,¹⁹ the distribution of the reaginic antibody in gradient fractions was homogeneous. Since the polymer (10.5S) and monomer (7S) forms of γ A isoagglutinins were completely separated under the experimental conditions, it is unlikely that the reaginic antibodies are composed of both 7S and 10.5S components. It was also found that the γ A isoagglutinin had faster electrophoretic mobility than reaginic antibodies when a mixture of these antibodies were analyzed by zone electrophoresis in Sephadex G-25 gel. These findings show that the reaginic antibody is different from γ A antibody in both physicochemical properties and in antigenic structure.

The possibility may be considered that reaginic antibodies are heterogeneous with respect to immunoglobulin class and that part of the antibodies is associated with γ A-immunoglobulin. In fact a "pure" γ A fraction, which was obtained from reaginic sera by the zinc-precipitation method³², and gave a single precipitin band with antihuman serum, contained high reaginic activity.³³ When the γ A fraction was further fractionated by DEAE-cellulose column chromatography, however, the reaginic activity in subfractions did not parallel γ A concentration. Proteins were eluted stepwise from the column with pH 8.0 phosphate buffers of increasing molarity, i.e., 0.02M, 0.035M, and 0.08M. The major portion of γ A was eluted in the second fraction, whereas most of the reaginic activity was concentrated in the first fraction (fraction γ A₁). Furthermore, essentially all reaginic activity in the γ A preparation remained in the supernatant fluid after the precipitation of all γ A by the antibody specific for the immunoglobulin. Those results indicated that reaginic activity in a so-called "pure" γ A preparation did not belong to γ A-immunoglobulin itself but to the other protein not detectable by immunodiffusion.³³ Our previous experiments on blocking of passive sensitization by γ A preparation were also repeated. Indeed, a γ A preparation from normal serum blocked passive sensitization. However, all A-myeloma proteins tested failed to do so. It was also found that the activity of γ A fraction to block passive sensitization did not correlate with the concentration of the protein. When a γ A fraction from a ragweed-sensitive serum was subfractionated by DEAE-cellulose column chromatography and subfractions were tested for the activity to block passive sensitization with reaginic antibody to egg albumin, the blocking activity was detected in the first fraction (fraction γ A₁) which contained antiragweed-reaginic antibody.³³ It

seems that blocking activity is due to the protein carrying the reaginic activity rather than γ A itself. We have also confirmed the findings by Yagi and associates²⁷ that the γ A-precipitin band formed with ragweed-sensitive sera bound radioactive ragweed allergen. However, the presence of γ A antibody did not have any correlation with the skin-sensitizing activity of the patients' sera.³⁴ Similar results have been reported by Reisman and associates.³⁵ Furthermore, the antiragweed γ A antibody in the patients' sera was precipitated by rabbit anti- γ A antibody leaving essentially all the skin-sensitizing activity in the supernatant fluids.^{34, 36}

A critical difference between the previous findings and our data was that reaginic activity was precipitated by a sheep anti- γ A serum by Fireman and associates,²⁴ whereas our rabbit anti- γ A serum did not precipitate reaginic activity. Since the difference is important for the conclusion, we have repeated the experiments with the same sheep anti- γ A serum used in their experiments. The antiserum was supplied by Dr. Vannier. Indeed, the antiserum removed reaginic activity from a reaginic serum. However, the activity of the antiserum did not have any connection with anti- γ A antibody. When the antibody in the antiserum was precipitated with A-mycloma protein and the supernatant fluid was tested for the ability to neutralize reaginic activity, the supernatant fluid and the original antiserum were comparable with respect to the neutralizing activity. The results indicated that anti- γ A in the sheep antiserum was not responsible for the precipitation of reaginic activity. In summarizing the results described, one may notice that all the previous findings are experimentally reproducible. However, the data do not support the γ A hypothesis. Our findings seem to indicate that reaginic antibodies are associated with a minor component which contaminated "pure" γ A preparations.

Evidence was also obtained that the reaginic antibodies are not IgD (γ D) immunoglobulin. When the patients' sera were fractionated, reaginic activity of the fractions did not correlate with the concentration of this protein. The precipitation of γ D from reagin-rich fractions by antibody specific for the protein did not result in any detectable decrease in reaginic activity.³¹ Lack of correlation between reaginic activity and either the γ G, γ A, or γ D concentration in chromatographic fractions from reaginic sera was confirmed by Perelmutter and associates.^{37, 38} The reaginic antibody is probably not γ M because the sedimentation coefficient of the antibody is 8S and the reagin-rich fraction does not contain a detectable amount of γ M. In order to confirm this, a pathological γ M protein was added to the fraction and then precipitated with anti- γ M.³⁹ As expected, reaginic activity was not coprecipitated with γ M-anti- γ M complexes. The presence of 7S γ M-immunoglobulin has been reported by Rothfeld and associates.⁴⁰ However, the results of the absorption experiments showed that reaginic antibody is neither 7S nor 19S γ M-immunoglobulin.

In collaboration with Dr. W. D. Terry, the relation between reaginic antibody and γ G subclasses was also studied.³⁹ The reaginic activity in chromatographic fractions of patients' sera did not parallel either γ G2 or γ G3 concentration, and the anti- γ G3 serum did not precipitate reaginic activity. Furthermore,

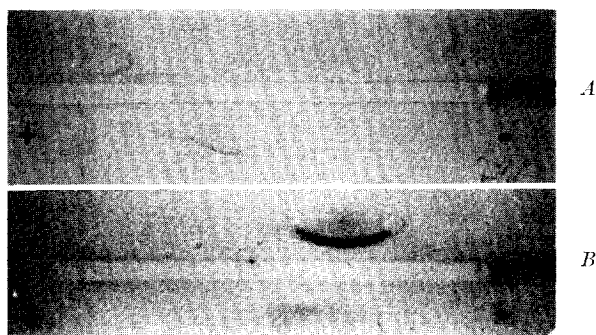


Fig. 1

Radioimmunoelectrophoresis of a reagin-rich fraction. Both the stained slide, *A*, and the radioautograph, *B*, are shown. After electrophoresis of a reagin-rich fraction in agarose, antibody trough was filled with anti- γ E antiserum. A γ_1 (γ E) precipitin band combined radioactive antigen.

our anti- γ G antibody precipitated both γ G2- and γ G4-myeloma proteins previously added to a reaginic fraction. However, reaginic activity in the fraction did not diminish by the precipitation. From these experiments, it seems that the reaginic antibodies are not associated with any of the γ G2, γ G3-, and γ G4-globulins. In summarizing these experimental results, we have speculated that reaginic antibody belongs to a unique immunoglobulin class which had not been described.

In order to prove the hypothesis that reaginic antibodies may belong to a distinct immunoglobulin class, we have tried to prepare rabbit antibodies which react with the carrier protein of reaginic activity. A rabbit was immunized with a reagin-rich fraction from a ragweed-sensitive serum, and the antiserum was absorbed with normal γ G and with A-myeloma and D-myeloma proteins.^{34, 36} The supernatant fluids did not give a precipitin band with any of the γ G-, γ A-, γ M-, or γ D-immunoglobulins but gave a γ_1 -precipitin band with a reagin-rich fraction in immunoelectrophoresis (Fig. 1, *A*). After washing the slide, radioactively labeled purified ragweed antigen (antigen E of King and associates⁴¹ was applied to the antibody trough. The radioactive antigen specifically combined to the γ_1 -precipitin band, indicating that the γ_1 -globulin had antibody activity (Fig. 1, *B*). As the γ_1 band was different from γ G, γ A, γ M, and γ D bands in specificity, the protein responsible for the band was tentatively designated γ E.³⁴ In radioimmunodiffusion, all of the sera containing high reaginic activity against the purified ragweed allergen gave the radioactive γ E band, whereas the sera lacking the reagin did not (Fig. 2, *A*).³⁶ More recently, the γ E antibodies against rye-grass allergen and horse dandruff have been detected in the sera of atopic patients who are sensitive to these allergens. The antibodies against these allergens were not found in the sera from ragweed-sensitive patients who are not sensitive to either rye grass or horse dandruff. These findings indicated that the combination of radioactive antigens to the γ E-precipitin band

was specific and correlated with the presence of skin-sensitizing activity in the sera. The anti- γ E serum actually precipitated reaginic antibody in the patients' sera. Incubation of reaginic sera with anti- γ E resulted in a formation of a trace amount of precipitate. The supernatant fluids did not give either a radioactive γ E band in Ouchterlony plates (Fig. 2, *B*) or P-K reactions. Representative results of the skin reactions by the supernatant fluid and by the original serum before absorption are shown in Fig. 3, *A*. So far, sera from 10 ragweed-sensitive patients and 10 serum samples from atopic patients sensitive to other allergens, such as grass, egg, and horse dandruff, have been tested. The reaginic activity in all of the sera studied was removed by the precipitation of γ E.

The correlation between γ E antibody and reaginic activity was studied by fractionating reaginic sera from ragweed-sensitive patients.³⁶ Throughout the experiments, distribution of γ G, γ A, and γ E antibodies was studied by radioimmunodiffusion, and reaginic activity was tested by P-K reactions. The globulin fraction of the sera was obtained by precipitation with 50 per cent saturation of ammonium sulfate at pH 7.0 and further fractionated by DEAE-cellulose column chromatography. Proteins were eluted stepwise with phosphate buffers of in-

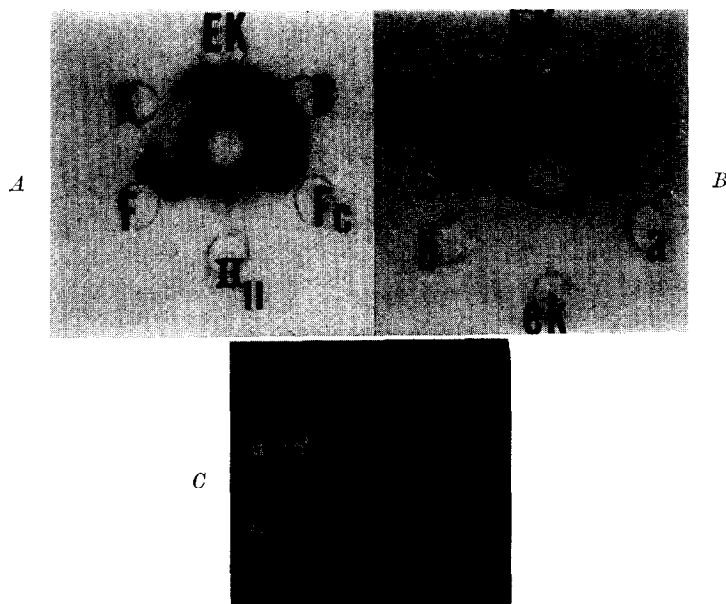


Fig. 2

Radioimmunodiffusion analyses of reaginic sera and a γ E fraction. The center wells in *A* and *B* were filled with the mixture of anti- γ E and radioactive antigen E. *A*, Peripheral wells were filled with reaginic sera. K and II₁₁ did not contain reaginic antibody against antigen E. *B*, Effect of absorption of reaginic sera with anti- γ E antibody. Peripheral wells were filled with the supernatants after the absorption (small letter) and the original sera diluted in saline (capital letter). *C*, Radioimmunodiffusion of γ E fraction placed in the center well. Peripheral wells were filled with antisera specific for one of the 5 immunoglobulin classes (γ G, γ A, γ M, γ D, and γ E) and the antibody against F_{ab} portion of γ G (a-F_{ab}). After washing, the slide was washed and peripheral wells were filled with radioactive antigen E.

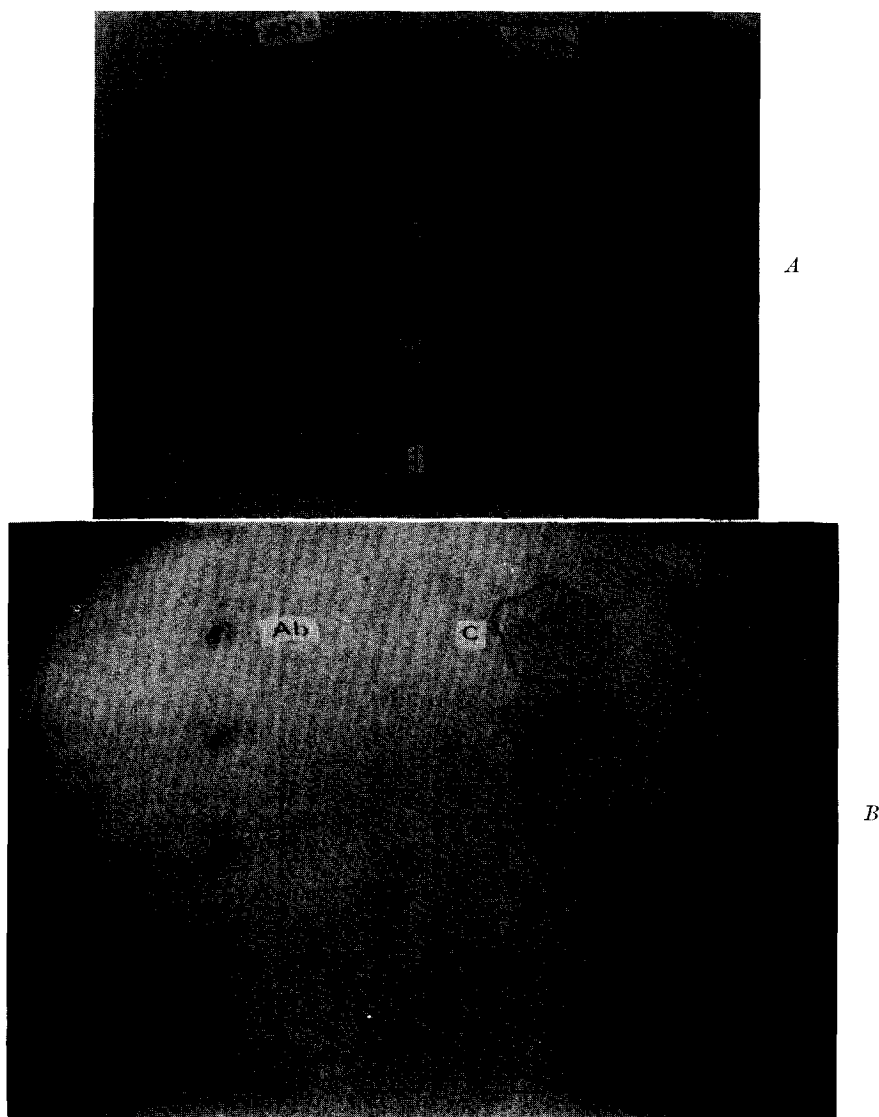


Fig. 3

A, Absorption of reaginic activity by anti- γ E antiserum. A reagin-rich fraction was incubated with anti- γ E serum. Specific precipitate was removed and serial dilutions of the supernatant were injected intracutaneously to left side (abs) of the back. Right side (cont) received the same dilutions of the unabsorbed fraction. All sites were challenged by allergen 24 hours later. *B*, The same patient's serum was absorbed with anti-E myeloma (anti-ND). Serial dilutions of the supernatant fluids were injected into left side (ab). Right side (c) received same dilutions of the unabsorbed serum.

Table I. *Distribution of antibodies associated with different immunoglobulins and of reaginic activity*

Fraction	Phosphate buffer for elution* (M/l)	Relative concentration of γA (per cent)	Presence of antibodies against antigen E in†			Minimum skin-sensitizing dose (μgN)
			γG	γA	γE ‡	
I	0.005	0	++	-	-	> 1.6
II	0.025	32	+	-	+++	0.05
III	0.035	61	±	+	+	0.4
IV	0.05	39	-	-	-	> 1.6
V	0.08	10	-	-	-	> 1.6

*pH of the buffer was 8.0 (J. Immunol. 97: 840, 1966).

†Radioactivity of precipitin bands was compared in radioautographs.

‡Protein concentrations of fractions I-V applied in radioimmunodiffusion were 1 per cent. (J. Immunol. 97 849, 1966.)

creasing molarity, and the fractions were tested for the presence of γG , γA , and γE antibodies by radioimmunodiffusion and for skin-sensitizing activity. As shown in Table I, both reaginic activity and γE antibody against ragweed antigen were detected in fractions II and III; and fraction II had the highest reaginic activity and the highest γE -antibody concentration. The γG antibody was concentrated in fraction I, and γA antibody was detected only in fraction III. The second fraction (fraction II), having the high reaginic activity and the highest level of γE antibody, was further fractionated by recycling gel filtration through Sephadex G-200 columns.⁴² The reaginic activity and γE antibody against purified ragweed allergen were detected in the first and second peaks, whereas the third peak, composed of γG -globulin, lacked both activities (Fig. 4). The reagin-rich fraction, thus obtained (fraction II₂ in Fig. 4), was then analyzed by sucrose-density gradient ultracentrifugation. Some γG anti-A isoagglutinin was added as a marker. After centrifugation, distribution of reaginic activity and of γE antibody were studied. The results indicated that the sedimentation velocity of reaginic antibody was higher than that of γG isoagglutinin, and γE antibody was detected in the fractions having the highest reaginic activity. As described previously, the sedimentation coefficient of the reaginic antibody was approximately 8S. The electrophoretic mobility of γE antibody was also in agreement with that of reaginic antibody, when a reagin-rich fraction was analyzed by electrophoresis in agarose gel. After electrophoresis, segments of the center piece of the gel were extracted with saline and tested for the presence of reaginic activity. Troughs were cut along with the direction of electrophoresis, and anti- γE serum was placed in a trough. The distribution of reaginic activity corresponded with the place of γE -precipitin-band formation.

Perelmutter and associates³⁶ applied DEAE-Sephadex column chromatography for the fractionation of reaginic sera. In their experiments, most of the γA was distinct from the reagin-containing fractions. Therefore, we have applied this method to study the correlation between γE antibody and reaginic activity. In our experiments, reaginic sera were fractionated by DEAE-cellulose column chromatography and gel filtration, and a reagin-rich fraction, thus obtained, was

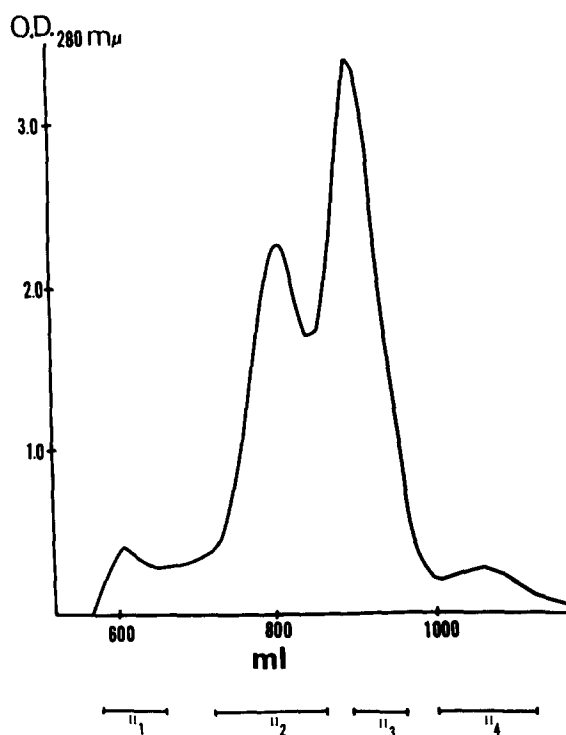
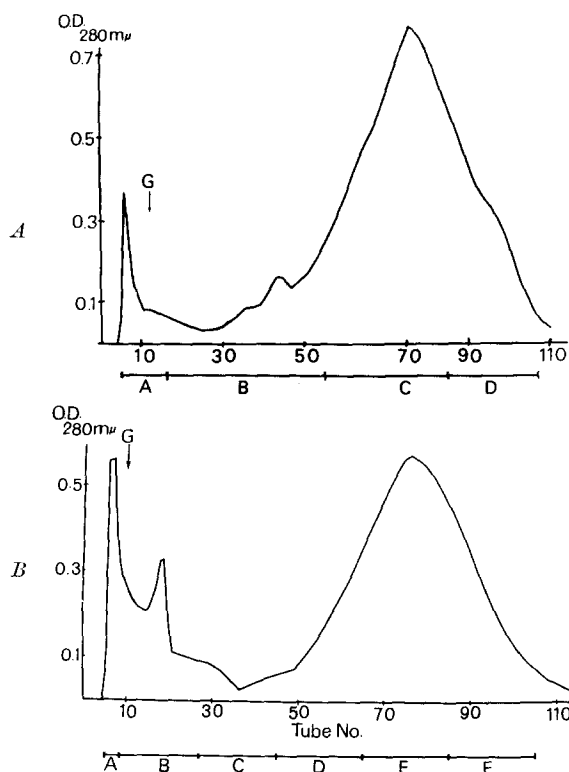


Fig. 4

Gel filtration of a reagin-rich fraction through a Sephadex G-200 column. The major protein component in fraction II₂ and fraction II₃ were γ A- and γ G-globulin, respectively. Reaginic activity was detected in fractions II₁ and II₂. (J. Immunol. 99: 1187, 1967.)

applied to a DEAE-Sephadex column which was equilibrated with 0.1M Tris-HCl buffer at pH 8.0 (Fig. 5, A). After gradient elution with a 0.5M Tris buffer of the same pH, the fractions were analyzed. It was found that the major protein component in the first and last peaks were γ G and γ A, respectively, and the highest reaginic activity was observed between the 2 peaks. By radioimmuno-diffusion, the intensity of radioactivity of the γ E-precipitin band paralleled reaginic activity, whereas distribution of both γ G and γ A antibodies exhibited no correlation with that of reagin. The parallel distributions between reaginic activity and γ E antibody to the same allergen in all the fractionations described strongly support the concept that reaginic activity is associated with γ E.

Direct proof for γ E as a carrier of reaginic activity was obtained after purification of the protein from reaginic sera.⁴² The globulin fraction was obtained from the sera by precipitation with ammonium sulfate and further fractionated successively by DEAE-cellulose column chromatography and recycling gel filtration followed by DEAE-Sephadex column chromatography. On a weight basis, reaginic activity in the most active fraction was 150 to 200 times higher than that in the original sera. However, the fraction still contained γ G- and γ A-globulins. These proteins were absorbed with purified rabbit anti- γ A and anti-

**Fig. 5**

DEAE-Sephadex chromatography of fraction II₂ (cf. Fig. 4). The starting buffer was 0.1M Tris-HCl buffer, pH 8.0. After the first peak was obtained, proteins remaining in the column were eluted with a gradient increase of molarity. G represents the start of gradient elution. In A, anti-ragweed γ E-antibody was detected in fractions A, B, C, whereas all fractions, A through F, contained the γ E antibody in B. The highest antibody activity was in fraction B in both cases. (*J. Immunol.* 99: 1187, 1967.)

γ G antibodies. The purified rabbit antibodies were precipitated with goat anti-body specific for the F_c portion of rabbit γ G, and the specific precipitates were incubated with the reagin-rich fraction for the absorption. By this procedure, almost all the γ G and γ A in the preparations were removed, leaving reaginic activity in the supernatant fluid. On a weight basis reaginic activity in the final preparations was about 1,000 times more active than the original serum. The preparation gave a positive P-K reaction with 0.0001 μ gN, while the P-K titer of the preparation was 1:80,000. The preparation gave γ E- and γ D-precipitin bands when it was analyzed by immunodiffusion with antisera specific for each immunoglobulin class. The presence of anti-ragweed antibodies was analyzed by radioimmunodiffusion. As shown in Fig. 2, C, only γ E antibody but none of γ G, γ A, γ M, or γ D antibody was detected in the preparation. As will be discussed, the radioactive band formed by anti-F_{ab} is due to the presence of light chains in γ E. Furthermore, both reaginic activity and γ E antibody in the most active preparation were completely removed by precipitation of γ E with

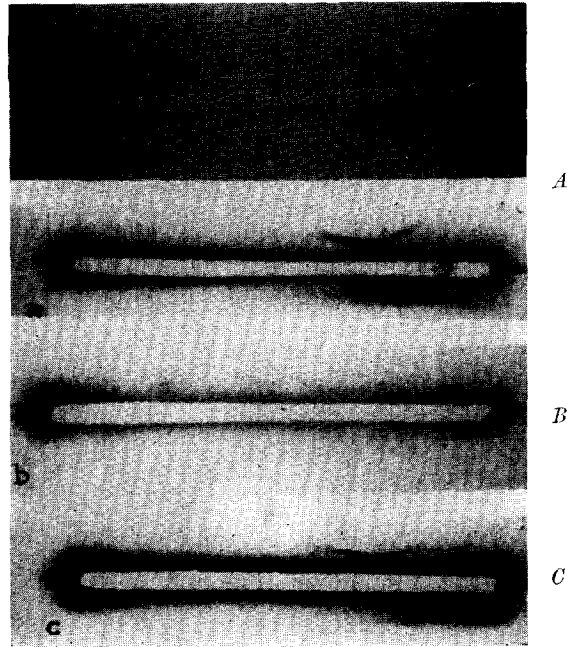


Fig. 6

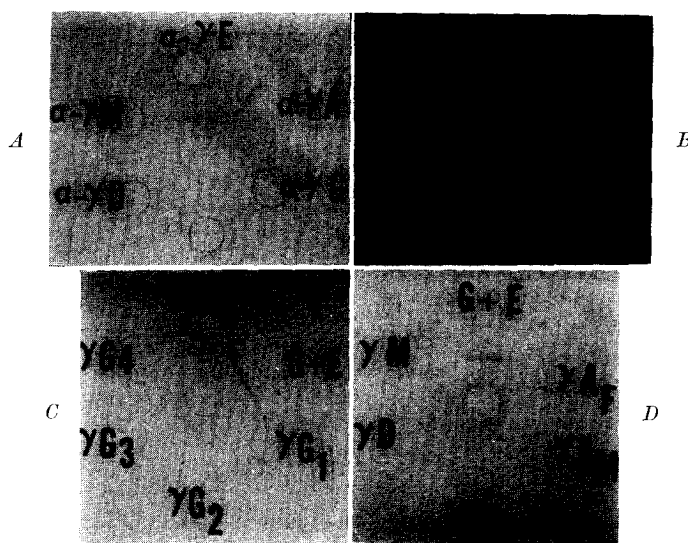
Radioimmuno-electrophoresis showing the presence of light chains in γ E. Both stained slide (top) and the radioautographs (*A, B, C*) are shown. The γ E-precipitin band was developed by immunoelectrophoresis (top). After washing 3 slides (*A, B, C*) showing the γ E-precipitin band, each slide was treated with normal rabbit serum, *A*, unlabeled anti- κ , *B*, or anti- λ antibody, *C*, followed by application of radioactive anti- κ -chain antibody. The radioactive antibody combined with the γ E precipitin band, *A, C*, and the combination was specifically blocked by unlabeled anti- κ antibody, *B*. (J. Immunol. 99: 849, 1967.)

anti- γ E. These results clearly show that γ E antibody is responsible for the reaginic activity in the purified preparation and thus identified γ E-globulin as a carrier of reaginic activity.

CHARACTERIZATION OF γ E-IMMUNOGLOBULIN

Antigenic structure

As described before, γ E was first detected by radioimmunodiffusion. Since the antiserum used in the immunoelectrophoresis did not contain any antibodies specific for heavy chain of 4 known immunoglobulins and lacked antibodies reacting with light chains, the protein was expected to be a unique immunoglobulin. However, more evidence is required for the conclusion that γ E actually represents a distinct immunoglobulin class. All immunoglobulins are composed of heavy and light polypeptide chains, and the presence of κ and λ light polypeptide chains is a common property of immunoglobulins. Therefore, we have investigated the presence of these light chains in γ E by radioimmuno-electrophoresis (Fig. 6).³⁹ Immunoelectrophoresis was set up between a reagin-rich fraction and anti- γ E antiserum. After the precipitin band developed (Fig. 6), the slides were washed and radioactively labeled anti- κ or anti- λ antibody was

**Fig. 7**

Antigenic analysis of γE . *A*, A γE -globulin fraction of a reaginic serum was placed in the center well against the antisera specific for the 5 immunoglobulins placed in peripheral wells. Only the anti- γE serum gave a precipitin band with the fraction. The slide, *A*, was washed and radioactive antigen E was applied to peripheral wells. Radioautograph, *B*, of the slide shows that the γE -precipitin band combined radioactive antigen. *C*, A monospecific anti- γE serum was placed in the center well of the plate against a reaginic serum (S), a fraction composed of γG - and γE -globulins (G + E) and γG -myeloma proteins of 4 subclasses. *D*, The center well was filled with the same anti- γE serum against γA -myeloma proteins of 3 subclasses, γD -myeloma and γM -macroglobulin. γA_F , γA_M represent Fu type and Ma type by Terry and Robert.⁴⁸ γA_H represents He type by Vaerman and Hieremaus.⁴⁶ Both Fu and Ma types belong to Le type by the latter investigators. The results in *C* and *D* show that the γE -antigenic determinant is not shared by any of the other immunoglobulins of known classes and subclasses.

applied to the antibody trough. The radioactive antibody combined with the γE -precipitin band (Fig. 6, *A*). The combination of the labeled antibody was specifically blocked by pretreatment of the slides with unlabeled antibody of the same specificity but not with antibody of the other specificity. For example, the combination of radioactive anti- κ antibody was blocked by pretreatment with unlabeled anti- κ antibody but not by treatment with anti- λ antibody (Fig. 6, *B* and *C*). These findings show that γE possesses antigenic determinants in both κ and λ polypeptide chains and indicate that γE is an immunoglobulin.

Since γE was detected with the use of a rabbit antiserum from which antibodies specific for γA , γG , and γD globulins were precipitated with myeloma proteins, it is possible that the antiserum contained antibodies directed toward minor antigenic determinants in the known immunoglobulins and that " γE "-globulin was detected by one of these antibodies. If this is the case, " γE "-globulin could represent a subclass of known immunoglobulins rather than a distinct class. Subclasses of γG were described by Grey and Kunkel⁴³ as well as by Terry and Fahey,⁴⁴ and 4 subclasses, $\gamma G1$, $\gamma G2$, $\gamma G3$, and $\gamma G4$, are known. Gamma A subclasses have been reported by Kunkel and Prendergast,⁴⁵ Vaerman

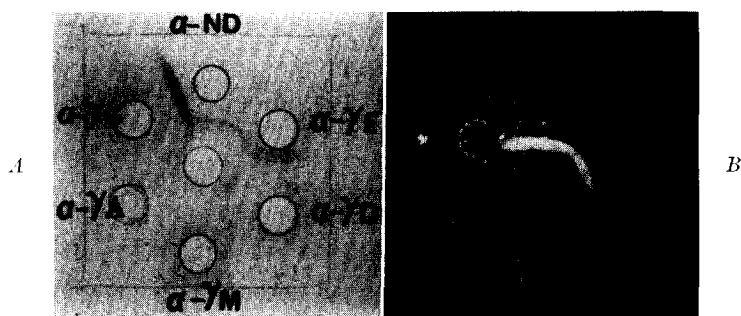


Fig. 8

Radioimmunodiffusion showing the antigenic identity of myeloma protein ND with γ E-globulin. A reagin-rich fraction was placed in the center well and antisera specific for each of the 5 immunoglobulins and that specific for myeloma ND (α -ND) were placed in center well, *A*. Anti- γ E and anti-ND showed a precipitin band of identical specificity with the reagin-rich (γ E) fraction. The slide was washed and radioactive antigen E was applied to peripheral wells. A radioautograph (*B*) of the slide showed that the precipitin band combined radioactive antigen.

and Heremans,⁴⁶ and Feinstein and Franklin,⁴⁷ as well as by Terry and Robert.⁴⁸ The subclasses described by the first 3 groups are exactly the same. However, γ E-globulin is not a subclass of the known immunoglobulins because of the following evidence. When a purified γ E fraction from a ragweed-sensitive serum was analyzed by immunodiffusion, only the anti- γ E serum, but none of the anti- γ G, anti- γ A, anti- γ M, or anti- γ D sera, gave a precipitin band, and the γ E band combined with radioactive ragweed antigen in radioimmunodiffusion (Fig. 7, *A* and *B*). The results indicate that γ E-globulin lacks major antigenic determinants present in γ , α , μ and δ chains. It was also found by immunodiffusion experiments that anti- γ E serum failed to give a precipitin band with myeloma proteins of all γ G and γ A subclasses, γ D-myeloma protein, and γ M-macroglobulin (Fig. 7, *C* and *D*). It is evident that γ E-globulin has antigenic determinants which are not shared by any of the known immunoglobulins. From these findings on antigenic structure of γ E, as well as the presence of antibody activity in γ E, it was concluded that γ E represents a distinct immunoglobulin class.

This conclusion was supported by the presence of a E-myeloma protein. An atypical type L-myeloma protein, which was called myeloma ND, was found by Johansson and Bennich in Sweden.⁴⁹ As the physiochemical properties of this protein were similar to those of γ E in our experiments, antisera specific for the myeloma protein and for γ E were compared. The anti-ND serum used in the experiment was specific for the F_c portion of the myeloma protein. In our laboratory, it was found that the anti-ND and anti- γ E gave a single precipitin band of identical specificity with a γ E fraction from a reaginic serum, and the precipitin band combined radioactive ragweed allergen (Fig. 8, *A* and *B*).⁵⁰ Since the γ E preparation did not contain a detectable amount of other immunoglobulins, it is apparent that both anti-ND and anti- γ E react with the same protein having antibody activity. It was also found that anti-ND precipitated reaginic activity in a ragweed-sensitive serum (Fig. 3, *B*). Johansson and Bennich found

that both anti- γ E and anti-ND gave a precipitin band of identical specificity with the myeloma protein. These results indicate that myeloma ND is a E-myeloma protein and that the γ E-antigenic determinants are probably present in the F_c portion of the γ E-immunoglobulin molecules. An interesting observation on the γ E-antigenic determinants is that the protein loses the activity to give a precipitin reaction with anti- γ E by heating at 56° C. for 4 hours. This loss of precipitating property following heat treatment was not observed for the other immunoglobulins and indicated that the γ E-antigenic determinants are heat labile.⁵¹

Physicochemical properties and structure of γ E-immunoglobulin

The physicochemical properties of γ E antibodies against ragweed allergen have been studied during purification and are summarized in Table II. The properties of γ E are in agreement with those of reaginic antibodies reported by previous investigators and are different from those of the 4 other immunoglobulins. As shown in the table, E-myeloma protein has similar properties to γ E antibodies. In addition, Johansson and Bennich⁴⁹ have measured the molecular weight and carbohydrate content of the myeloma protein. It is clear that the protein has slightly higher molecular weight than those of γ G and monomer form γ A. The carbohydrate content of the myeloma protein is similar to that of γ M.

The chromatographic properties of γ E in Table II represent the molarity of phosphate buffer (pH 8.0) by which the major part of γ E is eluted from a DEAE-cellulose column. However, γ E is chromatographically heterogeneous. When the γ E-rich fraction from a ragweed-sensitive serum was applied to a DEAE-Sephadex column and eluted by increase of molarity of Tris-HCl buffer, γ E antibody was detected in all fractions shown in Fig. 5, *B*. As expected, all fractions had reaginic activity which paralleled the intensity of radioactivity of the γ E-precipitin band. Precipitation of γ E with anti- γ E in fractions A, B, and D was accompanied by loss of reaginic activity. The chromatographic heterogeneity of γ E might explain heterogeneity of reaginic antibodies observed by other investigators.^{52, 53} In the rabbit, Sela and Mozes⁵⁴ have shown that the

Table II. *Physicochemical properties of human immunoglobulins*

	γ G	γ M	γ A	γ D	γ E (myeloma ND)	Reagin
Electrophoretic mobility	γ 1- γ 2	γ 1	γ 1	γ 1	γ 1	γ 1
Sedimentation coefficient S _{20,w}	6.6	18	6.6	7.0	8.0	Ca 8.0
DEAE cellulose chromatography (M)*	0.005	0.15	0.035	0.035	0.02	0.02
Molecular weight	150,000	900,000	180,000	—	(200,000)	—
Carbohydrate per cent	2.9	11.8	7.5	—	(10.7)	—

Parentheses indicate myeloma ND.

*Stepwise elution with phosphate buffers, pH 8.0. The numerals indicate molarity of phosphate buffer for elution of the major component.

chromatographic distribution of γ G antibodies differs depending on the antigen for immunization. One can expect that the chromatographic distribution of γ E antibodies may also be different depending on the allergen.

The sedimentation coefficient of the major component of γ E in atopic patients' sera and an E-myeloma protein is 8S. However, Girard⁵⁵ has reported that gel filtration of serum from a patient with penicillin hypersensitivity gave 2 types of reaginic antibodies. One corresponded to γ M and another was in the ascending portion of γ G peak. Our recent experiments confirmed the findings with a ragweed-sensitive serum. When the serum was applied to a Sephadex G-200 column, the major part of reaginic antibody was eluted with γ A, but a part of the antibody came off the column between γ M and γ A. Both fractions were rechromatographed for further purification. By radioimmunodiffusion γ E antibodies against ragweed antigen were detected in both fractions. The active fractions were analyzed by sucrose-density gradient ultracentrifugation. The sedimentation coefficient of the γ E antibody in the major fraction was 8S, whereas that in the minor component was approximately 11S. The 11S γ E antibody may be a polymer form γ E, although the possibility still exists that the antibody may be a complex of 8S γ E with some other protein. In any event, the heterogeneity of reaginic antibody in molecular size does not neces-

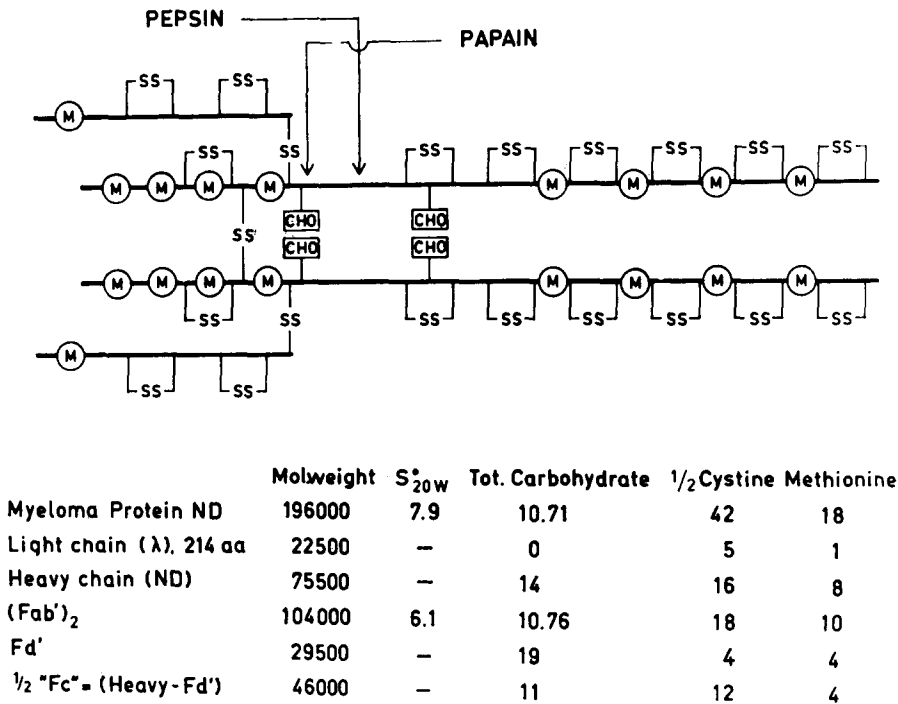


Fig. 9
Scheme of E-myeloma protein. M represents methionine and CHO represents carbohydrate. (Bennich and Johansson, Nobel Symposium, 1967.)

sarily indicate heterogeneity of immunoglobulin class possessing reaginic activity.

Gamma E-immunoglobulin is a very minor component of serum proteins. Johansson, Bennich and Wide,⁵⁶ who measured the concentration of γ E in sera of normal and atopic individuals, reported that the concentration of the protein in normal sera is in the range of 0.1 to 0.7 μ g per milliliter, with the average concentration of 0.33 μ g per milliliter. A significantly raised level of the protein was found in the sera of patients with allergic asthma.⁵⁷ Among the 16 cases studied by them, the highest level was 4 μ g per milliliter, and the average concentration was 1.6 μ g per milliliter. Increase of γ E concentration in atopic individuals is an interesting observation in view of the fact that reaginic antibodies belong to this protein.

In any event, there is too little γ E in serum to be isolated for structural studies. Therefore, all available information on the structure of this protein depends on an E-myeloma protein. Bennich and Johansson⁵⁸ have reduced the E-myeloma protein (myeloma ND) with mercaptoethanol followed by alkylation with iodoacetamide and isolated 2 components which correspond to heavy and light chains. The yield of light chain was 20 per cent of the total protein, and its molecular weight, calculated from the amino acid composition, was 22,500. Since the molecular weight of the original molecule is 200,000, the results indicate that the molecule contained 2 light chains. Assuming 2 heavy chains per molecule, the molecular weight of each heavy chain was calculated to be approximately 75,500, which is significantly higher than those of γ , μ , and α chains. The myeloma protein was subjected to papain or pepsin digestion. The papain digestion produced 2 fragments which correspond to F_c and F_{ab} fragments from γ G. The F_{ab} fragment contained λ -chain determinants, indicating the presence of λ chains, whereas the F_c fragment did not. The γ E-antigenic determinant(s) were present in F_c but not in F_{ab} fragments. Sedimentation coefficient of the F_c fragment was 5S, and its molecular weight was estimated to be 100,000. These data indicate that the F_c fragment of the E-myeloma protein represents a portion of heavy (ϵ) chains. Although the isolated ϵ chain did not have the γ E-antigenic determinant, the presence of the determinant in the F_c fragment indicates that the determinants were originally present in heavy chains. After pepsin digestion of the protein followed by gel filtration, approximately 52 per cent of the material was recovered as a 6S fragment. The fragment contained λ chains and corresponded to $(F_{ab}')_2$. Both F_c and $(F_{ab}')_2$ contained 11 per cent carbohydrate, whereas λ chain did not. Distribution of half cystine and methionine residues in heavy and light chains was estimated from chemical analysis of the isolated heavy and light chains and $(F_{ab}')_2$ fragment. From these findings Bennich and Johansson⁵⁸ proposed a schematic model of the E-myeloma protein as shown in Fig. 9. Apparently the great number of methionine residues per heavy chain and of half cystine residues in the F_c portion of the molecules are characteristics of this immunoglobulin. As will be discussed later, this structure might be related to peculiar biological properties of γ E antibodies.

Immunological properties of γ E antibodies

The most important property of γ E antibodies is the ability to sensitize human skin for erythema-wheel type allergic reactions. The question arose as to how much γ E antibody is required for giving Prausnitz-Küstner reactions. The characteristic antigenic structure of γ E suggested the possibility that quantitation of the antibody may be estimated in vitro by measuring the allergen-binding activity of γ E antibody in serum samples. Thus, a method for measuring antibodies associated with each immunoglobulin class was devised.⁵⁹ The principle of the method is shown in Table III. An excess amount of radioactively labeled purified ragweed allergen (antigen E) was incubated with the patients' sera. Aliquots of the mixture were incubated with the antibody specific for one of the immunoglobulins or anti-light chain to precipitate each immunoglobulin or all immunoglobulins. Since the γ E concentration on patients' sera is very low, the γ E-anti- γ E precipitates were formed in 0.5 per cent agarose, and the agarose containing the immune precipitate was washed to remove free antigen. Radio-

Table III. *Principle of quantitative measurements of antibodies*

Patient's serum + large excess of I^{131} -allergen Formation of allergen*-antibody complexes precipitation with				
Anti- γ G ↓ Ag*-Ab(γ G) + γ G	Anti- γ A ↓ Ag*-Ab(γ A) + γ A	Anti- γ M ↓ Ag*-Ab(γ M) + γ M	Anti- γ E+ ↓ Ag*-Ab(γ E) + γ E	Anti-L ↓ Ag*-Ab(lg) + lg
wash precipitate Radioactivity measurements				

+In agarose.

*Radioactive.

Table IV. *Allergen-binding activity of antibodies in patients' sera**

Serum	Allergen-binding activity					P-K titer
	γ G	γ A	γ E	IG†	IG cal‡	
EK	0.40	0	0.23	0.62	0.63	1600
B	0.26	0	0.38	0.66	0.64	3200
F _c §	0.16	0.082	0.14	0.33	0.38	800
F§	0.14	0.016	0.097	0.25	0.27	600
A	0.23	0	0.10	0.34	0.33	800
W	0.61	0.033	0.099	0.79	0.74	600
P	1.8	0	0.07	1.9	1.9	400
I	1.9	0	0	1.9	1.9	0

*Numerals in the table indicate μ g of antigen E bound by immunoglobulins in 1 ml. of serum.

†Measured by precipitation of all immunoglobulins with antilight-chain antibodies.

‡Allergen binding activities of γ G, γ A, and γ E antibodies were added.

§Untreated patients, all other patients were treated.

(J. Immunol. 98: 497, 1967.)

activity in the precipitates represents the amount of antigen combined to the antibody associated with each immunoglobulin class. The dose of antigen specifically bound to γ G, γ A, and γ E antibodies and to all antibodies is shown in Table IV. It is apparent that the antigen-binding activity of γ E antibody bears no correlation with those of either γ G or γ A antibodies and that the sum of the antigen bound by γ G, γ A, and γ E antibodies in the serum specimens is in agreement with that of total antibody, which was measured by precipitation with anti-light chain antibodies. An important finding in this experiment is that skin-sensitizing activity of the serum samples correlated with γ E-antibody concentration as measured by antigen-binding activity (Table IV). In order to estimate the concentration of γ E antibody in patients' sera, the antigen-binding value of γ E antibody in the sera was compared with that of rabbit antibody against the same antigen. The antigen-binding value of the rabbit antibody was measured by precipitating antigen-rabbit antibody complexes by anti-rabbit γ G goat antibodies, and the relation between the antigen-binding value and the antibody concentration was obtained (Fig. 10). The antigen-binding value of γ E antibody in the patients' sera were then interpolated to the relationship. By this method, one can estimate the concentration of rabbit antibody which has the same antigen-binding value as γ E antibody in the patients' sera (Table V). As the antigen-binding activity of antibodies is dependent on their avidity and valency, the concentration of rabbit antibody in the table does not necessarily represent the actual concentration of γ E antibody but gives a general idea on the

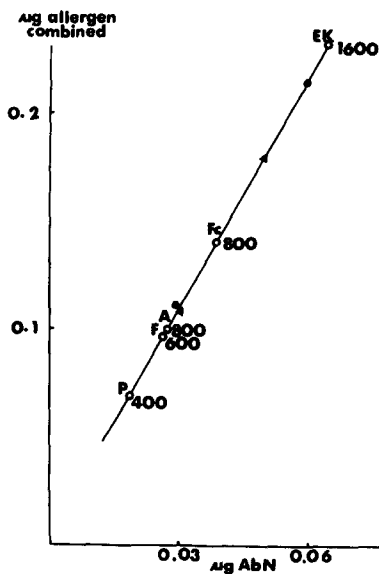


Fig. 10

Relation between antibody concentration and allergen-binding activity of rabbit antibody (●, ▲ represent two different measurements). The allergen-binding values of γ E antibodies in serum EK, Fc, F, and P were interpolated. The numerals in the figure represent the Prausnitz-Küstner titer of the serum samples. (*J. Immunol.* 98: 490, 1967.)

range of γ E-antibody concentration in the patients' sera. The recent studies which will be described later indicated that both human γ E and rabbit γ G antibodies are divalent. As P-K titers of these sera are known, one can calculate the dose of rabbit antibody which corresponds to that of γ E antibody in the minimum sensitizing dose of the sera. The calculated doses in 6 different sera are very close to one another and in the range of 2×10^{-6} μ gN (Table V). If one assumes that the avidity of γ E antibody is comparable to that of the rabbit γ G antibody used in the experiments, the minimum dose of γ E antibody required for giving positive P-K reactions would be on the order of 10^{-6} to 10^{-5} μ gN, which is about 1/1,000 of the minimum dose of guinea pig γ_1 and rabbit γ G antibodies for the induction of PCA reactions in the guinea pig. In an early stage of this investigation we speculated that P-K reactions in human beings by reaginic antibodies are much more sensitive than the PCA reactions in the guinea pig. The findings described above definitely proved this hypothesis. The results also showed a complete correlation between γ E antibody concentration and reaginic activity and supported the concept that at least the major part of the skin-sensitizing antibody in the patients' sera is γ E-immunoglobulin.

The γ E antibodies give PCA reactions in the monkey. It has been reported by Layton and his coworkers⁶⁰ that human reaginic sera induced PCA reactions. Their findings strongly suggested that the reaginic antibodies are responsible for the reactions. However, the problem remained as to whether the reaginic antibodies and/or some other antibodies in the reagin-rich fraction were involved in the reactions. Thus, we studied this problem by fractionating reaginic sera.⁶¹ The antigen-binding activity of the γ G, γ A, and γ E antibodies in the fractions was measured, and the ability of the same fractions to sensitize monkey skin was tested. The results showed that the fractions containing γ E antibody gave PCA reactions, whereas the fractions containing γ G or γ A antibodies but no γ E antibody failed to sensitize monkey skin (Table VI). The γ E antibody, of which the antigen-binding value was comparable to that of 10^{-4} to 10^{-5} μ gN of rabbit γ G antibody, was sufficient to give a positive reaction, but 1,000 times as much γ G antibody or 20 times as much γ A antibody was incapable of giving

Table V. Estimation of the minimum sensitizing dose of γ E antibody in Prausnitz-Küstner reactions

Serum specimen	Allergen-binding value of γ E antibody	Rabbit-antibody concentration*	Rabbit antibody corresponding to minimum P-K dose
	μ g/ml.	μ gN/ml.	$\times 10^{-6}$ μ gN
EK	0.23	0.065	2.0
F _c	0.14	0.038	2.4
F'	0.097	0.026	2.1
A	0.10	0.028	1.8
W	0.099	0.027	2.2
P	0.07	0.019	2.4

*The concentration of rabbit antibody which is comparable to γ E antibody with respect to allergen-binding activity.

(J. Immunol. 98: 500, 1967.)

Table VI. Allergen-binding activity of γG -, γA -, and γE -globulins in serum samples giving PCA reaction

Serum	Fraction	PCA		Allergen-binding activity*			Rabbit antibody† corresponding to minimum PCA dose
		Dilution	Reaction	γG	γA	γE	
				$\times 10^{-3} \mu g$			$\times 10^{-5} \mu gN$
EK	I	Undiluted	-	30	0	0	-
	II ₁₁	1:112	+	0	0	0.15	4
	II ₂₂	Undiluted	-	0.45	0	0	-
	III	1:16	+	0.15	0.12	0.25	7
Tr	γG	Undiluted	-	200	0	0	-
	γA	Undiluted	-	0	4	0	-
EK		1:80	+	0.25	0	0.14	4
F _c		1:40	+	0.2	0.1	0.17	5
W		1:20	+	1.5	0.09	0.22	7
P		1:10	+	9	0	0.35	9

*Allergen-binding activity in the minimum dose of each sample giving positive PCA reactions was calculated. In the samples giving a negative PCA reaction, the allergen-binding activity in the samples injected was listed.

†The dose of rabbit antibody which is comparable to γE antibody in the minimum PCA dose with respect to allergen-binding activity.

a positive reaction. Furthermore, the ability of the reagin-rich fraction and original sera to induce the PCA reaction was absorbed by anti- γE , but the absorption of γG and γA in the active fractions did not reduce the skin-sensitizing activity. It was also found that the presence of excess γG antibody with γE antibody in the sensitizing sample neither enhanced nor inhibited the PCA reaction. These findings show that γE antibodies in atopic patients' sera are responsible for PCA reactions in the monkey.

Arbesman and associates⁶² have reported that isolated monkey ileum, passively sensitized in vitro with human reaginic sera, contracted upon exposure to allergen. In collaboration with Dr. Arbesman, we have studied the possibility that γE antibodies may passively sensitize monkey ileum. The results showed that a γE -rich fraction of atopic patients' sera actually sensitized ileum, and the sensitizing activity of the fraction was removed by anti- γE .⁶³ However, the findings obtained by Wicher and associates⁶⁴ suggest the possibility that the antibodies associated with other immunoglobulins as well may sensitize the ileum. Further studies are necessary to elucidate whether immunoglobulins other than γE may also be involved in the reaction.

The reaginic antibodies were considered to be univalent because the sera from atopic individuals do not give a precipitin reaction with allergen despite high skin-sensitizing activity. However, the problem has to be re-evaluated because the actual concentration of reaginic antibodies in patients' sera is very low on a weight basis. From the measurement of the antigen-binding value of γE antibodies, the concentration of γE antibody seems to be comparable to 0.4 μgN per milliliter of rabbit antibody even when the P-K titer is 1:10,000. It is

Table VII. *Effect of anti-immunoglobulin on hemagglutination*

<i>Anti-ragweed</i>	<i>Anti-Ig</i>	<i>Antibody concentration*</i>						
		<i>0.05</i>	<i>0.025</i>	<i>0.0125</i>	<i>0.006</i>	<i>0.003</i>	<i>0.0015</i>	<i>0</i>
γ G	None	3	3	1	0	0	0	0
	Anti- γ G	3	3	3	3	2	1	0
	Anti- γ A	3	2	1	0	0	0	0
	Anti- γ E	3	3	1	0	0	0	0
γ E	None	3	2	1	0	0	0	0
	Anti- γ G	nd	2	1	0	0	0	0
	Anti- γ A	nd	2	1	0	0	0	0
	Anti- γ E	nd	3	3	3	2	1	0

*Antibody concentration of γ G and γ E antibodies was estimated by antigen-binding activity and represented by the concentration of rabbit antibody having the comparable antigen-binding activity.

possible that the lack of the precipitin reaction may be due to low concentration rather than univalent nature of the antibody. In fact, our recent studies have shown that γ E antibodies are capable of agglutinating red cells coated with antigen. As described previously, the preparations of γ E antibodies were obtained by fractionating reaginic sera followed by absorption of immunoglobulins other than γ E with rabbit antibodies specific for each immunoglobulin class. In radio-immunodiffusion with I^{131} -labeled antigen E, only the γ E antibodies were detected in the preparations. After measuring antigen-binding activity of the γ E antibodies, serial dilutions of the preparations were incubated with rabbit erythrocytes coated with ragweed allergen. The results showed that the γ E-antibody preparation agglutinated the cells (Table VII). On the basis of antigen-binding activity, the γ E and γ G antibodies obtained from the same serum had comparable hemagglutinating activity. The reactions by both γ G and γ E antibodies were completely inhibited if the antibody preparation were incubated with one microgram per milliliter of antigen E before the addition of the antigen-coated cells, indicating that the reactions were specific for the antigen. Furthermore, the hemagglutination by γ E antibody was enhanced if the sensitized cells were incubated with anti- γ E (Table VII). The enhancement was specific because both anti- γ G and anti- γ A failed to enhance the hemagglutination. As expected, the agglutination of the same antigen-coated cells by γ G antibody preparation was enhanced by anti- γ G but not by either anti- γ A or anti- γ E. The results showed that γ E antibodies were actually responsible for the hemagglutination and indicated that the antibodies are multivalent with respect to their antibody-combining sites. As previously described, Bennich and Johansson⁵⁸ indicated that γ E is composed of 2 heavy and 2 light chains. If the antibody-combining sites of γ E antibody are located in the F_{ab} portion of the molecules, one can speculate that γ E antibodies are probably divalent.

The other immunochemical properties of γ E antibodies have not been established. It has been believed that reaginic antibodies lack C'-fixing activities and are unable to sensitize guinea pig skin for PCA reactions. In order to reach a firm conclusion, a γ E preparation containing a high concentration of γ E antibodies but neither γ G nor γ A antibody is necessary. For example, it was found

that a reagin-rich fraction having a P-K titer of 1:3,200 did not give a positive PCA reaction in the guinea pig. The antigen-binding value of the γ E antibody in the fraction was comparable to that of 0.12 μ gN per milliliter of rabbit γ G antibody. The results indicate the sensitizing activity of γ E antibodies is less than that of rabbit γ G antibody, if active at all, and suggest the unlikelihood of the involvement of γ E antibody in the guinea pig PCA reactions induced by reaginic sera. However, one cannot exclude the possibility that the sensitizing activity of γ E antibody might be comparable to that of human γ G antibody because 1 μ gN per milliliter of human γ G antibody is necessary for PCA reactions in the guinea pig.

MOLECULAR BASIC OF REAGINIC HYPERSENSITIVITY REACTIONS BY γ E ANTIBODIES

Structural basis of sensitizing activity

In anaphylactic reactions in the guinea pig, it has been shown that guinea pig γ I- and γ G-globulins from certain animal species nonspecifically block passive sensitization. The mechanism of the blocking is considered to be due to competition between antibody and nonantibody immunoglobulins for combination with certain guinea pig tissues with which sensitizing antibodies combine upon sensitization.²⁶ Similar findings were obtained in P-K reactions in human beings. Nonantibody γ E blocked passive sensitization of human skin when the protein was mixed with reaginic serum and the mixtures were injected intracutaneously into normal individuals for passive sensitization.²⁹ The blocking of passive sensitization is one of the characteristic properties of γ E-immunoglobulin. As shown

Table VIII. *Blocking effect of immunoglobulins on passive sensitization with reaginic antibody*

<i>Proteins injected with reaginic antibody (μgN)</i>			<i>Wheal (mm.)</i>	<i>Erythema (mm.)</i>
γ G	γ G ₁	2.0	8.5	30×32
	γ G ₂		9.5	35×42
	γ G ₃		10.5	30×43
	γ G ₄		10.0	30×49
γ A	Fu	2.0	9.5	30×41
	Ma		9.5	34×37
	He		10.0	31×45
γ D		1.6	9.5	31×34
γ M		2.0	9.5	34×36
γ E + γ G*		0.06	0	0
		0.03	5.0	20×22
		0.015	6.0	21×25
γ E + γ G (heated)		2.8	9.0	30×32
Saline		0	9.5	36×42

* γ E-globulin comprises 25 per cent or less of total protein.
(J. Immunol. 99: 855, 1967.)

in Table VIII, myeloma proteins of the other 4 classes and subclasses failed to block the passive sensitization. Blocking of passive sensitization by E-myeloma protein was also reported.⁶⁵ The fact that only γ E blocked passive sensitization indicates that the protein has affinity for human skin tissues to which reaginic antibodies combine upon sensitization. As light chains of immunoglobulin are common for all immunoglobulins, one can expect that the structures responsible for the passive sensitization probably exist in the heavy chains of the molecules.

One of the characteristic properties of human reaginic antibodies is that the sensitizing activity of antibodies is lost by heating at 56° C. for 2 to 4 hours. In fact, skin-sensitizing activity of γ E antibody was lost by heating. The heat inactivation of the γ E antibodies may be explained by the loss of affinity of γ E molecules for tissue sites, because the blocking activity of nonantibody γ E was lost by heating at 56° C. for 4 hours (Table VIII).⁵¹ On the other hand, evidence was obtained that antibody-combining sites in γ E antibodies remained after heating. When the antigen-binding activity of γ G, γ A, and γ E antibodies was measured before and after heating at 56° C. for 4 hours, more than 50 per cent of the allergen-binding activity of γ G and γ A antibodies remained after heating, whereas γ E antibody was not detected after heat treatment (Table IX). However, the apparent loss of antigen-binding activity of γ E antibodies is due to the nonprecipitability of γ E. As described before, the γ E-antigenic determinants are heat labile, and heated γ E is not precipitated by anti- γ E. When antigen-antibody complexes in the same mixture were precipitated by antilight chain antibody, the antigen-binding value of the heated γ E-antibody preparation was more than 50 per cent of the value of the native preparation. As the original preparation did not contain a significant amount of either γ G or γ A antibody and the antigen-binding value of total antibody, determined by precipitation with anti-light chain, was comparable to that of γ E antibody in the preparation, almost all antibody in the preparation should be γ E-immunoglobulin. Thus, it seems that γ E antibody was responsible for the antigen binding of the heated preparation.

Table IX. *Effect of heat on antigen-binding activity of γ G, γ A, and γ E antibodies*

<i>Fraction</i>	<i>Heat</i>	<i>Antibody for precipitation</i>	<i>AgE combined</i>	<i>Activity remaining (per cent)</i>
γ G	-	anti- γ G	$\mu\text{g} \times 10^{-2}$ 2.0	75
	+		1.5	
γ A	-	anti- γ A	1.5	78
	+		1.2	
γ E	-	anti- γ E	6.0	8
	+		0.5	
	-	anti-F _{ab}	6.7	66
	+		4.4	
	-	anti- γ G	0	
	-	anti- γ A	0	

(*J. Immunol.* 99: 610, 1967.)

In fact, the heated γ E preparation showed blocking antibody activity. It is apparent that heat inactivation of reaginic antibody is not due to the loss of antibody-combining sites but to degradation of certain structures in the γ E molecules involved in the fixation to skin tissues.⁵¹

Another characteristic of reaginic antibodies is inactivation by reduction and alkylation.^{66, 67} In agreement with the previous observations, skin-sensitizing activity of γ E antibodies greatly diminished by reduction in 0.1M mercaptoethanol for one hour followed by alkylation with iodoacetamide, although 0.01M mercaptoethanol did not change the skin-sensitizing activity. Further studies on the effect of reduction-alkylation on antiragweed γ E antibodies indicated that antigen-binding activity of the antibodies diminished to about one third by the reduction in 0.1M mercaptoethanol followed by alkylation. No effect was detected on the γ E-antigenic determinant. The reduced-alkylated γ E maintained affinity for skin tissues to which reaginic antibody combines. However, the affinity of the treated molecules was less than that of native γ E. Approximately 5 times more reduced-alkylated material than untreated sample was required for blocking passive sensitization with reaginic antibodies against heterologous allergen. It was also found that sensitization with the reduced-alkylated γ E antibody did not persist for a long time. The minimum dose of the treated antibody required for giving a positive P-K reaction increases when the sensitization period was changed from 2 hours to 48 hours. It is apparent that reduction and alkylation affects 2 different portions of γ E antibody molecule, i.e., antibody-combining sites and tissue fixation site.

As already discussed, the affinity of γ E-molecules for target cells is ascribed to certain structures in ϵ chains. In PCA reactions in guinea pigs, evidence has been presented that structures in rabbit γ G molecules essential for the fixation to the tissues are present in F_c portion of the molecules.^{8, 9} If F_c portion of γ E molecules is responsible for the affinity to human tissues, one might speculate that disulfide linkages in the F_c portion of the molecule may be essential for maintaining the structure, because reduction and alkylation of γ E diminished the affinity. It is interesting that the F_c portion of the ϵ chain contains 5 to 6 intraheavy-chain disulfide bonds, and a single inter- ϵ -chain bond is present in F_d (Fig. 9). In rabbit γ G, Hong and Nisonoff⁶⁸ have shown that reduction in 0.01M mercaptoethanol was sufficient to reduce an inter- γ -chain disulfide bond in almost all molecules. If susceptibility of the inter- ϵ -chain bond to the reducing reagent is comparable to that of the inter- γ -chain bond, reduction in 0.01M mercaptoethanol, which does not affect the sensitizing activity of γ E antibody, may be sufficient for the reduction of inter- ϵ -chain bond. Although the importance of the inter- ϵ -chain bond cannot be excluded, it is possible that some of the intrachain disulfide bonds are susceptible to reduction in 0.1M mercaptoethanol, and cleavage of these bonds may be responsible for decrease in tissue affinity of γ E molecules.*

*After submitting this review, Stanworth and associates reported that F_c fragment of an E-myeloma protein blocked passive sensitization. Their findings support the idea that cleavage of intra- ϵ -chain disulfide bond(s) rather than interchain bond is responsible for the decrease of tissue affinity.

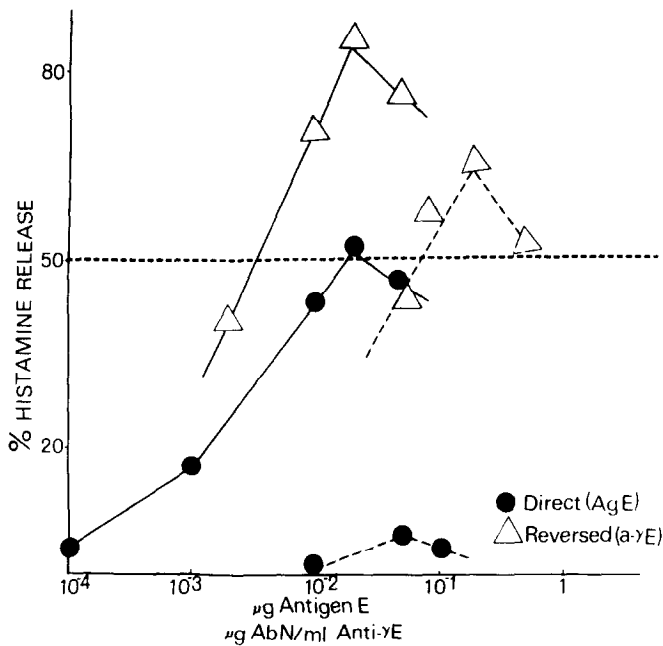
Mechanisms of reaginic hypersensitivity reactions

Although reaginic hypersensitivity reactions in human beings are different from anaphylactic reactions in the guinea pig with respect to the quantity of antigen and antibody involved, both reactions are induced by the antibodies of a certain immunoglobulin class, and passive sensitization of the skin by the antibodies is blocked by nonantibody immunoglobulin of the same class. In anaphylactic reactions in the guinea pig, it is well known that injections of non-antibody rabbit γ G into guinea pig skin followed by anti-rabbit γ G gives reversed PCA reactions. As normal individuals have γ E, one can expect that the protein is present on target cells to which reagin combines upon sensitization and therefore that an intracutaneous injection of the antibody specific for γ E into normal individuals would give a reversed type allergic reaction. In fact, normal individuals gave erythema-wheal reactions upon injection of anti- γ E. The minimum dose of the antibody for giving the reaction was on the order of 10^{-5} μ gN°. None of the antibodies specific for other immunoglobulins, i.e., anti- γ G, anti- γ A, anti- γ M, and anti- γ D, gave reactions even with 10^{-2} μ gN. Anti-light-chain antibodies prepared from anti- γ G antisera gave a positive reaction with 0.01 μ gN. Since anti- γ chains failed to give the reaction, it seems that anti-light chain reacts with γ E on the target cells and induces the reaction. The requirement of 1,000-fold more of anti-light chain than anti- γ E is probably due to the combination of anti-light chain with immunoglobulins of other classes present in the skin tissues. An interesting fact is that the dose of anti- γ E for the reversed reaction is comparable to that of γ E antibodies required for a positive P-K (direct) reaction.⁵⁰

The sensitivity of human skin to anti- γ E differs depending upon individuals. An atopic patient who has an extremely high γ E concentration in his serum gave a positive reaction with 10^{-8} μ gN of anti- γ E, whereas 2 agammaglobulinemic patients did not react to 10^{-3} μ gN of anti- γ E. In all of these cases, the sensitivity to histamine was at a normal level. It was found that the activity of anti- γ E serum was removed by precipitation of anti- γ E antibody with γ E. The induction of reversed type allergic reactions by anti- γ E antibody is a direct proof that normal individuals possess γ E on target cells.

It was also found that intracutaneous injection of anti- γ E to normal monkeys increased permeability of their skin capillaries. With Evans blue injected intravenously, skin sites receiving anti- γ E showed a bluing reaction. The amount of rabbit anti- γ E required for the positive reaction was 10^{-5} μ gN. Again, none of the antibodies specific for other immunoglobulin gave the reaction with 0.04 μ gN. These findings indicate that the monkey has a protein which cross-reacts with human γ E and that the protein is present on the cells to which human reaginic antibodies are fixed upon passive sensitization. The monkey protein was detected in normal monkey serum. Although the protein has not been isolated, distribution of the protein in chromatographic fractions in normal monkey serum as well as its size was similar to human γ E and suggested that the protein is " γ E"-immunoglobulin of the monkey.⁵⁰

The reversed type allergic reactions by anti- γ E enabled us to study target cells. Pretreatment of monkey skin sites with compound 48/80 prevented the skin reaction by anti- γ E without changing the sensitivity to histamine. On the other

**Fig. 11**

Histamine release by ragweed allergen (AgE) and by anti- γ E from leukocytes passively sensitized with a reaginic serum. Normal leukocytes from the donor released 50 per cent of total histamine by 0.08 μ gN per milliliter of anti- γ E (Δ - - Δ) but not by ragweed allergen ($\bullet$ - - $\bullet$). After sensitization, 0.004 μ gN per milliliter of anti- γ E (Δ — Δ) or 0.018 μ g per milliliter of the allergen ($\bullet$ — $\bullet$) released 50 per cent histamine.

hand, pretreatment with anti- γ E prevented the reaction to 48/80. If one accepts that the compound specifically attacks mast cells,⁶⁹ the results indicate that mast cells are involved in the reversed type allergic reactions.

Lichtenstein and Osler⁷⁰ have observed histamine release from isolated leukocytes of atopic patients upon exposure to allergen to which the donors are sensitive. Their results suggest that reaginic antibodies are involved in the reaction. Levy and Osler⁷¹ succeeded in passively sensitizing leukocytes with the patients' sera for histamine release. If reaginic antibodies are actually involved in the histamine release, γ E should be present on leukocytes, and therefore one can expect reversed type histamine release from leukocytes by the exposure to anti- γ E. In fact, anti- γ E released histamine from leukocytes of atopic patients. Similar to the skin reaction, neither anti- γ G, anti- γ A, anti- γ M, nor anti- γ D released histamine from the cells, whereas antilight-chain antibodies did. It was also found that the sensitivity of normal leukocytes to anti- γ E for histamine release increased by incubation of leukocytes with a γ E-rich serum containing antiragweed reaginic antibody. As demonstrated by Levy and Osler,⁷¹ the same cells released histamine by exposure to ragweed antigen (Fig. 11). It is evident that γ E-immunoglobulin is fixed to leukocytes, and the reaction of the cell-fixed γ E with anti- γ E is responsible for histamine release. These findings

Table X. *Skin reactions by γ E antibody-antigen complex**

Dilution of complex	Antigen (μ g per milliliter)						
	32	10	2	0.5	0.1	0.025	0
Undiluted†	5.5 (22)	7.0 (24)	8.0 (27)	9.0 (29)	11.0 (35)	8.0 (30)	0
2×	0	5.5 (20)	7.0 (22)	8.0 (28)	10.5 (33)	7.0 (27)	0
8×	0	0	0	6.5 (22)	9.0 (30)	5.0 (20)	0

*Numerals in this table represent average diameters of wheal in millimeters, and those in parentheses represent average diameters of erythema in millimeters.

†The antigen-binding value of the antibody preparation was 0.09 μ g per milliliter.

strongly support the hypothesis that γ E antibodies are responsible for histamine release from leukocytes by allergen.

In our previous studies on soluble antigen-antibody complexes, it has been shown that preformed antigen-antibody complexes induce an increased permeability of guinea pig skin capillaries. The skin reactivity of the complexes depends on the nature of antibody involved. Only the antibodies having sensitizing activity for guinea pigs formed skin-reactive complexes, indicating that the formation of skin-reactive complexes in vivo is responsible for PCA reactions in guinea pigs.⁷² The results also showed that the skin reactivity of the complexes differs depending on the antigen-antibody ratio in the complexes and indicated a requirement of at least 2 antibody molecules for the formation of a skin-reactive complex. The similarity between PCA reactions in the guinea pig and P-K reactions in human beings suggested the possibility that soluble complexes composed of γ E antibody may give erythema-wheal reactions in normal human skin. This possibility was therefore studied in the following experiments.⁷³ The γ E-rich fraction containing γ E antibody but no γ G, γ A, or γ M antibody against ragweed allergen was obtained from atopic patients' sera, and the antigen-binding activity of the γ E antibody in the preparation was measured. The preparation was incubated with varying concentrations of purified ragweed allergen for 15 minutes at 37° C. and the mixtures were injected into normal human skin. The mixture of γ E antibody with the ragweed allergen provoked erythema-wheal reactions, whereas those of either γ G or γ A antibody with the same antigen failed to do so. The intensity of the skin reaction was different depending on the antigen-antibody ratio in the mixture. The maximal reaction was obtained when a slight excess of antigen was added, and the activity of the mixture became lower by the addition of a greater excess of antigen (Table X). At the optimal antigen-antibody ratio, the amount of antibody in the minimal skin-reactive dose of the complex was approximately 30 to 50 times more than the minimal dose of the same preparation required for a positive P-K reaction at the sensitization period of 24 hours. However, the dose of the γ E antibody in the mixture was on the same order of magnitude as that required for giving a positive reaction when the skin sites were challenged within 5 minutes. When the antibody and antigen were injected separately, however, a much higher con-

centration of antigen than that present in the mixture was required for a positive reaction, and inhibition of the skin reaction by excess antigen was not observed. Since the above findings strongly suggested that the skin reactions by antigen- γ E antibody mixtures are due to preformed complexes, experiments were undertaken to prove that γ E antibodies actually formed complexes with antigen in vitro. Purified ragweed antigen (antigen E) was labeled with I^{131} and added to a γ E-antibody preparation. The antigen-antibody ratio in the mixtures was in extreme antigen excess (800 times excess as compared with the antigen-binding value of γ E antibody) in one mixture and in moderate antigen excess ($5\times$ excess) in the other. The latter mixture gave a positive erythema-wheal reaction at a dilution of 1 : 128, whereas the mixture containing a great excess antigen did not show the reaction even at a dilution of 1 : 8. Both of the mixtures were then analyzed by sucrose-density gradient ultracentrifugation. As controls, the γ E antibody preparation or radioactive antigen E was applied to separate tubes and analyzed. After centrifugation, distribution of radioactivity in the gradient fractions was studied. Free antigen gave a single peak of 3.3S, and the sedimentation coefficient of γ E antibody was 8S. The mixture of γ E antibody with a great excess of antigen gave 2 radioactive peaks. A slower sedimenting peak corresponded to free antigen (3.3S), and the sedimentation coefficient of the faster component was 9.7S, which was higher than that of γ E antibody alone. The mixture with a moderate excess antigen showed 3 peaks, i.e., 3.3S, 9.7S, and 13.1S. In order to confirm that the 9.7S and 13.1S components represent antigen-antibody complexes, the gradient fractions were analyzed by radioimmuno-diffusion. The fractions were mixed with nonantibody γ E and placed in peripheral wells of Ouchterlony plates. The center well was filled with anti- γ E. The γ E-precipitin band was formed by the nonantibody γ E. Radioautographs of the plates showed that the γ E-precipitin band formed with the 9.7S and 13.1S fractions had radioactivity, whereas the band formed with the 3.3S fraction plus carrier protein did not. Fixation of the radioactive antigen by anti- γ E indicates that the antigen in these peaks was in the form of complex with γ E antibody and therefore that both 9.7S and 13.1S fractions represent antigen- γ E antibody complexes. As the antigen-antibody mixture containing 13.1S complex had high skin reactivity while the activity of the antigen excess mixture containing only 9.7S complex was very low, it seems that 13.1S complex has the activity whereas 9.7S complex does not.

Since γ E antibody is probably divalent, one can expect that the major part of complexes formed in great antigen excess are probably composed of 2 antigen and one antibody molecules (Ag_2Ab complex), the molecular weight of which is 270,000. It appears likely that the 9.7S complex represents an Ag_2Ab complex. As γ E antibody is 8S, the complex should not contain more than one γ E-antibody molecule, whereas the 13.1S complex is probably composed of two antibody molecules. It seems that two or more γ E-antibody molecules are required for the formation of a skin reactive complex.

The requirement of two or more γ E-antibody molecules for the formation of skin-reactive complex is comparable to the previous observations on rabbit γ G antibody complexes having skin reactivity in the guinea pig. In the case of the

γ G-antibody complexes, it was suggested that an interaction between antibody molecules brought into apposition by antigen and possible consequent structural changes in the F_c portion of the antibody molecules might be involved in the induction of the biological activity.⁷² This hypothesis was supported by the fact that nonspecifically aggregated γ G and aggregated F_c fragments have the ability to induce increased permeability of skin capillaries. Recent studies by Henney and Ishizaka⁷⁴ showed that a new antigenic determinant is revealed in the F_c portion of the γ G molecule upon formation of antigen-antibody complexes and by nonspecific aggregation, indicating that structures in F_c portion of the molecules are actually changed by the formation of antigen-antibody complexes. The requirement of 2 γ E-antibody molecules for the formation of skin-reactive complexes suggests that induction of skin-reactive properties by the formation of these complexes may also involve interaction between the γ E-antibody molecules and/or structural change in the molecules.

If reaginic hypersensitivity reactions are actually due to the formation of skin-reactive complexes in vivo, one may speculate that at least 2 γ E-antibody molecules in the form of doublet on a cell surface is necessary to sensitize target cells, and the reaction is induced by bridging the γ E-antibody molecules by antigen. With benzylpenicilloyl (BPO) haptens in penicillin hypersensitivity, Levine and Fellner⁷⁵ have found that BPO-polymer conjugates of widely different molecular sizes are equally effective when compared at equimolar concentrations. From the data they suggested that wheal and erythema reactions were initiated by simple bridging of 2 or small numbers of tissue-fixed skin-sensitizing antibody molecules by multivalent haptens.

Investigations on the mechanisms of reaginic hypersensitivity reactions suggest 2 possibilities for inducing the reactions without participation of allergen. If structural changes in γ E molecules which may be induced by interaction of the molecules are actually responsible for the hypersensitivity, one might speculate about the possibility that nonspecific aggregation or slight denaturation of γ E may induce the same symptoms without allergen. In connection with this view, biologic activities of nonspecifically aggregated γ E have to be studied. Another possibility is the formation of autoantibody against γ E. It has been shown that aggregated human γ G is immunogenic in human beings, and antibodies specific for aggregated γ G react with antigen- γ G antibody complexes. Repeated exposure of atopic patients having γ E antibodies to specific allergen may result in the formation of autoantibody against denatured γ E. Once such autoantibodies are formed, the antibody will induce or enhance allergic reactions. It should be noticed that only 10^{-5} μ gN of heterologous anti- γ E was sufficient to induce erythema-wheal reactions in normal individuals. Although direct evidence is lacking, it may be worthwhile to consider these possibilities for future studies on so-called intrinsic type of allergic disorders.

SUMMARY

Reaginic activity in sera of atopic patients is associated with γ E-immunoglobulin rather than the other 4 immunoglobulins, i.e., γ G, γ A, γ M, and γ D. This conclusion is based on the facts that (1) reaginic activity in the patients' sera

was precipitated by anti- γ E but not by the antibodies specific for heavy chains of the other 4 immunoglobulins; (2) the distributions of reaginic activity and of γ E antibody paralleled each other when the patients' sera were fractionated by anion-exchange column chromatography, gel filtration, density gradient ultracentrifugation, and zone electrophoresis; (3) the γ E fraction of the patients' sera, in which only γ E antibody but neither the γ G, γ A, γ M, nor γ D antibodies was detected, possessed high reaginic activity, and the activity in the preparation was removed by anti- γ E.

The protein has κ and λ light chains of immunoglobulins, possesses characteristic antigenic determinants which are not shared by the other immunoglobulins of known classes and subclasses, and lacks the major antigenic determinants in the other classes. The antigenic structure of γ E as well as association of antibody activity with the protein indicate that γ E represents a distinct immunoglobulin class. Physicochemically, γ E-immunoglobulin is a γ_1 glycoprotein with a sedimentation coefficient of 8S and a molecular weight of 200,000. Structural studies of an E-myeloma protein showed that the protein is composed of 2 heavy (ϵ) and 2 light polypeptide chains. The heavy (ϵ) chains of the protein have a peculiar structure, and γ E-antigenic determinants are present in F_c portion of the heavy chains. The concentration of the protein in normal serum is 0.1 to 0.7 μ g per milliliter, and the level is elevated in atopic patients.

The γ E antibodies are responsible for P-K reactions in human beings, PCA reactions in the monkey by reaginic sera, and probably for antigen-induced histamine release from leukocytes of atopic individuals. The minimum dose of γ E antibody required for giving positive P-K reactions in human beings was estimated to be 10^{-6} to 10^{-5} μ gN. The γ E-antibody agglutinated red cells coated with antigen, indicating that the antibody is multivalent (probably divalent). Nonantibody γ E and an E-myeloma protein blocked passive sensitization of human skin with reagin, indicating that the protein has affinity for the tissue constituents to which reagin combines upon sensitization. Structures of the protein responsible for the affinity were heat labile, whereas antibody-combining sites in the γ E antibodies remained after heating at 56° C. Reduction of the antibody in 0.1M mercaptoethanol followed by alkylation partially degraded both antibody-combining sites and the tissue fixation site in the molecules. It was also found that antibodies specific for γ E induced erythema-wheal reactions in normal human beings and monkeys and released histamine from leukocytes of atopic individuals. Induction of the reversed type reactions by the anti- γ E antibody indicated that γ E-immunoglobulin is present on target cells to which reaginic antibodies combine upon sensitization.

With respect to the immune mechanisms of reaginic hypersensitivity reactions by γ E antibodies, it was found that soluble antigen- γ E antibody complexes preformed in vitro gave erythema-wheal reactions in normal individuals. Analysis of the antigen-antibody complexes strongly suggested the requirement of 2 or more γ E-antibody molecules for the formation of a skin-reactive complex. This finding indicated that bridging of 2 cell-fixed γ E molecules initiates the hypersensitivity reactions.

The authors wish to express their gratitude to Drs. M. W. Chase, H. Fudenberg, J. L. Fahey, W. D. Terry, C. E. Arbesman, and L. M. Lichtenstein for antigen E, myeloma proteins, and reaginic sera to accomplish this work. We also thank Dr. D. S. Rowe for his work and arrangement to decide on the nomenclature of IgE.

REFERENCES

1. Prausnitz, C., and Küstner, H.: Studien über die Überempfindlichkeit, *Zentralbl. Bakt.* **86**: 160, 1921.
2. Cohen, S., and Porter, R. R.: Structure and Biological Activity of Immunoglobulins, *Advances Immunol.* **4**: 287, 1964.
3. Cohen, S., and Milstein, C.: Structure of Antibody Molecules, *Nature* **214**: 449, 1967.
4. Franklin, E. C.: The Immunoglobulins—Their Structure and Function and Some Techniques for Their Isolation, *Progr. Allergy* **8**: 58, 1964.
5. Ishizaka, K., Ishizaka, T., Lee, E. M., and Fudenberg, H.: Immunochemical Properties of Human γ A Isohemagglutinin. I. Comparisons with γ G and γ M Antibodies, *J. Immunol.* **95**: 197, 1965.
6. Ovary, Z., and Karush, F.: Studies on the Immunologic Mechanism of Anaphylaxis. II. Sensitizing and Combining Capacity In Vivo of Fractions, Separated From Papain Digests of Antihapten Antibody, *J. Immunol.* **86**: 146, 1961.
7. Ishizaka, K., Ishizaka, T., and Sugahara, T.: Biological Activity of Soluble Antigen-Antibody Complexes. VII. Role of an Antibody Fragment in the Induction of Biological Activities, *J. Immunol.* **88**: 690, 1962.
8. Immunoglobulin E. A New Class of Human Immunoglobulin, *Bull. World Health Organization* **38**: 151, 1968. In press.
9. Scherrer, E. L.: The Distribution of Reagins in the Blood Plasma, *J. ALLERGY* **2**: 467, 1930.
10. Stull, A., Sherman, W. B., and Cooke, R. A.: The Association With Pseudoglobulin of the Skin-sensitizing Substance of Allergic (Hay Fever) Serum, *J. Allergy* **9**: 7, 1937.
11. Vaughan, J. H., Favour, C. B., and Jaffee, I. H.: Partition of the Skin-Sensitizing Antibody Among Alcohol-Fractionated Plasma Proteins, *J. ALLERGY* **23**: 489, 1952.
12. Cooke, R. A., Sherman, W. B., Menzel, A. E. O., Chaplin, H. B., Howell, C. M., Scott, R. B., Myers, P. A., and Downing, L. M.: ACTH and Cortisone in Allergic Diseases, *J. ALLERGY* **22**: 211, 1951.
13. Campbell, D. H., Cann, J. R., Friedman, T. B., and Brown, R.: The Relation of Allergy Reagins to Electrophoretic Components of Serum, *Science*, **119**: 289, 1954.
14. Schon, A. H., Fyles, T. W., and Rose, B.: Electrophoretic Separation of Skin-Sensitizing Antibody From the Sera of Ragweed Sensitive Patients, *J. ALLERGY* **26**: 329, 1955.
15. Cann, J. R., and Loveless, M. H.: Biophysical Characterization of Reaginic and Blocking Sera, *J. ALLERGY* **28**: 379, 1957.
16. Humphrey, J. H., and Porter, R. R.: Reagin Content of Chromatographic Fractions of Human γ Globulin, *Lancet* **1**: 196, 1957.
17. Stanworth, D. R.: Studies on the Physicochemical Properties of Reagin to Horse Dandruff, *Immunology* **2**: 384, 1959.
18. Gynes, L., Gordon, J., and Schon, A. H.: Ultracentrifugal Characterization of Antibodies in Sera of Ragweed-Sensitive Individuals, *Immunology* **4**: 177, 1961.
19. Anderson, B. R., and Vannier, W. E.: The Sedimentation Properties of the Skin-Sensitizing Antibodies of Ragweed Sensitive Patients, *J. Exper. Med.* **120**: 31, 1964.
20. Heremans, J. F., Heremans, M. T., and Schultze, H. E.: Isolation and Description of a Few Properties of the β_{2A} Globulin of Human Serum, *Clin. chim. acta* **4**: 96, 1959.
21. Heremans, J. F., and Vaerman, J. P.: β_{2A} Globulin as a Possible Carrier of Allergic Reaginic Activity, *Nature* **193**: 1091, 1962.
22. Augustin, R., Connolly, R. C., and Lloyd, G. M.: Atopic Reagin as a Prototype of Cytophilic Antibodies, *Protides Biol. Fluids* **11**: 56, 1963.
23. Vaerman, J. P., Epstein, W., Fudenberg, H., and Ishizaka, K.: Direct Demonstration of Reagin Activity in Purified γ_{1A} Globulin, *Nature* **203**: 1046, 1964.
24. Fireman, P., Vannier, W. E., and Goodman, H. C.: The Association of Skin-Sensitizing Antibody With the β_{2A} Globulins in Sera From Ragweed Sensitive Patients, *J. Exper. Med.* **117**: 603, 1963.
25. Ishizaka, K., Ishizaka, T., and Hornbrook, M. M.: Blocking of Prausnitz-Küstner Sensitization With Reagin by Normal Human β_{2A} Globulin, *J. ALLERGY* **34**: 395, 1963.
26. Biozzi, G., Halpern, B. N., and Binaghi, R.: The Competitive Effect of Normal Serum Proteins From Various Animal Species on Antibody Fixation in Passive Cutaneous Anaphylaxis in the Guinea Pig, *J. Immunol.* **82**: 215, 1959.
27. Yagi, Y., Maier, P., Pressman, D., Arbesman, C. E., and Reisman, R. E.: The Presence of the Ragweed-binding Antibodies in the β_{2A} , β_{2M} and γ Globulins of the Sensitive Individuals, *J. Immunol.* **91**: 83, 1963.

28. Loveless, M. H.: Reagin Production in a Healthy Male Who Forms No Detectable β_{2A} Immunoglobulins, *Fed. Proc.* **23**: 403, 1964.
29. Ovary, Z.: Immediate Reactions in the Skin of Experimental Animal Provoked by Antigen-Antibody Interaction, *Progr. Allergy* **5**: 640, 1958.
30. Ishizaka, K., and Ishizaka, T.: Physicochemical Properties of Reaginic Antibody. I. Association of Reaginic Activity With an Immunoglobulin Other Than γA - or γG -Globulin, *J. ALLERGY* **37**: 169, 1966.
31. Ishizaka, K., Ishizaka, T., and Lee, E. H.: Physicochemical Properties of Reaginic Antibody. II. Characteristic Properties of Reaginic Antibody Different From γA Isohemagglutinin and γD Globulin, *J. ALLERGY* **37**: 336, 1966.
32. Vaerman, J. P., Heremans, J. F., and Vaerman, C.: Studies of the Immunoglobulins of Human Serum. I. A Method for the Simultaneous Isolation of the Three Immune Globulins (γ_{ss} , γ_{1M} and γ_{1A}) From Individual Small Serum Samples, *J. Immunol.* **91**: 7, 1963.
33. Ishizaka, K., and Ishizaka, T.: Physicochemical Properties of Reaginic Antibody. III. Further Studies on the Reaginic Antibody in γA -Globulin Preparation, *J. ALLERGY* **38**: 108, 1966.
34. Ishizaka, K., Ishizaka, T., and Hornbrook, M. M.: Physicochemical Properties of Reaginic Antibody. IV. Presence of a Unique Immunoglobulin as a Carrier of Reaginic Activity, *J. Immunol.* **97**: 75, 1966.
35. Reisman, R. E., Arbesman, C. E., and Yagi, Y.: Radioimmuno-electrophoresis of Ragweed-Binding Antibodies in Allergic Sera, *J. ALLERGY* **36**: 362, 1965.
36. Ishizaka, K., Ishizaka, T., and Hornbrook, M. M.: Physicochemical Properties of Reaginic Antibody. V. Correlation of Reaginic Activity With γE Globulin Antibody, *J. Immunol.* **97**: 840, 1966.
37. Perelmutter, L., Rose, B., and Goodfriend, L.: The Relationship Between the Skin-Sensitizing Antibody and γA Protein in the Sera of Ragweed-Allergic Individuals, *J. ALLERGY* **37**: 236, 1966.
38. Perelmutter, L., Rose, B., and Goodfriend, L.: The Relationship Between Skin-sensitizing Antibodies and γD Globulin in the Sera of Ragweed Sensitive Individual, *Internat. Arch. Allergy* **29**: 364, 1966.
39. Ishizaka, K., Ishizaka, T., and Terry, W. D.: Antigenic Structure of γE Globulin and Reaginic Antibody, *J. Immunol.* **99**: 849, 1967.
40. Rothfeld, N. F., Frangione, B., and Franklin, E. C.: Slowly Sedimenting Mercaptoethanol Resistant Anti-Nuclear Factors Related Antigenically to Macro-Immunoglobulins (γM -globulin) in Patients with Systemic Erythematodes, *J. Clin. Invest.* **44**: 62, 1965.
41. King, T. P., Norman, P. S., and Connell, J. T.: Isolation and Characterization of Allergen From Ragweed Pollen. II, *Biochemistry* **3**: 458, 1964.
42. Ishizaka, K., and Ishizaka, T.: Identification of γE Antibodies as a Carrier of Reaginic Activity *J. Immunol.* **99**: 1187, 1967.
43. Grey, H. M., and Kunkel, H. G.: H. Chain Subgroups of Myeloma Proteins and Normal 7S γ Globulin, *J. Exper. Med.* **120**: 253, 1964.
44. Terry, W. D., and Fahey, J. L.: Subclasses of Human $\gamma 2$ Globulin Based on Differences in the Heavy Polypeptide Chains, *Science* **146**: 400, 1964.
45. Kunkel, H. G., and Prendergast, R. A.: Subgroups of γA Immune Globulins, *Proc. Soc. Exper. Biol. & Med.* **122**: 910, 1966.
46. Vaerman, J. P., and Heremans, J. F.: Subclasses of Human Immunoglobulin A Based on Differences in the Alpha Polypeptide Chains, *Science* **153**: 647, 1966.
47. Feinstein, D., and Franklin, E. C.: Two Antigenically Distinguishable Subclasses of Human A Myeloma Proteins Differing in Their Heavy Chains, *Nature* **212**: 1496, 1966.
48. Terry, W. D., and Robert, M. S.: Antigenic Heterogeneity of Human Immunoglobulin A Protein, *Science* **153**: 1007, 1966.
49. Johansson, S. G. O., and Bennich, H.: Immunological Studies of an Atypical (Myeloma) Immunoglobulin, *Immunology* **13**: 381, 1967.
50. Ishizaka, K., and Ishizaka, T.: Reversed Type Allergic Skin Reactions by Anti- γE Globulin Antibodies in Humans and Monkey, *J. Immunol.* **100**: 554, 1968.
51. Ishizaka, K., Ishizaka, T., and Menzel, A. E. O.: Physicochemical Properties of Reaginic Antibody. VI. Effect of Heat on γE , γG , and γA Antibodies in the Sera of Ragweed Sensitive Patients, *J. Immunol.* **99**: 610, 1967.
52. Reid, R. T., Minden, P., and Farr, R. S.: Reaginic Activity Associated With IgG Immunoglobulin, *J. Exper. Med.* **123**: 845, 1966.
53. Fireman, P., Boesman, M., and Gitlin, D.: Heterogeneity of Skin-Sensitizing Antibodies, *J. ALLERGY* **40**: 259, 1967.
54. Sela, M., and Mozes, E.: Dependence of the Chemical Nature of Antibodies on the Net Electrical Charge of Antigens, *Proc. Nat. Acad. Sc.* **55**: 455, 1966.
55. Girard, J.-P.: Antibody Synthesis In Vitro by Human Peripheral Lymphocytes, *Internat. Arch. Allergy* **32**: 294, 1967.

56. Johansson, S. G. O., Bennich, H., and Wide, L.: A New Class of Immunoglobulin in Human Serum, *Immunology* 14: 265, 1968.
57. Johansson, S. G. O.: Raised Levels of a New Immunoglobulin Class (IgND) in Asthma, *Lancet* 2: 951, 1967.
58. Bennich, H., and Johansson, S. G. O.: Studies on a New Class of Human Immunoglobulins. II. Chemical and Physical Properties, Nobel Symposium III.
59. Ishizaka, K., Ishizaka, T., and Hornbrook, M. M.: Allergen-Binding Activity of γ E, γ G and γ A Antibodies in Sera From Atopic Patients; In Vitro Measurements of Reaginic Antibody, *J. Immunol.* 98: 490, 1967.
60. Layton, L. L., Yamanaka, E., Lee, S., and Kenko, C. W.: Demonstration of Human Reaginic Antibodies to Foods, Cat Dander, an Insect, and Ragweed and Grass Pollens, *J. ALLERGY* 33: 271, 1962.
61. Ishizaka, K., Ishizaka, T., and Arbesman, E. C.: Induction of Passive Cutaneous Anaphylaxis in Monkeys by Human γ E Antibody, *J. ALLERGY* 39: 254, 1967.
62. Arbesman, C. E., Girard, J. P., and Rose, N. R.: Demonstration of Human Reagin in the Monkey. II. In Vitro Passive Sensitization of Monkey Ileum With Sera of Untreated Atopic Patients, *J. ALLERGY* 35: 535, 1964.
63. Wicher, K., Kobayashi, S., Arbesman, C. E., and Ishizaka, K.: Demonstration of Human Reagin in Monkey Tissues. V. In Vitro Sensitization of Ileum With Absorbed Globulin Fractions From Sera of Patients Sensitive to Ragweed, *J. ALLERGY* 41: 74, 1968.
64. Wicher, K., Girard, J.-P., Reisman, R. E., Yagi, Y., and Arbesman C. E.: Demonstration of Human Reagin in Monkey Tissues. IV. In Vitro Sensitization of Ileum With Globulin Fractions From Sera of Patients Sensitive to Ragweed, *J. ALLERGY* 41: 63, 1968.
65. Stanworth, D. R., Humphrey, J. H., Bennich, H., and Johansson, S. G. O.: Specific Inhibition of the Prausnitz-Küstner Reaction by an Atypical Human Myeloma Protein, *Lancet* 2: 330, 1967.
66. Rockey, J. H., and Kunkel, H. G.: Unusual Sedimentation and Sulfhydryl Sensitivity of Certain Isohemagglutinins and Skin-sensitizing Antibody, *Proc. Soc. Exper. Biol. & Med.* 110: 101, 1962.
67. Leddy, J. P., Freeman, G. L., Luz, A., and Todd, R. H.: Inactivation of the Skin-sensitizing Antibodies of Human Allergy by Thiols, *Proc. Soc. Exper. Biol. & Med.* 111: 7, 1962.
68. Hong, R., and Nisonoff, A.: Relative Labilities of the Two Types of Interchain Disulfide Bond of Rabbit γ G Immunoglobulin, *J. Biol. Chem.* 240: 3883, 1965.
69. Mota, I., Beraldo, W. T., Ferri, A. G., and Junqueira, L. C. U.: Action of 48/80 on the Mast Cell Population and Histamine Content of the Wall of the Gastro Intestinal Tract of the Rat, Histamine, Ciba Foundation Symposium: 1956, Little, Brown & Company, p. 47.
70. Lichtenstein, L. M., and Osler, A. G.: Studies on the Mechanisms of Hypersensitivity Phenomena. IX. Histamine Release From Human Leukocytes by Ragweed Pollen Antigen, *J. Exper. Med.* 120: 507, 1964.
71. Levy, D. A., and Osler, A. G.: Studies on the Mechanisms of Hypersensitivity Phenomena. XIV. Passive Sensitization In Vitro of Human Leukocytes to Ragweed Pollen Antigen, *J. Immunol.* 97: 203, 1966.
72. Ishizaka, K.: Gamma Globulin and Molecular Mechanisms in Hypersensitivity Reactions, *Progr. Allergy* 7: 32, 1963.
73. Ishizaka, K., and Ishizaka, T.: Induction of Erythema-Wheal Reactions by Soluble Antigen- γ E Antibody Complexes in Humans, *J. Immunol.* 101: 68, 1968.
74. Henney, C. S., and Ishizaka, K.: Antigenic Determinants Specific for Aggregated γ G Globulin, *J. Immunol.* 100: 718, 1968.
75. Levine, B. B., and Fellner, M. J.: The Nature of Immune Complexes Initiating Allergic Wheal-and-Flare Reactions, *J. ALLERGY* 36: 342, 1965.