

The potency and antigen E content of commercially prepared ragweed extracts

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This study compares several methods for their ability to evaluate the potency of commercial extracts of ragweed pollen extract. Samples of short ragweed pollen allergenic extracts were purchased from six commercial manufacturers and were assayed for antigen E and PNU content, as well as for reactivity in ragweed-sensitive human beings by direct skin test and by in vitro histamine release. Human reactivity correlated extremely well with the antigen E content of the extracts despite great variations in the amounts of antigen E present. The PNU content as labeled or as assayed in our laboratory showed no correlation with either antigen E content or degree of reactivity. There was a hundredfold or greater difference between the antigen E content and corresponding biologic activity of extracts labeled to have the same PNU content. Results indicate that double diffusion determinations of antigen E content would be a satisfactory method for the standardization of the potency of ragweed pollen allergenic extracts.

Standardization of allergenic extracts of pollens, molds, danders, etc., has been a continuing problem since the introduction of prophylactic inoculation (desensitization). Wide variations in potency, as measured by reactivity in the skin of sensitive individuals, of different lots of extracts of a particular species of pollen are observed commonly. Hence, when a new lot of an extract is to be used for desensitization, it is customary to initiate treatment with a lower dose although the new lot is the same labeled strength as the lot used previously.

Allergenic extracts of whole pollens are usually complicated mixtures, and it has been difficult to determine which constituent(s) is responsible for eliciting either an allergic reaction or therapeutic effect during immunotherapy. Methods of expressing potency have been largely indirect and based either on a simple ratio of weight of raw source material to volume of extracting fluid or on the total or the protein nitrogen of the extract.¹

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Received for publication Aug. 1, 1969.

Since most allergens appear to be protein in nature, designation of potency by protein nitrogen units (PNU) (defined as 0.01 μ g of phosphotungstic acid-precipitable nitrogen) has gained wide acceptance. PNU content, however, is not necessarily related to biologic activity, as these crude extracts contain proteins which are not allergenically active.^{2, 3} Furthermore, specific protein allergens may be denatured during extraction or storage with loss of their biologic activity and yet still appear as protein in chemical determination.

The identification of individual allergens and their isolation in a high degree of purity provide a new approach to the problem of potency standardization. An example of such an allergen is antigen E of ragweed pollen. This antigen represents a major portion (90 per cent or more³) of the biologic activity of the extract as measured by skin tests. Two trials of immunotherapy of hay fever with purified antigen E have demonstrated clinical efficacy with adequate doses: in one,⁴ results were similar to those obtained with crude extract; in the other,⁵ antigen E appeared to be slightly less effective than crude extract.

Such considerations prompted an exploration of the relationship between the antigen E content of commercially prepared extracts and their biologic activity.

In the present study 6 lots of commercially prepared short ragweed allergenic extracts were examined for the relationship between antigen E content, PNU content, skin reactivity, and ability to release histamine from leukocytes of ragweed-sensitive persons. The results indicate an excellent correlation within the measurements of the two biological activities and between these and antigen E content, but no correlation was noted between these three parameters and the PNU content.

MATERIALS AND METHODS

Extracts of short ragweed pollen were purchased from six United States licensed manufacturers. Antigens E and K and rabbit anti-E serum were obtained from Dr. Merrill Chase acting for the National Institute of Allergy and Infectious Diseases Committee on Standardization of Allergens. The antigen E content of extracts was determined by gel diffusion on microscope slides.³ A dose-response curve was prepared by measuring the distance between the front of the gel-diffusion band and the center of the antigen well for 4 serial twofold dilutions of antigen E. The concentration of antigen E in an extract was then determined by the distance traveled by its antigen E band relative to the reference curve. Antiserum to whole short ragweed extract (anti-SRE) was prepared in rabbits by Mr. Donald Hill of the Division of Biologics Standards.

Gel diffusion also was carried out in 60 mm. plastic Petri dishes containing 8 ml. of 1 per cent agar and 1 per cent sodium azide. The antiserum (0.2 ml.) was placed in the center well and permitted to diffuse for about 20 hours prior to addition of antigen to the outer wells.⁶

Skin reactivity was determined as the highest dilution of each of the 6 products giving a 1+ reading when 0.05 ml. of serial tenfold dilutions were injected into the skin of the back of 5 untreated ragweed-sensitive human beings. If a 1+ reading was not obtained, interpolation was made between the dilution giving a 1+ or greater reaction and the dilution giving no reaction.

Histamine release was carried out with the use of the isolated peripheral leukocytes of 5 ragweed-sensitive patients; these patients were different from those used for the skin tests. Several dilutions of an extract were employed and a dose-response curve constructed. The concentration of each extract required to liberate 50 per cent of the histamine was estimated graphically as previously described.^{7, 8}

In order to free these relationships from a dependence on PNU or other laboratory-determined parameters, the minimum volume of each extract (as received from the manufacturer) required to produce a skin reaction or 50 per cent histamine release was used as a measure of reactivity. The minimum volume was computed from the dilution employed and the volume injected intradermally or added to the reaction mixture used for histamine release.

PNU were determined by analyzing the precipitate obtained with phosphotungstic acid by the method of Stull, Cooke, and Tennant⁹ and compared with the PNU on the label of the lot of extract.

RESULTS

The presence of antigen in the 6 ragweed extracts was determined by gel diffusion (Figs. 1 and 2). The upper portion of Fig. 1 shows that extracts 2 and 5 gave an intense gel-diffusion band. The band of extract 5 joined directly with the band produced by antigen E, and the band of extract 2 joined the antigen 5 band, showing that both of these extracts had antigen E. Extract 6 had a very small quantity of antigen E and also a small quantity of antigen K as revealed by the anti-short ragweed serum bands in the lower portion of the Fig. 1. In Fig. 2 in which the center well contained anti-short ragweed serum, 5 of the 6 extracts gave one very intense band; extracts 1 and 2 had several other bands of lesser intensity, while extract 6 had only one rather weak band. The joining intense bands were shown to be due to antigen E by a gel-diffusion procedure similar to that employed for the test shown in Fig. 1. The measured amount of antigen E in each of the 6 extracts may be found in Table I.

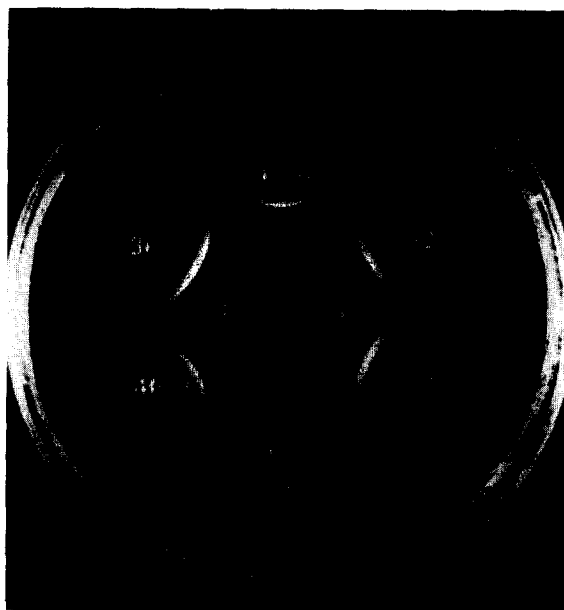
Fig. 3 demonstrates the direct relationship between skin reactivity and histamine release for the 6 extracts. In both tests extract 1 was the most reactive and extract 6, the least reactive. The other 4 extracts were intermediate in reactivity.

The relationship between the antigen E content of each extract and skin reactivity and histamine release is shown in Figs. 4 and 5. Extract 1 had the highest concentration of antigen E and had the highest skin- and histamine-release activities. Extract 6 had the lowest concentration of antigen E and was least reactive in both tests, while extracts 2, 3, and 4 were of intermediate reactivity and fell in the same order as that of their antigen E content.

The lack of a correlation between PNU and antigen E content is shown in Fig. 6. The claimed value was obtained from the label, while the found value was determined in our laboratories. In either case, the relationship between the antigen E content and PNU was completely random. Table I gives the PNU and antigen E values and demonstrates that the ratio of PNU to antigen E may vary more than a hundredfold.

**Fig. 1**

Method for identification of antigens E and K in ragweed extracts.

**Fig. 2**

Bands produced when 6 ragweed extracts are diffused against *anti-SRE* serum.

DISCUSSION

Only a few studies have been made on the variation in antigen content of different crops of ragweed pollen or the effect of small variations in extraction procedures.^{10, 11} Also lacking is information on whether or not variation in antigen E content accounts for variation in reactivity in human beings.

In the present study each of 6 commercial extracts was found to contain antigen E, although its concentration in one of them was very low. In the 5 extracts with substantial quantities, antigen E appeared to be the major antigen as judged by the gel-diffusion test; in two of these preparations other precipi-

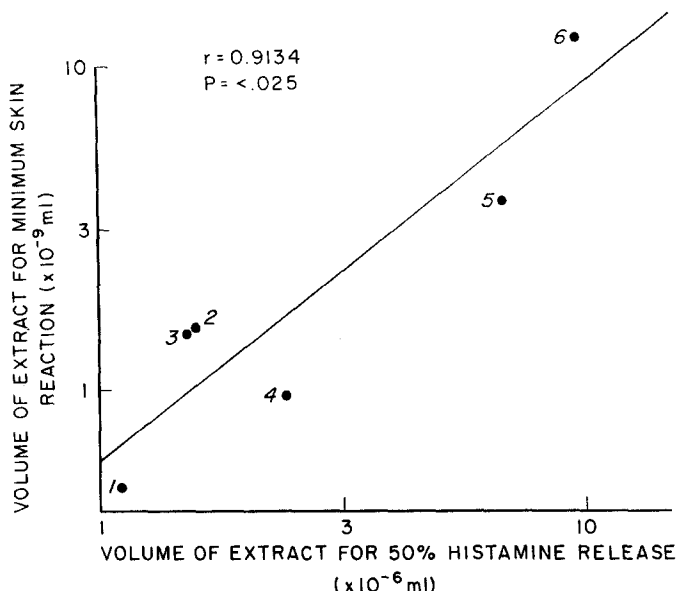


Fig. 3

The relationship between the geometric mean skin reactivity and the geometric mean histamine release activity of ragweed extracts.

Table I. The antigen E content and PNU of extracts

Extract	PNU		Antigen E ($\mu\text{g}/\text{ml.}$)	PNU Antigen E	
	Claimed	Found		Claimed	Found
1	10,000	12,000	577	17	20
2	29,000	9,000	354	57	25
3	40,000	24,000	341	117	71
4	50,000	17,000	146	342	117
5	50,000	19,000	101	495	190
6	40,000	8,000	20	2,000	416

tating antigens were represented only by faint lines. The one extract with little antigen E seemed to contain more of antigen K, a second allergen of ragweed isolated by King and associates.³

In this study of 6 extracts, the antigen E content, determined by a relatively simple double diffusion test, successfully predicted the reactivity of the extract in human patients. This does not establish that the reactivity is exclusively the consequence of antigen E, but it agrees with earlier evidence that antigen E accounts for a major proportion of the reactivity of ragweed pollen extracts.⁷

On the other hand, PNU determination (either by the manufacturer or in our laboratory) showed a total lack of correlation with either antigen E or biologic reactivity. Since antigen E comprises only about 6 per cent of the

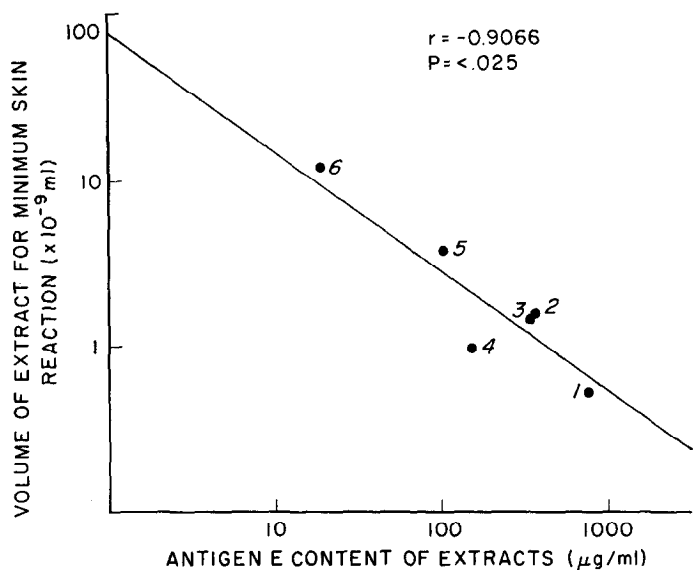


Fig. 4
The relationship between skin reactivity and antigen E content.

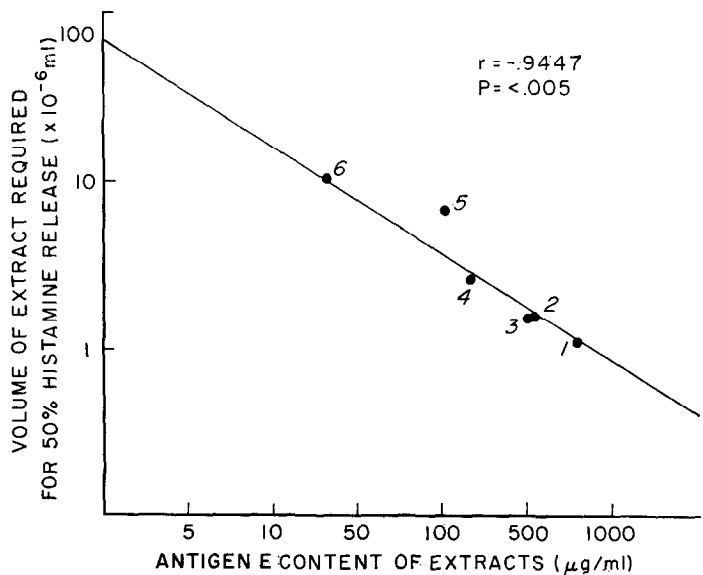


Fig. 5
The relationship between histamine release activity and antigen E content.

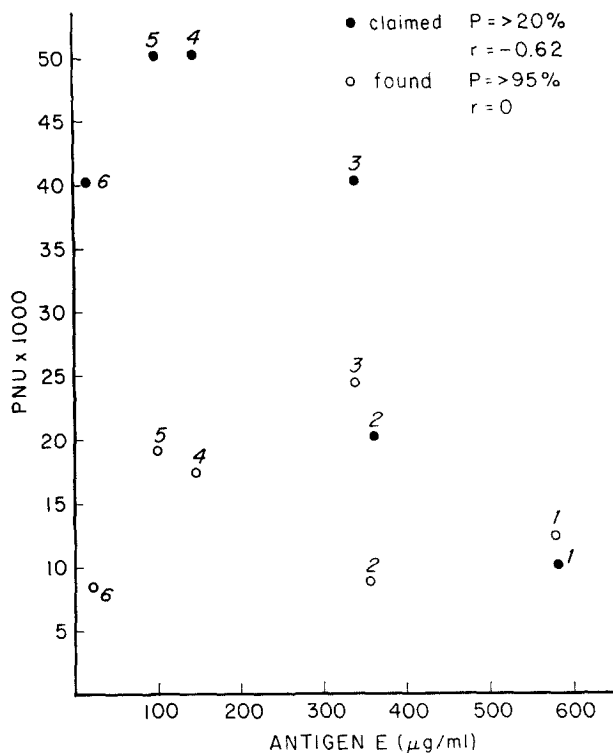


Fig. 6

The relationship between PNU and antigen E content.

protein in most samples of pollen extract,³ this finding is not surprising. It appears that immunologically unreactive proteinaceous material or nonproteinaceous nitrogen-containing material is precipitated by phosphotungstic acid, thus leading to total "protein" values which have little relationship to biologic activity in man.

The data should not be interpreted to mean that some manufacturers regularly prepare "poor" extracts while others prepare "good" extracts. A second sample of low ragweed extract was obtained from the supplier of the extract with the least antigen E and the least reactivity in human beings. This sample of a different lot had a high antigen E content and good biologic activity. Such variation is not surprising since no methods have been available for controlling the active specific antigen in different collections of pollen in the process of extraction or in the storage of the allergenic extracts.

Since there is considerable evidence that the results of immunotherapy depend on the dosage of active allergen, standardization becomes an increasingly important problem. Our data demonstrate that there can be a hundredfold difference between the active allergen in two batches of ragweed-allergenic extract bearing a label stating identical PNU content. As long as this continues to be the case it will be impossible to compare the efficacy of immunotherapeutic

trials carried out in different centers or even in the same center with different ragweed extract preparations.

In the case of ragweed pollen extracts, our evidence indicates that PNU determination based on phosphotungstic acid-precipitable protein is an unsatisfactory method for potency evaluation, whereas double diffusion measurement of antigen E shows promise of being a useful method. The double diffusion method employed here is simple and could be accomplished in different laboratories with comparable results if the same standard antiserum and antigen E were employed in each laboratory.

The advantages of a reasonably accurate method of standardization of allergenic extracts which is based on the constituent(s) responsible for reactivity in man are sufficiently obvious not to be listed here. Although ragweed is only one of a variety of allergenic extracts in use, it is, nevertheless, one of the more important ones. Considerable work will have to be done before similar standardization can be recommended for extracts other than ragweed. No other purified allergen has been as well characterized as antigen E, and it is possible that other extracts will not have single constituents which so accurately predict biologic activity.

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