

Capacity of purified antigens and whole pollen extracts to release histamine from leukocytes of hay fever patients*

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Eighty-seven consecutive patients appearing with complaints suggestive of spring and fall hay fever were subjected to basophil histamine release study with 4 antigenic preparations: whole ragweed extract, antigen E of ragweed, mixed grass pollen extract, and Group I antigen of rye grass. Among the 87 patients, 12 failed to release histamine to ragweed extract or antigen E and 16 failed to release histamine to grass extract or Group I. Among the remaining patients 5 reacted to whole ragweed extract but not antigen E and 2 reacted to mixed grass extract but not Group I. The patients with this pattern were all at the lower end of the spectrum of sensitivity for the crude extracts. These data tend to confirm that antigen E and Group I are the antigens of prime importance in the majority of hay fever patients with ragweed and grass pollen sensitivity respectively.

Since the isolation and partial characterization of antigen E of ragweed pollen,¹ considerable work has been directed toward assigning its proper role in the reaginic sensitivity that confers fall hay fever. A major role was originally suggested on the basis that (1) nearly every ragweed-sensitive patient reacted strongly to antigen E by skin test and histamine release and (2) absorption of antigen E from pollen extracts by immune precipitation removed 90 per cent or more of the skin test activity of the pollen extract in the patients tested.² That other proteins in pollen also had biologic activity seemed likely as other fractions of pollen showed skin test activity.² Furthermore, passive transfer sites of ragweed reaginic serum in the skin of normal volunteers could have their reactivity to antigen E exhausted but still show reactivity to whole pollen extract.³

Several such proteins have now been identified. Antigen K, a second antigen of ragweed, was subsequently isolated and shown to be highly reactive in hay fever patients.⁴ In almost all patients it was about one-half as active on a weight basis as antigen E. It is also less abundant in pollen so that it appears to play

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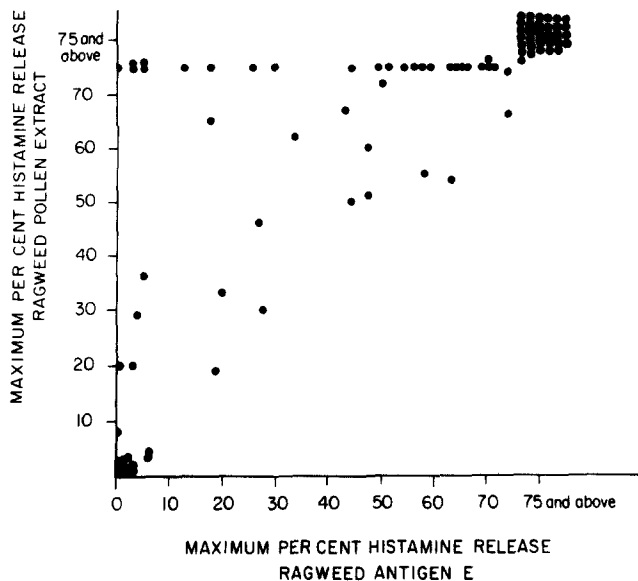


FIG. 1. Maximum per cent histamine release from ragweed antigen E (abscissa) vs. maximum per cent histamine release from ragweed pollen extract (87 unselected patients with spring and fall hay fever symptoms).

a relatively minor role. Ra3 and Ra5 are additional antigens of lower molecular weight that are highly active in some ragweed patients and relatively or totally inactive in others.^{5, 6} The situation is complex in that antigens E and K have considerable immunologic cross-reaction and that antigen E probably cross-reacts with other proteins (as yet not isolated) of ragweed.⁴ Ra3 and Ra5, on the other hand, clearly do not cross-react with antigen E.

Antigen E appears to be an important constituent of extracts used for immunotherapy. Purified antigen E is an adequate agent for injection therapy and in our hands gave clinical results as good as those obtained with whole extract.⁷⁻⁹ Others using low doses of antigen E found it to be somewhat less effective than whole extract but nevertheless noted therapeutic effects.³ The addition of antigen K to antigen E offered no therapeutic advantage.¹⁰ Although antigen E is therapeutically effective, it offers no advantage over whole extracts and, consequently, appears unlikely to be generally used because of the expense of isolation. However, subsequent studies have shown that determinations of antigen E content by a simple double diffusion in gel technique closely correlate with biologic activity of commercial ragweed extracts as measured by skin test or histamine release.¹¹ This has led to the suggestion that all extracts should be labeled for their content of antigen E, as protein nitrogen units (PNU) determinations have been shown to be unrelated to the biologic activity of commercial extracts.

The importance of antigen E was called into question by Melam, Pruzansky, and Patterson,¹² who reported that 4 of 12 ragweed-sensitive patients reacted by leukocyte histamine release to crude extract but not at all to antigen E. We

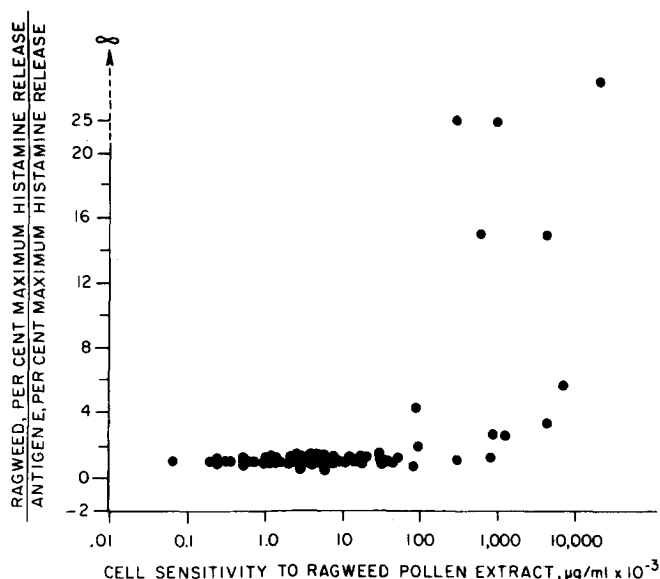


FIG. 2. Cell sensitivity to ragweed pollen extract vs. ratio of maximum per cent histamine release, ragweed, to maximum per cent histamine release, antigen E, in 63 patients with measurable cell sensitivity to ragweed extract.

were thereby prompted to restudy not only the question of antigen E in ragweed but also to collect data on the more general question of whether it is regularly true that one or a small number of specific proteins found in a pollen will account for its allergenic activity. The isolation of Group I antigen of rye grass pollen^{13, 14} and evidence that it stands in the same relationship to several grass pollens as antigen E does to ragweed^{15, 16} offered a chance to determine if the experience with ragweed could be applied to another common cause of hay fever.

Our data confirm that antigen E and Group I are of primary importance in ragweed and grass pollen sensitivity respectively in patients highly sensitive to the whole extracts. Exceptional patients may be sensitive to other constituents without being sensitive to the "major" constituent, but these patients are relatively insensitive to the whole pollen extract.

MATERIALS AND METHODS

Patients

Individuals who responded to a newspaper story asking for volunteers with "hay fever" were sent a simple questionnaire requesting information concerning their symptoms. Patients who responded indicating that they had stuffy or runny nose with or without eye symptoms during the late spring as well as the early fall but not perennially were included in the study if they agreed to participate. No further selection was exercised for the purpose of this study. Data was collected on 87 patients.

Allergens

Whole short ragweed pollen extract. Whole short ragweed pollen extract was prepared by the Hollister-Stier Labs., Spokane, Washington, for the Committee on Standardization of

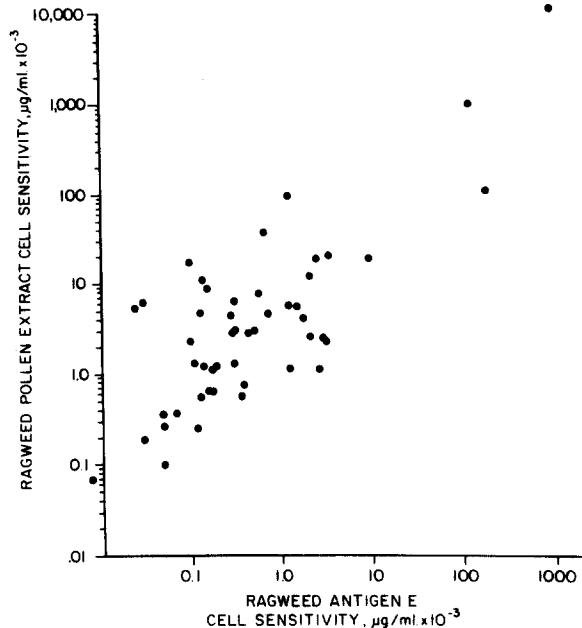


FIG. 3. Antigen E cell sensitivity (abscissa) vs. ragweed extract cell sensitivity in 48 patients with measurable cell sensitivity to both.

Allergens of the National Institute of Allergy and Infectious Diseases. It was furnished as a lyophilized extract containing 27 μg AgE per 10,000 PNU.

Whole grass pollen extract. Whole grass pollen extract was a gift from Dr. David G. Marsh and contained a mixture in the proportions: 30 per cent timothy, 30 per cent orchard, 20 per cent sweet vernal, 10 per cent red top, and 10 per cent June grass. It was furnished as a lyophilized extract containing 50 μg Group I per milligram nondialyzable solids by radial-immunodiffusion using rye Group I antiserum and rye Group I as a standard. It contained 4,400 PNU per milligram nondialyzable solid. This might be a figure quite different than would be obtained by PNU determinations on similar nondialyzed extracts.

Antigen E. Antigen E prepared by the method of King, Norman, and Connell² was furnished by Dr. Floyd McIntire of Abbott Laboratories, North Chicago, Ill.

Group I. Group I was a gift of Dr. D. G. Marsh.¹⁴ It was prepared from perennial rye grass pollen by the method of Johnson and Marsh.¹³

Histamine release studies. Histamine release studies were carried out by the techniques developed by Lichtenstein and Osler,¹⁷ following the micromodification described by May and associates.¹⁸ All studies were done with well washed cells in a serum-free medium except as indicated. Each of the allergen preparations was made up in one batch at the start of the study at a concentration of 10 γ protein per milliliter in tris-ACM buffer. They were aliquoted and stored at -20°C . until used. A fresh aliquot was thawed and used for each day's experimentation.

Symptom evaluation

Daily symptom diaries. Daily symptom diaries during the seasons of grass and ragweed pollination were scored as reported previously.⁸

Pollen counts

Daily pollen counts. Daily pollen counts during the grass and ragweed season were taken from an Ogden rotating slide sampler placed in a residential section of the city of Baltimore.

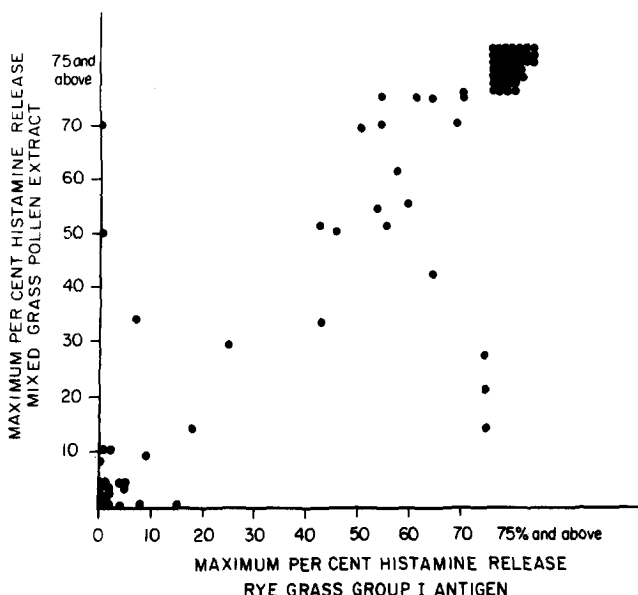


FIG. 4. Maximum per cent histamine release from Group I (abscissa) vs. maximum percent histamine release from mixed grass pollen extract (87 unselected patients with spring and fall hay fever symptoms).

RESULTS

The 87 patients who form the group reported in this study had histamine release determinations of leukocyte sensitivity performed to 4 antigens: whole ragweed extract, antigen E, whole mixed grass pollen extract, and Group I of rye grass. Complete dose-response curves using serial threefold dilutions were performed with each of these antigens twice on each patient. If the patient responded fully (80 per cent release or more), the concentration of antigen required for 50 per cent histamine release was interpolated. If there was less than a full response at any antigen concentration, the maximal per cent release was recorded.

Fig. 1 shows the relationship between histamine release to ragweed extract and to antigen E in the entire group. Twelve patients showed less than 15 per cent histamine release to *both* antigens and, therefore, cannot be considered further by this technique. In the remaining patients, there is strong relationship between the maximal histamine release to the two antigens. As might be expected, in some patients there is a tendency for maximal histamine release to be greater with whole ragweed extract than with antigen E. In only 5 (5.7 per cent) instances did this reach the extreme case described by Melam, Pruzansky, and Patterson¹² of no (or virtually no) release from antigen E but good release from ragweed extract.

There were 63 individuals with the 50 per cent or more histamine release to whole pollen extract required to determine a cell sensitivity. In these individuals the ratio

$$\frac{\% \text{ maximum histamine release, ragweed extract}}{\% \text{ maximum histamine release, antigen E}}$$

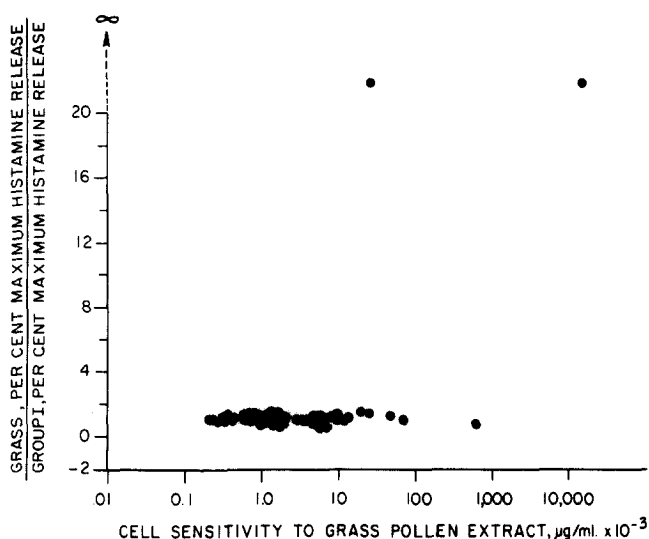


FIG. 5. Cell sensitivity to grass pollen extract (abscissa) vs. ratio of maximum per cent histamine release, mixed grass extract, to maximum per cent histamine release, rye Group I, in 60 patients with measurable cell sensitivity to grass pollen extract.

is plotted against cell sensitivity to whole extract (Fig. 2). It will be noted that ratios greater than one, i.e., better release to whole extract, occurred only in patients who were at the insensitive end of the scale. The 5 patients with highest ratios are those with good release to whole extract but poor release to antigen E. It will be seen that these patients lie at the extreme lower end of the scale of cell sensitivity.

Fig. 3 shows the relationship between sensitivity to antigen E and ragweed in the 48 patients in whom cell sensitivity to both antigens could be determined. These points lie remarkably close to a straight line and there is correlation coefficient of 0.73 ($p < 0.001$) between the two determinations. Such a picture does, of course, exclude patients with poor reactivity to antigen E.

As these patients were selected also to have spring hay fever during the season of grass pollination, it was of interest to examine similar relationships between a purified grass pollen allergen (i.e., the Group I allergen of rye grass isolated by Johnson and Marsh^{13, 14}) and the mixture of grass pollen extracts employed by us in the immunotherapy of grass hay fever. Fig. 4 shows the maximum per cent histamine release for the entire group of 87 patients. As with ragweed, some patients had virtually no histamine release to either crude or purified allergen and cannot be considered further. There were only 2 patients who had virtually no release to Group I but greater than 50 per cent release to whole grass extract. In this instance for reasons that are not clear there were 3 patients in whom release was more complete with the purified allergen than with crude material. Fig. 5 shows data from those 60 patients with 50 per cent or more release to mixed grass pollen extract and examines the ratio

$$\frac{\% \text{ maximum release, mixed grass poll extract}}{\% \text{ maximum release, Group I}} \text{ plotted}$$

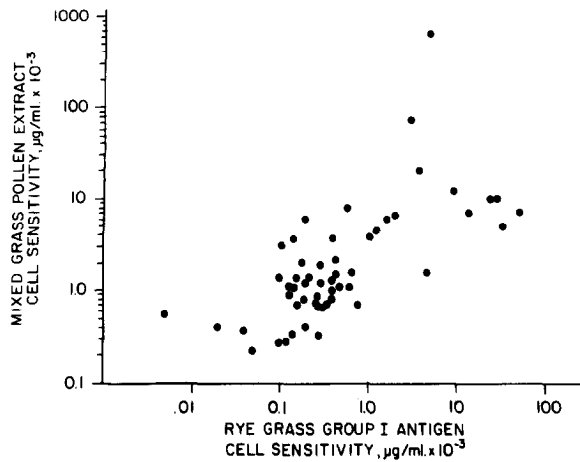


FIG. 6. Cell sensitivity to rye Group I (abscissa) vs. cell sensitivity to mixed grass pollen extract in patients with measurable cell sensitivity to both.

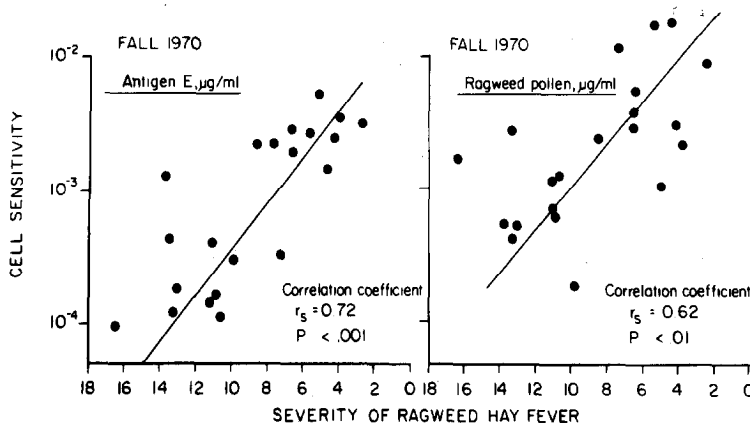


FIG. 7. Correlation between cell sensitivity to antigen E or ragweed pollen extract and symptoms during the 1970 ragweed pollination season.

against cell sensitivity to mixed grass pollen extract. Again, it is only in 2 patients at the lower end of the cell sensitivity spectrum that the ratio departs significantly from one. As with ragweed and antigen E, when the relationship between cell sensitivity to mixed grass pollen extract is plotted against cell sensitivity to Group I (Fig. 6), most points lie near a straight line with a correlation coefficient of +0.683 ($p < 0.001$).

Following these initial studies, most of these patients underwent immunotherapy so that it is impossible to consider the relationship between cell sensitivity and disease in the entire group. The results of the immunotherapy will be considered in a separate report. Twenty-one patients of the group were, however, given placebo treatment, and the relationship between cell sensitivity measurements and actual symptomatology during exposure may be considered. Fig. 7 shows the relationship between both cell sensitivity to antigen E and

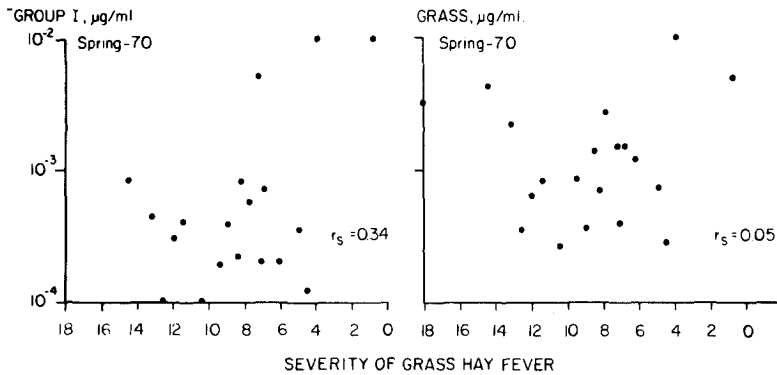


FIG. 8. Correlation between cell sensitivity to Group I or mixed grass pollen extract and symptoms during the 1970 grass pollination season.

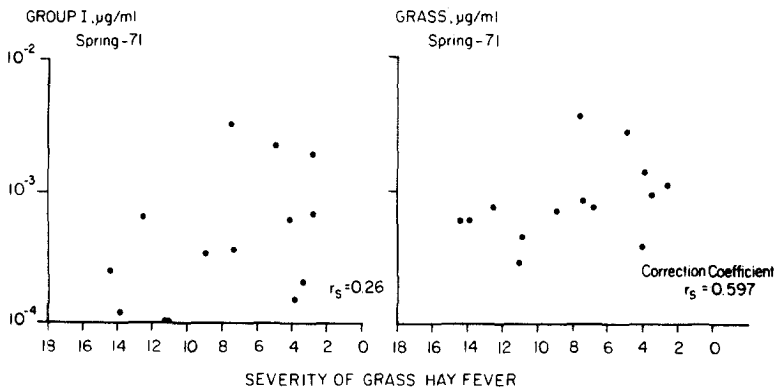


FIG. 9. Correlation between cell sensitivity to Group I or mixed grass pollen extract and symptoms during the 1971 grass pollination season.

ragweed pollen extract to symptom scores collected during the pollination season of 1969. There is good relationship between both in vitro measurements and symptom scores. Measurements with whole pollen extract appear not to represent any improvement over measurements with antigen E, and the correlation coefficient (Spearman rank correlation coefficient) with pollen extract is actually slightly lower.

Similar relationships between Group I and mixed grass extract are shown in Fig. 8 for the same patients during the grass pollination season of 1970. Although cell sensitivity measurements and symptoms appear to show some relationship, it is less close than that shown with ragweed. The correlation coefficient is lower and fails to meet the usual test of significance. Several reasons for this apparent lack of correlation may be mentioned: (1) In Maryland there is considerable overlap of grass pollination with the preceding oak pollination season and with the following plantain pollination and mold spore seasons. Consequently, patients' symptom scores in the spring may to some extent represent reactions to allergens other than grass. (2) The grass pollen mixture used for testing may

not be representative. The virtual impossibility of identifying individual grass pollens by morphology precludes the development of a combination that accurately reflects actual exposure. (3) Grass pollen, being heavier, may spread shorter distances than ragweed pollen. Exposure over an area as large as Baltimore may therefore be less homogenous, making it less feasible to rank patients for severity of sensitivity by symptoms score methods.

A better relationship appeared when these patients were followed through the spring season in 1971 (Fig. 9). It is, however, not significant with these numbers or as good as that observed during the ragweed season.

DISCUSSION

In an earlier report we noted that in 250 hay fever patients skin-tested with antigen E and whole ragweed extract, 2 patients were responsive to ragweed extract but not to antigen E.¹⁹ Although not reported fully at that time, these 2 patients were relatively *insensitive* to whole pollen extract, showing wheal reactions only at concentrations of 1,000 PNU per milliliter. At that time and subsequently our testing with these reagents has been performed on patients with clear histories of typical ragweed sensitivity and moderate to severe symptomatology. The report by Melam, Pruzansky, and Patterson¹² that 4 of 12 patients with ragweed hay fever from their clinic failed to respond with histamine release to antigen E¹² gave us a reason to study a group of patients with rhinitis who were less carefully selected to have a high degree of sensitivity. In 87 patients selected only to be symptomatic in some way during the fall season, 5 (5.6 per cent) were found who are similar to those noted by Melam, Pruzansky, and Patterson. When a similar study of mixed grass pollen extract and Group I was carried out, only 2 (2.3 per cent) patients were found who reacted fully with whole pollen extract but not to a purified allergen. With both grass and ragweed the patients who fit this pattern were at the very lower end of the sensitivity spectrum to the crude extracts. These patients were also relatively insensitive by skin test. The data presented by Melam, Pruzansky, and Patterson do not allow us to decide whether the same is true in their series of patients, as histamine release studies to crude extract are reported only on those patients who did not respond to antigen E. If it is correct that patients without reactions to antigen E are nearly all very low in sensitivity to whole extract, then the frequency with which this pattern is found will depend very much on the selection of patients for study through rigid (or not so rigid) criteria for diagnosis. Our methods of recruiting patients by calls for volunteers tend to bring in patients with "ordinary" or "uncomplicated" hay fever who less often consult the allergy specialist than the more chronically symptomatic patients with complex disease. In a subsequent paper we intend to present data on those patients whom we found to be relatively insensitive to ragweed by histamine release and skin test. These patients appear to have disease for the most part unrelated to exposure to ragweed.

Such data make us believe that the diagnosis of ragweed hay fever should be questioned in patients who fail to react to antigen E and that the diagnosis should be accepted only when symptoms have been demonstrated to follow pollen

exposure closely. A quantitative challenge test with ragweed pollen might be another way to verify the diagnosis in such patients.

The data presented here also bear on the use of purified allergens as standards for crude extracts. The strong correlation between biologic activity of the purified allergens of either ragweed or grass and their respective crude extracts indicates strongly that immunologic quantitation of the concentration of allergen in an unknown extract should predict the biologic activity of that extract. This has already been put to the test with ragweed extract where immunodiffusion determinations of antigen E have accurately predicted the activity by both skin test and histamine release of a variety of commercial extracts.¹¹ A similar study is under way relating Group I to grass pollen extracts.

Leaving aside questions of the primacy of antigen E or Group I in accounting for the activity of the pollen in patients, it can be pointed out that the presence of one particular antigen in an extract in appropriate quantity suggests that the procedures of preparation and storage have maintained the integrity of the other extractable antigens of pollen. The immunodiffusion techniques and materials for quantitation of antigen E and Group I in crude extracts are relatively simple and inexpensive so that labeling based on the content of these substances should be easy to achieve.

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