Studies of reagins to α gliadin in a patient with wheat hypersensitivity

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Serum from a markedly wheat-sensitive patient was studied and found to contain a high titer of reaginic antibody. This is believed to represent a major pathogenetic mechanism in the subject's allergy. Of particular interest was the finding that P-K sites responded to both oral and intradermal challenge. In terms of potency, α-gliadin appeared to be the important allergen as determined by the wheal and flare it elicited and by its ability to neutralize reagin in vitro. Skin-sensitizing antibodies eluted from DEAE-Sephadex showed heterogeneity in molecular charge, as well as variability in ability to fix to human skin.

There is little question about the importance of reaginic antibodies in atopic disease. By definition, reagins are passively transferable, heat-labile antibodies which fix to the skin and lead to a wheal and erythema reaction on challenge with specific allergen. Over the last several years, many studies have been done in an effort to characterize skin-sensitizing antibodies, but relatively little has been written about reagins to food proteins. In this study we report on a serum having a reagin titer of 1:10,000 to wheat α-gliadin by P-K testing. The subject was a 16-year-old boy who had severe gastrointestinal and pulmonary symptoms upon exposure to wheat. The studies document certain characteristics of one kind of food sensitivity.

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MATERIALS AND METHODS

Preparation of allergens

Whole gliadin* and gliadin fractions isolated by starch-gel electrophoresis†[1] were used as allergens in this study. One milligram of each allergen was dissolved in 10 ml. of normal saline to make an initial concentration of 0.1 mg. per milliliter. Serial tenfold dilutions were made from the initial concentration, and the solutions were sterilized by passage through 0.22 μ Millipore filters in Swinney adapters.

Preparation of antisera

Antisera against human IgG, IgA, IgM, and IgD were prepared by immunizing rabbits with 5 to 15 mg. of isolated human myeloma or macroglobulinemia proteins emulsified in complete Freund's adjuvant. Three weeks after the initial dose, the rabbits were given a booster injection which was repeated at 2 month intervals thereafter to maintain the antibody titer. Bleedings were performed at appproximately 10 day intervals, beginning after the booster injection. Antisera were made specific for a given immunoglobulin heavy chain by absorption with kappa and lambda light chains and appropriate immunoglobulins of the other major classes. Specificity was established by Ouchterlony analyses and immunoelectrophoresis.

Gel-diffusion techniques

Oudin tubes. Specific antisera to human immunoglobulin heavy chains were mixed in appropriate concentration with agar at 55° C. and placed in capillary tubes. A sample to be tested for immunoglobulin content was placed in a capillary tube of smaller diameter which was then inserted into the larger tube, the sample being overlaid onto the antiserum-containing agar. Eighteen hours later, the distance of migration of the specific precipitate line from the gel interface was read in tenths of a millimeter, and the results were converted to milligrams per cent with the use of a graph prepared from the values of standard sera of known immunoglobulin content tested at the same time.

Ouchterlony analysis. This was carried out on microscopic slides with reservoirs 2 mm. in diameter and 4 mm. center to center for routine analyses. A second previously described technique[2] with plexiglas matrices and specific immunoglobulin reference lines permitted an approximate quantitation of immunoglobulins in concentrations as low as 1 μg per milliliter of IgG, IgA, or IgD and 5 μg per milliliter of IgM.

Immunoelectrophoresis. Immunoelectrophoresis was carried out on microscopic slides by a modification of Scheidegger's technique[3] with the use of National Instrument Company apparatus.

Determination of allergen specificity

Sites sensitized with 1:50 reaginic serum were challenged with 0.02, 0.2, or 2 μg of whole gliadin, α-gliadin, or β-gliadin in order to determine their relative

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potency. One site was challenged 3 times with 0.2 μg of α-gliadin until there was no further response to that allergen. After all reaction had subsided, the same site was challenged with whole gliadin and finally with oral wheat in order to determine if either of these contained allergens, other than α-gliadin, to which the sensitized site would still respond.

Neutralization of reaginic serum with gliadin allergen

Whole reaginic serum was diluted 1:5 with normal saline, and 0.1 ml. aliquot parts were mixed with an equal volume of alpha, beta, or whole gliadin in various concentrations. The mixtures were allowed to stand at room temperature for 30 minutes and then were diluted with normal saline to make a final serum concentration of 1:50. After standing at 4° C. overnight, the mixtures were centrifuged at 10,000 r.p.m. for 30 minutes. Supernatant fluids were removed, sterilized by filtration, and used for passive-transfer studies. Sensitized sites were challenged with 0.2 μg of α-gliadin to observe if reagin neutralization had taken place.

Fractionation with ammonium sulfate

Five milliliters of reaginic serum was diluted with an equal amount of 0.1M sodium phosphate buffer, pH 7.4, and proteins were precipitated in sequence by adding ammonium sulfate in stages to obtain the following concentrations: 20 per cent, 35 per cent, 45 per cent, and 55 per cent. Precipitates obtained after each addition of ammonium sulfate were washed with a fresh solution of ammonium sulfate of the appropriate concentration, and then each precipitate was dissolved in 0.5 ml. of normal saline. The resulting solutions were dialyzed against phosphate buffer and tested for immunoglobulin content. Reaginic activity was assayed after diluting each fraction 1:100.

Effects of pH, heat, and 2-mercaptoethanol

pH. In order to determine the effect of pH upon skin-sensitizing activity, 1 ml. aliquot parts of 1:10 serum were dialyzed for a 24 hour period against 0.1M buffers of the following pH values: 2.2, 4.5, 7.4, 10, and 11. After the prescribed exposure, the samples were dialyzed against 0.1M sodium phosphate, pH 7.4, and tested for P-K activity by intradermal challenge with 0.02 μg of α-gliadin.

Heat. The effect of heat on skin-sensitizing activity was studied by placing duplicate 0.4 ml. aliquot parts of whole serum diluted 1:5 in constant-temperature water baths at 52 and 56° C. One aliquot part was removed after one hour and one after 4 hours at each temperature and was subsequently tested for reaginic activity.

2-Mercaptoethanol (2-ME). An aliquot part of reaginic serum was mixed with 2-ME to make a 1:10 serum dilution with a final concentration of 0.1M 2-ME and was left at room temperature for 4 hours in a nitrogen atmosphere to attain maximum reduction. The sample was not alkylated but was exhaustively dialyzed against several changes of 0.1M tris-HCl buffer, pH 8.0, and tested for the presence of reagins by intradermal challenge with α-gliadin. A

sample treated in the same manner but without exposure to 2-ME served as a control.

Gel filtration

Gel filtration was carried out by applying 2 ml. of serum in 0.1M sodium phosphate buffer, pH 7.4, to a 100 ×2.5 cm. column of Sephadex G-200* in the same buffer with the use of upward flow. Ten milliliter samples were collected and protein content monitored by reading absorption at 280 mμ on a Beckman DU Spectrophotometer. Aliquots from several parts of each protein peak were sterilized by Millipore filtration and tested for P-K activity by challenge with 0.02 μg α-gliadin, intradermally. The ascending and descending portions of each protein peak were then pooled and concentrated to 2 ml. by pervaporation and were analyzed for immunoglobulin content.

Column chromatography

A 34 × 2 cm. glass column was packed by gravity with DEAE-Sephadex A-50* equilibrated with 0.01M sodium phosphate buffer, pH 8.0. Three milliliters of whole serum was dialyzed against the buffer used for equilibration and then applied to the column. Stepwise elution was carried out with the use of sodium phosphate buffer, pH 8.0, of increasing molarity, i.e., 0.01, 0.03, and 0.1M, after which 1.5M NaCl was added to elute the remaining proteins. Several aliquot parts from each protein peak were tested for P-K activity. Pools were then made and concentrated to 3 ml. with an Amicon ultrafiltration apparatus. Concentrated samples were analyzed for immunoglobulin content. A second chromatographic study was carried out with 15 ml. of serum fractionated by precipitation with 40 per cent saturated ammonium sulfate. The precipitated protein was redissolved in normal saline and dialyzed against 0.01M sodium phosphate, pH 8.0. Ten milliliters of solution (360 mg. of protein) was applied to a column of DEAE-Sephadex similar to the one already described. The technique of elution, P-K testing of protein pools, and immunoglobulin determinations were as already described.

Passive-transfer studies

Prausnitz-Küstner (P-K) studies were performed on subjects who had been on a wheat-free diet for 3 or more days prior to testing. Wheat had to be eliminated from the diet to minimize the neutralization of sensitized sites by circulating dietary gliadin allergen. Either diluted whole serum or chromatographic fractions of serum, 0.02 ml., were injected intradermally, and 6 hours later the sites were challenged. The 6 hour interval was selected for convenience since reactions of similar intensity could be obtained at 6 hours or at the customary 24 to 48 hours following passive transfer. In some studies the allergen was administered orally as ½ to 1 slice of bread, and in other experiments antigenic challenge was made by the intradermal route with 0.02 ml. of a solution of either alpha, beta, or whole gliadin administered with a tuberculin syringe. Reactions

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were recorded as a P-K score, defined as one half the mean diameter of the erythema plus the diameter of the wheal in centimeters (E/2 + W). This method recognizes the contribution of both erythema and wheal to the skin reactions but imparts a double importance to the wheal. Readings were taken at 15 minutes and 30 minutes if challenge was intradermal and at 30 minute intervals for 3 hours if challenge was by the oral route.

Tanned-cell hemagglutination

Tanned-cell hemagglutination was carried out with the use of a modification of the technique described by Boyden.[4] Sheep red blood cells (R.B.C.) suspended in Alsever's solution were centrifuged at 1,500 r.p.m. for 10 minutes and then washed 3 times with normal saline. The packed R.B.C. were then diluted 1:60 with normal saline and allowed to incubate at 37° C. for 10 minutes with tannic acid in a dilution of 1:20,000 w/v. The cells were then washed, centrifuged, and resuspended in phosphate-buffered saline, pH 7.4. The sensitizing allergen was α-gliadin, and after trying several concentrations it was found that 0.1 mg. of allergen dissolved in 1 ml. solution and added to 11 ml. of suspended R.B.C. gave optimal results. The mixture of cells and allergen was allowed to incubate at 37° C. for 15 minutes. The coated cells were then centrifuged, washed with one per cent normal rabbit serum in saline, and resuspended in 6 ml. of the same solution. Serial twofold dilutions of either whole serum or concentrated chromatographic serum fractions were made starting from an initial concentration of 1:20. To 1 ml. in each tube was added 0.1 ml. of the 2.5 per cent suspension of sensitized cells. The tubes were agitated and allowed to sit overnight at room temperature. The results were read the next morning and the end point recorded as the highest dilution to show unequivocal hemagglutination.

RESULTS

Allergen specificity

When sites sensitized with 1:50 reaginic serum were challenged with 0.2 μg alpha, beta, or whole gliadin, only α-gliadin produced a significant reaction (P-K score, 2.5, Fig. 1, *A*). A tenfold greater amount of either whole gliadin or beta gliadin was required to elicit a wheal and flare reaction, and the P-K scores were only 1.4 and 1.7, respectively. In addition, a reaginic site was challenged repeatedly with α-gliadin until there was no further response. After all reaction had subsided, the site was challenged with whole gliadin and later with oral wheat. No further wheal and flare reaction was elicited by either, suggesting that α-gliadin caused the reactions seen and that there was contamination of the β-gliadin preparation used with α-gliadin. In fact, it was demonstrated in a previous report that such contamination does exist.[18]

Reagin neutralization in vitro

The results of reagin neutralization with whole gliadin, α-gliadin, and β-gliadin are shown in Fig. 1, *B*. A site sensitized with 0.02 ml. of 1:50 serum, to which 0.2 μg of α-gliadin had been added, showed no response on subsequent challenge with 0.2 μg of α-gliadin, indicating that reagin neutralization had

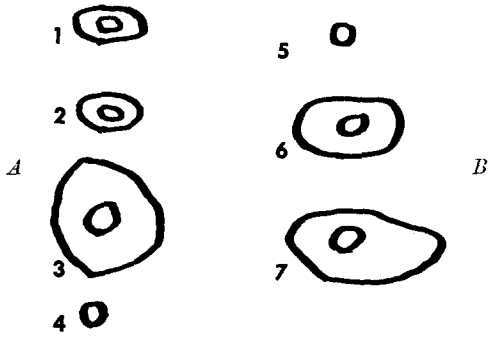


Fig. 1

P-K reactions traced on transparent film 20 minutes after challenge with antigen. *A*, Sites *1* to *3* were sensitized with serum diluted 1:50 and Site *4* was an unsensitized control site. Challenges were as follows: *(1)* 2 μg whole gliadin; *(2)* 2 μg β-gliadin; *(3)* 0.2 μg α-gliadin; *(4)* 0.2 μg α-gliadin. *B*, Sites *5* to *7* were sensitized with 0.02 ml. aliquot parts of 1:50 serum which had been preincubated with antigen and then challenged with 0.2 μg of α-gliadin. Antigens were mixed with the sera as follows: *(5)* 0.2 μg α-gliadin; *(6)* 0.2 μg whole gliadin; *(7)* 0.2 μg β-gliadin.

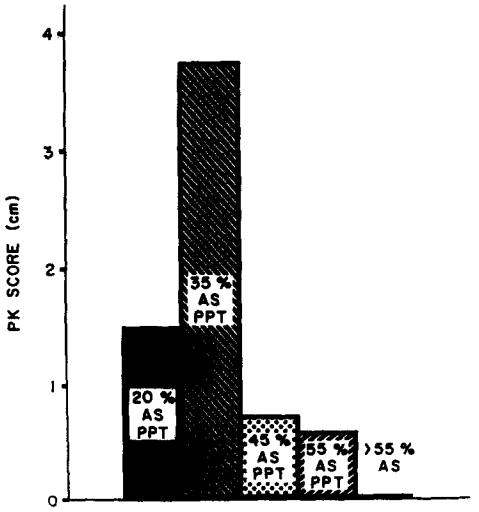


Fig. 2

Sequential fractionation of reaginic serum with ammonium sulfate. Sites were sensitized with 0.02 ml. of each fraction and were challenged by oral ingestion of wheat. It can be seen that most of the reaginic activity is salted out by 35 per cent ammonium sulfate, but traces of reaginic activity are also present in 3 other fractions.

taken place. The same quantity of whole gliadin or β-gliadin added to a similar serum dilution failed to inhibit the wheal and flare response.

Ammonium sulfate fractionation

The bulk of reaginic activity was precipitated from whole serum with 35 per cent saturated ammonium sulfate followed by dialysis against 0.1M phosphate, pH 7.4. This fraction contained IgG in high concentration, but IgA,

IgM, and IgD were also detectable in small amounts. A P-K score of 3.7 cm. was recorded 90 minutes after a site sensitized with a 1:100 dilution of this fraction was challenged by oral ingestion of wheat (Fig. 2).

Effect of pH, heat, and reduction on reaginic activity

Sera diluted 1:10 and exposed for a 24 hour period to 0.1M buffers of different pH values retained their ability to respond with wheal and flare to antigenic challenge after exposure to pH values ranging from 4.5 to 10 and subsequent return to neutrality. There was increasing loss of activity at more extreme pH values. The sample exposed to pH 2.2 retained no reaginic activity.

Reaginic activity was heat labile and disappeared completely after exposure to 56° C. for one hour.

The effect of 0.1M 2-mercaptoethanol on the reaginic activity was appreciable since a sample exposed for 4 hours lost all activity; whereas a control sample treated in an identical manner, except for the omission of mercaptoethanol, retained full activity.

Gel filtration

The three usual effluent peaks were obtained by filtration of whole serum on Sephadex G-200 (Fig. 3). IgM was located in the first peak, and IgA and IgG were located in the second. Representative effluent samples tested for P-K activity showed the bulk of the reagins to be between the first two peaks. The most

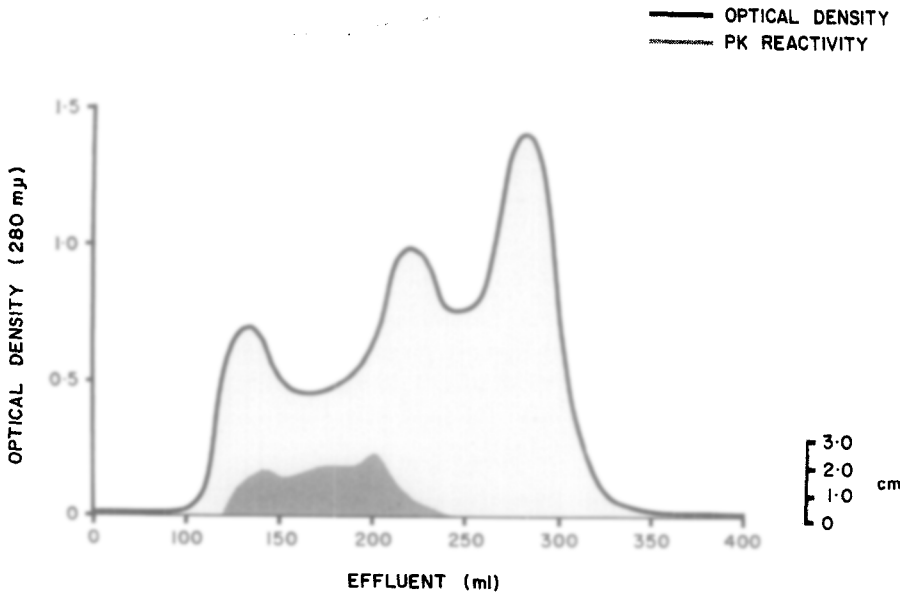


Fig. 3

Gel filtration of 2 ml. whole reaginic serum with the use of Sephadex G-200. The distribution of reagins was studied by sensitizing skin sites with unconcentrated effluent from individual tubes. Challenge was with intradermal α-gliadin.

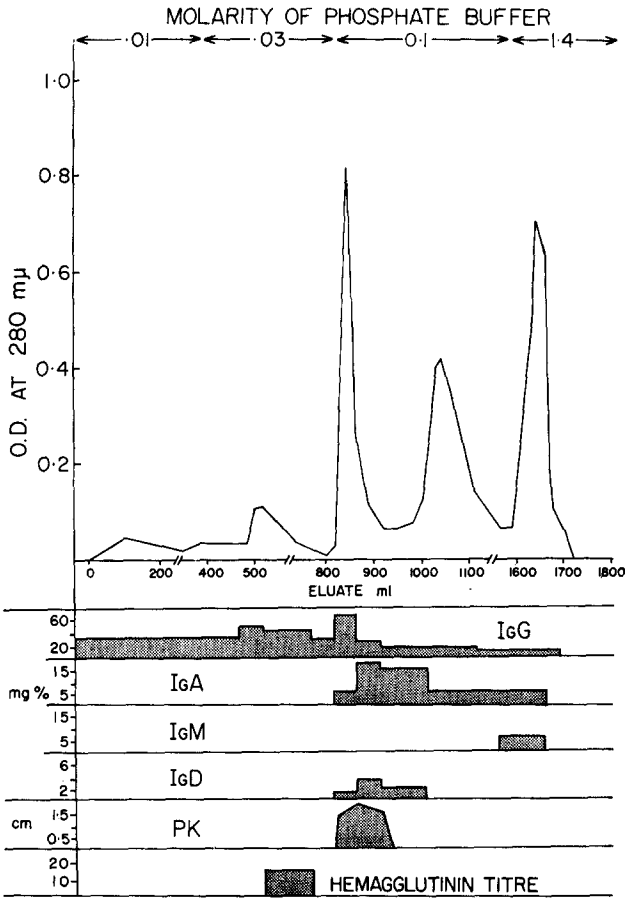


Fig. 4

Chromatographic study of 3 ml. of whole reaginic serum applied to DEAE-Sephadex A-50. Stepwise elution was carried out with sodium phosphate buffer, pH 8.0. Aliquot parts from unconcentrated pooled fractions were tested for P-K activity by intradermal challenge with α-gliadin antigen. The immunoglobulin content and hemagglutination titer of the fractions after concentration are shown.

active aliquot part from this area, having a protein concentration 1/300 that of whole serum, gave a 2.7 cm. P-K score on challenge, compared with a simultaneous score of 2.5 cm. with the use of a 1:50 serum dilution in the same test subject. The concentrated pools with the most skin-sensitizing activity contained both IgA and IgG but no IgM or IgD.

Column chromatography with DEAE-Sephadex A-50

The elution pattern of whole serum from DEAE-Sephadex is shown in Fig. 4. Reagins were detected in a single protein peak eluted with 0.1M phosphate buffer. Concentrated samples from this area contained IgG, IgA, and IgD. In this experiment in which only 3 ml. of serum was used, reagins too dilute to detect by P-K testing may have been present elsewhere. Hemagglutinins to α-

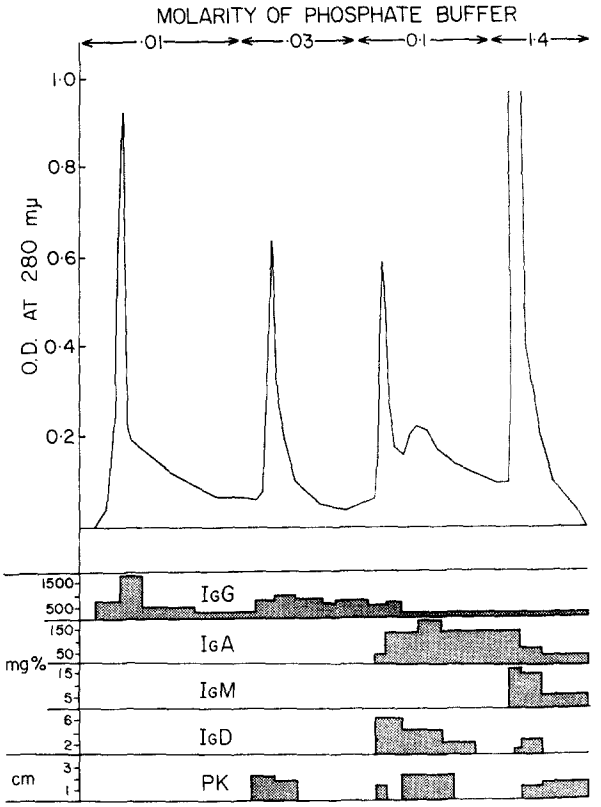


Fig. 5

DEAE-Sephadex A-50 chromatography of 15 ml. whole reaginic serum precipitated with 40 per cent ammonium sulfate. Stepwise elution was carried out with sodium phosphate buffer, pH 8.0. Aliquot parts of pooled fractions were analyzed for immunoglobulin content and tested for P-K activity by intradermal challenge with α-gliadin antigen.

gliadin were eluted from the column earlier than reagins and were in a fraction in which only IgG was detectable.

When 15 ml. of whole serum was precipitated with 40 per cent ammonium sulfate and applied to a second column, reagins were eluted at 3 separate points on the chromatogram as demonstrated by passive-transfer studies (Fig. 5). The early peak of P-K activity was associated with detectable IgG only, whereas reagin-containing fractions eluted later contained IgG, IgA, IgM, and IgD.

Precipitating and hemagglutinating antibodies to gliadin

Fig. 6 shows an Ouchterlony study comparing precipitating antibodies in the sera of 3 patients with gliadin-induced celiac disease with those of the wheat-sensitive subject reported herein. Previous work has demonstrated antibodies to one constituent of gliadin in about 40 per cent of celiac subjects, as detected by gel diffusion.[5] In the present study, a precipitin line formed opposite the 3 celiac sera in a reaction of identity. Two lines of a different identity formed between the wheat-sensitive serum and the allergen, which indicates that the

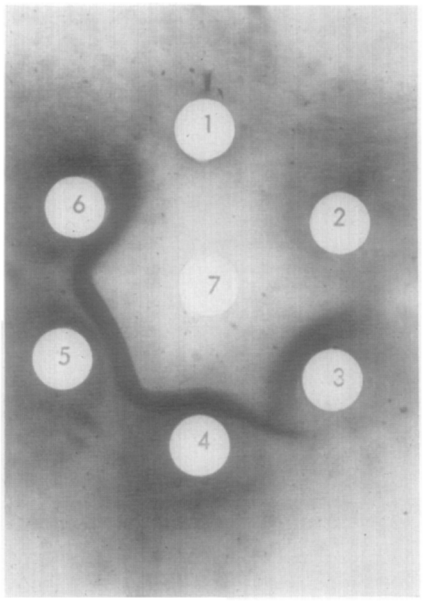


Fig. 6

Ouchterlony analysis comparing precipitating antibodies to gliadin in three celiac sera with those in the wheat-sensitive serum reported here. *1* and *2*, Normal sera; *3*, wheat-sensitive reagin-containing serum; *4*, *5*, and *6*, selected celiac sera with precipitins to gliadin but no reagins; *7*, crude gliadin (1 mg. per milliliter).

precipitins in the reaginic serum were directed against different allergens in the crude gliadin preparation. Whereas the precipitins of the celiac subjects were directed toward antigenic specificities present in purified α-gliadin, no precipitins could be demonstrated in the reaginic serum when either purified α- or β-gliadin was used as the allergen. The precipitins in the reaginic serum were therefore clearly directed to different antigenic molecules than the precipitins of the celiac subjects and presumably to allergens not present in α-gliadin, although the possibility has not been ruled out that certain α-gliadin constituents were masked or denatured by the conditions used for purification of the crude gliadin.

Whole serum was found to have hemagglutinating antibodies to α-gliadin in a titer of 1:320. Three sera from volunteers with no history of wheat intolerance were selected as controls, and all three had negative results at 1:20 and greater dilutions. Examination of DEAE-Sephadex fractions of whole serum for hemagglutination titer revealed activity in one sample. This fraction contained only IgG by immunoelectrophoresis and, as was indicated previously, was eluted at a point on the chromatogram distinct from the reagins.

DISCUSSION

Reagins to food proteins have been known since the early studies of fish hypersensitivity by Prausnitz and Küstner. Because high reagin titers are some-

what unusual in food allergy and since dietary exposure to allergen in P-K recipients may complicate interpretation of results, most of the recent laboratory studies of skin-sensitizing antibody have been carried out with the use of sera from subjects with inhalant allergy. Studies on wheat sensitivity have been particularly infrequent.

Early workers[6] suggested that reagins could be removed from serum by selective precipitation of IgA, but subsequent work has shown the situation to be more complex. A serum was reported to contain reaginic antibodies but no detectable IgA.[7] Later, reagins were shown to have a heterogenous distribution on DEAE-Sephadex chromatography,[8] suggesting that perhaps more than one type of antibody was involved. In some studies, skin-sensitizing antibody was found in chromatographic fractions which appeared to contain only IgG.[9, 10] Recently Ishizaka and associates[11] described a new class of antibody possessing skin-fixing properties and showed that reaginic activity was greatly diminished by specific precipitation of the new immunoglobulin with rabbit antiserum. The immunoglobulin, called IgE, has γ_1 mobility on electrophoresis, is composed of heavy chains and either kappa or lambda light chains, and has an 8S sedimentation coefficient.[12] It has been reported to passively sensitize monkey ileum[13] and to sensitize monkey skin for passive cutaneous anaphylaxis.[14]

The findings of the current study indicate that reagins were present in this wheat-sensitive subject in high titer and were directed to a purified food allergen present in α-gliadin preparations. This allergen is implicated because α-gliadin elicited a wheal and flare reaction in the smallest amounts in P-K recipients and had the ability to neutralize reagin in vitro. One aspect of this situation which differs from inhalant atopy is that P-K sites responded to either oral or intradermal challenge. A wheal and flare could be anticipated 60 to 120 minutes after oral challenge, the delay presumably representing the time required for antigenic material to be absorbed from the gastrointestinal tract and to arrive at the sensitized site via the bloodstream. The oral route appears to provide a slow but more or less continuing supply of antigen, resulting in a prolonged wheal and flare response, while a typical, rapid, short-lived reaction was obtained on direct intradermal challenge.

Of interest was the fact that reagins were eluted from one DEAE-Sephadex column with buffers of three different ionic strengths in three separate areas of the chromatogram. This finding suggests the heterogenous nature of skin-sensitizing antibody in agreement with other workers.[9, 10, 15] Furthermore, when testing the serum fractions on different volunteers, most of the samples gave comparable P-K scores on challenge, but several repeatedly gave positive responses in only certain volunteers. This finding could conceivably be explained on the basis of a slightly lower concentration of reagin in these fractions associated with an increased skin sensitivity to reagin on the part of certain volunteers, or it could represent a heterogeneity of reaginic skin-fixing properties. However, many of the fractions induced positive responses of similar magnitude in the different recipients, and the findings were reproducible. Thus it is possible that the fractions in question may have contained substances which were inhibitory to P-K reactions when injected into certain recipients (Table I).

Table I. *P-K scores using DEAE-Sephadex fractions containing reagins to α-gliadin*

Fraction No.	*Molarity of eluting buffer*	*P-K scores**		
		Y. B.	*G. H.*	*E. L.*
7	0.03	2.0	1.6	1.6
8	0.03	1.6	2.6	1.8
9	0.03	1.0	0	1.0
10	0.1	1.3	0	1.0
11	0.1	0	0	0
12	0.1	2.0	3.0	2.1
13	0.1	2.0	2.5	2.0
14	0.1	0	0	0
15	1.3	0	0	0
16	1.3	1.0	0	0
17	1.3	1.3	0	0

*Three volunteers, Y. B., G. H., and E. L., were used; scores represent measurements of erythema/2 + wheal (in cm.).

Recently, Reid and associates[16] have suggested that reagin heterogeneity may be associated with that portion of the molecule responsible for skin fixation. These workers found that the skin-sensitizing activity of some fractions could be blocked by pretreatment of P-K sites with a normal human serum fraction containing only detectable IgG. However, the P-K activity of other reagin-containing fractions from the same allergic serum could not be inhibited in like manner. Our finding that reaginic fractions showed variability in their capacity to induce skin reactivity in P-K volunteers, some fractions sensitizing only one recipient and others 2 or 3 different recipients, adds further evidence of heterogeneity in reaginic or in blocking activity. One kind of blocking activity that should be considered is the low molecular weight skin-fixing factor recently described by Goodfriend and colleagues.[17]

The high reagin content of this subject's serum provides a reasonable explanation for the symptoms he had on exposure to minute amounts of wheat protein. That the presence of low concentrations of skin-sensitizing antibody does not correlate with clinical hypersensitivity has been observed frequently. However, when reagins are present in high titer and when a specific allergen in minute amounts induces marked symptoms in the patient and strongly positive P-K reactions in normal recipients of that patient's serum, the association of reagins with symptoms is indeed likely to be meaningful. It is of interest that precipitins and hemagglutinins to wheat gliadin were also present in this serum and conceivably could have played some role in the pathogenesis of symptoms, although demonstration of this is difficult. Additional evidence for the importance of reagins was provided by 2 volunteers who received serum from this subject in undiluted form for P-K studies and, after challenge with oral wheat, developed large local wheal and flare reactions which were accompanied by respiratory symptoms (pronounced rhinorrhea in one subject and bronchospasm in another). These systemic symptoms disappeared in 2 to 12 hours. At the next subsequent ingestion of bread, minor local swelling and erythema reactions recurred at the site of passive transfer without systemic symptoms. Neither of the volunteers

had these symptoms previously, nor did either have a history of atopic disease. Since the symptoms were related temporally to a vigorous local reaction at sensitized sites, it seems reasonable to attribute them to a small amount of wheat reagin, which may have been absorbed from the local site and later become fixed to respiratory epithelium, or to the effects of mediators released locally producing a subsequent effect on the respiratory tract.

The pathogenesis of the kind of gliadin sensitivity which this subject had is quite different from the sensitivity to gliadin in celiac patients. In the latter subjects reaginic antibodies are seldom demonstrable, whereas precipitins to a particular constituent of gliadin are rather frequently encountered.[18] In celiac disease, larger amounts of wheat are generally required to produce symptoms, and there is an absence of bronchospasm on exposure to airborne wheat flour. In addition, the onset of symptoms following the ingestion of allergen is less precipitous than was the case with this subject, where the feeding of solutions containing as little as 5 mg. of wheat flour produced a sensation of nausea followed almost immediately by propulsive emesis. Indeed, the question as to whether or not immunologic mechanisms are of pathogenetic significance in celiac disease is far from settled and beckons new investigative approaches.

Recently, the presence of precipitins to food substances has been demonstrated in the stools of children with a variety of clinical manifestations of food intolerance, including gastrointestinal blood loss, anemia, diarrhea, and failure to thrive.[19-21] Similar antibodies could not be detected in the sera of the patients. The potential diagnostic significance of these findings became apparent when the offending foods were eliminated from the diet and clinical symptoms abated. Unfortunately at the time of this study, neither the secretions nor the stool from the subject reported in this paper was examined for antibody content. However, determinations of this nature may well be in order for this patient as well as others with clinical manifestations of food intolerance or allergy. By use of radioimmunodiffusion and labeled food allergens, antibodies of any of the five immunoglobulin classes may now be identified providing specific antisera are at hand. It is likely that helpful information will result from quantitative studies of the antibodies belonging to each immunoglobulin class, and preliminary studies in this laboratory suggest that interesting correlations do indeed exist.

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Erratum

In the article, "Migratory atelectasis in an asthmatic child following steroid withdrawal," by William F. Hughes and Robert E. Reisman, in the "Brief communication" section of the May, 1969, issue of the JOURNAL, Fig. 1, on page 302, should be Fig. 2, and Fig. 2, on page 303, should be Fig. 1. The legends are correct for the corresponding corrected figure numbers.