

# Some antigenic relationships between crude extracts and purified fractions of ragweed pollen

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*The capacities of two crude ragweed pollen extracts and of two purified fractions to inhibit the specific antibody binding of  $I^{131}$ -labeled ragweed pollen extract (I\*RW) have been studied. Although the majority of antigenic groups capable of inhibiting the I\*RW binding capacity had been removed from the purified fractions (Pool C and Fraction IV<sub>2</sub>), both probably contained at least trace amounts of most, if not all, of the antigens present in crude ragweed extracts. Pool C and Fraction IV<sub>2</sub> had similar but demonstrably different capacities to inhibit the I\*RW-antiserum reaction. Whether the antigenic differences between Pool C and Fraction IV<sub>2</sub> were associated with skin-test reactive molecules is not known. The antigenic differences between Pool C and Fraction IV<sub>2</sub> were amplified by the use of related, but not identical, antibody populations in the inhibition test used for these studies. The useful role which antibody and antigen heterogeneity plays in the interpretation of the inhibition test has been discussed and is applicable to other  $I^{131}$ -labeled antigen-antibody inhibition test systems.*

Ragweed pollen extract is a complex mixture of antigens. Recent efforts have produced purified fractions with high biologic activity as measured by their capacity to elicit positive skin tests when injected intradermally into ragweed-sensitive individuals.<sup>1, 2</sup> These purified antigens from ragweed have been characterized both chemically and immunologically with respect to composition and purity of the end products, and some aspects of the antigenic relationships between the purified fractions and the original crude extracts

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have also been studied. In the present study, the antigenic relationships between two of the purified ragweed pollen fractions and the antigenic mosaic of crude ragweed pollen extracts have been further explored by comparing the inhibitory effects of graded amounts of unlabeled purified ragweed pollen fractions and crude ragweed pollen extracts on the primary interaction between  $I^{131}$ -labeled ragweed pollen extract and antisera against crude ragweed pollen. Also, in this study, an attempt has been made to assess the influence of antibody heterogeneity on the interpretation of such antigen comparisons.

## **MATERIALS AND METHODS**

### **$I^{131}$ -labeled ragweed pollen extract**

The preparation of a  $I^{131}$ -labeled pollen extract has been described previously.<sup>3</sup> This extract, designated as I\*RW, contained 0.02  $\gamma$  nitrogen (N) per 0.5 ml. of solution.

### **Unlabeled ragweed pollen antigens**

*Crude ragweed pollen extracts.* One extract was prepared from a mixture consisting of equal amounts (by weight) of defatted short and tall ragweed pollen.\* After extraction in 5 per cent Coca's solution and fractionation with 50 per cent saturated ammonium sulfate (SAS), the supernatant was dialyzed, concentrated, lyophilized, and designated as RW extract. Another preparation, designated as NIH extract,† was a dialyzed, concentrated, and lyophilized crude short ragweed pollen extract which had not been fractionated with ammonium sulfate.

The lyophilized crude pollen extracts were dissolved in borate buffer and a 1 mg. per milliliter solution of each preparation contained approximately 60  $\gamma$  total ragweed extract nitrogen (N) per milliliter. These concentrated solutions were then accurately diluted in 1:100 normal rabbit serum (NRS)-borate buffer diluent so that the initial concentration of these extracts when used in the inhibition test was exactly 6  $\gamma$  N per milliliter. Subsequent dilutions of these extracts were also made with the NRS-borate buffer solution. The diluents containing serum were used to prevent adsorption of unlabeled antigens onto the surface of containers and pipettes. No significant binding of I\*RW by the normal rabbit serum-buffer solution could be demonstrated.

*Purified ragweed pollen fractions.* Fraction IV<sub>2</sub>,‡ was prepared from short ragweed pollen extract by ammonium sulfate fractionation and chromatography on DEAE cellulose and on Sephadex.<sup>1</sup> Fraction IV<sub>2</sub> represented the most skin-reactive preparation derived from this procedure.

Similarly, a fraction also derived from short ragweed pollen,§ a highly skin-reactive material, has been designated as Pool C.<sup>2</sup>

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†The NIH extract was obtained from the National Institute of Allergy and Infectious Diseases through the courtesy of Dr. R. P. Wodehouse and Dr. C. Gussoni of Lederle Laboratories.

‡Obtained through the courtesy of Dr. T. P. King of the Rockefeller Institute.

§Obtained through the courtesy of Dr. A. R. Goldfarb, of the Sinai Hospital in Detroit.

Each of the purified ragweed pollen fractions was diluted with 1:100 NRS-borate buffer solutions, in the same fashion as employed for the crude extracts. The initial concentration of these fractions used for inhibition tests was 7.5  $\gamma$  N per milliliter.

### Antisera

A human antiserum, designated as 43D, was obtained from a ragweed-sensitive patient who attended the Immunology Clinic of the Falk Clinic, of the University of Pittsburgh School of Medicine. This patient's previous immunization had consisted of one injection of mixed tall and short ragweed pollen extract in an incomplete Freund's adjuvant three months prior to bleeding.

Antisera 1030-9 and 1031-9 were obtained from rabbits immunized with a series of mixed ragweed pollen extracts in incomplete Freund's adjuvant as previously described.<sup>3</sup> The rabbits bled for antisera 328, 332, 337, and 338\* had been repeatedly immunized with either a short ragweed pollen extract (328 and 332) or tall ragweed pollen extract (337 and 338) in a complete Freund's adjuvant.<sup>4</sup>

### The inhibition of I\*RW-antiserum interaction by the prior addition of unlabeled ragweed antigens

The experimental procedure to determine the inhibitory effects of various unlabeled antigens on the primary interaction between I\*RW and ragweed pollen antiserum has been discussed in detail previously.<sup>5</sup> Unlabeled test antigens were allowed to interact with a ragweed pollen antiserum prior to the addition of I\*RW, and if the unlabeled ragweed antigens occupied antiragweed binding sites, a decrease in the subsequent binding of I\*RW became manifest after the precipitation of I\*RW-antibody complexes with 40 per cent saturated ammonium sulfate. Prior to use of an antiserum in an inhibition test, it was first diluted so that it bound only 35 per cent of 0.02  $\gamma$  I\*RW N after the addition of 40 per cent SAS when borate buffer was added in place of the unlabeled pollen antigens. This P-35 end point (35 per cent of 0.02  $\gamma$  I\*RW N precipitated) was arbitrarily selected to establish a base line for comparing the degree of inhibition with different antisera.

In order to compare the unlabeled purified fractions and crude extracts, the amount of each unlabeled antigen required to reduce by 50 per cent the specific binding of I\*RW by each antiserum was determined. The end point at which a 50 per cent inhibition of a specific I\*RW-antiserum interaction had occurred was termed the  $I_{50}$  and was calculated as follows:

$$I_{50} \text{ in } \% = \left[ \frac{\% \text{ I*RW}_E - \% \text{ I*RW}_C}{2} + \% \text{ I*RW}_C \right] = \frac{\% \text{ I*RW}_E + \% \text{ I*RW}_C}{2}$$

where  $\% \text{ I*RW}_E$  was the per cent of I\*RW precipitated by an experimental serum with borate buffer added in place of unlabeled antigens, and  $\% \text{ I*RW}_C$  was

\*Obtained through the courtesy of Dr. C. Gussoni of Lederle Laboratories.

the per cent of I\*RW precipitated by normal rabbit serum with borate buffer added.

## **EXPERIMENTAL RESULTS**

### **Graded inhibition of the I\*RW-antiserum interaction by the addition of increasing amounts of unlabeled antigens**

The inhibitory effect of increasing amounts of two unlabeled purified ragweed pollen fractions (Fraction IV<sub>2</sub> and Pool C) and two unlabeled crude ragweed pollen extracts (RW and NIH) upon the interaction between seven antisera and I\*RW are presented in Table I. The data for the human antiserum (43D) and for one of the six rabbit antisera (1031-9) are also presented in Figs. 1 and 2, respectively. In a previous study,<sup>5</sup> it was shown that the standard deviation of the mean I\*RW binding determinations with a single lot of I\*RW did not exceed 0.8 per cent. Inasmuch as the data presented in Table I were also obtained with one lot of I\*RW, the degree of reproducibility in the present experiments was assumed to be the same.

From Table I it can be seen that the addition of increasing amounts of any of the unlabeled ragweed antigen preparations resulted in a progressive decrease in the per cent of 0.02  $\gamma$  I\*RW N precipitated by all the antisera. The unlabeled crude ragweed extracts were capable of inhibiting the specific binding of I\*RW, i.e., inhibition of the precipitation of I\*RW to a value approximately equal to that of the NRS control. In general, when compared on the basis of nitrogen content, the purified ragweed fractions had less inhibiting effect on the I\*RW-antiserum system than did the crude extracts. In fact, the human antiserum 43D was the only one whose specific binding for I\*RW could be almost completely inhibited by the prior addition of the maximum amount of either of the unlabeled purified fractions which were used in these studies.

### **A comparison of the inhibitory efficiencies of the purified and crude ragweed pollen preparations**

From graphs similar to those in Figs. 1 and 2, the  $\gamma$  N of each unlabeled ragweed pollen preparation required to reach the I<sub>50</sub> end point was calculated for each antiserum and these data are presented in Table II. It can be seen that neither of the purified fractions had inhibitory capacities comparable to those of the crude fractions. For example, 0.18  $\gamma$  N of RW or 0.44  $\gamma$  N of NIH was as effective as 3.5  $\gamma$  N of Fraction IV<sub>2</sub>, or 6.3  $\gamma$  of Pool C in causing a 50 per cent reduction of specific I\*RW binding by antiserum 332.

In order to compare in greater detail the relative efficiencies of the various individual antigen preparations with respect to inhibiting I\*RW binding, the  $\gamma$  N of purified ragweed fractions needed to inhibit as well as 1  $\gamma$  N of the crude ragweed extracts were calculated and are presented in Table III. It can be seen that between 8 and 92  $\gamma$  N of the purified fractions were required to inhibit as well as 1  $\gamma$  N of the crude extracts. These high values for the purified fraction/crude extract ratios presented in Table III indicate that the majority of antibody responsible for I\*RW binding in the human and rabbits immunized

**Table I.** *Per cent 0.02  $\gamma$  I\*RW N precipitated by antisera in presence of unlabeled ragweed antigens and 40% SAS*

| Serum source        | Unlabeled antigens       |                |      | Unlabeled antigens                                   |                     |        |
|---------------------|--------------------------|----------------|------|------------------------------------------------------|---------------------|--------|
|                     | RW and NIH<br>$\gamma$ N | % Precipitated |      | Fraction IV <sub>2</sub><br>and Pool C<br>$\gamma$ N | % Precipitated      |        |
|                     |                          | RW             | NIH  |                                                      | Fr. IV <sub>2</sub> | Pool C |
| Normal rabbit serum | None added               | 7.2            | 7.2  | None added                                           | 7.2                 | 7.2    |
| Human 43D*          | 3.0                      | 7.0            | 7.0  | 3.75                                                 | 10.5                | 7.5    |
|                     | 0.3                      | 8.1            | 7.6  | 1.88                                                 | 13.8                | 8.5    |
|                     | 0.03                     | 14.9           | 11.1 | 0.75                                                 | 17.9                | 10.1   |
|                     | 0.015                    | 21.1           | 14.7 | 0.38                                                 | 23.1                | 14.3   |
|                     | 0.0015                   | 31.0           | 29.7 | 0.038                                                | 32.5                | 31.5   |
|                     | 0.00015                  | 33.8           | 32.4 | 0.0008                                               | 34.2                | 34.4   |
|                     | None added               | 34.7           | 34.7 | None added                                           | 34.7                | 34.7   |
| Rabbit 1030-9*      | 3.0                      | 8.6            | 9.7  | 3.75                                                 | 21.7                | 19.9   |
|                     | 0.3                      | 14.9           | 16.0 | 1.88                                                 | 26.7                | 20.5   |
|                     | 0.03                     | 26.7           | 22.2 | 0.75                                                 | 29.2                | 20.4   |
|                     | 0.015                    | 28.4           | 25.5 | 0.38                                                 | 30.7                | 26.7   |
|                     | 0.0015                   | 37.5           | 33.6 | 0.038                                                | 35.2                | 33.7   |
|                     | 0.00015                  | 36.8           | 36.5 | 0.0008                                               | 37.2                | 35.4   |
|                     | None added               | 36.4           | 36.4 | None added                                           | 36.4                | 36.4   |
| Rabbit 1031-9*      | 3.0                      | 6.6            | 8.2  | 3.75                                                 | 19.0                | 21.4   |
|                     | 0.3                      | 16.2           | 20.1 | 1.88                                                 | 25.3                | 24.1   |
|                     | 0.03                     | 27.6           | 27.6 | 0.75                                                 | 26.6                | 25.7   |
|                     | 0.015                    | 31.0           | 29.9 | 0.38                                                 | 29.6                | 29.5   |
|                     | 0.0015                   | 33.3           | 33.6 | 0.038                                                | 33.9                | 33.0   |
|                     | 0.00015                  | 35.0           | 34.1 | 0.0008                                               | 36.0                | 34.9   |
|                     | None added               | 34.6           | 34.6 | None added                                           | 34.6                | 34.6   |
| Rabbit 328†         | 3.0                      | 7.8            | 7.5  | 3.75                                                 | 21.6                | 22.6   |
|                     | 0.3                      | 18.9           | 22.6 | 1.88                                                 | 26.2                | 25.3   |
|                     | 0.03                     | 30.5           | 30.3 | 0.75                                                 | 29.9                | 27.9   |
|                     | 0.015                    | 32.0           | 31.7 | 0.38                                                 | 31.9                | 30.3   |
|                     | 0.0015                   | 34.4           | 33.2 | 0.038                                                | 34.3                | 33.3   |
|                     | 0.00015                  | 35.8           | 34.9 | 0.0008                                               | 34.4                | 35.1   |
|                     | None added               | 33.7           | 33.7 | None added                                           | 33.7                | 33.7   |
| Rabbit 332‡         | 3.0                      | 8.0            | 6.7  | 3.75                                                 | 19.1                | 22.9   |
|                     | 0.3                      | 16.6           | 22.1 | 1.88                                                 | 26.9                | 25.8   |
|                     | 0.03                     | 30.5           | 28.9 | 0.75                                                 | 29.9                | 27.9   |
|                     | 0.015                    | 31.8           | 31.8 | 0.38                                                 | 31.1                | 31.0   |
|                     | 0.0015                   | 32.9           | 33.4 | 0.038                                                | 33.8                | 32.5   |
|                     | 0.00015                  | 35.2           | 33.9 | 0.0008                                               | 33.1                | 33.4   |
|                     | None added               | 33.3           | 33.3 | None added                                           | 33.3                | 33.3   |
| Rabbit 337‡         | 3.0                      | 6.2            | 10.2 | 3.75                                                 | 19.3                | 21.3   |
|                     | 0.3                      | 14.4           | 20.6 | 1.88                                                 | 27.0                | 24.3   |
|                     | 0.03                     | 29.7           | 27.7 | 0.75                                                 | 30.0                | 27.9   |
|                     | 0.015                    | 32.1           | 31.3 | 0.38                                                 | 32.1                | 29.2   |
|                     | 0.0015                   | 34.4           | 33.9 | 0.038                                                | 33.8                | 34.0   |
|                     | 0.00015                  | 34.4           | 34.5 | 0.0008                                               | 35.8                | 34.9   |
|                     | None added               | 34.8           | 34.8 | None added                                           | 34.8                | 34.8   |
| Rabbit 338‡         | 3.0                      | 6.7            | 8.6  | 3.75                                                 | 17.1                | 17.6   |
|                     | 0.3                      | 13.2           | 15.3 | 1.88                                                 | 24.1                | 23.3   |
|                     | 0.03                     | 26.8           | 25.6 | 0.75                                                 | 26.7                | 24.8   |
|                     | 0.015                    | 29.9           | 26.0 | 0.38                                                 | 29.5                | 26.4   |
|                     | 0.0015                   | 32.6           | 31.6 | 0.038                                                | 32.6                | 32.1   |
|                     | 0.00015                  | 32.7           | 29.6 | 0.0008                                               | 34.1                | 33.2   |
|                     | None added               | 33.2           | 33.2 | None added                                           | 33.2                | 33.2   |

\*Anti-mixed ragweed pollen extract.

†Anti-short ragweed pollen extract.

‡Anti-tall ragweed pollen extract.

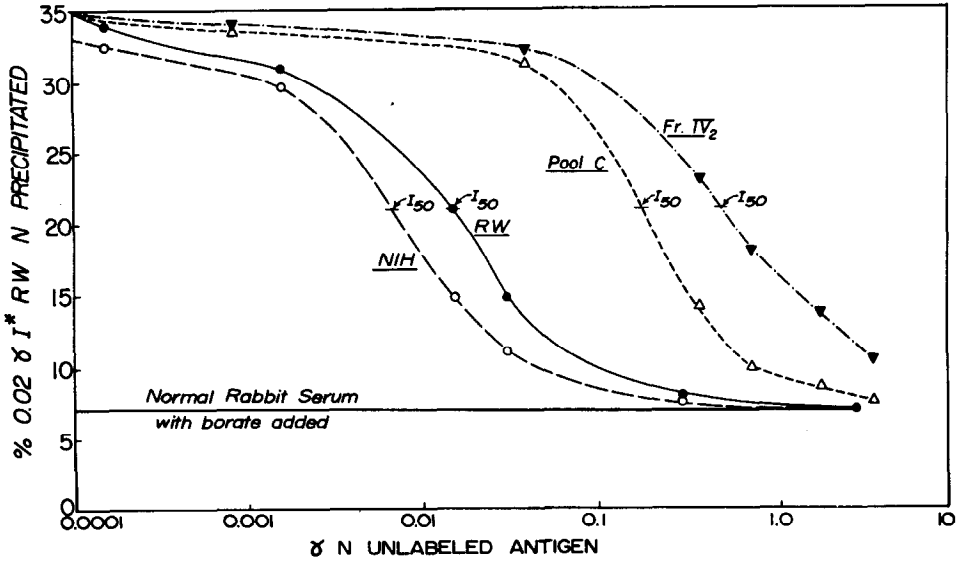


Fig. 1

The inhibition of  $I^*RW$ -antibody interaction by the prior addition of unlabeled ragweed antigens using antiserum from a human (43D) immunized with mixed ragweed pollen (see text).

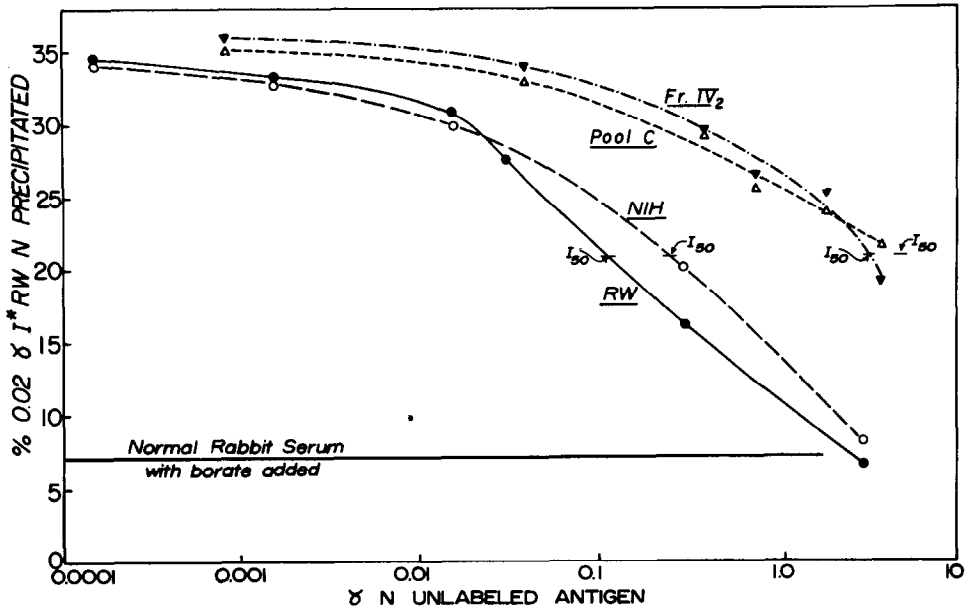


Fig. 2

The inhibition of  $I^*RW$ -antibody interaction by the prior addition of unlabeled ragweed antigens using antiserum from a rabbit (1031-9) immunized with mixed ragweed pollen (see text).

**Table II.** *Amounts of unlabeled ragweed antigens required for 50% inhibition of specific I\*RW binding*

| Sera          | Unlabeled antigens expressed in terms of $\gamma$ nitrogen added |        |                          |        |
|---------------|------------------------------------------------------------------|--------|--------------------------|--------|
|               | RW                                                               | NIH    | Fraction IV <sub>2</sub> | Pool C |
| <i>Human</i>  |                                                                  |        |                          |        |
| 43D           | 0.015                                                            | 0.0065 | 0.5                      | 0.17   |
| <i>Rabbit</i> |                                                                  |        |                          |        |
| 1030-9*       | 0.08                                                             | 0.04   | 3.7                      | 1.3    |
| 1031-9*       | 0.12                                                             | 0.23   | 3.0                      | 4.1    |
| 328†          | 0.22                                                             | 0.37   | 4.1                      | 5.0    |
| 332†          | 0.18                                                             | 0.44   | 3.5                      | 6.3    |
| 337‡          | 0.12                                                             | 0.24   | 3.3                      | 4.0    |
| 338‡          | 0.11                                                             | 0.10   | 2.9                      | 2.0    |

\*Anti-mixed ragweed pollen extract.

†Anti-short ragweed pollen extract.

‡Anti-tall ragweed pollen extract.

**Table III.** *Comparative inhibitory efficiencies of unlabeled ragweed antigens*

| Sera          | Fraction IV <sub>2</sub> /NIH extract | Pool C/NIH extract | Fraction IV <sub>2</sub> /RW extract | Pool C/RW extract | Fraction IV <sub>2</sub> /Pool C | RW extract/NIH extract |
|---------------|---------------------------------------|--------------------|--------------------------------------|-------------------|----------------------------------|------------------------|
| <i>Human</i>  |                                       |                    |                                      |                   |                                  |                        |
| 43D           | 76.9                                  | 26.2               | 33.3                                 | 11.3              | 2.9                              | 2.3                    |
| <i>Rabbit</i> |                                       |                    |                                      |                   |                                  |                        |
| 1030-9*       | 92.5                                  | 32.5               | 46.3                                 | 16.3              | 2.8                              | 2.0                    |
| 1031-9*       | 13.0                                  | 17.8               | 25.0                                 | 34.2              | 0.7                              | 0.5                    |
| 328†          | 11.1                                  | 13.5               | 18.6                                 | 22.7              | 0.8                              | 0.6                    |
| 332†          | 8.0                                   | 14.3               | 19.4                                 | 35.0              | 0.6                              | 0.4                    |
| 337‡          | 13.8                                  | 16.7               | 27.5                                 | 33.3              | 0.8                              | 0.5                    |
| 338‡          | 29.0                                  | 20.0               | 26.4                                 | 18.2              | 1.5                              | 1.1                    |

\*Anti-mixed ragweed pollen extract.

†Anti-short ragweed pollen extract.

‡Anti-tall ragweed pollen extract.

with crude ragweed pollen was directed against antigenic groups which had in large part been removed during the purification of Pool C and Fraction IV<sub>2</sub>. In addition, and discussed in greater detail below, the observation that the ratios in any given column in Table III are not constant indicate heterogeneity between all four unlabeled antigen preparations as well as heterogeneity among the antibody populations in the antisera studied.

## DISCUSSION

The human and rabbit antisera used in these studies were obtained from subjects immunized with whole ragweed extract in adjuvant. This method of immunization results in the production of antibody populations directed against a variety of different antigens known to exist in crude ragweed extracts. The I\*RW test antigen was also a heterogeneous mixture. The observa-

tion that it required between eight and ninety times more of the purified fraction than of the crude extract to reach the  $I_{50}$  end point indicates that most of the antibody measured by the I\*RW was directed against  $I^{131}$ -labeled antigen moieties in the crude extract which had been removed during the preparation of Pool C and Fraction IV<sub>2</sub>. Because these purified fractions constitute only a minor proportion of the total antigen mosaic and yet contain much of the skin-test reactivity of crude ragweed pollen extract, it is clear why a previous study failed to distinguish between the sera of untreated symptomatic ragweed-sensitive individuals and sera of ragweed skin-test negative persons on the basis of I\*RW binding.<sup>3</sup> In contrast, it is now possible to detect increased binding of  $I^{131}$ -labeled Fraction IV<sub>2</sub> in the serum of most untreated skin-test positive symptomatic individuals.<sup>6, 7</sup>

Despite the high degree of purity of Fraction IV<sub>2</sub> and Pool C, Table I and Fig. 1 demonstrate that both fractions completely inhibited the I\*RW binding by the human antiserum. This finding suggests that both Fraction IV<sub>2</sub> and Pool C still contained trace amounts of most, if not all, of the antigens present in crude ragweed extracts.

Significantly smaller amounts of both crude ragweed extracts and purified fractions were needed to effect a 50 per cent inhibition of the I\*RW-human antiserum interaction than were needed to reach the  $I_{50}$  end point with the I\*RW-rabbit antiserum interactions. This was not simply a function of a higher binding capacity in the rabbit antisera than in the human antiserum, because all sera were diluted to approximately the P-35 level prior to the inhibition studies. It is possible that this variance was a reflection of the necessarily minimal immunization of this human, ragweed-sensitive subject, as contrasted to the relatively high concentration of ragweed antigen in adjuvant used to immunize the rabbits. Immunization with high concentrations of antigens in adjuvant<sup>8</sup> probably resulted in the production of rabbit antisera having relatively higher concentrations of antibodies to some of the minor components of ragweed pollen than were present in the human antiserum. If this were the case, then the rabbit antisera would require relatively higher concentrations of the unlabeled antigens to provide enough of the trace antigens to reach the  $I_{50}$  end point.

The data in Table II demonstrate that roughly comparable amounts of both purified antigens were required to reach the  $I_{50}$  end point for each antiserum studied. These observations are in accord with the studies of Pruzansky and Patterson<sup>9</sup> and suggest some antigenic similarities between Fraction IV<sub>2</sub> and Pool C. However, these data also clearly demonstrate antigenic differences between the two purified preparations, a subject recently reviewed in considerable detail by Goldfarb.<sup>10</sup> Table III demonstrates Fraction IV<sub>2</sub>/Pool C inhibitory efficiency ratios ranging from 0.6 to 2.9 among the antisera studied. If the two purified fractions had been antigenically identical, these ratios should have approached unity for each of the I\*RW-antiserum interactions. Further, if the assumption were made that the two purified antigens were different but the antibody populations were constant in each pair of antisera from rabbits immunized with the same crude extracts, then the Fraction IV<sub>2</sub>/Pool C ratios should have

been constant for each pair of antisera at some value other than unity. A diversity of antibody response was not detected between the two rabbits immunized with short ragweed pollen extract (328 vs. 332), but a diversity of antibody was clearly evident between the two rabbits immunized with tall ragweed extract (337 vs. 338), and between the two rabbits receiving the mixed ragweed pollen extract (1030-9 vs. 1031-9). Thus, the two- to fourfold variation in the Fraction IV<sub>2</sub>/Pool C ratios from one to the other member of a pair of antisera indicated that a diversity of antibody response from individual to individual, and antigen dissimilarities between Fraction IV<sub>2</sub> and Pool C were both important factors in determining the results of these inhibition experiments.

A comparison of the RW extract/NIH extract inhibition ratios in Table III also demonstrate a diversity of antigen composition between the two crude extracts and a diversity of antibody response among the pairs of animals injected with the same extract. Not only did the ratios between these two crude fractions vary over a fourfold range, but it was noted that the RW extract/NIH extract ratios appeared to vary in direct proportion with the Fraction IV<sub>2</sub>/Pool C ratios. This linear relationship suggests that Fraction IV<sub>2</sub> differed from Pool C in a manner which was similar to the difference between RW extract and NIH extract. The reasons for these similarities and differences are not known, but a review of the methods of preparation of these four antigen solutions suggests that both Pool C and the NIH extract probably contained more antigens which were insoluble in 50 per cent saturated ammonium sulfate than were present in either Fraction IV<sub>2</sub> or RW extract. Although it is clear that the antigenic mosaic of Pool C differed from that of Fraction IV<sub>2</sub> it should be emphasized that the difference may not have been related to skin-test reactive molecules, but could have been associated with nonreactive antigens.

The use of antisera against similar but somewhat different antigen preparations in these inhibition studies amplified antigenic differences between the unlabeled test-antigen preparations. Conversely, the antigenic differences between the unlabeled test-antigen preparations amplified the diversity of the immune response which occurred with two of the three rabbit pairs studied. The deliberate use of closely related but not identical antigens, as well as antisera, should be a useful technique for obtaining clearer definitions of either antigen or antiserum differences during future inhibition studies with this or other I\* antigen-antibody systems.

The able technical assistance of Mr. Richard Mays is gratefully acknowledged.

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## ERRATUM

In the article, "Hypergammaglobulinemia—The Role of the Immunoglobulins ( $\gamma G$ ,  $\gamma A$ ,  $\gamma M$ )," which appeared in the *JOURNAL OF ALLERGY*, vol. 36, pp. 463-471, September—October, 1965, the following change should be made. The fifth percentile for normal  $\gamma A$  should read 68 mg. per cent rather than 84 mg. per cent. This value appears in line 9 of the abstract and on the right-hand side of Table 1A.