

Association of IgE with reaginic activity in sera from grass pollen- and horse dander-sensitive individuals

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Immunoglobulin E is associated with reagin-rich chromatographic fractions in sera of patients sensitive to rye grass pollen and to horse dander. Thus, in a patient (W. G.) who is sensitive to both allergens, the peak reaginic activity to both allergens was associated with IgE antibody. Absorption of reagin-rich fractions with anti-IgE serum removed reagins to several allergens upon passive transfer (P-K) testing. The inactivation of reagins and probably IgE upon treatment with thiols or heat was confirmed.

Human atopic reagins in serum of patients who are allergic to ragweed and egg white have been associated with a new class of immunoglobulins, IgE.^{1, 2a, 2b} Titers of IgE antibody in such sera, as evidenced by binding of radioiodinated allergen (¹³¹I-AgE of ragweed pollen) parallel skin-sensitizing activity; other Ig antibody titers do not.³ Absorption of reagin-rich serum fractions with anti-IgE serum results in complete removal of the IgE precipitin band, its allergen-binding capacity, and skin-sensitizing activity. However, absorption of such fractions with anti-IgA and anti-IgG, while removing IgA and IgG, fails to reduce skin-sensitizing activity.^{1, 2a, 3} Wheal and flare reactions in reversed Prausnitz-Küstner (P-K) experiments,⁴ resulting from injections of anti-IgE serum into the skin of nonatopic individuals, demonstrate the presence of IgE in normal persons.⁵

An atypical myeloma protein (IgND) described recently by Johansson and Bennich was identified as E myeloma protein.^{6a, 6b} With antisera to this protein

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Supported by United States Public Health Service Research Grants No. AI 08333 and AI 04985.

Presented at the 24th annual meeting of the American Academy of Allergy, Boston, Mass., Feb. 5, 1968.

Received for publication May 29, 1969.

they demonstrated markedly increased amounts of this immunoglobulin in serum of atopic individuals.⁷ This E myeloma protein (IgE) has been physico-chemically characterized as being an 8S γ_1 -globulin with a molecular weight of about 196,000, a high carbohydrate content (~ 11 per cent),⁸ and a serum concentration in nonatopic persons of about 248 (range 66 to 1830) ng. per milliliter.⁹

While IgE appears to be intimately associated with atopic reagin, it is not yet certain whether only IgE has skin-sensitizing activity. Reid and associates¹⁰ found reaginic activity to different allergens associated with different DEAE-cellulose fractions, and such activities showed a different order of lability in 2-mercaptoethanol (2-ME). They suggested that there may be a considerable heterogeneity among immunoglobulins with reaginic activity.

The present study was undertaken to determine if reaginic activity to allergens other than ragweed is associated with IgE and to study the question of reaginic heterogeneity.

METHODS

Allergens

The allergens used in this study were: a highly active "purified" fraction or Group I of rye grass pollen (*Lolium perenne*) prepared by D. Marsh¹¹; a DEAE-cellulose fraction of horse dander extract prepared by D. Stanworth¹²; and crude extracts of house dust (Hollister-Stier, 1:100), mixed grasses (Hollister-Stier, 1:20), and horse dander (Hollister-Stier, 1:20).

Table 1. Symptoms and skin test results in grass pollen-sensitive patients

<i>Symptoms</i>	<i>Direct skin tests (I. D.)</i>		<i>P-K titer</i>	<i>Serum (ml. for fractions)</i>
<i>W. G. (35)</i>				
Asthma and hay fever, grass season 20 yr.	Grass	6+*	4,000	240
	Artemisia	4+	0	
Diphtheria, Child—Rx, diphtheria antitoxin	Horse dander	2+	100	
Blacksmith, 10 yr.				
Auto acc., 1962—Rx, tetanus antitoxin— anaphylaxis				
Untreated				
<i>C. S. (32)</i>				
Hay fever, grass season 15 yr.	Grass	6+	500	300
Sneezes with house dust 5 yr.	House dust	3+	100	
Treated 1 year				
<i>S. P. (17)</i>				
Severe vernal conjunctivitis	Grass	6+	1,000	70
Hay fever, grass season 8 yr.	Artemisia	2+	0	
Treated 5 years	Medicago	3+	0	
	House dust	2+	0	

*Grading of skin tests: 1+, erythema < 20 mm.; 2+, erythema > 20 mm.; 3+, wheal < 5 mm. + erythema; 4+, wheal < 10 mm. with pseudopods + erythema; 5+, wheal < 15 mm. with pseudopods + erythema; 6+, wheal > 15 mm. with pseudopods + erythema.

Serum

Serum was collected in large volume (70 to 300 ml.) from three patients (W. G., C. S., S. P.) who had known sensitivity to grass pollen and several other allergens; 8 ml. was obtained from another patient (E. R.) who was sensitive to horse dander only. The results of direct and passive transfer (P-K) skin tests in these patients and a brief clinical summary are reported in Table 1.

DEAE-cellulose column chromatography

The globulins were precipitated 3 times by adding equal volumes of saturated ammonium sulfate (550 Gm. of $(\text{NH}_4)_2\text{SO}_4$ per liter) and serum and then dialyzed for 24 hours at 4° C. against 0.005M phosphate buffer, pH 8.0. Fifty milliliter aliquots of globulins (~ 1 Gm. of protein) were then fractionated on a DEAE-cellulose column (45 $\times$ 2.5 cm.) by stepwise elution with phosphate buffers of increasing molarity (fraction I, 0.005M; fraction II, 0.025M; fraction III, 0.035M; fraction IV, 0.05M; fraction V, 0.08M; and fraction VI, 0.15M). The fractions were eluted with each buffer after which their ultraviolet absorption was determined at 280 $m\mu$. They were then pooled separately and concentrated by negative pressure dialysis. Hypertonic saline was added to bring the fractions to isotonicity.

Gel filtration

Sephadex G-200 was suspended in borate-buffered saline (BBS), pH 8.0, after sieving and equilibration for 24 hours. The gel was then deaerated and poured into 2 columns (90 $\times$ 3.2 cm.) mounted in tandem to make an effective column length of 180 cm. Ten milliliter aliquots of DEAE-cellulose fractions II and III were applied through a flow adapter at the bottom of one column; an upward flow with BBS through both columns was maintained by a peristaltic pump at a flow rate of 12 to 14 ml. per hour.^{2b} Ultraviolet absorption at 280 $m\mu$ was determined in 4 ml. fractions on a Beckman DU2 spectrophotometer. The peaks were pooled either as a whole or into ascending and descending portions; the pools were concentrated by negative pressure dialysis.

Protein concentrations were measured by the Nessler method¹⁴; antisera specific for IgE, IgG, IgM, and IgA were those employed in a previous study.⁵ Since the anti-IgE serum did not precipitate with myeloma proteins of any of the known immunoglobulins or their subgroups, this antiserum was considered to be specific for IgE. It gave a specific precipitin line with E myeloma protein.^{6b}

Radioimmunodiffusion

Immunodiffusion was performed by the Ouchterlony method¹⁵ with the use of a one per cent agarose gel in Veronal-buffered saline (VBS), pH 8.4. Precipitin bands formed after 48 hours' moist incubation at room temperature, after which the plates were washed for 48 hours in normal saline.

Purified rye grass fraction I or horse dander was radioiodinated with ¹³¹I by the chloramine T method.¹⁶ This antigen was placed into the anti-immunoglobulin wells, incubated for 24 hours at room temperature, and washed for 48 hours in saline. The slides were then dried, and x-ray film (Kodak No Screen) was applied for 2 to 4 days for radioautography.

IgG and IgA concentration in serum fractions was measured by the Mancini method¹⁷ with the use of agar gel plates which contained antiserum specific for IgG or IgA. These were standardized against a known concentration of IgA or IgG globulin run at the same time on the same plate. The concentrations in milligrams per milliliter of all immunoglobulins present in a fraction were added, and the percentage contribution of IgG and IgA was determined and is shown in Tables II to V.

Passive transfer (P-K) reactions

Dilutions of whole serum or chromatographic fractions were brought to pH 7.0, and 0.1 ml. aliquots were injected intradermally (on the back, arm, or thigh) into nonatopic individuals who gave no skin reactions with the purified rye grass, horse dander, or crude house dust allergens. Twenty-four to 72 hours later, the sites were challenged with allergen: 0.2 μ g purified rye grass Group I in 0.02 ml. of saline; 2 μ g purified horse dander in 0.02 ml. of saline; or 1:100 crude house dust in 0.05 ml. of saline. Itching, erythema, and wheal reactions appeared within 20 minutes. The P-K titer was expressed as the maximum dilution of the serum required to give a positive reaction (erythema, 5 mm.). The total nitrogen (N) in the fraction (milligrams of N per milliliter) divided by the P-K titer times 10 (0.1 ml. fraction per test site) represented the minimum skin-sensitizing dose in micrograms of total N per site for positive P-K test.

Monkey P-K tests were performed with *Macaca irus* monkeys.¹⁸ Aliquots of 0.05 ml. of twofold serial dilutions of whole serum or fractions, absorbed and unabsorbed with anti-IgE, anti-IgA, and anti-IgG serum, were injected intradermally into the abdominal skin. Aliquots of 0.05 ml. of normal saline and serum from nonatopic persons served as controls. Twenty-four hours later, 4 ml. of one per cent Evans blue dye was injected intravenously, followed by purified allergen (0.2 to 2 μ g in 0.02 ml. of saline) given intradermally into sensitized sites. Positive reactions occurred as blue spots of dye leakage within 5 to 20 minutes.

Absorption studies

The whole serum or the fractions which produced positive P-K reactions in human beings or monkeys were mixed with one-half, one-third, or one-fourth volume of guinea pig anti-IgE serum. After standing for 24 hours at 4° C., the precipitate was removed by centrifugation (10,000 r.p.m.). IgA or IgG were repeatedly absorbed from whole serum or the fractions with equivalent amounts of antibody for the homologous protein; supernatants were checked with appropriate antiserum for completeness of absorption. Serial dilutions of the supernatant fluids or unabsorbed samples were injected intradermally into nonatopic subjects or monkeys for the passive transfer tests.

Heat inactivation and thiol treatment

Heat inactivation of fractions was accomplished by incubating at 56° C. for 4 hours. In the thiol treatment performed by the method of Leddy and associates,¹⁹ the fraction was diluted 1:5 or 1:50 in tris-buffered saline (TBS)

pH 7.35, 0.15M, and added to an equal volume of 0.2M thiol:2-mercaptoethanol (2-ME), DL-penicillamine (PA), or L-cysteine (Cys), which had been freshly prepared. Thus, the final serum dilution was 1:10 or 1:100 with 0.1M thiol. Untreated controls were diluted in TBS alone and handled in a similar manner. All samples were sterilized by Millipore filtration (0.45 μ M pore size) and incubated for 24 hours in closed, sterile tubes. The 2-ME group was kept at room temperature, the PA and Cys groups at 37° C. After incubation, treated and control fractions were dialyzed separately against TBS for 48 hours at 4° C, with at least 16 changes of buffer to remove the thiol. The dialyzed fractions were resterilized by Millipore filtration, cultured, and then used in P-K tests in a human recipient.

RESULTS

DEAE-cellulose fractionation

The serum of Patient W. G. produced 6 DEAE-cellulose fractions (Fig. 1 and Table II). Fractions I to V all had some P-K skin activity with both rye grass pollen and horse dander allergens; fraction IV had a minimum dose for a positive P-K reaction of 1 μ g of N per milliliter for rye grass and 10 μ g of N per milliliter for horse dander. This was given a value of 1 in

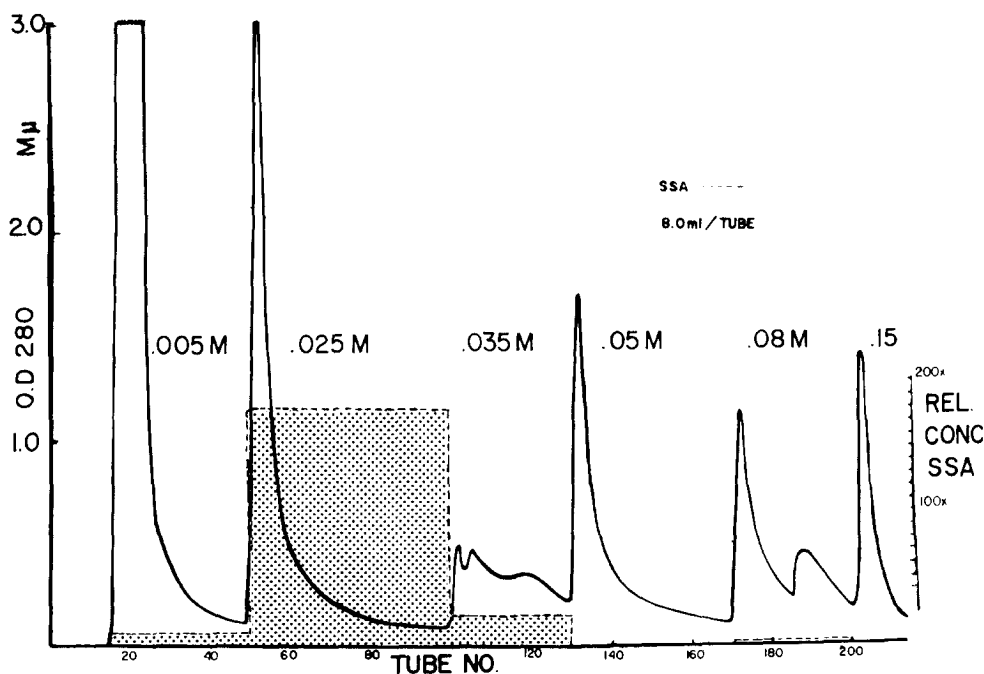


Fig. 1

Stepwise chromatographic fractionation of serum W. G. on DEAE-cellulose with phosphate buffers, pH 8.0, with increasing ionic strength. Pooled fraction includes all material eluted with a given buffer; e.g., fraction I comprised all material eluted with 0.005M PO_4 buffer.

order to compare the relative skin activity of the other fractions; the activity in other fractions was expressed as a multiple of this value. Fraction I contained primarily IgG and skin activity slightly higher than that of fraction IV. Fraction II consisted of about $\frac{1}{4}$ IgG and $\frac{3}{4}$ IgA with sufficient IgE to give precipitation on immunodiffusion against anti-IgE; it had the highest skin activity and could be diluted 40,000 times and still produce a positive grass P-K reaction. Fraction III with similar relative concentrations of immunoglobulins had considerable skin activity. Fraction V had about the same activity as fraction IV, and fraction VI had no detectable skin activity.

Fraction I of Patient C. S. serum contained minimal activity and was given the value of 1 for purposes of comparison. Patterns of skin activity were similar to those of the serum of Patient W. G. (Table III).

Fraction IV of Patient S. P. serum was used as the reference point. Fraction II carried the major skin activity but fraction III also contained considerable rye grass activity (Table IV).

Table II. Serum W. G.: Skin-sensitizing activity of DEAE-cellulose fractions

Fraction	Phosphate buffer for elution (mole/L.)	Relative conc. (%)		IgE by immuno-diffusion	Total N (mg./ml.)	Minimum dose N for positive P-K			
						Rye grass pollen		Horse dander	
		IgG	IgA			μg	Relative	μg	Relative
I	0.005M	100	0	0	4.7	0.094	10×	17	1.7×
II	0.025M	23	77	+	2.2	0.0055	180×	0.07	140×
III	0.035M	27	73	±	1.25	0.042	24×	0.4	25×
IV	0.05M	31	69	0	1.7	1.0	1×	10	1×
V	0.08M	31	69	0	2.1	0.5	2×	10	1×
VI	0.15M	27	73	0	0.76	None		None	•

Table III. Serum C. S.: Skin-sensitizing activity of DEAE-cellulose and Sephadex G-200 fractions

Fraction	Phosphate buffer for elution (mole/L.)	Relative conc. (%)		Total N (mg./ml.)	Minimum dose N for positive P-K			
					Rye grass pollen		House dust	
		IgG	IgA		μg	Relative	μg	Relative
I	0.005M	100	0	6.52	10	1×	None	
II	0.025M	39	61	1.8	0.026	400×	1.8	6×
III	0.035M	45	55	0.9	0.09	100×	9	1×
IV	0.05M	47	53	0.8	None		None	
V	0.08M	47	53	0.65	None		None	
<i>Sephadex G-200</i>								
IIB		38	62	0.47	0.12	80×	4.7	2×
IIC ₁		93	7	0.56	None		None	
IIC ₂		95	5	0.70	None		None	

Table IV. Serum S. P.: Skin-sensitizing activity of DEAE-cellulose and Sephadex G-200 fractions

Fraction DEAE- cellulose	Phosphate buffer for elution (mole/L.)	Relative conc. (%)		Total N (mg./ml.)	Minimum dose N for positive P-K with rye grass pollen	
		IgG	IgA		μg	Relative
I	0.005M	100	0	1.65	None	
II	0.025M	84	16	0.7	0.07	7×
III	0.035M	60	40	0.35	0.35	1.5×
IV	0.05M	63	37	0.72	0.5	1×
V	0.08M	46	54	0.66	None	
<i>Sephadex</i> <i>G-200</i>						
IIB		45	55	0.2	0.013	80×
IIC ₁		83	17	0.29	None	
IIC ₂		88	12	0.36	None	

Table V. Serum W. G.: Skin-sensitizing activity of Sephadex G-200 fractions of DEAE-cellulose fractions II and III

Fraction	Relative conc. (%)		Total N (mg./ml.)	Minimum dose N for positive P-K			
	IgG	IgA		Rye grass pollen		Horse dander	
				μg	Relative	μg	Relative
IIB	2	98	1.2	0.0044	240×	0.6	16×
IIC ₁	74	26	0.62	0.41	2.5×	None	
IIC ₂	94	6	0.44	None		None	
IIIA	24	76	0.23	0.23	5×	None	
IIIB	13	87	0.53	0.033	30×	5	2×
IIIC	97	3	0.24	None		None	

Gel filtration

Fractions II and III of Patient W. G. serum revealed 4 peaks: A, B, C, and D (Fig. 2, *A* and *B*, and Table V). Relative skin activity, when compared with the use of DEAE-cellulose fraction IV as unity, showed that the IgA-rich fractions IIB and IIIB contained the highest skin activity (240× and 30×, respectively, for rye grass pollen and 16× and 2×, respectively, for horse dander).

After filtration of Patient C. S. serum, fraction IIB carried both rye grass and house dust skin activity. Similarly, rye grass activity in serum of Patient S. P. and horse dander activity in serum of Patient E. R. were concentrated in fraction IIB.

Radioimmunoassay

With Patient W. G. serum and its DEAE-cellulose fractions diffused against guinea pig anti-IgE serum and radioiodinated rye grass pollen, both a precipitin line and binding of the allergen occurred with whole serum and fraction II (Fig. 3). There was no allergen binding by other fractions. Binding of radio-

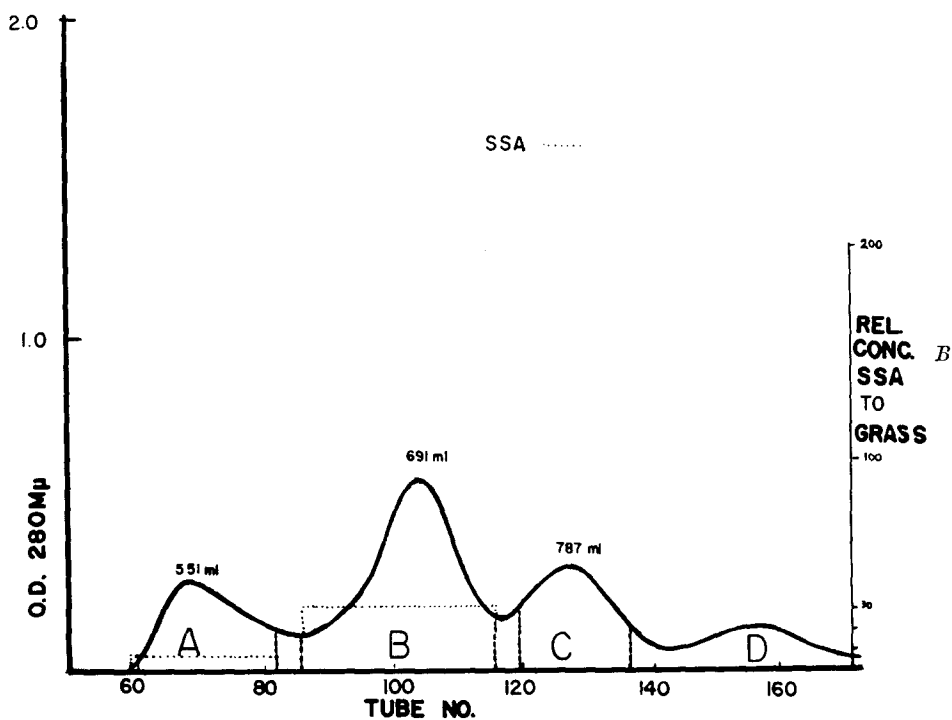
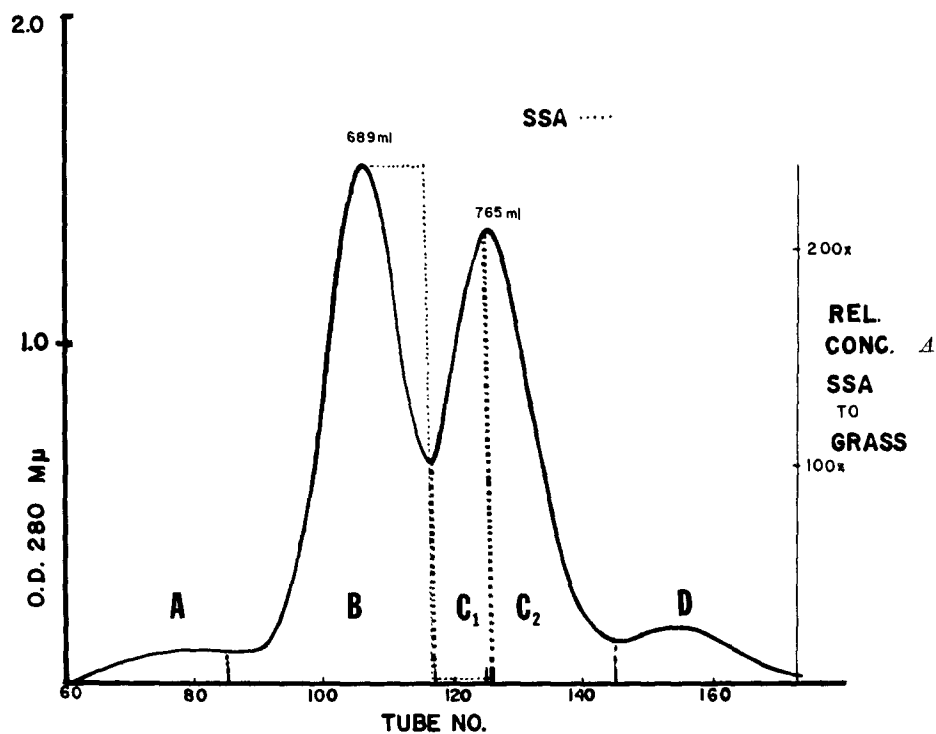
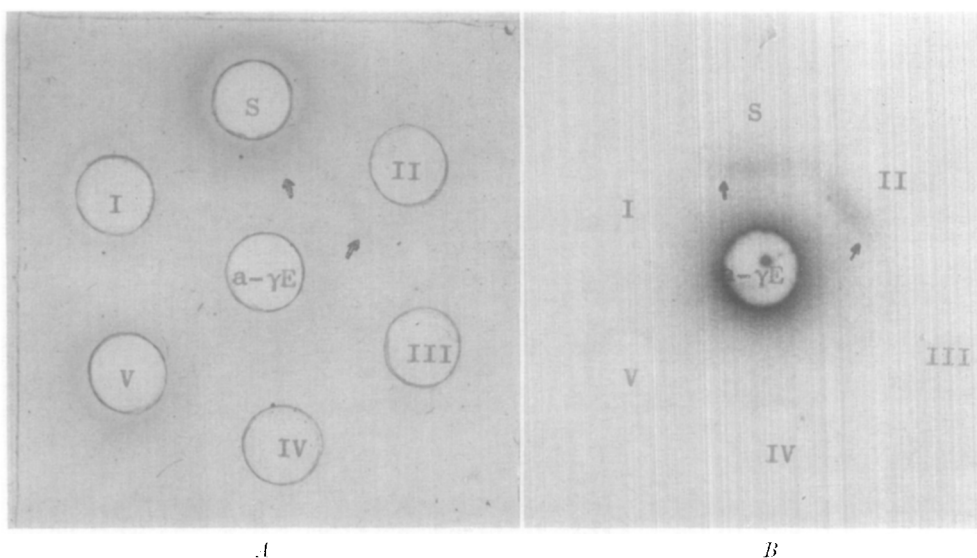


Fig. 2
Gel filtration fractionation of serum W. G. DEAE-cellulose fraction II, 0.025M (A), and III, 0.035M, (B) on Sephadex G-200 in borate-buffered saline, pH 8.0.

**Fig. 3**

A, Precipitation in agar gel of whole serum (*S*) of patient W. G. and its DEAE-cellulose fractions *I* to *V* against anti-IgE serum.

B, Radioautograph of same plate. Radioactively labeled (^{125}I) rye grass pollen Group I extract was placed in center well along with anti-IgE.

iodinated horse dander failed to be demonstrated, probably because the antibody was present in too small a concentration in all fractions. For similar reasons, no grass allergen binding could be demonstrated with fractions of serum from Patients C. S. and S. P. Binding of horse dander to fraction II occurred only in the serum of Patient E. R.

Absorption studies

Absorption of whole serum of Patient W. G. and its fractions II, IIB, and IIIB with anti-IgE serum resulted in complete removal of rye grass and horse dander P-K activity (Figs. 4 and 5). Absorption of the most active fraction IIB with anti-IgA and anti-IgG failed to affect the skin activity. Furthermore, absorption of whole serum with anti-IgE removed all the skin activity, whereas absorption with anti-IgG failed to affect the P-K titer.

Skin-sensitizing activity of whole serum of Patient S. P. and its fraction II, fraction IIB of Patient C. S. serum, and whole serum of Patient E. R. were absorbed with anti-IgE serum (Table VI).

Thiol and heat inactivation

DEAE-cellulose fractions II and III of Patient W. G. serum were diluted 1:100; gel filtration fraction IIB, IIC₁, and IIIB were diluted 1:10 and incubated with 0.1M concentrations of one of the thiols for 24 hours or heated at 56° C. for 4 hours (Table VII). After dialysis, the filtration fractions were

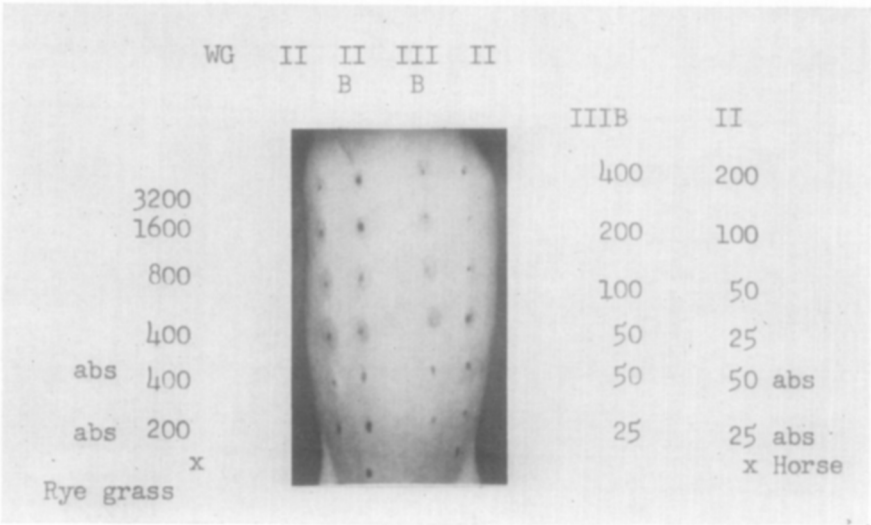


Fig. 4
Effect of absorption of serum W. G. and its fractions by anti-IgE serum upon the monkey passive transfer test. Left 3 columns are rye grass pollen reactions; right column is horse dander reaction.

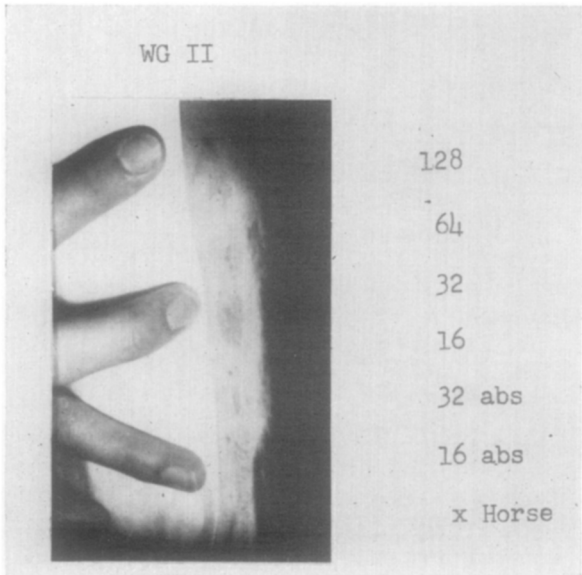


Fig. 5
Effect of absorption on the horse dander reaction of serum W. G. by anti-IgE serum in the monkey passive transfer test. Repeat of right hand column of Fig. 4 for better resolution in a second monkey.

Table VI. *Effect of absorption of reagin-containing fractions with anti- γ E on skin-sensitizing activity with rye grass (10 μ g/ml.)*

Serum	Fraction	Absorption material	Monkey P-K reaction: Dilutions injected			
			5 $\times$	10 $\times$	20 $\times$	40 $\times$
C. S.	IIB	Unabsorbed	8 $\times$ 7*	6 $\times$ 6	0	0
	(0.47 mg./ml. N)	a- γ E, 4:1	0	0	0	0
S. P.	II	Unabsorbed	ND†	10 $\times$ 8	9 $\times$ 8	Trace
	(0.7 mg./ml. N)	a- γ E, 4:1	0	0	0	0

*Diameter of blue dye spot (mm.).

†ND = not done.

Table VII. *Effect of thiols and heat on skin-sensitizing activity of serum fractions of Patient W. G. with rye grass pollen*

Fraction	Dilution of untreated and treated fractions	P-K end-point titer (undiluted fraction)	Untreated	Treatment with 0.1M thiol.			Heat 56° C., 4 hr.
				2-Mercaptoethanol	Penicillamine	Cysteine	
II	1:100	40,000	15 $\times$ 10* (40 $\times$ 35)	< 100†	< 100	< 100	< 100
III	1:100	3,000	10 $\times$ 10 (20 $\times$ 15)	0	0	0	0
IIB	1:10	27,000	10 $\times$ 8 (30 $\times$ 20)	10-100	10-100	< 10	< 10
	1:100		8 $\times$ 6 (20 $\times$ 15)	0	0	0	0
IIIC ₁	1:10	500	10 $\times$ 8 (35 $\times$ 20)	< 10	< 10	< 10	< 10
IIIB	1:10	1,600	12 $\times$ 8 (30 $\times$ 20)	10-100	10-100	< 10	< 10
	1:100		8 $\times$ 8 (15 $\times$ 12)	0	0	0	0

*Dimensions of wheal (mm.); numbers in parentheses = dimensions of flare (mm.).

†Presence of activity in serum dilution.

diluted 1:100. All thiol- or heat-treated or untreated fractions were injected into the skin of a nonatopic individual, and all sites were challenged with either rye grass pollen or horse dander after 48 hours. Heating or thiol treatment inactivated all fractions diluted 1:100. Fractions IIB and IIIB diluted 1:10 and treated with 2-ME or PA still showed considerable activity, while Cys treatment caused complete inactivation. Since P-K titers of fractions IIB and IIIB were 27,000 and 1,600, respectively, 2-ME and PA treatment reduced these titers by more than 90 per cent. Challenge with horse dander failed to elicit any reactions in thiol- or heat-treated skin-reactive fractions of serum of Patient W. G. Similarly, treated fractions of sera of Patients C. S. and S. P. diluted 1:10 were also inactive when challenged with grass pollen.

DISCUSSION

The results of these experiments with sera of 4 patients allergic to rye grass pollen, horse dander, and house dust confirmed similar observations in ragweed-sensitive individuals.^{2a} The highest reaginic activity to these allergens in all 4 sera was found in the DEAE-cellulose fraction II (and its gel filtration subfraction, IIB). Gel filtration, used to separate γ G from γ E in the reagin-rich fractions, showed reaginic antibody concentrated in fraction IIB of sera from Patients W. G. and S. P. Some skin activity in fraction II of serum of Patient C. S. was lost on gel filtration, although fraction IIB remained the most active of the gel filtration fractions. As in a previous study,^{2b} the most active fractions were richest in IgE.

Several DEAE-cellulose fractions adjacent to fraction II also contained lesser amounts of skin-sensitizing activity. This was attributed to chromatographic heterogeneity of IgE molecules, since the widest distribution of reagin occurred in those sera with the highest skin-sensitizing activity, e.g., in Patient W. G. serum. A similar observation was made with the ragweed reaginic system.^{2a, 2b}

Sufficient IgE was present in fraction II (0.025M) of serum of Patient W. G. to give a precipitin line on gel diffusion and binding of radioiodinated rye grass Group I with anti-IgE antiserum. Therefore, the distribution of IgE antibody to rye grass pollen and horse dander in this serum was associated with the reagin-rich fraction. Since at least 10 μ g per milliliter of IgE is required for precipitation, sera with extremely high IgE concentrations are required to demonstrate precipitin lines with anti-IgE sera. Because radioimmunodiffusion is considerably more sensitive than simple precipitating immunodiffusion,²⁰ binding of radioiodinated horse dander allergen to IgE antibody in the reagin-rich fraction (fraction II) of serum of Patient E. R. was demonstrated by co-precipitation with a non-antibody IgE. Complete removal of detectable skin-sensitizing activity to both rye grass pollen and horse dander from whole serum and its most active fractions was accomplished by absorption with specific anti-IgE antiserum. This indicates further that the major portion of reagin is associated with IgE.

Absorption of whole serum with anti-IgA or anti-IgG failed to affect the P-K titer with either rye grass pollen or horse dander allergens. Similarly, absorption of reagin-rich fractions IIB and IIIB of serum of Patient W. G. with anti-IgA and anti-IgG caused no reduction in skin-sensitizing activity. The predominately IgG-containing fraction I of serum of Patient W. G. had a small amount of rye grass pollen reagin which was removed by absorption with anti-IgE serum but was unaffected by absorption with anti-IgG serum. However, because anti-IgE serum itself causes a wheal and flare reaction with the recipient's own normal IgE in the skin,⁵ the histamine released on the initial passive skin sensitization may render the site refractory to subsequent specific antigenic challenge.⁴ Therefore, these absorption experiments generally were done with fractions containing high reaginic activity to avoid this difficulty. Since the skin activity in fraction I is low, a portion of skin-sensitizing

activity (~ 20 per cent) may be associated with another immunoglobulin (although all the visible skin activity was removed by the anti-IgE serum absorption). It may be possible to further delineate this problem by the use of specific immunosorbents. These absorption experiments indicate that the major portion of reagin is associated with IgE.

In a previous study,²¹ IgE antibody (as evidenced by binding of radioiodinated ragweed) was inactivated by heating at 56° C. for 4 hours. In a similar manner, heating of skin-reactive fractions of all sera in this study caused complete skin inactivation and confirms the heat lability of reagin.²²

Leddy and associates¹⁹ observed that skin-sensitizing activity in allergic sera was labile in thiols. Reid and associates¹⁰ reported a difference in the degree of skin-sensitizing activity following incubation of DEAE-cellulose fractions with 0.1M 2-ME of serum from a patient sensitive to 2 allergens: Bermuda grass pollen (*Cynodon dactylon*) and bovine serum albumin (BSA). In their experiments, the weaker skin activity to Bermuda grass pollen was more readily inactivated than the high concentration of BSA skin-sensitizing antibody. Nonalkylated fractions with high skin activity from the 3 grass pollen-sensitive patients in this study were incubated 24 hours in 0.1M thiols (2-ME, PA, Cys), which caused loss of most of their skin activity. Dilutions of 1:100 of serum of Patient W. G. Sephadex fractions were completely inactivated. However, 1:10 dilutions of such fractions incubated with 0.1M 2-ME or PA still retained some activity; 0.2M 2-ME and PA caused complete inactivation. This indicates that 0.1M concentration of these thiols is insufficiently strong to inactivate all reaginic activity in these highly active fractions. Horse dander activity in all these fractions was completely inactivated by incubation in all thiols at 0.1M concentration. Rye grass pollen and house dust skin activity in all fractions of the other 2 sera were labile upon 0.1M thiol treatment. The lability of IgE in thiols is currently under investigation. Since the major part of the reaginic activity in this study belongs to IgE, the results strongly suggest that IgE antibody is sensitive to reducing agents, i.e., thiols.

Addendum

More recent studies by one of the authors (K. I.) have shown that both the antibody-combining sites and the structures essential for passive sensitization in anti-ragweed IgE antibodies were degraded by reduction in 0.1M mercaptoethanol followed by alkylation.²³

The authors wish to express their gratitude to David G. Marsh, Ph.D., for supplying purified Group I rye grass pollen allergen; to D. R. Stanworth, Ph.D., for supplying purified horse dander; and to R. T. Reid, M.D., for supplying serum of Patient E. R.

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