

Measurement of ragweed antigen E by double antibody radioimmunoassay

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Antigen E (AgE), the principal allergen of short ragweed pollen, has previously been quantitated by histamine release from sensitized leukocytes or by immunodiffusion. To measure AgE activity a double antibody radioimmunoassay (RIA) was developed. With this assay as little as 7 ng. of AgE was measurable, and by logit transformation of inhibition curves AgE activity in various allergy extracts could be compared immunologically. AgE reactivity was detected in extracts of several Ambrosia and Franseria species, but only AgE from short ragweed produced inhibition curves parallel to AgE IV-C. With giant ragweed this cross-reactivity was confirmed with immunodiffusion analysis. Certain lots of extracts from botanically unrelated pollens contained small amounts of AgE with inhibition curves parallel to AgE from short ragweed. No dialyzable AgE activity was found in freshly prepared short ragweed extracts. Extraction of short ragweed pollen with 50 per cent glycerin yielded extracts with greater AgE content than extraction with Coca's solution. AgE activity from short ragweed survived freezing and thawing (10 times) and was stable for months at 4° C. in concentrated aqueous solutions. The quantities of AgE coupled to solid-phase polymers used in the radioallergosorbent test could also be estimated with RIA.

Since the isolation and characterization of antigen E (AgE) from short ragweed pollen,^{1, 2} AgE has been measured by histamine release from peripheral leukocytes³ or by immunodiffusion.^{2, 4} In this report we describe a sensitive double antibody radioimmunoassay (RIA) for AgE. The assay has been used to measure AgE activity and, by logit transformation of inhibition curves, to compare immunologically AgE activity in various allergen extracts.

MATERIALS AND METHODS

AgE protein

Purified AgE IV-C² (3.8 mg. per milliliter) was kindly supplied by Dr. F. C. McIntire of Abbott Laboratories. Analysis of AgE IV-C by disc electrophoresis revealed the presence of two rapidly migrating major bands and a series of faint minor bands. When studied by immunoelectrophoresis with potent rabbit antisera to crude ragweed pollen, a single band was seen with one serum and two bands, one major and one minor, with another. This AgE preparation produced positive wheal and flare skin reactions in patients with ragweed hay fever at concentrations as low as 0.2 pg. per milliliter.

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Allergen extracts

Aqueous (nonglycerinated) and glycerinated extracts were purchased from or were donated by Center Laboratories, Hollister-Stier Laboratories, and Greer Laboratories.

Cellulose-ragweed polymers

Several cellulose-short ragweed polymers were prepared by reaction of partially purified short ragweed extracts with cyanogen bromide-activated cellulose. The details of preparation of the partially purified AgE and the coupling procedure are presented elsewhere.⁵ Briefly, 500 mg. of microcrystalline cellulose was reacted with 1 to 2 Gm. of cyanogen bromide in a volume of 10 to 20 ml. at pH 11 for 30 minutes at 4° C. by addition of 4N sodium hydroxide. After washing, the activated cellulose was reacted with 10 to 20 ml. of ragweed extracts, either 50 mg. of protein (containing 25 mg. of AgE) prepared by gel filtration of aqueous ragweed extract through Sephadex G-25 and G-100 or 426.5 mg. of extract (containing approximately 25 mg. of AgE) purified only through Sephadex G-25 columns. These mixtures were reacted overnight at 4° C. with magnetic stirring and thoroughly washed (up to 15 times) in 0.2M H_3BO_3 -0.04M NaOH-0.16M NaCl (pH 8.0). The final preparations were analyzed by RIA.

Antiserum to AgE

Rabbits were immunized with AgE IV-C in complete Freund's adjuvant by intradermal injection of 0.2 mg. at time zero and 1.0 mg. at 10 weeks and again at 14 weeks. The animals were bled 2 weeks after the last injection. Analysis of the rabbit serum (R2) used in the RIA by immunoelectrophoresis with whole short ragweed extract revealed numerous bands, while with AgE IV-C at a concentration of 3.8 mg. per milliliter one major band and a very faint second band were seen. In micro-Ouchterlony gel diffusion this antiserum gave a single band with AgE IV-C.

Precipitin studies

Various allergen extracts were analyzed with antiserum to AgE by micro-Ouchterlony gel diffusion.⁶ Precipitin bands were photographed after incubation in a moist chamber for 24 hours at room temperature.

Dialysis of short ragweed pollen

Two experiments were performed to determine whether low molecular weight AgE was present in ragweed pollen. In the first experiment undefatted short ragweed pollen (5.3 Gm.) was ground in a mortar and pestle, then washed into a Visking No. 27 dialysis casing with 30 ml. of 0.01M K_2HPO_4 - KH_2PO_4 -0.15M NaCl (PBS), pH 7.4. Care was taken to avoid contamination of the ends of the casing with the ragweed slurry. The material was dialyzed for 40 hours at 4° C. against 1,000 ml. of PBS. In a second experiment 1 Gm. of ragweed pollen suspended in 6.6 ml. of PBS was dialyzed for 48 hours at 4° C. against 16.4 ml. of PBS. Aliquots of dialysate and retentate from both experiments were assayed for AgE activity by RIA.

Radioiodination of AgE IV-C

Radioiodination was carried out according to the method of Greenwood and associates.⁷ AgE IV-C (19 μ g) in a volume of 0.01 ml. was added to ^{125}I or ^{131}I (2 mc. in about 0.004 ml.) in a polyvial and buffered at pH 7.4 with 0.5M K_2HPO_4 - KH_2PO_4 (0.03 ml., pH 7.4). After addition of chloramine-T (100 μ g, 0.44 μ moles) in a volume of 0.01 ml., the solution was stirred thoroughly and incubated for 60 seconds at room temperature before addition of sodium metabisulfite (90 μ g, 0.48 μ moles) in a volume of 0.02 ml. After a rapid additional stirring the material in the polyvial was immediately transferred to a Sephadex G-25 column (1.2 $\times$ 15 cm.) equilibrated with 0.1M K_2HPO_4 - KH_2PO_4 buffer (pH 7.4) containing one per cent bovine serum albumin and 0.1 per cent sodium azide (hereafter abbreviated PO₄-alb). The

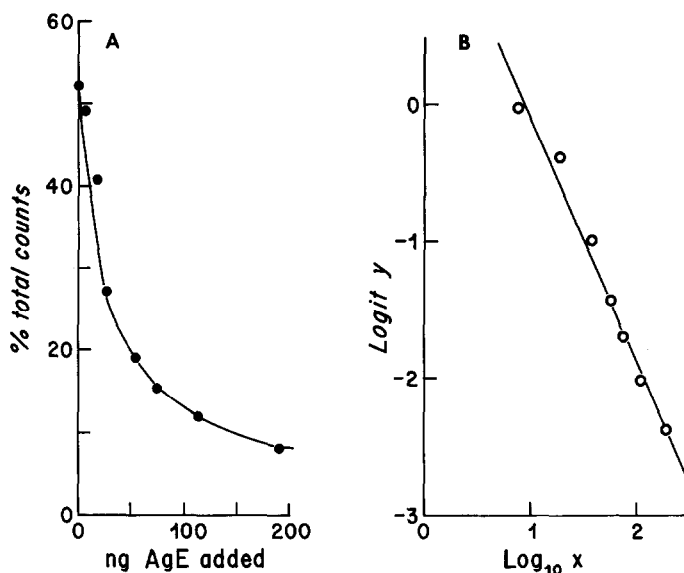


FIG. 1. Measurement of AgE by RIA. **A**, Standard inhibition curve for RIA of AgE IV-C. **B**, Logit transformation of the AgE IV-C standard curve.

radioiodinated AgE IV-C was separated on this column using additional PO_4 -alb as the eluting buffer. Fractions of approximately 1 ml. were collected and the radioactive counts in each tube were monitored. The peak tube from the first peak (void volume) was fractionated on a second column (1.2×15 cm.) of Sephadex G-100, which had also been equilibrated with PO_4 -alb, and the peak tube from the second column was used in the RIA. Between 93 and 98 per cent of the radioactivity was precipitated with 10 per cent tungstic acid.

Performance of the radioimmunoassay

Antiserum to AgE IV-C and material to be tested were diluted in PO_4 -alb. Reagents were added to disposable 10×75 mm. glass tubes in the following order: (1) AgE IV-C standard, or material to be tested, in a volume from 0.02 to 0.5 ml.; (2) PO_4 -alb to bring the resultant volume to 0.5 ml.; (3) rabbit anti-AgE IV-C (0.1 ml.) diluted 1:1,000 in PO_4 -alb; (4) radiolabeled AgE IV-C (approximately 0.3 ng.) in a volume of 0.1 ml. After incubation overnight at 4°C . the following were added: (1) goat antiserum to rabbit IgG (0.15 ml.); (2) a 1:20 dilution of normal rabbit serum (0.15 ml.). The goat antiserum was added in moderate excess as determined by preliminary quantitative precipitin analysis. After additional incubation overnight at 4°C . the tubes were centrifuged at $3,400 \times g$ for 30 minutes at 4°C . Supernatant fluids were decanted, and the tubes were drained. The radioactivity in the precipitate was measured in a gamma scintillation counter. A standard inhibition curve was constructed for each assay covering the range from 7 to 190 ng. of AgE IV-C (Fig. 1, A). From the per cent of radiolabeled AgE IV-C precipitated in the presence of unknown samples, the concentration of AgE was read from the standard curve. When multiplied by the dilution factor, this figure represented AgE content per milliliter of allergen extract. In each assay tubes with radiolabeled AgE IV-C were tested in the absence of anti-AgE to determine the number of counts nonspecifically trapped in the precipitate; these were always less than 3 per cent of the total radioactive counts added. In every assay a material of known AgE concentration was analyzed to determine the precision of the assay. The coefficient of variation between assays was 4.5 per cent; the coefficient of variation of replicate tubes within the assay ranged from 2.0 to 5.1 per cent. When cellulose-ragweed polymers were analyzed, the assay tubes were usually rotated to ensure adequate contact between anti-AgE and the solid phase AgE.

Measurement of AgE activity

Allergy extracts, either 40,000 protein nitrogen units (PNU) per cubic centimeter or 1:10 (food) or 1:20 (pollen) weight/volume, were tested for AgE activity by adding up to 0.2 ml. of the concentrated extract to a RIA tube. If no inhibition was observed with this amount of antigen, the extract was considered to have no AgE activity. If inhibition was demonstrated, various concentrations were assayed in an attempt to construct an inhibition curve for that particular extract. Potent short ragweed extracts were diluted up to 1:1,000 for testing in the assay. In order to investigate parallelism between the inhibition curves of extracts and the curves of the AgE IV-C standard, the data were logit transformed.⁸ Logit transformations were carried out with the aid of the Monroe 1785 calculator. A minimum of four points on the inhibition curve were measured to establish the regression line, and the minimum acceptable logit linear correlation coefficient was arbitrarily set at 0.98. Application of this transformation to an immunoassay is illustrated in Fig. 1, B, with the use of the same data contained in Fig. 1, A. Because the resulting plot is a straight line, with measurable slope, Y-intercept, and standard error, one can statistically analyze differences between inhibition curves. Differences between logit-transformed curves were investigated with the t-test, using the "special d-solution" to the Behren's-Fisher problem as discussed by Scheffé.⁹ Parallelism between inhibition curves is taken as evidence of antigenic identity, while lack of parallelism indicates partial antigenic identity.¹⁰ It is important to emphasize that antigenic *relatedness* and not antigenic *content* is measured by the parallelism of the logit plots. Antigenic *content*, in terms of micrograms per milliliter of AgE content, can be expressed only for those extracts whose logit curves parallel the AgE IV-C standard curve.

RESULTS

Antigen E activity of various allergy extracts

Table I lists the various extracts assayed for AgE activity. Group A extracts showed no inhibitory activity and were considered devoid of AgE activity. Group B extracts showed slight but consistent inhibitory effects, but logit plots of the inhibition curves showed poor linearity. Group C extracts showed strong inhibitory activity, and logit plots of the inhibition curves showed good linearity.

Comparison of AgE activity of various allergy extracts with AgE IV-C

Fig. 2 shows logit plots for four different short ragweed extracts from the same manufacturer. Extract Nos. 1A and 1C were both extracted and diluted in Coca's solution. Extract Nos. 1B and 1D were both extracted and diluted in Coca's solution containing 50 per cent glycerin. Moreover, Extract Nos. 1C and 1D were obtained from the same lot of short ragweed pollen. The parallelism between the inhibition curves produced by these extracts and the AgE IV-C standard indicates that they all possess the same antigenic determinants.

All but one short ragweed extract from the other two manufacturers also exhibited parallelism with the AgE IV-C standard. The single exception was an aqueous preparation assayed one month prior to the expiration date shown on the label. This extract produced an inhibition curve with a lower slope, suggesting that there was a loss of some antigenic determinants in this extract with "aging." Unfortunately, earlier assays on this same extract lot had not been done. Logit plots of inhibition curves for mixed ragweed extracts (equal parts giant and short ragweed) from two different suppliers also exhibited parallelism with the AgE IV-C standard curve.

TABLE I. Antigen E antigenic activity of various allergy extracts.

Group A	Group B	Group C
Western water hemp	Slender ragweed	Short ragweed (All extracts)
Carelessweed	Giant ragweed (Extract	Mixed ragweed (All extracts)
Pigweed	No. 2)	Giant ragweed (All extracts
		but No. 2)
Narrow leaf marsh elder		Prairie sage (Extract Nos.
		3A, 3B, 3C)
Rough marsh elder		Western ragweed (All
Sagebrush		extracts)
Coastal sage		Southern ragweed
Mugwort		False ragweed
Annual wormwood		Burweed marsh elder
Lambs quarters		Cocklebur
Kochia		
Russian thistle		
English plantain		
Prairie sage (Extract Nos.		
1A and 1B)		
Elm		
Oak		
Black locust		
Hormodendrum		
Alternaria		
Pyrethrum		
House dust		
Dog epithelium		
Cat epithelium		
Mixed feathers		
Banana*		
Watermelon*		
Cantaloupe*		

Group A extracts showed no inhibitory effect at 8,000 PNU in the RIA. Group B extracts showed slight inhibitory activity with poorly linear logit plots. Group C extracts showed strong inhibitory activity with good linear logit plots, although they did not necessarily parallel the AgE IV-C standard curve.

*0.2 ml. of a 1:10 extract.

In Fig. 3 logit plots of inhibition curves for extracts of giant ragweed are shown. Extract Nos. 1A and 1B were, respectively, aqueous and glycerinated extracts from the same company, while Extract No. 3 was from a second company. All three preparations produced inhibition curves with slopes differing from that of AgE IV-C, but no difference in slopes among these three preparations was detected.

In Fig. 4 logit plots for three other aqueous ragweed extracts are shown. Two different lots of western ragweed from the same company (only one of which is illustrated) yielded inhibition curves which were not significantly different from that of AgE IV-C. Western ragweed extracts from other suppliers were not examined. Southern ragweed and false ragweed, though active, had inhibition curves with lower slopes and were clearly antigenically distinct from AgE IV-C.

In Fig. 5 logit plots obtained with other aqueous extracts from Compositae family pollens are shown. Both burweed marsh elder and the two prairie sage extracts gave inhibition curves which were not significantly different from that of AgE IV-C, while the slope of the curve with cocklebur was markedly lower.

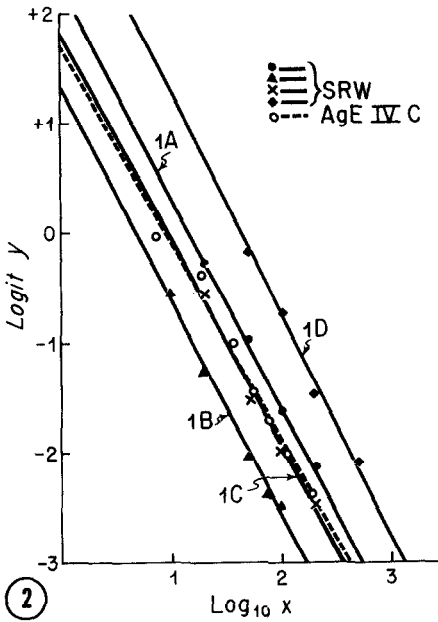


FIG. 2. Logit transformation of inhibition curves for four different short ragweed (SRW) extracts.

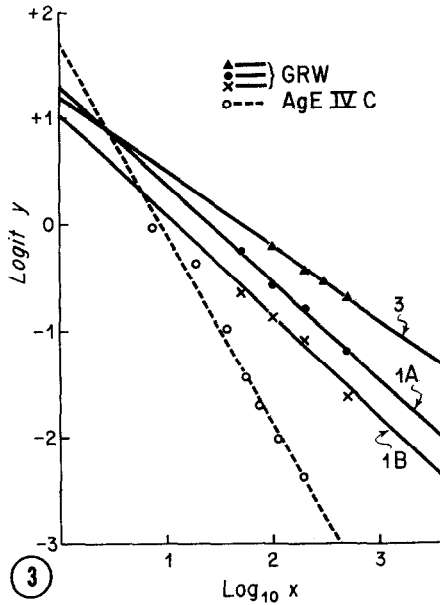


FIG. 3. Logit transformation of inhibition curves for three different giant ragweed (GRW) extracts.

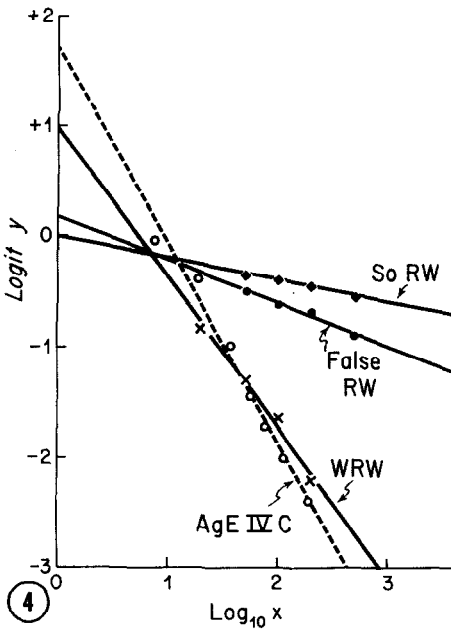


FIG. 4. Logit transformation of inhibition curves for southern ragweed (So RW), false ragweed, and western ragweed (WRW).

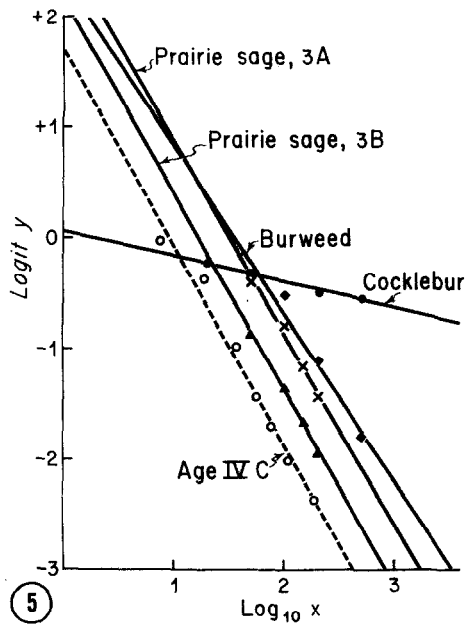


FIG. 5. Logit transformation of inhibition curves for other Compositae family pollen extracts.

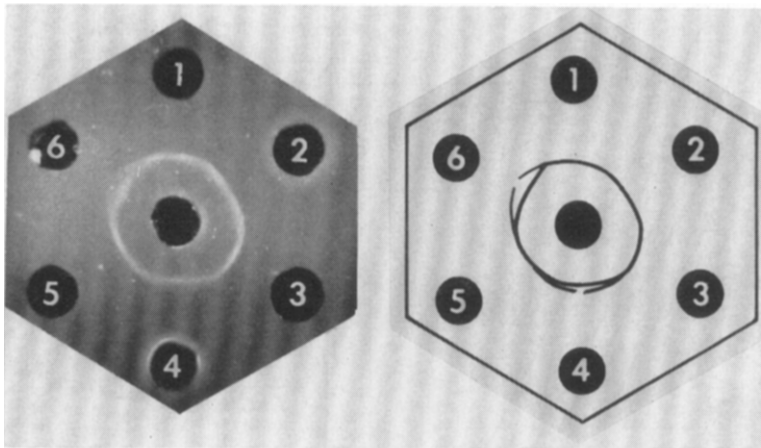


FIG. 6. Gel diffusion analysis of ragweed antigens. Rabbit antiserum (R2) to AgE IV-C is in the center well. AgE IV-C, 190 μ g per milliliter, is in Wells 1, 3, and 5. Well 2 contains 40,000 PNU per cubic centimeter short ragweed extract (glycerinated). Well 4 contains giant ragweed extract (glycerinated), 40,000 PNU per cubic centimeter. Well 6 contains giant ragweed extract (aqueous), 40,000 PNU per cubic centimeter. The photograph of the original agar slide is on the left and a drawing of it on the right.

Other marsh elder extracts from the same manufacturer failed to show any AgE activity; burweed marsh elder extracts from other suppliers were not examined. Although not shown in Fig. 5, a third aqueous prairie sage lot from the same manufacturer also produced an inhibition curve which paralleled that of AgE IV-C. Both an aqueous and a glycerinated prairie sage extract from another supplier failed to show any AgE activity.

Analysis of extracts by immunodiffusion

AgE activity in the various extracts was also compared by Ouchterlony gel diffusion for those materials containing sufficient AgE to produce a visible precipitin band. All of the short ragweed and mixed ragweed extracts produced bands with the same rabbit antiserum used in the RIA, and all of these fused in reactions of identity. The reactions between giant and short ragweed extracts and antiserum to AgE are shown in Fig. 6. The band formed by AgE from short ragweed gave a reaction of identity with AgE IV-C, while the AgE IV-C bands gave reactions of partial identity with the bands produced by giant ragweed. This confirmed the antigenic cross-reactivity between AgE IV-C and giant ragweed extract found by RIA. Extracts of southern ragweed and false ragweed failed to produce precipitin bands.

Quantitation of AgE in various allergy extracts

The AgE content of various concentrated (40,000 PNU per cubic centimeter) allergy extracts is listed in Table II for those extracts whose inhibition curves paralleled the AgE IV-C standard curve. A glycerin extract (No. 1D in Table II) showed 2 to 3 times the AgE content of an aqueous extract (No. 1C in Table

TABLE II. Antigen E content of various allergy extracts

Extract	Aqueous (A) or glycerinated (G)	AgE potency
Short ragweed		
No. 1A	A	80 μ g/ml.
No. 1B	G	185 μ g/ml.
No. 1C	A	109 μ g/ml.
No. 1D	G	275 μ g/ml.
No. 2	A	195 μ g/ml.
No. 3B	A	106 μ g/ml.
Mixed ragweed		
No. 2A	A	185 μ g/ml.
No. 2B	A	212 μ g/ml.
No. 3A	A	40 μ g/ml.
No. 3B	A	100 μ g/ml.
Prairie sage		
No. 3A	A	300 ng./ml.
No. 3B	A	550 ng./ml.
No. 3C	A	160 ng./ml.
Western ragweed		
No. 3A	A	800 ng./ml.
No. 3B	A	4,600 ng./ml.
Burweed marsh elder	A	205 ng./ml.

The extracts listed had inhibition curves paralleling that of the AgE IV-C standard curve.

II) from the same lot of short ragweed pollen. However, the difference between these extracts might have reflected differences in extraction procedures. Ragweed pollen is extracted by this company with aqueous media for 3 days at 4° C. with periodic shaking by hand, while with 50 per cent glycerin pollen is extracted for 7 days at 20° C. Because these variables might have altered the quantity of AgE extracted, we compared the quantities of AgE obtained from the same lot of ragweed pollen under the same conditions. Two aliquots of 0.5 Gm. of pollen were washed 5 times with 5 ml. of ethyl ether, dried, and extracted for 26 hours at 23° C. with 20 ml. of either Coca's solution or Coca's solution containing 50 per cent glycerin.¹¹ Supernatants of these suspensions were analyzed by RIA for AgE activity. The 50 per cent glycerin extract contained 130 μ g per milliliter, while the aqueous extract contained 71 μ g per milliliter. Control assays of glycerin alone (up to 0.5 ml. of a 50 per cent solution) did not produce inhibition, showing that glycerin by itself did not interfere with the RIA.

There was a two- to fivefold difference in AgE content among the mixed ragweed extracts of comparable PNU per cubic centimeter strength. Short ragweed extracts varied similarly by a two- to threefold factor. The non-short ragweed extracts listed in Table II were 100 to 1,000 times less potent in AgE content than extracts of short ragweed.

Stability of AgE at various temperatures

A partially purified concentrated extract of short ragweed containing 2.3 mg. per milliliter of AgE prepared in our laboratory⁵ was frozen and thawed up to 10 times; aliquots assayed after 5, 7, and 10 freeze-thawings showed no change in AgE potency as compared to the nonfrozen preparation. In a second experiment a vial of commercial aqueous mixed ragweed extract (equal parts giant and short ragweed), 40,000 PNU per cubic centimeter, was stored at 4° C.

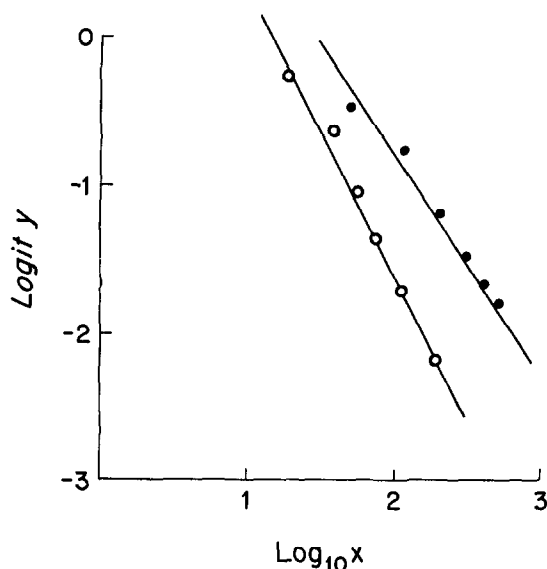


FIG. 7. Comparison of AgE IV-C and cellulose-ragweed extract. The open circles designate the AgE IV-C points, while the dots show the points with the cellulose-ragweed extract. The slopes of the two lines differ ($p < 0.01$) by the Scheffé test.

for several months. Aliquots were periodically withdrawn and frozen at -70°C . and later analyzed for AgE in the same RIA. Aliquots withdrawn at 15 months, 12 months, 11 months, and 10 months prior to the expiration date shown on the label showed no change in AgE activity and had virtually identical AgE content. Thus, there was no change in either the AgE determinants or in AgE content under these conditions of storage.

Dialysis of short ragweed pollen

To determine whether low molecular weight AgE material was present in short ragweed, slurries of pollen were dialyzed, and the dialysate tested for AgE activity. In the first experiment (5.3 Gm. of pollen in 30 ml. of PBS dialyzed against 1,000 ml. of PBS) the AgE content of the material inside the dialysis casing was $440\text{ }\mu\text{g}$ per milliliter, and no AgE activity was found in the dialysate. In the second experiment (1.0 Gm. of pollen in 6.6 ml. of PBS dialyzed against 16.4 ml. of PBS) the AgE content of the material inside the casing was $415\text{ }\mu\text{g}$ per milliliter, while no AgE was detected in 0.5 ml. of the dialysate. Thus, the quantity of dialyzable AgE was less than 15 ng. per milliliter, or less than 246 ng. per gram of pollen.

Cellulose-ragweed polymers

Solid-phase ragweed extract was analyzed on several occasions, and inhibition curves with slopes approximating those obtained with AgE IV-C were found. In one such experiment with the more highly purified AgE, containing 50 per cent AgE by weight, the slopes of the regression lines were not significantly different by the Scheffé test. In two other experiments with ragweed ex-

tract purified by gel filtration on Sephadex G-25 alone, a difference between the slopes of the polymer and AgE IV-C was found in one experiment, but not in the second. In Fig. 7 the results of the experiment in which the slopes differed are shown. Although the slopes of the regression lines differ statistically ($p < 0.01$ by the Scheffé test), the difference is slight.

DISCUSSION

In this study we have described a radioimmunoassay for AgE, the major antigenic material from short ragweed pollen.^{1,2} Although AgE is present in ragweed pollen in sufficient quantity to be measured by radial immunodiffusion,⁴ nonetheless RIA offers several advantages. Among these are the need for only microgram quantities of purified AgE and for very small amounts of antiserum. The sensitivity of the assay permits the detection of nanogram quantities of AgE activity and in this study enabled us to detect activity in extracts of pollen usually considered unrelated to short ragweed. By logit transformation of inhibition curves the antigenic relationships of the various active materials could be compared, even though some of these extracts did not form precipitin bands in agar gel diffusion with antiserum to AgE. Finally, the AgE activity associated with solid phase materials, as used in the radioallergosorbent test, could be estimated.⁵

Our results indicate that short ragweed preparations from various manufacturers, both aqueous and glycerinated, possess AgE activity that is immunologically identical to AgE IV-C; this AgE activity in concentrated extracts seems to be quite stable when stored at 4° C. However, analysis of one short ragweed extract one month prior to the expiration date revealed a loss of reactivity suggesting that aging may alter AgE antigenic determinants in some instances. There were wide quantitative differences in AgE content between various extracts related both to different manufacturers and even different lot numbers from the same manufacturer, a finding previously reported by Baer and associates.⁴ These variations in the quantity of AgE might be due to differences in extraction procedures. Extracts of ragweed pollen may be prepared by various means. The defatted pollen may be extracted in Coca's solution, followed by addition of an equal volume of glycerin to produce a "50 per cent glycerin extract," or the defatted pollen may be extracted directly with 50 per cent glycerin solution. An extract destined for lyophilization may be produced by extraction in distilled water alone. Our results indicate that extraction with 50 per cent glycerin increases the quantity of AgE in the extraction fluid by a factor of two- to threefold without altering the AgE antigenic determinants, a finding with obvious implications for the various suppliers of allergy extracts.

Analysis of antigenic relationships between AgE IV-C and AgE activity in different extracts revealed that extracts of short ragweed pollen and of mixed ragweed pollen (giant and short ragweed) contained AgE which was not distinguishable immunologically from AgE IV-C. In contrast the AgE activity in extracts of giant ragweed yielded inhibition curves with lower slopes than AgE IV-C, and this cross-reactivity was confirmed by Ouchterlony analysis. AgE activity detected in southern ragweed (*Ambrosia bidentata*) and false rag-

weed (*Franseria acanthicarpa*) also differed from AgE IV-C. On the other hand, the finding of AgE activity in these different genera suggests a botanical relationship between them, a suggestion previously made by Payne,¹² who proposed that the Ambrosia members may have evolved from the franseroid complex of weeds and suggested a new nomenclature for these plants. Finally, certain lots of extracts of western ragweed, burweed marsh elder, and prairie sage contained trace quantities of AgE activity which produced inhibition curves parallel to AgE IV-C. This was not a consistent finding in all lots and suggests contamination of some of these extracts by trace amounts of short ragweed. Nonetheless these quantities of AgE could produce positive wheal and flare skin reactions in sensitive patients and result in the patient being considered allergic to the designated extract, when in fact he is reacting to the trace quantities of AgE.

Some ragweed-sensitive patients have been reported to show intolerance to banana, melon, and cantaloupe, reacting with oral puritis. Anderson and associates¹³ have demonstrated a common antigen between cantaloupe and ragweed by passive cutaneous anaphylaxis in the guinea pig. However, the lack of inhibition shown by these food extracts in RIA suggests that any common antigen present is not AgE.

Our findings indicate that no low molecular weight, dialyzable substance is present in short ragweed extracts that contains antigenic determinants in common with AgE and which are recognized by our antiserum. Feigen and associates¹⁴ have obtained a dialyzable low molecular weight antigen from short ragweed pollen (*Ambrosia elatior*) which inhibited the anaphylactic reaction of passively sensitized guinea pig hearts on challenge with crude aqueous nondialyzable ragweed extracts. Our results suggest that any such dialyzable antigen is not likely AgE. Our results also stand in contrast to the situation with grass pollen extracts, in which a low molecular weight, dialyzable allergen has been found¹⁵ which behaves as a hapten in precipitation-inhibition experiments.

Finally, the RIA described here has permitted the detection of AgE activity in cellulose-ragweed polymers. Our experiments suggest that AgE determinants are largely preserved following coupling, although in one experiment a significant difference in the slope of the regression line was detected. It is possible that certain antigenic determinants are denatured following coupling to cellulose, although it is clear that this occurs to a relatively slight extent, if at all.

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