

## ANTIGENS OF AMBROSIA ELATIOR (SHORT RAGWEED) POLLEN

### I. Crossed Immunoelectrophoretic Analyses<sup>1</sup>

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A crude extract of short ragweed pollen was obtained by extraction, centrifugation, dialysis and freeze-drying. Crossed immunoelectrophoresis, with a high-titer purified rabbit antibody fraction, revealed that the extract contained at least 52 antigens, of which 33 migrated toward the anode, 16 toward the cathode, and 3 toward both the anode and cathode. The electrophoretic precipitation pattern indicated a great deal of electrophoretic heterogeneity in several of the antigens. The overall distribution of apparent m.w. for 35 of the 52 antigens and the approximate m.w. for 19 identified antigens were determined by a combination of gel filtration and immunochemical analysis. Among antigens with m.w. weights less than or equal to 10,000, 8 of 10 were basic; on the other hand, all 5 identified antigens with m.w. above 40,000 were acidic. The apparent pI distribution for 30 antigens, and more precise pI values for 20 of 52 antigens, were determined by means of sucrose-gradient isoelectric focusing. The data obtained provide a reproducible reference system of considerable use in ragweed allergen chemistry and enable one to predict conditions for isolation of ragweed antigens.

The pollen of short ragweed (*Ambrosia elatior*) is one of the most important allergen sources in North America and much effort has been employed in identifying and characterizing the antigenic components that commonly act as allergens in man. The antigenic composition of ragweed pollen components was first investigated by Wodehouse in 1954 (1) using double-diffusion analysis against rabbit antiserum (see also Richter and Sehon, (2)). Subsequently, Gussoni in 1966 (3) showed that there are at least 14 antigens in short ragweed pollen extract by immunoelectrophoretic analysis. Recently, isoelectric focusing has revealed more than 20 proteins (4), including several that are known to be closely related antigenically.

Several highly purified ragweed antigens, AgE,<sup>3</sup> AgK, Ra3, BPA-R (Ra4), and Ra5, are now established as being allergenic in humans (4–10) and, recently, a further allergen, Ra6, has been isolated (11). Antigen E, in particular, is a potent immunogen both in terms of its ability to stimulate IgG antibody production in rabbits and IgE (as well as IgG) antibody response in man (5).

Immunodiffusion, immunoelectrophoresis, and isoelectric focusing have limited resolving power, and it seemed probable that many additional antigenic components exist in ragweed pollen extract, several of which might prove to be important allergens. Quantitative immunoelectrophoretic (QIE) techniques, particularly crossed immunoelectrophoresis (CIE), has already proved to be very useful in the characterization of antigens in complex allergen extracts, as recently reviewed by Löwenstein (12). Subsequently, crossed radioimmunoelectrophoresis (CRIE) has been used to identify the important allergens in these systems.

It is, therefore, the aim of the present series of studies to introduce QIE techniques into ragweed immunochemistry in order to extend the antigenic analysis of short ragweed pollen extract. We have also estimated the m.w. and isoelectric points of the major antigenic components. Subsequently, we have identified within our CIE reference pattern all of the characterized allergens (Löwenstein *et al.*, in preparation). Finally, we have utilized CRIE to investigate which of the various ragweed antigens commonly act as allergens in allergic humans (Löwenstein and Marsh, in preparation), and have analyzed genetic association between IgE response to specific antigens and HLA and total IgE phenotypes (Marsh *et al.*, in preparation).

#### MATERIALS AND METHODS

**Antigens.** Short ragweed (*A. elatior*) pollen samples were obtained from Greer Laboratories, Lenoir, NC as dried nondefatted materials, which had been stored at 4°C in tightly sealed containers. The samples were delivered to our laboratory a few weeks after collection and stored in their original containers at –20° until used. According to the manufacturer, the samples were greater than 95% pure short ragweed pollen grains plus ca. 4% nonpollen fragments of short ragweed.

Dialyzed freeze-dried extracts from 1976 and 1978 collections of this pollen, denoted Am.e.76 and Am.e.78, were prepared by a procedure similar to those previously described for grass pollens (13, 14). Briefly, the method consisted of extracting the pollen (1:10 w/v) at 5°C for 20 hr with a buffer solution (0.125 M  $\text{NH}_4\text{HCO}_3$  and 0.015 M  $\text{NaN}_3$ , adjusted to pH 7.5), centrifugation (50,000 × G, 1 hr, 0°C), dialysis (3 times, 24 hr, 5°C, using Union Carbide (Visking) dialysis tubing with limiting pore size corresponding to approximate m.w. <5000), and freeze-drying. Solutions (20 mg freeze-dried material/ml) were freshly prepared in 0.15 M sodium chloride/glycerol (50%, v/v) for use in QIE techniques, or in 0.1 M sodium bicarbonate, 0.015 M sodium azide solution for immunization of rabbits. Analysis of Am.e.76 by rocket electrophoresis and radial immunodiffusion<sup>4</sup> showed a content of 23.9 µg AgE, 7.5 µg Ra3, and 2.5 µg Ra5/mg freeze-dried material.

**Antibodies.** Anti-Am.e.76 antibodies were raised by immunizing 3 rabbits with Am.e.76. The immunization and subsequent isolation of the immunoglobulins were performed according to Harboe and Ingild (Reference 15, pp. 161–164). During this isolation the concentration of the immunoglobulins was increased about 5-fold. Anti-Am.e.78 was produced in a similar way. The concentration of the specific antibodies was about 1 to 3 mg/ml, being one-tenth of the total IgG concentration. The antibodies were stored at 4°C in a solution containing 0.015 M sodium azide and 1% (v/v) Aprotinin (Novo, Mainz, Federal Republic of Germany) to avoid proteolytic degradation of the antigens in the second dimension gel immunoelectrophoresis (16).

**Quantitative immunoelectrophoresis.** QIE methods (15), including crossed, crossed-line, and fused-rocket immunoelectrophoresis, were per-

<sup>4</sup> Marsh, D. G., L. Belin, L. M. Lichtenstein, C. A. Bruce, and R. Hussain. 1980. Rapidly released allergens from short ragweed pollen: I. Kinetics of release of known allergens in relation to biological activity. Submitted.

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<sup>3</sup> Abbreviations used in this paper: Ag, antigen; Am.e.76 and Am.e.78, 1976 and 1978 collections of the pollen of *Ambrosia elatior* (short ragweed); QIE, quantitative immunoelectrophoresis; CRIE, crossed radioimmunoelectrophoresis; CLIE, crossed-line immunoelectrophoresis.

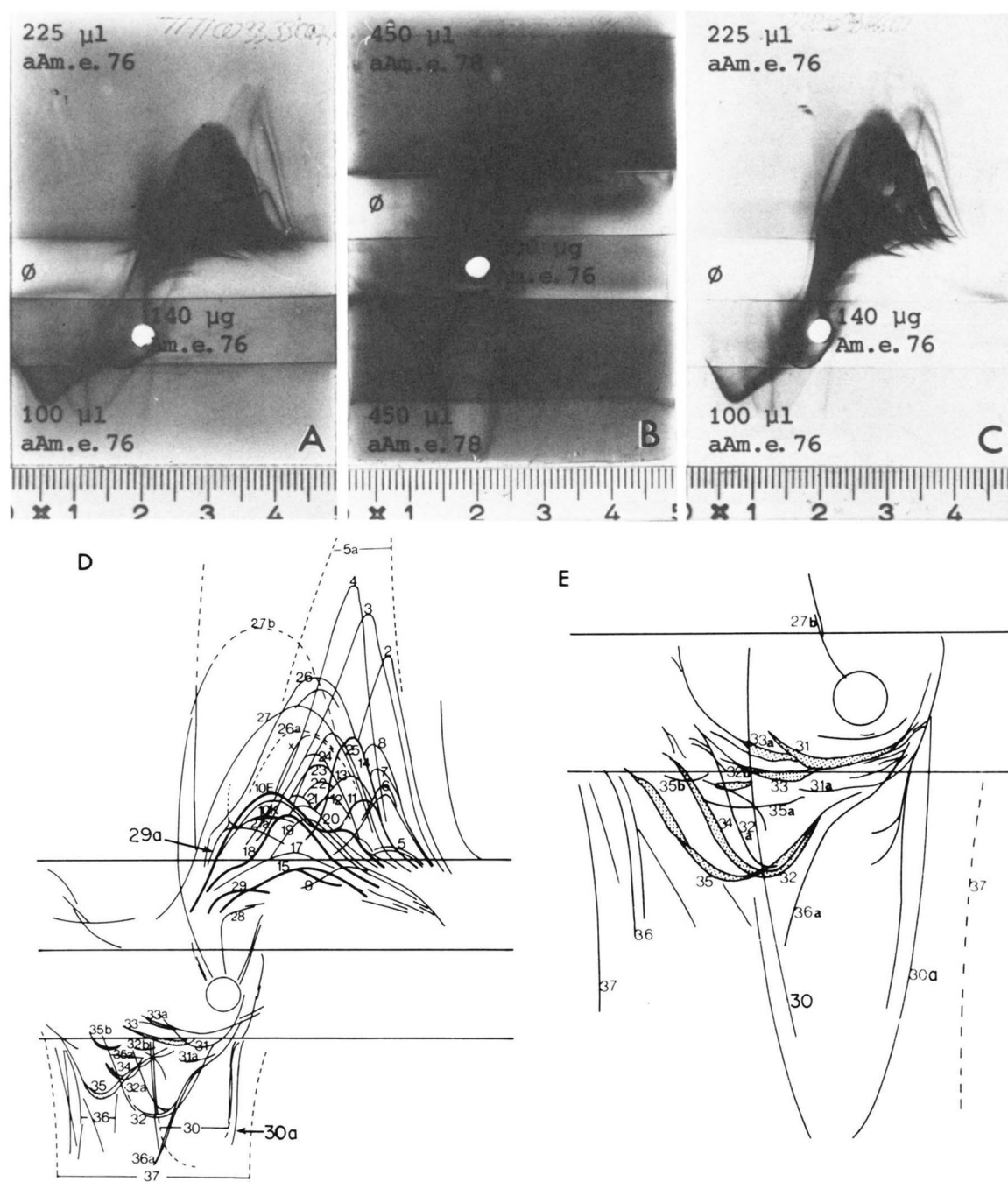


Figure 1. Crossed immunoelectrophoretic (CIE) analysis of the reference ragweed pollen extract, Am.e.76, against anti-Am.e.76 and anti-Am.e.78. CIE was performed in 1% agarose gel containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate, and 0.0003 M sodium azide (pH 8.6, 25°C) at 15°C using 10 V/cm for 30 min in the 1st dimension and 2 V/cm for 15 hr in the 2nd dimension (12). Anodes were to the right for the 1st dimension and at the top for the 2nd dimension. An intermediate gel containing no antigen or antibodies was placed between the 1st dimension gel and the anodic antibody-containing gel in order to improve resolution in the 2nd dimension. A, B, and C, amounts and types of antigens and antibodies are indicated on the figures. Figure B is overexposed in order to reveal the maximum number of basic precipitates. D, drawing of the reference precipitation pattern (Am.e.76 vs. antiAm.e.76) with classification numbers for the precipitates and corresponding antigens. E, expanded drawing of the precipitated cathodic-moving antigens.

formed as described recently (12) and as specified in the figures. In brief, crossed immunoelectrophoresis (CIE) was performed as follows: electrophoresis of ragweed extract was carried out in agarose gel at pH 8.6, followed by a 2nd dimension electrophoresis into a gel containing purified IgG fractions of anti-ragweed antibodies. For individual antigens, the sensitivity of the method is about 10 to 100 ng/ml with a coefficient of variation  $\pm 3$  to 8% (15).

Crossed-line immunoelectrophoresis (CLIE) was performed in order to identify the antigens present in a purified fraction relative to the reference crossed immunoelectrophoretic precipitation pattern. The procedure was like CIE except that, in the 2nd dimension run, a gel containing the purified fraction was introduced between the first dimension gel and the antibody-containing gel. Fused line- and crossed-immunoprecipitates were then compared with the reference immunoprecipitates.

Fused rocket immunoelectrophoresis was performed in order to monitor the column fractionations used to determine the isoelectric points and m.w. of ragweed antigens. Rows of wells were punched in a 2-cm strip of gel on a glass plate and antibody-containing gel was poured onto the free parts of the plate. Aliquots from the fractionation experiments were transferred to the sample wells in the order they were collected. After allowing the antigens to diffuse into the gels (1 hr), electrophoresis was performed, and the resultant fused rockets gave the immunochemical profile of the eluted fractions.

**Molecular weights and *pI* determinations.** Molecular weight determinations and associated immunochemical analyses of ragweed pollen antigens were performed essentially as described previously (17). A Sephadex G-75 column (70 cm  $\times$  2 cm<sup>2</sup>), flow rate 4 ml/hr, was equilibrated and run at 20°C by using a buffer solution consisting of 0.05 M sodium phosphate,

0.5 M sodium chloride, 0.015 M sodium azide, ionic strength 0.615, pH 7.2. It was, first, calibrated with 1.0 ml of a solution containing 1 mg of each of the following marker proteins:  $\alpha$ -chymotrypsinogen, myoglobin, cytochrome c, and 100  $\mu$ l human serum (containing the IgG, haptoglobin, serum albumin and  $\alpha_1$ -antitrypsin markers), and 0.5 mg blue dextran. The elution volumes for the marker proteins and blue dextran were determined by UV absorption, and for the human serum proteins by fused rocket immunoelectrophoresis with monospecific antibodies against IgG, haptoglobin, albumin and  $\alpha_1$ -antitrypsin (Dako, Copenhagen, Denmark). A solution of Am.e.76 (10 mg in 1 ml) was then fractionated on the column, and an immunochemical elution profile of the ragweed pollen antigens was obtained by means of fused rocket immunoelectrophoresis. Individual antigens (designated in the reference pattern shown in Figs. 1D and 1E) were identified by using CLIE with Am.e.76 as the reference antigenic mixture.

Determination of the pI values of ragweed pollen antigens was performed essentially as described previously (17, 18). Samples containing 10 mg Am.e.76 were subjected to isoelectric focusing with an Ampholine 8101 column (LKB, Sweden). An ampholyte pH gradient, 3.5 to 10.0, was distributed in a sucrose gradient (0 to 50%, w/v) at 15°C over a period of 40 hr, by using a potential of 200 V at 2.2 mA (falling to 1.0 mA). The pH values of the eluted fractions were measured and the pI values corresponding to antigens in the immunochemical elution profile of the fractions were determined by fused rocket immunoelectrophoresis and, finally, the individual antigens were identified by using CLIE.

## RESULTS

Figure 1A and B shows examples of the CIE reference precipitation patterns obtained with water-extractable, nondialyzable short ragweed pollen preparation Am.e.76 against anti-Am.e.76 and anti-Am.e.78. The 2 polyspecific antibody pools were judged to be similar in terms of their ability to precipitate the same antigens in similar proportions. Appropriate separation of the antigens was obtained by varying the time and position of the application well for electrophoresis in the 1st dimension, and optimal antigen-antibody balance was attained by varying the proportions of the reactants. By fixing and staining the 1st dimension gel (15), it was confirmed that all protein-staining components were included in the portion of the gel transferred to the 2nd dimension run. Very weak precipitates were detected either by increasing the amounts of antigen and antibody and over-exposing negative of the resultant stained plate (Fig. 1B) or after performance of CRIE (Løwenstein and Marsh, in preparation), and are indicated in the drawings (Fig. 1D and E) by dashed lines. Attempts to induce all the antigens to migrate in the same direction by selecting an agarose gel having a higher degree of electroendosmosis or by changing the mobility of the antibodies (e.g., by carbamylation) did not succeed. The reproducibility of the CIE reference precipitation patterns is illustrated by comparing Figure 1A with C, which was performed 1 yr later.

The analysis revealed a total of 52 antigens (Fig. 1D and E, the antigens include several designated as "5a", etc.) of which 33 (Ag 1-27b) moved toward the anode and 16 (Ag 30-37) toward the cathode; 3 antigens (Ag 28, 29, 29a) moved toward both the anode and the cathode. The electrophoretic mobilities of some of the antigens relative to Ag 10E (main peak) are given in Table I.

The precipitation patterns for certain antigens (e.g. Nos. 10E and 30a) were asymmetric, suggesting electrophoretic heterogeneity. Certain poorly defined precipitation lines were seen around the application well for antigens having isoelectric points near pH 8.6. The precipitates of Ag 10E, 10K, 11, 13, 14, 15, 18, 24, and 31 were very dense and well stained. In contrast, Ag 1, 5a, 26a, X, 27a, 29a, 33, 34, 36, and 37 were only faintly stained with Coomassie Brilliant Blue or were only visible after performance of CRIE. The clearance of the protein stain within the area enclosed by the precipitate of Ag 30a, especially noticeable in Figure 1B, is probably due to the proteolytic activity of this antigen.

The physicochemical analyses of ragweed pollen antigens by gel filtration and isoelectric focusing were performed on the crude mixture (Am.e.76), and the antigenic compositions of the individual fractions were evaluated mainly by CLIE. The approximate m.w. were calculated on the basis of a calibration curve by using marker proteins, where m.w. were plotted against their corresponding  $K_{av}$  values (Table I). About 35 distinctly visible precipitates were obtained by fused rocket immunoelectrophoresis, of which 19 could be identified in the CIE reference pattern and characterized in terms of apparent m.w. Most of the antigens, including 6 which

migrated toward the cathode, were eluted with  $K_{av}$  values corresponding to m.w. between 10,000 and 40,000. Of the antigens having m.w. less than or equal to 10,000 8 of 10 migrated toward the cathode. Four of these low m.w. components, Ag 27, 32, 36, and 37, could be identified in the CIE reference pattern (Table I and Fig. 1D). (It should be noted that unequivocal identification of low m.w. components proved to be difficult because of their high rates of diffusion in the gels.) Five antigens having m.w. over 40,000 were identified as acidic Ag 2, 6, 15, 20, and 28 in the CIE pattern.

The immunochemical analyses of fractions from the Sephadex G-75 gel filtration column are shown in Figure 2A and examples of antigen identification are given in Figure 2B-E. The irregularities of the precipitation peaks in Figure 2A are caused partly by the discontinuity in the sample application, and are more pronounced for antigens of higher m.w. It is, therefore, not possible to evaluate size heterogeneity from these patterns. Figure 2B and C illustrates the identification by using CLIE of Ag 32 and 36 in fraction No. 54 as line precipitates fused with curved precipitates. Figure 2D and E illustrates the indirect identification of Ag 27 in fraction No. 55 by absorption *in situ* in CLIE. Among antigens with m.w. below 10,000, 8 were basic and only 2 were acidic; whereas all the antigens with m.w. above 40,000 (at least 6) were acidic. The large number of antigens made it difficult to recognize any partial identity between antigens, or any protein-protein associations, especially for molecules above 20,000 daltons. The only situation

TABLE I  
Physicochemical characterization of ragweed pollen antigens

| Antigen No. | Migration <sup>a</sup> | Approximate m.w. (daltons $\times 10^{-3}$ ) <sup>b</sup> | pI <sup>c</sup>            |
|-------------|------------------------|-----------------------------------------------------------|----------------------------|
| 2           | 3.4                    | 43                                                        |                            |
| 3           | 2.9                    |                                                           | 4.9                        |
| 4           | 2.7                    |                                                           | 4.8                        |
| 5           | 3.4                    |                                                           | 4.6                        |
| 5a          | 2.9                    |                                                           |                            |
| 6           | 3.3                    | 41                                                        | 4.5                        |
| 7           | 3.1                    |                                                           |                            |
| 8           | 3.1                    |                                                           |                            |
| 9           | 2.6                    |                                                           | 4.5                        |
| 10E         | 1.0 and 2.5            | 33 and 18 <sup>d</sup>                                    | 5.0, 5.8, and 4.4          |
| 10K         | 1.2                    | 33 and 18 <sup>d</sup>                                    | 5.8 and 5.0                |
| 11          | 2.9                    | 33 and 18 <sup>d</sup>                                    | 4.3                        |
| 12          | 2.2                    |                                                           |                            |
| 13          | 2.6                    | 34                                                        | 4.8                        |
| 14          | 2.7                    |                                                           | 4.6                        |
| 15          | 1.7                    | 43                                                        | 4.6                        |
| 17          | 1.5                    |                                                           |                            |
| 18          | 1.2                    | 26                                                        | 5.4                        |
| 19          | 1.7                    |                                                           |                            |
| 20          | 1.7                    | 58                                                        | 4.5                        |
| 21          | 1.6                    |                                                           |                            |
| 22          | 2.1                    |                                                           |                            |
| 23          | 2.0                    |                                                           |                            |
| 24          | 1.9                    |                                                           |                            |
| 25          | 2.2                    |                                                           |                            |
| 26          | 1.9                    | 26                                                        | 4.6                        |
| 26a         | 1.6                    |                                                           |                            |
| X           | 1.8                    | 26                                                        | 4.6                        |
| 27          | 1.6                    | <10                                                       | ca. 3.0, 5.0, 5.8, and 6.7 |
| 27a         | 0.9                    |                                                           |                            |
| 28          |                        | 58                                                        |                            |
| 29          | 0.3                    |                                                           |                            |
| 29a         | 0.0                    |                                                           |                            |
| 30          | -0.6                   | 26                                                        | 9.5                        |
| 31          | -0.5                   | 17                                                        | 7.7                        |
| 32          | -1.4                   | 10                                                        | 8.9                        |
| 33          |                        |                                                           |                            |
| 34          | -2.1                   |                                                           |                            |
| 35          | -2.5                   | 17                                                        | 8.8                        |
| 36          | -2.5                   | <10                                                       |                            |
| 37          | -1.4                   | <10                                                       |                            |

<sup>a</sup> The migration velocity relative to that of the main component of Ag 10E in 1% (w/v) agarose gel.

<sup>b</sup> Determined by Sephadex G75 gel filtration by using purified proteins of known m.w. as references; see text.

<sup>c</sup> Determined by column isoelectric focusing in a sucrose gradient. Values corresponding to the main fraction are italicized.

<sup>d</sup> Possibly the lower m.w. fractions correspond to break down products (Løwenstein *et al.*, in preparation).

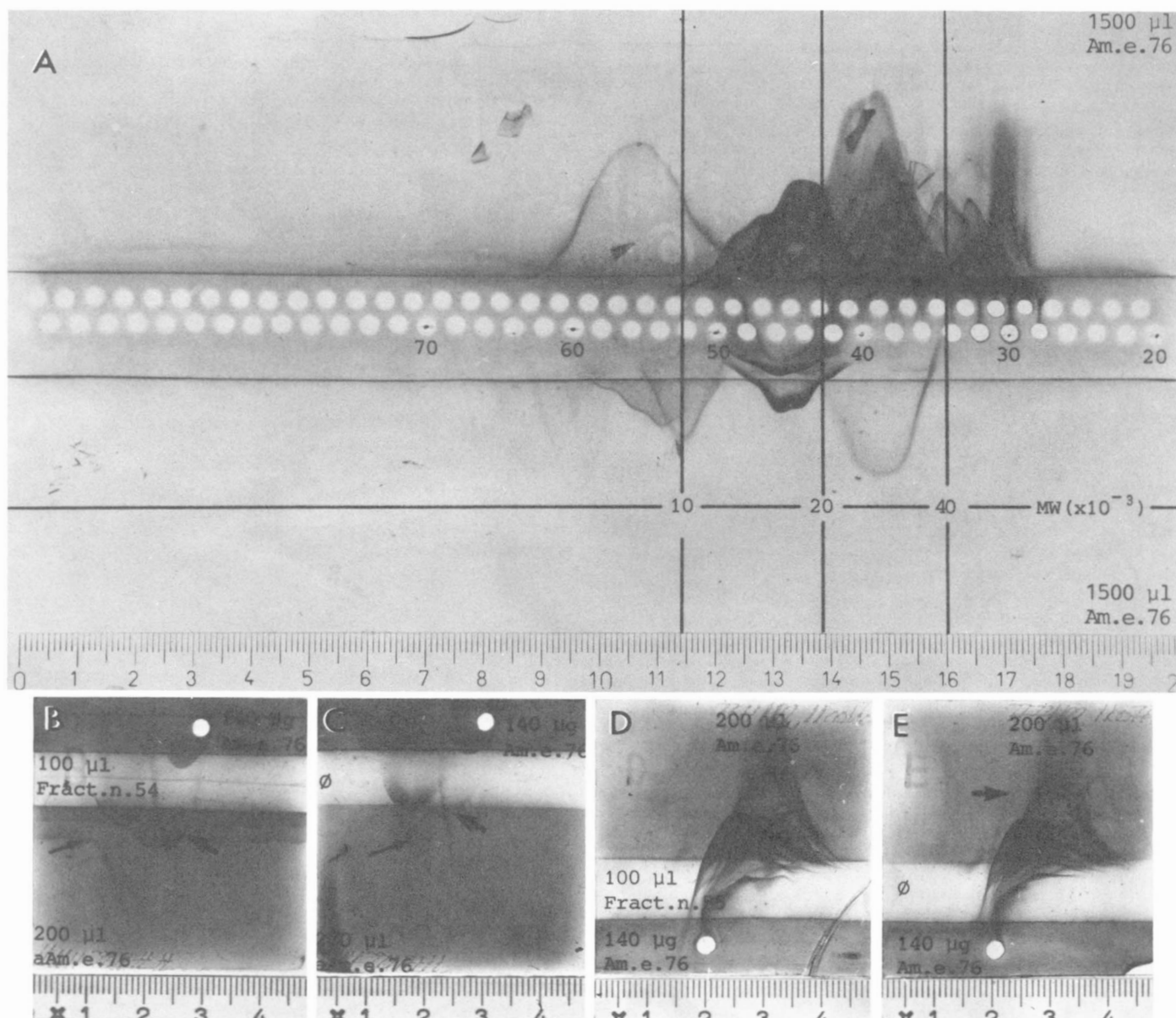


Figure 2. Quantitative immunoelectrophoretic (QIE) analysis of fractions of Am.e.76 (against anti-Am.e.76, 13  $\mu\text{l}/\text{cm}^2$ ) after chromatography on Sephadex G75. A, aliquots (7  $\mu\text{l}$ ) from the numbered column fractions were applied in adjacent wells and allowed to diffuse (1 hr) before being subjected to fused-rocket immunoelectrophoresis (15 hr at 2 V/cm) into gels containing anti-Am.e.76. Approximately m.w. ( $\times 10^{-3}$ ) of the antigens are indicated. B, CLIE: fraction No. 54 was incorporated into the intermediate gel between 1st dimension gel and the cathodic antibody-containing gel. Am.e.76 was placed in the well. C, control for B without antigen incorporation in the intermediate gel. The fused-line- and crossed-immunoprecipitates (B), compared with the control crossed-immunoprecipitates (C), demonstrate immunochemical reactions of identity of Ags 32 (indicated by a large arrow) and 36 (indicated by a small arrow) in the reference extract. D, CLIE: fraction No. 55 was incorporated into the intermediate gel between 1st dimension gel and the anodic antibody-containing gel. Am.e.76 was placed in the well. E, control for D without antigen incorporation into the intermediate gel. *In situ* absorption of Ag 27 in the reference extract (marked by arrow in E, control plate) by fraction No. 55 incorporated into the intermediate gel in D indirectly demonstrates a reaction of immunochemical identity.

in which antigenic cross-reactivity could be studied in detail was the AgE-AgK system, which will be discussed in the next paper of this series (Löwenstein *et al.*, in preparation).

A similar immunochemical approach was used to determine the pI values of some antigens in fractions obtained after column isoelectricfocusing of Am.e.76 (Fig. 3). Forty-five fractions, containing about 30 detectable antigens were distributed over the pH range of 4 to 9. Most of the antigens had pI between 4 and 6, but 9 migrated toward the cathode and, of these, 4 had isoelectric points above pH 9. Twenty of the antigens were identified by means of CLIE as components of the CIE reference pattern (Table I). Figure 3B and C provides an example of antigen identification: fraction No. 28 was found to contain Ag 29a, 27a, 19, 10K, 10E, 11, 27, and 26, of which Ag 10E, 27, and 26 are indicated by arrows in Fig. 3B and C. These figures also show the partial identity between Ag 10E, 10K and 11. Several antigens exhibited isoelectric heterogeneity; e.g., Ag 10E possessed 2 distinct peaks, a

major one at pH 5.0 and a minor at 5.8, with a shoulder at pH 4.4. Antigen 27 had 4 distinct peaks at pH 3.0, 5.0, 5.8, and 6.7. Some antigens were distributed across ranges of between 2 and 5 pH units without having any distinct maximum, e.g., Ag 17 and 29.

#### DISCUSSION

Crossed immunoelectrophoretic analyses revealed that short ragweed extract is composed of at least 52 antigens from which about 39 were easily visible by using standard experimental conditions, and could be characterized in terms of their migration velocity relative to the main component of Ag 10E [identified as AgE (Löwenstein *et al.*, in preparation)], as shown in Table I. We were able to estimate the m.w. of 19 of the antigens by a combination of gel filtration and crossed immunoelectrophoretic methods. The isoelectric points of the major isoelectric variants of 20 antigens were estimated by a combination of isoelectricfocusing and crossed immunoelectrophoresis. It would be necessary to

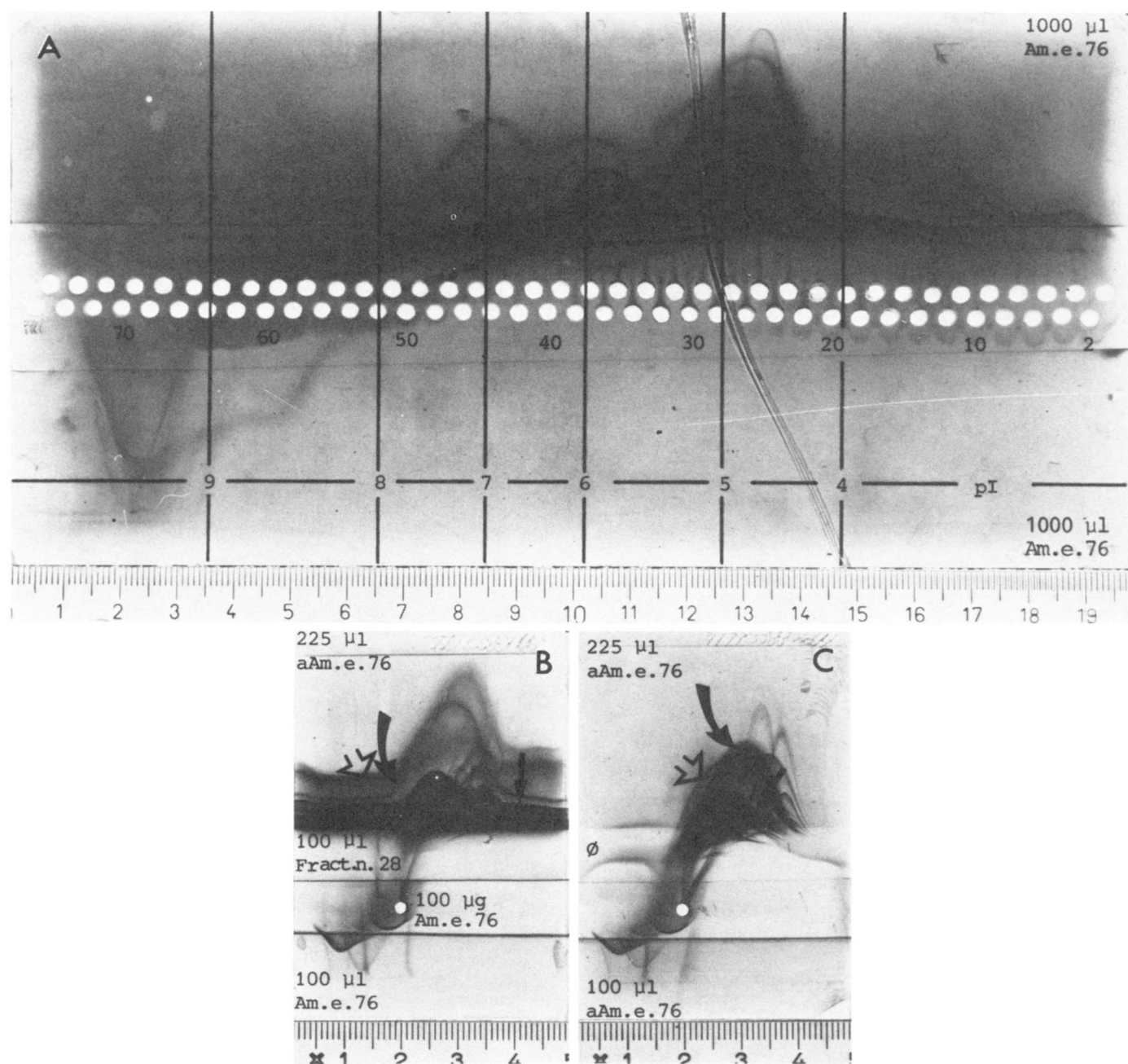


Figure 3. Quantitative immunoelectrophoretic analysis of fractions from Am.e.76 after isoelectricfocusing in a sucrose-gradient column (pH gradient 3–10). A, aliquots (10  $\mu$ l) of fractions subjected to fused rocket immunoelectrophoresis. Experimental conditions were as for Fig. 2A. The pI values corresponding to the fractions are indicated. B, CLIE: fraction No. 28 was incorporated into the intermediate gel between 1st dimension gel and the cathodic antibody-containing gel and Am.e.76 in the well. C, control for B without antigen incorporated into the intermediate gel. Arrows illustrate antigen identification as follows:  $\downarrow$ , 10E,  $\curvearrowright$ , 27, and  $\downarrow\downarrow$ , 26, which was obtained by comparing the fused-line- and crossed-immunoprecipitates in B with the crossed-immunoprecipitates in C.

increase the sensitivity of the method by increasing the concentrations of ragweed extract and antibody, or performing CRIE analysis, in order to reveal the remaining antigens.

These 52 components certainly do not represent all the antigens of ragweed pollen that can be extracted with aqueous buffer. The present technique, although much more sensitive than previous methods, may still not detect antigenic components present in small amounts against which the immunized rabbits made little or no antibody, because of their genetic incapacity to respond to low doses of such components. The precipitation pattern shown in Figure 1D is also an expression of the quantity of the antigens extracted from the pollen after 20 hr at 5°C, and the relative immunogenicities of these antigens. In other studies, 16-min extracts of ragweed pollen examined by CIE against anti-Am.e.(16-min) antisera have shown quite different precipitation patterns that emphasize basic antigenic components (19). The use of anti-

Am.e.(16-min) seemed not to reveal any new antigens in Am.e.76, but the titer against some antigens—in particular the relatively low m.w. cathodic ones—was increased, presumably because of lower antigenic competition during the immunization schedule. It might be argued, therefore, that the best antibodies for general analytical purposes and standardization would be produced by immunizing with antigens from various stages of the pollen extraction.

Our physicochemical data were also evaluated with the aim of predicting the optimal chromatographic conditions for the isolation of some important new allergens. Certain antigens, including those identified as allergens in CRIE (Löwenstein and Marsh, in preparation), will be relatively easy to isolate by conventional chromatographic techniques, because of their unique combination of size and charge. For example, antigen 27, toward which about half of ragweed-sensitive patients produce IgE antibody, could be isolated contaminated with antigens of much higher pI by using a Sephadex

G-75 column, and the impurities could then be removed by ion-exchange chromatography. Similarly, some IgE-binding cathodic antigens (e.g., Ag 30, 31, 32, 35, and 37) could be separated from the acidic allergens on a cationic exchanger and subsequently separated from each other by gel filtration chromatography.

This study illustrates the usefulness of QIE methods in allergy research. No other methods have such high resolving power. These techniques have revealed not only the great complexity of ragweed antigens, but also can be used to predict useful methods for isolating further ragweed components. It should, however, be emphasized that these methods of analysis have to be combined with physicochemical and chemical methods, which are not dependent on the ability of the ragweed components to induce antibody formation in animals. Furthermore, since not all ragweed components that are antigens in rabbits may act as allergens in man, the present techniques should be combined with CRIE analyses (12) in order to identify the allergens.

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